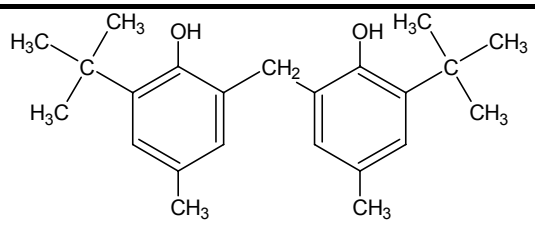
FOREWORD INTRODUCTION

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

CAS N°: 119-47-1

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	119-47-1
Chemical Name	6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol
Structural Formula	

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

Acute toxicity of this substance is low; oral LD_{50} values in animals is greater than 5,000 mg/kg. The substance is not irritating to skin and moderately irritating to eyes. There is no skin sensitization in humans. In repeated dose toxicity studies, including a rat 28-day repeated dose toxicity test [Japan TG], a preliminary reproduction toxicity screening test [OECD TG 421] and a rat 18 month chronic toxicity test, effects on sperm in the cauda epididymis and histopathological changes in the testis, such as degeneration of step 19 spermatids and vacuolation of Sertoli cells, were observed in the 42.3 mg/kg and higher dose groups. Based on the above results, the NOAEL for repeated dose toxicity is considered to be 12.5 mg/kg/day. In a reproductive/developmental toxicity study [OECD TG 421], the effects on reproductive parameters, such as decrease in number of corpora lutea, implantation scars and pups born, were observed in the 200 mg/kg/day and higher dose groups but not 50 mg/kg/day. Therefore, NOAEL for female reproductive toxicity is considered to be 50 mg/kg/day. However, NOAEL for male reproductive toxicity is 12.5 mg/kg/day because of the testicular toxicity as described above. As for the developmental toxicity, low body weight gain of offspring and increased number of stillbirths were observed at 800 but not 200 mg/kg/day. The teratogenic effects were not observed in a study with rats up to 375 mg/kg/day. Based on these findings, the NOAEL for developmental toxicity is considered to be 200 mg/kg/day. Three bacterial reverse mutation tests and mammalian chromosomal tests with and without metabolic activation show negative results [OECD TG 471, 472, 473]. No tumors were observed in a 18-month chronic feeding study with rats up to 1,000 ppm, however this study is not qualified to be regarded as a carcinogenicity study. Therefore, no conclusion could be reached on the carcinogenicity.

Environment

The substance is not readily biodegradable (MITI (I), corresponding to the OECD 301C ; 0 % after 28 days) and seemed not highly bioconcentrated according to OECD 305; BCF 23 – 125. The fugacity level III calculations suggest that the majority of the substance would distribute

into sediment if released to aquatic compartment. Relevant to the environmental hazard of the substance, only the toxicity to aquatic organisms (i.e. green algae, water flea and fish) has been actually measured. Due to low water solubility of the substance, homogenous solution could be attained only at the nominal concentration of 5.0 mg/L with the maximum allowable dispersant concentration of 100 mg/L. The acute $L(E)C_{50}$ values for these aquatic species are all greater than this solubilization limit (i.e. >4.8 – >5.0 mg/L). The lowest chronic value (i.e. 21day-NOEC) is 0.34 mg/L determined for the reproduction of *Daphnia magna*, and is also above the water solubility. There is some uncertainty connected to these toxicity values of apparent aquatic toxicity because they were all considerably above the water solubility. Assessment factor of 50 is used to this chronic value and the PNEC for the aquatic environment is estimated to be 0.0068 mg/L. Although no measured result on the toxicity to sediment dwelling organisms can be evaluated, the provisional PNEC for the sediment compartment is tentatively estimated to be 2.0 mg/kg according to the equilibrium partitioning method specified in the EU-TGD.

Exposure

Production volume of the substance is estimated ca. 1,000 - 1,200 t/year in Japan, and ca. 3,300-3,500 t/year world-wide.

The substance is applied exclusively for the use resulting in inclusion into or onto matrix; it is used in the polymers industry as antioxidants and/or stabilizers, and in the rubber industry as additives.

Consumer exposure: In consideration of the application of the substance (mostly for industrial use), consumer use is regarded not relevant in Japan (the migration of the substance is practically none; i.e. consumer exposure from the polymer/rubber is expected to be negligible).

Occupational exposure: During production, processing and use in Japan, occupational exposure at a production and industrial use sites is the only case for serious consideration; based on exposure monitoring at a production site, it was estimated as the worst case that the highest daily intake (EHE) is 0.0068 mg/kg/day if a worker is assigned to implement without any industrial hygiene protection.

Exposure to the environment: During production, processing and use in Japan, only the aquatic release of the substance at the production site seems to be possible.

NATURE OF FURTHER WORK RECOMMENDED

Exposure assessment at production, processing and use sites would be recommended at the national or regional levels because the low NOAEL of this chemical for repeated dose toxicity is established from the critical effect on testes.

Considering the distribution characteristics of the substance (i.e. distribution tendency to sediment if released to aquatic environment), and due to lack of measured toxicity value to the sediment dwelling organisms, further work characterizing aquatic hazards possibly including the sedimentary environment would be recommended. But this work would be conducted if significant emission to the environment is evidenced from the above exposure assessment.

FULL SIDS SUMMARY

CAS NO: 119-47-1		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point			130-131 °C
2.2	Boiling Point			187 °C (at 0.07 hPa)
2.3	Density			1.08 g/cm^3
2.4	Vapour Pressure		Calculated	4.7 x 10^{-11} Pa
2.5	Partition Coefficient (Log Pow)		Unknown	6.25
2.6 A	Water Solubility			0.02 mg/l
B	pH			No data
	pKa			No data
2.12	Oxidation: Reduction Potential			$E_{1/2}$ = 0.35 mV
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation		calc.(Atkinson)	In air $T_{1/2}$ = 3.141 hours
3.1.2	Stability in Water		/	$T_{1/2}$ = min
3.2	Monitoring Data		/	No data
3.3	Transport and Distribution		Calculated (Fugacity Level III type)	Release 100% to air (99.8% to soil and 0.1% to sediment), Release 100% to water (6.9% to water and 93.1% to sediment) Release 100% to soil (99.9% to soil and 0.1% to sediment)
3.5	Biodegradation		OECD 301C	No biodegradation observed
3.7	Bioaccumulation	Carp (Cyprinus carpio)	OECD 305C	BCF(8 weeks) = 23-37 (1.0 mg/L), 60-125 (0.1 mg/L)
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	Oryzias latipes	OECD 203	LC_{50} (24 hr) >5.0 mg/l, LC_{50} (48 hr) >5.0 mg/l, LC_{50} (72 hr) >5.0mg/l, LC_{50} (96 hr) >5.0 mg/l, NOEC (96 hr) = 5.0 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates *Daphnia*	Daphnia magna	OECD 202	EC_{50} (24 hr) >4.8 mg/l, EC_{50} (48 hr) >4.8 mg/l, NOEC (48 hr) = 0.74 mg/L.
4.3	Toxicity to Aquatic Plants e.g. Algae	Selenastrum capricornutum (ATCC22662 strain)	OECD 201	Biomass: EC_{50} (0-72 hr) >5.0 mg/l, NOEC (0-72 hr) = 0.63 mg/l. Growth rate: EC_{50} (24-72 hr) >5.0 mg/l, NOEC (24-72 hr) = 1.3 mg/l.
4.5.2	Chronic Toxicity to Aquatic Invertebrates (*Daphnia*)	Daphnia magna	OECD 211	Mortality: LC_{50} (21 d) = 1.0 mg/l Reproduction: EC_{50} (21 d) = 1.1 mg/l NOEC (21 d) = 0.34 mg/l
4.6.1	Toxicity to Soil Dwelling Organisms		/	No data
4.6.2	Toxicity to Terrestrial Plants			No data

CAS NO: 119-47-1		SPECIES	PROTOCOL	RESULTS
(4.6.3)	Toxicity to Other Non-Mammalian Terrestrial Species (Including Birds)			No data
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat	Unknown	LD_{50} > 5,000 mg/kg
		Rat	Unknown	LD_{50} >10,000 mg/kg
		Rat	Unknown	LD_{50} 5,000 mg/kg
		Mouse	Unknown	LD_{50} 11,000 mg/kg
5.1.2	Acute Inhalation Toxicity	Other*	Unknown	LC_0 100 mg/m^3
5.1.3	Acute Dermal Toxicity	Rabbit	Unknown	LD_{50} > 10,000 mg/kg
5.2.1	Skin Irritation/Corrosion	Rabbit	Unknown	Not irritating
5.2.2	Eye Irritation/Corrosion	Rabbit	Unknown	moderately irritating
5.3	Skin sensitization	Human	Unknown	Not sensitizing
5.4	Repeated Dose Toxicity	Rat (18 month)	Unknown	NOAEL 12.7 mg/kg/day**
		Rat (12 week)	Unknown	LOAEL 60 mg/kg/day**
		Rat (90 day)	Unknown	NOAEL 16.5 mg/kg/day**
		Rat (53 day)	OECD 421	NOAEL 12.5 mg/kg/day
		Rat (28 day)	JP TG	LOAEL 50 mg/kg/day
		Dog (90 days)	Unknown	NOAEL 11 mg/kg/day**
5.5	Genetic Toxicity in vitro			
A.	Bacterial Test	*S. typhimurium*	OECD 471	Negative
		E. coli	OECD 472	Negative
B.	Non-Bacterial In Vitro Test	CHL/IU	OECD 473	Negative
5.6	Genetic Toxicity In Vivo			No data
5.7	Carcinogenicity	Rat	Unknown	No neoplastic lesions (the study is not regarded as a carcinogenicity study, therefore, inadequate)
5.8	Toxicity to Reproduction	Rat	OECD 421	NOAEL (female) 50 mg/kg/day NOAEL (male) 12.5 mg/kg/day (based on testicular toxicity in 5.4 repeated dose toxicity)
5.9	Developmental Toxicity/ Teratogenicity	Rat	OECD 421	NOAEL 200 mg/kg/day
		Rat	Unknown	Not teratogenic NOAEL for Maternal Toxicity: 93.5 mg/kg/day

* Species : Not specified in IUCLID/ original report: Not available
** NOAEL & LOAEL values were converted to mg/kg/day from ppm of dietary exposure

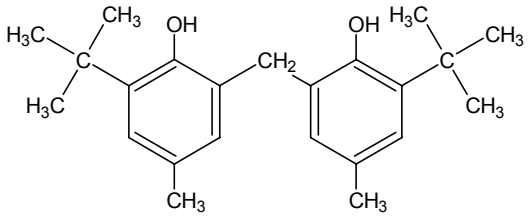
SIDS INITIAL ASSESSMENT REPORT (SIAR)

for

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol

1. IDENTITY

IUPAC name: 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
CAS number: 119-47-1
Molecular formula: $C_{23}H_{32}O_2$
Structural formula:



Synonyms: (Chemical Name)
2,2'-Methylenebis(4-methyl-6-*tert*-butylphenol); 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol; 2,2'-Methylene-bis(6-*tert*-butyl-*p*-cresol); 2,2'-Methylenebis(6-*tert*-butyl-4-methylphenol).

(Trade Name)
Sumilizer MDP; Lowinox 22M46; Vulkanox BKF; Yoshinox 2246; Cyanox 2246; Santowhite PC.

Purity: >= 98.0% weight/weight.
Impurities: None.
Additives: None.

Physical and chemical properties:

ITEMS	PROTOCOL	RESULTS
Melting Point	Unknown	130-131 °C
Boiling Point	Unknown	187 °C (at 0.07 hPa)
Vapour Pressure	Calculated	4.7×10^{-11} Pa
Partition Coefficient (Log Pow)	Flask shaking method	6.25
Water Solubility	Unknown	0.02 mg/l
pH / pKa		No data
Oxidation /Reduction potential	Anodic Voltammetry	$E_{1/2}$ = 0.35 mV

2. GENERAL INFORMATION ON EXPOSURE

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol is produced in a semi-open batch system. The production volume of the substance is estimated ca. 1,000 - 1,200 t/year in Japan, and ca. 3,300-

3,500 t/year world-wide; major produces are located in Germany, Japan, United Kingdom, France and the United States.

The substance is applied exclusively for the use resulting in inclusion into or onto matrix; it is used in the polymers industry as antioxidants and/or stabilizers, and in the rubber industry as additives. The exposure of the substance is limited to industrial use (such as rubber tire and engineering plastics) in Japan.

In consideration of the application of the substance (mostly for industrial use), consumer use is regarded not relevant in Japan.

During production, processing and use in Japan, occupational exposure at a production and industrial use sites is the only case for serious consideration. As for exposure to the environment, the aquatic release of the substance at the production site seems to be possible. But the estimated emission amount would be practically negligible, and thus, exposure to environmental organisms would be very low (Appendix 1).

2.1 Environmental Fate

The substance is not readily biodegradable (MITI (I), corresponding to the OECD 301C: 0% after 28 days based on BOD and 1% based on HPLC analysis).

The substance, if released to the air compartment, will react with photochemically-produced hydroxyl radicals with the half life of 3.141 hours (estimated with the Atkinson model).

Fugacity level III calculations suggest that the majority of the substance would be distributed into soil if released to soil and/or air compartment(s), and sediment if released to aquatic compartment.

When the substance is released to air, significant amount of the substance would be firstly photo-degraded, and then any undegraded substance would be deposited to soil where the degradation rate would be very slow. In contrast, when the substance is released to soil directly, it would remain in soil since its solubility is low, it is essentially non-volatile, and the degradation rate in soil would be low either. Thus, even though the resulting distribution tendency to soil is similar for both the air and soil emissions, the amount in the soil would be significantly different from one another (i.e. very little when released to air on a mass basis).

In spite of high hydrophobicity (e.g. log Pow ; 6.25), the substance is not so highly bioconcentrated (according to OECD 305; BCF 23 – 125) in aquatic organism (e.g. BCF 23 - 125), probably due to the restricted permeability through the fish gill.

(Note) Appendix 1 shows the Predicted Environmental Concentration (PEC) calculated with the worst case scenario in Japan.

2.2 Occupational Exposure

The following estimations are based on the situations in Japan.

Occupational exposures at production sites may occur through the inhalation (dust) route.

The atmospheric concentration was measured at a production site. The monitored data are shown in the Table 1.

Table 1: Workplace monitoring data for 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol

Industrial activity (Country)	Sampling area at a production site (equipment)	Operating conditions	Monitoring data	Source
Manufacturing of 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (Japan)	1) Dryer area (dryer) 2) Hopper area (product hopper) 3) Packing room (Packing machine)	All the equipment is operated automatically	1) <0.01 mg/m^3 2) <0.01 mg/m^3 3) 0.38 mg/m^3	Sumitomo Chemical (1997), unpublished report

(Monitoring method)
Air sample was suctioned at the breathing zone(1.5 m in height) of the worker at the suction rate of 500L/min for 30min. and was passed through a filter. The substance collected on the filter was dissolved in a solvent and analyzed quantitatively by HPLC method. The identity of the substance was confirmed by GC/MS.

This substance is a solid having extremely low vapor pressure (4.7 x 10^{-11} Pa), therefore, the dermal or vapor exposure is practically none.

The workers are exposed to the substance through the dust during the production. Among the production sites, the loading (packing) is the most dusty and expected to be at the highest exposure level (0.38 mg/m^3). If the worker (body weight; 70 kg, respiratory volume; 1.25 m^3/hr, exposure period; 1hour) is assigned to implement without protection, the highest daily intake (EHE) is calculated to be 0.0068 mg/kg/day as the worst case. Normally, workers wear respiratory protective equipment (mask) during the operation (Appendix 3).

As for the consumer products, the substance is used as antioxidant/stabilizer in the polymers and rubbers, of which contents are less than 1%. So, even if contact with the products, the migration of the substance is practically none; i.e. consumer exposure from the polymer/rubber is expected to be negligible.

Some of the substance might be released from the facilities through the waste water. The concentration in the surface water was calculated using the method in EU-TGD (1996) to find 3.8 x 10^{-7} mg/L (Appendix 1).

The consumer exposure through the drinking water was also calculated using the method in EU-TGD(1996), of which daily intake (EHE) is negligible (6.8 x 10^{-10} mg/kg/day) (Appendix 4).

NOTE: Other than the Japanese situations described above, no quantitative estimations could be made enough. Thus, in the SIAM-11, it was compromised that exposure assessment in national or regional levels (except Japan) should be recommended as a POST-SIDS work by taking the estimated possible hazard of this substance (especially on human health) into account.

3. EFFECTS ON HUMAN HEALTH

a) Toxicokinetics and metabolism

There is no available information on toxicokinetics and metabolism of this substance.

b) Acute toxicity

There is no key study. Available data are shown in Table 2. Acute oral and dermal data show that the LD_{50} values were greater than or equal to 5000 mg/kg. Inhalation data is poor in contents, especially, species is not specified. However, the LC_0 is not inconsistent with above finding. Based on these information, acute toxicity of this substance is considered to be low.

Table 2: Acute toxicity of 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol

Route	Animals	Values	Type	References
Oral				
	Rat	> 5,000 mg/kg	LD_{50}	Sumitomo Chemical (1977a)
	Rat	> 5,000 mg/kg	LD_{50}	Takagi, A. et al. (1994)
	Rat	>10,000 mg/kg	LD_{50}	Bayer AG (1988), ACC (1988)
	Rat	5,000 mg/kg	LD_{50}	Stasenkova et al (1977)
	Mouse	11,000 mg/kg	LD_{50}	Stasenkova et al (1977)
Inhalation				
	Other*	100 mg/m^3 (4 hr)	LC_0**	Bayer AG (IUCLID, 1996)
Dermal				
	Rabbit	> 10,000 mg/kg	LD_{50}	American Cyanamid (1988)

**: species is not specified, **:LC_0: no lethal concentration, in this case, no toxic symptom was observed*

There is no available information on humans.

Conclusions:
Acute toxicity of this substance is low because LD_{50} values by oral and dermal exposure routes are high; greater than 5000 mg/kg.

c) Repeated oral dose toxicity

Five studies were reviewed (Table 3). All the studies were carried out by oral administration (gavage or feeding), ranging from 28 days to 18 months in experimental period. Toxic effects were similar in the studies, showing the effects on testis, liver and body weight gain. The NOAELs or LOAELs are also consistent; the NOAELs ranging from 12.5 to 16.5 mg/kg/day and the LOAELs from 42.3 to 60 mg/kg/day. Among these studies, a rat 28-day study (MHW, Japan, 1996), a rat 53-day study (MHW, Japan,1999) and a rat 18-month study (Takagi et al.,1994) were considered to be the most reliable because the studies were conducted under the well-designed protocols and giving the detail information. The details of these studies are as follows.

A rat 28-day repeated oral dose toxicity test was conducted under the guideline of Japanese government (GLP). SD rats (6/sex/dose) received gavage doses of 0 (vehicle; 5% gum Arabic), 50, 200 and 800 mg/kg/day for 28 days. Hematological examination revealed prolongation of PT and APTT (male: more than 50 mg/kg, female: more than 200 mg/kg). In the pathological examination, increase in liver weight was observed (male: more than 50 mg/kg, female: more than 200 mg/kg). The histopathological examination showed hepatocyte hypertrophy (male & female: more than 200 mg/kg) and changes in testis, such as degeneration of step19 spermatids and vacuolation of Sertoli cells (male: more than 50 mg/kg).

Based on the above results, the NOAELs are considered to be less than 50 mg/kg/day for male and 50 mg/kg/day for female (MHW, Japan, 1996).

A rat 53-day oral dose toxicity test was conducted after the above study for confirming the NOAEL, adding a lower dose group and sperm examinations. Under OECD TG 421 (Preliminary Reproduction Toxicity Screening Test), SD rats (12/sex/dose) received gavage doses of 0, 12.5, 50, 200 and 800 mg/kg/day for 50-52 days in male and for 40-48 days from 14 days before mating, throughout pregnancy to day 3 of lactation in female. In male, increase in abnormal sperm ratio, decrease in sperm motility ratio and in the number of sperm in the cauda epididymis and histopathologically giant cell formation in the testis were observed in 50 mg/kg and higher dose groups. Atrophy and degeneration of seminiferous tubules, atrophy of the testis and epididymis, and decrease in the absolute and relative testis and epididymis weight were observed at 200 and 800 mg/kg. In female, suppression of body weight gain and lower food consumption were noted in the 200 mg/kg and higher dose groups.

Based on the above results, the NOAELs are considered to be 12.5 mg/kg/day for male and 50mg/kg/day for female (MHW, Japan, 1999).

A rat 18-month chronic oral dose toxicity study was conducted with Wistar rats (30/sex/dose), fed in doses of 0, 100, 300 and 1000 ppm. In the 300 ppm and lower dose groups, no significant effect was observed. Suppression of body weight gain and increase in liver weight were observed at 1000 ppm of both sexes. Decrease in testis weight and the histopathological changes in the testis and the epididymis were also noted in male at 1000 ppm, which is equivalent to 42.3 mg/kg/day.

Based on the above results, the NOAELs are considered to be 12.7 mg/kg/day (300 ppm) for male and 15.1 mg/kg/day (300 ppm) for female (Takagi et al., 1994).

Table 3: Repeated oral dose toxicity of 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol

Route	Animal	Period	Doses	NOAEL mg/kg/day	LOAEL mg/kg/day	Toxic effects	Reference
Oral	Rat						
		18 Month	100, 300, 1000 ppm	12.7	42.3	Testis, Liver, BW	Takagi (1994)
		12 Week	1200,6000,30000ppm		60	Testis, Liver, BW	Takagi (1994)
		90 day	330, 1000, 3000 ppm	16.5	50	Testis, Liver	ACC (1965)
		53 day	12.5,50,200,800 mg/kg	12.5	50	Testis, BW	MHW (1999)
		28 day	50, 200, 800 mg/kg		50	Testis, Liver, hematol.	MHW (1996)

ACC: American Cyanamid Co., hematol.; Hematology(PT & APTT)
BW: suppression of body weight gain
Some of NOAEL & LOAEL values were converted to mg/kg/day from ppm of dietary exposure

There is no available information on human toxicity.

Conclusions:
Toxic effects in repeated dose toxicity studies are effects on the sperm in the cauda epididymis and histopathological changes in testis.

The NOAEL for repeated dose toxicity in animal is 12.5 mg/kg/day, based on 53-day toxicity study

d) Reproduction/developmental toxicity

Two were considered to be key studies. One is a teratogenicity study and the other is Preliminary Reproduction Toxicity Screening Test [OECD TG 421]. Both were conducted under the well-designed protocols and giving the detail information. The details of these studies are as follows;

A teratogenicity test was conducted with Wistar rats by gavage administration at doses of 0, 93.5, 187 and 375 mg/kg for the day 7-17 of pregnancy (15-23 dams/ dose)[Tanaka et al., 1990]. The maternal toxic effects, such as suppression of body weight, were observed in 187 mg/kg and higher dose groups and fetal toxic effects, such as fetal death, were noted at 375 mg/kg. There was no teratogenic effect in external, visceral and skeletal observations

A preliminary reproduction toxicity study were carried out with SD rats by gavage administration at doses of 0 (vehicle; 5 % gum Arabic), 12.5, 50, 200 and 800 mg/kg from 14 days before mating to 14 days after mating in male and from 14 days before mating to the day 3 of lactation in female [MHW, Japan: 1999]. As for the female reproductive toxicity, decrease in number of corpora lutea, implantation scars and pups born were observed in the 200 mg/kg and higher dose groups. As for the developmental toxicity, low body weight gain of offspring and increased number of stillbirths were observed at 800 mg/kg/day, but not at 200 mg/kg/day.

Based on the above results, the NOAELs are considered to be 50 mg/kg/day for female reproductive toxicity and 200 mg/kg for developmental toxicity. As for male reproductive toxicity, the NOAEL is considered to be 12.5 mg/kg/day, based on testicular toxicity as described in c) Repeated oral dose toxicity.

There is no available information on humans.

Conclusions:
Effects on female reproductive parameters are decrease in corpora lutea, implantation scars, pups born, and those on developmental parameters are low body weight gain of offspring and increased number of stillbirths. The teratogenic effects were not observed.

The NOAEL for female reproductive toxicity is 50 mg/kg/day and the NOAEL for male reproductive toxicity is 12.5 mg/kg/day because of the testicular toxicity. The NOAEL for developmental toxicity is 200 mg/kg/day..

e) Genotoxicity

This substance has been investigated in in vitro tests, of which results showed negative in a DNA damage study with bacteria (Sumitomo Chemical, 1977b), 3 bacterial reverse mutation studies (MHW, Japan, 1996, Sumitomo Chemical, 1977b, Yamaguchi et al.,1991) and a chromosomal aberration study with mammalian cultured cells (MHW, Japan,1996), with and without an exogenous metabolic activation systems. Among these studies, MHW studies were identified to be key studies because they were well conducted and reported.

Reverse gene mutation assay was conducted by OECD TG 471 and TG 472. The substance was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 *uvr*A at concentration of up to 5000 μg /plate (The precipitation was observed at more than 313 mg/plate without S9 mix and at more than 625 mg/plate with S9 mix), with and without an exogenous metabolic activation system (MHW, Japan, 1996).

Chromosomal aberration test by OECD TG 473 was conducted in cultured Chinese hamster lung (CHL/IU) cells. Structural chromosomal aberrations and polyploidy were not induced up to a

maximum concentration of 0.008 mg/ml on continuous treatment, and 0.03 or 0.001 mg/ml on short-term treatment, with and without an exogenous metabolic activation systems, respectively (MHW, Japan, 1996).

There were no available data on genotoxicity *in vivo*.

Conclusions:
This substance is not genotoxic with and without an exogenous metabolic activation system in bacteria and mammalian cells.

f) Carcinogenicity

A 18 month chronic toxicity study was conducted with Wistar rats (30/sex/dose), fed in doses of 0, 100, 300 and 1000 ppm (male 42.3mg/kg/day, female 54.3mg/kg/day). Suppression of body weight gain and increase in liver weight were observed at the highest dose in both sexes. The histopathological examination revealed no neoplastic lesion attributable to the substance in any organs of either sex (Takagi et al., 1994).

Conclusions:
No tumors were observed in a 18-month chronic feeding study with rats up to 1, 000 ppm, however this study is not qualified to be regarded as a carcinogenicity study. Therefore, no definite conclusion could be reached on the carcinogenicity.

g) Other human health related information

Irritation and sensitization

- No skin irritation in rabbits was reported (American Cyanamid, 1988).
- Moderate eye irritation in rabbits was reported (Anon, 1986).
- No skin sensitization was observed in human maximization test with 25 adult men (American Cyanamid, 1980).

Conclusions*:*
This substance is not irritating to skin and moderately irritating to eyes in rabbits. There is no skin sensitization in human.

Data on the structurally related substance

Two repeated dose oral toxicity studies were carried out on the 4,4'-diethyl analog, 6,6'-di-tert-Butyl-4,4'-diethyl-2,2'-methylenediphenol (CAS No. 88-24-2) .

- In a 4 and 12 week-feeding rat study at doses of 2000, 10000, 50000 ppm, testicular atrophy and decrease of spermatogenesis were observed in the 10000 ppm and higher dose groups (Takagi et al., 1992).
- The other study was a 18 month-feeding rat study at doses of 300, 1000, 3000 ppm. Any histopathological and toxicological effects on testis were not observed even at the highest dose. (Takagi et al., 1996).

Conclusions*:*
A structurally-related compound, 4,4'-diethyl analog of the substance, is reported to have effects on testis. However, the effect is considered to be a little bit weaker.

3.2 Initial Assessment for Human Health

There is no available information on toxicokinetics and metabolism of this substance. Acute toxicity of the substance is low. In the repeated dose toxicity studies, effects on sperm in the cauda epididymis and histopathological changes in testis were observed in the 42.3 mg/kg and higher dose groups. The NOAEL for repeated dose oral toxicity is 12.5 mg/kg/day. In a reproductive/developmental toxicity study, effects on female reproductive toxicity were observed at 200 mg/kg/day and higher dose groups and those on developmental toxicity were at 800 mg/kg/day. The teratogenic effect was not observed up to 375 mg/kg/day. The NOAEL for female reproductive toxicity is 50 mg/kg/day and that for male reproductive toxicity is 12.5 mg/kg/day based on testicular toxicity as described above. The NOAEL for developmental toxicity is 200 mg/kg/day. The substance is not genotoxic with and without an exogenous metabolic activation system in bacterial and mammalian cells. No tumors were observed in a 18-month chronic feeding study with rats up to 1, 000 ppm, however this study is not qualified to be regarded as a carcinogenicity study. As for other human related information, this substance is not irritating to skin and moderately irritating to eyes in rabbits. There is no skin sensitization in human

4. EFFECTS ON THE ENVIRONMENT

4.1 Aquatic Effects

In the following table, the most relevant results from acute and chronic tests with aquatic organisms are presented:

Table 4: Aquatic Toxicity of 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol

Organism	Test duration	Result (mg/L)	Reference
Aquatic plants Green algae (*Selenastrum capricornutum*)	72 hr (cl)	EC_{50} (bms) >5.0 (nc*) EC_{50} (gr) >5.0 (nc*) NOEC (bms) =0.63 (nc*) NOEC (gr) =1.3 (nc*)	EA Japan (1999)
Invertebrates Water flea (*Daphnia magna*)	48 hr (op, s) 21 d (op, ss)	EC_{50} (imm) >4.8 (mc) NOEC (imm) =0.74 (mc) LC_{50} =1.0 (mc) EC_{50} (rep) =1.1 (mc) NOEC (rep) =0.34 (mc) LOEC (rep) =0.89 (mc)	EA Japan (1999) EA Japan (1999)
Fish Medaka (*Oryzias latipes*) Fathead minnow (*Pimephales promelas*)	96 h (op, ss) 48 h (op, s/ss) 96 hr (op, s)	LC_{50} >5.0 (nc*) NOEC =5.0 (nc*) LC_{50} >500 LC_{50} =0.31 (nc)	EA Japan (1999) CITI Japan (1992) ABC Lab. (1983)

cl ; closed system, op ;open system
s ; static, ss ;semi-static
nc; nominal concentration (actual concentration not measured), mc;measured concentration, nc; nominal concentration (actual concentration measured and greater than 80% of the nominal)*
bms ; biomass, gr ;growth rate, imm ;immobility, rep ;reproduction

Due to low water solubility of the substance, homogenous solution could be attained only at (and less than) the nominal concentration of 5.0 mg/L with the maximum allowable dispersant (i.e. DMF/Castor oil HCO-40, 3/1 (w/w)) concentration of 100 mg/L (see below NOTE). Therefore, in some of the studies listed in the table, no definite $L(E)C_{50}$ values could be determined (i.e. greater than the solubilization limit).

In the green algal test, the EC_{50} values on both growth rate and biomass were greater than 5.0mg/L. The NOEC values determined were 0.63 mg/L and 1.3 mg/L. Similarly, the acute EC_{50} value to water flea was greater than this solubilization limit (i.e. >4.8 mg/L based on the measured concentration). But in the chronic test with water flea, the definite $L(E)C_{50}$ values were evaluated to be around 1 mg/L (i.e. 1.0 mg/L and 1.1 mg/L). The NOEC for the reproduction of water flea was 0.34 mg/L (the lowest chronic NOEC in all the studies). For fish acute toxicity, three tests results were found, but the reported LC_{50} values were remarkably different from one to another (i.e. >5.0 mg/L and >500 mg/L for medaka and 0.31 mg/L for fathead minnow). Due to lack of essential data especially on the specification of test substance as well as the analytical monitoring, the LC_{50} value for fathead minnow (i.e. 0.31 mg/L) could not be employed in the hazard assessment described below. The LC_{50} value of more than 500 mg/L for Medaka was not either employed in the assessment because of its distinct study design. For fish chronic toxicity, no experimental data was found out, but from the bioconcentration study with carp where no toxic symptoms were observed at 1.0 and 0.10 mg/L during 8 weeks exposure, it is suggested that the chronic NOEC to fish would be greater than 1.0 mg/L.

There is no available information on the toxicity measured with sediment dwelling organisms.

NOTE:
Although the tested nominal concentrations were relatively high compared to the water solubility, the results of all the studies (employed in the assessment) are considered to be quite valid because:

(1) All the studies employed in the assessment were conducted according to the OECD guidelines.
(2) It is clarified that no precipitations as well as no emulsions have been observed by visual inspections for up to 5.0 mg/L of exposure concentrations (i.e. suggesting the substance bioavailable),
(3) Clear conc.-effect relationships as well as progressivity of the effects were observed in the definitive Daphnia acute/chronic and Algal inhibition studies (i.e. also suggesting the substance bioavailable),
(4) No toxic symptoms in fish during 96 hrs of exposure can be well understood by taking the restricted uptake through fish gill membrane of the steric hindered molecule into account as suggested in the bioconcentration study.

(See the corresponding parts in the robust summary for more detailed information.)

However, by the discussions with some authorities in the SIAM-11 (especially on the lowest chronic NOEC of 0.34 mg/L, being significantly above the solubility of 0.02 mg/L), it was compromised that the sentence “There is some uncertainty connected to these toxicity values of apparent aquatic toxicity because they were all considerably above the water solubility” should be added to the SIAP, and the further relevant ecotoxicity studies with aquatic organisms should be recommended if significant emission of the substance is evidenced by the POST-SIDS exposure assessment.

4.2. Terrestrial Effects

There is no available information.

4.3. Other

There is no available information.

4.4. Initial Assessment for the Environment

It is possible that the substance would be released into aquatic environment, and show tendencies to distribute into the sediment compartment. The substance is not readily biodegradable and is not highly bioconcentrated.

The PNEC for the aquatic compartment is estimated based on the reported chronic NOECs considered as reliable. Since two chronic NOECs from species representing two trophic levels (i.e. those from the chronic daphnid and algal tests) are obtained, the assessment factor of 50 is employed. By applying this assessment factor to the lowest NOEC (i.e. 0.34 mg/L), the PNEC is estimated as indicated below.

PNEC (aquatic) = 0.34 / 50 = 0.0068 mg/L

Additionally, since the majority of the substance would be distributed to the sediment compartment if released to the aquatic environment, the PNEC for the sediment compartment is also estimated tentatively. Due to lack of measured data on the toxicity to sediment dwelling organisms, the value is estimated by using the above PNEC for the aquatic compartment according to the equilibrium partitioning method specified in the EU-TGD (1996). In the equilibrium partitioning method, it is assumed that (1) the sensitivity to the substance is equivalent between sediment-dwelling organisms and water column organisms, and (2) the concentrations in sediment, interstitial water and benthic organisms are thermodynamically equilibrated. The provisional PNEC for the sediment compartment, thus obtained, is as follows:

Ksed-water = F water_sed + F solid_sed x (Kp sed / 1000) x RHO solid

where F water_sed : 0.8 m^3/m^3 (default),
F solid_sed : 0.2 m^3/m^3 (default),
Kp sed : 7500 L/kg (see Appendix 1)
RHO solid : 2500 kg/m^3 (default).
Thus, Ksed-water = 3800 m^3/m^3.

PNEC (sediment) = (Ksed-water / RHOsed) x PNEC (aquatic) x COR x 1000

where RHO sed : 1300 kg/m^3 (default).
COR : 1/10 (An additional factor for correcting the uptake via ingestion of sediment for such hydrophobic chemicals with logP of more than 5)
Thus, PNEC (sediment) = 2.0 mg/kg.

The risk quotients (i.e. the ratio of the PEC to the PNEC) estimated with the worst case scenario in Japan are shown in Appendix 2.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Physical/chemical property, production, use and distribution
This substance is a solid substance with very low volatility. The substance is of low solubility in water (0.02 mg/L), not readily biodegradable (OECD TG301C: 0% after 28 days) but not so highly bioaccumulative in fish (previous OECD TG305C: BCF = 23-37 (1.0 mg/L) / 60-125 (0.1 mg/L)).

A fugacity calculation shows that this substance would be distributed mainly to soil and sediment.

As this substance is used in polymers industry as antioxidant and/or stabilizer, and in rubber industry as additives, worker exposure may take place at production sites and user sites in the industry. Consumer use is not relevant in Japan.

During production, processing and use in Japan, only the aquatic release of the substance at the production site seems to be possible. But the estimated emission amount would be practically negligible, and thus, exposure to environmental organisms would be very low.

Human health
Acute toxicity of this substance is low; oral LD50 values in animals are greater than 5,000 mg/kg. The substance is not irritating to skin and moderately irritating to eyes. There is no skin sensitization in human. In repeated dose toxicity studies, including a rat 28-day repeated dose toxicity test [Japan TG], a preliminary reproduction toxicity screening test [OECD TG 421] and a rat 18- month chronic toxicity test, effects on sperm in the cauda epididymis and histopathological changes in the testis, such as degeneration of step 19 spermatids and vacuolation of Sertoli cells, were observed in the 42.3 mg/kg and higher dose groups. Based on the above results, the NOAEL for repeated dose toxicity is considered to be 12.5 mg/kg/day. In a reproductive / developmental toxicity study [OECD TG 421], the effects on reproductive parameters, such as decrease in number of corpora lutea, implantation scars and pups born, were observed in the 200 mg/kg and higher but not 50 mg/kg/day. Therefore, NOAEL for female reproductive toxicity is considered 50 mg/kg/day. However, NOAEL for male reproductive toxicity is 12.5 mg/kg/day because of the testicular toxicity as described above. As for the developmental toxicity, low body weight gain of offspring and increased number of stillbirths were observed at 800 but not 200 mg/kg/day. The teratogenic effects were not observed in a study with rats up to 375 mg/kg/day. Based on these findings, the NOAEL for developmental toxicity is considered to be 200 mg/kg/day. Three bacterial reverse mutation tests and mammalian chromosomal tests with and without metabolic activation show negative results [OECD TG 471, 472, 473]. No tumors were observed in a 18-month chronic feeding study with rats up to 1, 000 ppm, however this study is not qualified to be regarded as a carcinogenicity study. Therefore, no conclusion could be reached on the carcinogenicity.

Environment
Based on the evaluated NOECs for chronic endpoints (i.e. those from the chronic daphnid and algal tests), and by applying the assessment factor of 50 to the lowest NOEC (i.e. 0.34 mg/L), the PNEC for the aquatic environment is estimated to be 0.0068 mg/L. The PNEC for the sediment environment is also tentatively estimated to be 2.0 mg/kg according to the equilibrium partitioning method in the EU-TGD

5.2 Recommendations

Exposure assessment at production, processing and use sites would be recommended at the national or regional levels because the low NOAEL of this chemical for repeated dose toxicity is established from the critical effect on testes.

Considering the distribution characteristics of the substance (i.e. distribution tendency to sediment if released to aquatic environment), and due to lack of measured toxicity value to the sediment dwelling organisms, further work characterising aquatic hazards possibly including the sedimentary environment would be recommended. But this work would be conducted if significant emission to the environment is evidenced from the above exposure assessment.

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Appendix 1. The predicted Environmental Concentration (PEC) with the worst case scenario expected in Japan.

Under conditions of production, processing and use in Japan, the following worst case scenario for emission would be probable:

(1) Life cycle
During production, a release of the substance into the aquatic environment seems to be possible.

The use pattern as well as the properties of the substance suggests that a release into the environment at processing site (user's facility) in polymer and rubber industries should be negligible. Since the substance will be included into or onto matrix such as polymers and rubbers tightly, the emission into the environment from the matrix is unlikely.

(2) Environmental compartment receiving the emission
During production, the substance is in contact with the process water. Thus, the emission into the aquatic compartment would be possible. Indirect release through WWTP sludge application into soil compartment is unlikely, since such industrial sludge at production site will be incinerated generally. Due to low vapour pressure (4.7×10^{-11} Pa) of the substance, the release to the atmosphere would be negligible.

Other than an accident at production (as well as processing) site, direct release of the substance to the soil compartment is unlikely.

Thus, only the aquatic release of the substance at production site seems to be possible. Since there exists only very few possible point sources (e.g. two production sites in Japan), it is not opportune to determine a background concentration (i.e. regional or global concentration).

Thus, in this Appendix, only the local PECs in aquatic and sediment compartments at around production site is estimated. The data utilized in the estimation is from one major production company in Japan (Sumitomo Chemical, 1998, 1999).

1. Local water concentration at production site

According to the equations specified in the EU-TGD (1996), the "PEC (local-water)" is estimated with the worst case default parameters as follows:

(1) Concentration in WWTP (Waste Water Treatment Plant) inflow

$$C(\text{local\_WWTP\_inflow}) = E(\text{local\_water}) \times 10^6 / \text{Effluent (WWTP)}$$
(eq (17) in the EU-TGD)

where E (local_water): 0.073 (kg/Y) / 365 = 2×10^{-4} kg/day (Sumitomo Chemical, 1998),
Effluent (WWTP): 3000 m^3/day = 3×10^6 L/day (Sumitomo Chemical, 1999).

Thus, C (local_WWTP_inflow) = 6.7×10^{-5} mg/L.

(2) Concentration in WWTP outflow

$$C(\text{local\_WWTP\_effluent}) = C(\text{local\_WWTP\_inflow}) \times F(\text{WWTP\_water})$$
(eq (18) in the EU-TGD)

where F (WWTP_water): 0.07 (estimated with the physico-chemical properties (logH = -6 (Pam^3/mol) and logPow = 6.25) and the biodegradability (not degradable) according to the method specified in the EU-TGD).

Thus, C (local_WWTP_effluent) = 4.7×10^{-6} mg/L.

(3) PEC (local_water)

PEC (local_water) = C (local_WWTP_effluent) / [(1 + Kp susp x Susp water x 10^{-6}) x Dilution]
(eq (30) in the EU-TGD)

where Kp susp: 1.5×10^4 L/kg (see Note 1),
Susp water: 15 mg/L (default),
Dilution: 10 (default).

Thus, PEC (local_water) = 3.8×10^{-7} mg/L (worst case).

2. Local sediment concentration at production site

PEC (local_sediment) = (K susp-water / RHO susp) x PEC (local_water) x 1000
(eq (35) in the EU-TGD)

where K susp-water: 3750 m^3/m^3 (estimated according to the eq (10) in the EU-TGD),
RHO susp: 1150 kg/m^3 (default).

Thus, PEC (local_sediment) = 1.2×10^{-3} mg/kg (worst case).

(Note 1) Based on the Koc 1.5×10^5, the partition coefficient between water and soil, sediment or suspended mater can be estimated by applying default organic carbon contents specified in the EU-TGD (1996):

System	**OC in solid phase**	**Partition coefficient**
Soil-Water	2%	Kp soil = 3000 L/kg
Sediment-Water	5%	Kp sed. = 7500 L/kg
Suspended matter-Water	10%	Kp susp = 1.5×10^4 L/kg

Appendix 2. The risk quotient (PEC/PNEC) with the worst case scenario expected in Japan.

For the potential aquatic release of the substance during production in Japan, the quotient PEC (with the worst case, see Appendix 1) / PNEC for aquatic and benthic (sediment) organisms under local conditions can be estimated as indicated below:

Compartment	**PEC / PNEC**
Aquatic	0.000056 (=3.8 x 10^{-7} /0.0068)
Sediment	0.00060 (=1.2 x 10^{-3} /2.0)

Thus, even with the worst case scenario, no immediate concern in aquatic and sediment compartments is suggested. Although the PNEC for the sediment compartment is only the provisional one estimated according to the equilibrium partitioning method, this very small quotient strongly supports that there is at present no need for further testing for the sediment compartment.

Furthermore, due to the negligible release of the substance to the atmospheric and terrestrial compartments, "no immediate concern" would be also expected in these environments.

The 2ndary poisoning through food-web would be unlikely because of the low bioaccumulation potential of the substance.

Appendix 3. Occupational exposure with the worst case scenario and risk assessment

Based on the highest air concentration (0.38 mg/m^3) at a production site, and the maximal exposure period (1 hr/day), the daily intake (EHE) is calculated to be 0.0068 mg/kg/day as follows;

$$EHE = Cair \times IHair \times period \times BW^{-1}$$

Where	Cair	concentration at a production site	:	0.38 mg/m^3
	Ihair	inhalation rate	:	1.25 m^3/hr
	Period	exposure period	:	1 hr/day
	BW	adult body weight (default)	:	70 kg

Thus

$$EHE = 0.38 \text{ mg/m}^3 \times 1.25 \text{ m}^3\text{/hr} \times 1 \text{ hr/day} \times 70 \text{ kg}^{-1} = 0.0068 \text{ mg/kg/day}$$

Based on the daily intake (EHE) calculated in the worst case scenario, the margin of safety (MOS) for occupational exposure was estimated as follows;

$$MOS = NOAEL / EHE$$

where	NOAEL :	12.5 mg/kg/day	based on 53-day oral dose toxicity test
	EHE :	0.008 mg/kg/day	worst case daily intake

Thus

$$MOS = 12.5 / 0.0068 = 1800$$

The MOS of 1500 is based on the worst case scenario. Actual MOS is expected to be higher and normally workers wear respiratory protective equipment (mask) during the operation.

Appendix 4. Consumer exposure through the drinking water and risk assessment

The concentration in the surface water (PEC in Appendix 1) is estimated to be 3.8×10^{-7} mg/L in the worst case scenario. Using the method by EU-TGD (1996), EHE for the drinking water (DOSEdrw) is calculated to be 6.8×10^{-10} mg/kg/day as follows;

$$DOSEdrw = C_{drw}\times F_{pur}\times IH_{drw}\times BW^{-1}$$: e.g. (17,18,19) in the EU-TGD

where

C_{drw}	concentration in drinking water	:	3.8×10^{-7} mg/L
F_{pur}	purification factor (worst case)	:	1/16
IH_{drw}	drinking water (default)	:	2 L/day
BW	Adult body weight (default)	:	70 kg

Thus

$$DOSEdrw = 3.8\times10^{-7} \text{ mg/L}\times1/16\times2 \text{ L/day}\times70 \text{ kg}^{-1} = 6.8\times10^{-10} \text{ mg/kg/day}$$

Based on the DOSEdrw, the margin of safety (MOS) for the drinking water was calculated as follows;

$$MOS = NOAEL / EHE$$

where

NOAEL: 12.5 mg/kg/day based on 53-day oral dose toxicity test
EHE: $6.8 \text{ X } 10^{-10}$ mg/kg/day (DOSEdrw)

Thus

$$MOS = 12.5 / (6.8 \text{ X } 10^{-10}) = 1.8 \text{ X } 10^{10}$$

REVISED OECD HPV FORM 1

SIDS DOSSIER
ON THE HPV CHEMICAL

6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol

CAS No. 119-47-1

Sponsor Country : Japan

DATE: Oct. 6, 2000

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Appendix I. Proofs of the calculations for theoretical distribution of 6,6-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol.

Note: *; Data elements in the SIDS
†; Data elements specially required for inorganic chemicals

1. GENERAL INFORMATION

1.01 SUBSTANCE INFORMATION

***A. CAS number** 119-47-1

B. Name *(IUPAC name)* 6,6'-Di-*tert*-butyl-2,2'-methylenedi-*p*-cresol

***C. Name** *(OECD name)* 6,6'-Di-*tert*-butyl-2,2'-methylenedi-*p*-cresol

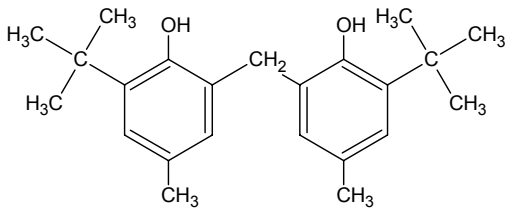
†D. CAS Descriptor *(where applicable for complex chemicals)*

Oc1c(C(C)(C)C)cc(C)cc1Cc2cc(C)cc(C(C)(C)C)c2O

E. EINECS-Number 204-327-1

F. Molecular Formula $C_{23}H_{32}O_2$

***G. Structural Formula**



H. Substance Group Not applicable

I. Substance Remark None

J. Molecular Weight 340.51

1.02 OECD INFORMATION

A. Sponsor Country: Japan

B. Lead Organization:

Name of Lead Organization: Sumitomo Chemical Company, Limited
Contact person: Mr. Koji Tomita
Address: Ministry of Foreign Affairs
Economic Affairs Bureau
Second International Organisations Div.
2-2-1 Kasumigaseki, Chiyoda-ku
Tokyo 100, Japan
Tel: 81-3-3581-0018
Fax: 81-3-3581 9470
E-mail: seiichi.urauchi@mofa.go.jp

C. Name of responder: the same as above.

1.1 GENERAL SUBSTANCE INFORMATION

A. Type of Substance

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

B. Physical State *(at 20°C and 1,013 hPa)*

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

C. Purity >= 98.0% weight/weight (Sumitomo Chemical, 1999a)

1.2 SYNONYMS

(Chemical Name) 2,2'-Methylenebis(4-methyl-6-*tert*-butylphenol); 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol; 2,2'-Methylene-bis(6-*tert*-butyl-para-cresol); 2,2'-Methylenebis(6-t-butyl-4-methylphenol).

(Trade Name) Sumilizer MDP; Lowinox 22M46; Vulkanox BKF; Yoshinox 2246; Cyanox 2246; Santowhite PC.

1.3 IMPURITIES None (Sumitomo Chemical, 1999a)

1.4 ADDITIVES None (Sumitomo Chemical, 1999a)

***1.5 QUANTITY** In Japan, ca. 1,000-1,200 tons/Y, produced by two Japanese companies.

Remarks: Estimated global production is 3,300-3,500 tons/y; major producers are located in the United States, Germany, Japan, United Kingdom and France.

Reference: Sumitomo Chemical (1999b)

1.6 LABELLING AND CLASSIFICATION

Labeling Not assigned according to the EC Directive 67/548/EEC

Classification Not classified

1.7 USE PATTERN

A. General:	**Type of Use:**	**Category:**
	main	Use resulting in inclusion into or onto matrix, 100%
	(a)industrial use	Polymers industry; antioxidant Antioxidant / Stabilizer
	(b)industrial use	Rubber Industry; additives Antioxidant

Remarks: Stabilizer used in styrenic and olefin polymers and polyoxymethylene homo and copolymers.

Antioxidant (ABS, polypropylene, polyacetal, rubber, latex, adhesives).

Reference: Kuney, J.H and J.M. Mullican (1994).
Ashford, R. D. (1994)

B. Uses in Consumer Products

No important use as a direct ingredient of any consumer products.

Remarks: Indirect exposure to consumers via migration from a polymer containing the substance as stabilizer or antioxidant may be considered; it is however regarded as not relevant; the polymers for which the substance is applied mostly to industrial use (such as rubber tire and engineering plastics).

In the USA, such use as for food packaging material seems exist. The use is approved by the FDA (21CFR 178.2010 Antioxidants and/or stabilizers for polymers) as shown below:

2,2'-Methylenebis(4-methyl-6- tert-butylphenol).
For use only:
1. At levels not to exceed 0.1 percent by weight of olefin polymers complying with sec. 177.1520(c) of this chapter, items 1.1, 1.2, 1.3, 2.1, 2.2, 2.3, 3.1, 3.2, 3.3, or 4 used in articles that contact food of the types identified in sec.176.170(c) of this chapter, table 1, under Categories I, II, IV-B, VI, VII-B, and VIII.
2. At levels not to exceed 1 percent by weight of polyoxymethylene copolymer as provided in sec.177.2470 (b) (1) of this chapter.
3. At levels not to exceed 0.5 percent by weight of polyoxymethylene homopolymer as provided in Sec. 177.2480(b)(1) of this chapter.

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

Exposure limit value: None

Short term exposure limit value:None

1.9 SOURCES OF EXPOSURE

(A) Potential human exposure: Can be negligible by applying protective measures as written below:

(a) At a production site:
Exposure is possible, other than sampling and analysis, during drying and packing procedure; a worker may be exposed to dust for an hour a day. The workplace is provided with a air ventilator and the worker is equipped with protective gear such as mask, rubber gloves and goggles to prevent exposure. Spill is collected and burnt.

Exposure monitoring data
Measured in 1997 at a production site in Japan (producing ca. 800 t of the substance in 1998):

Method:
Air of workplace atmosphere was suctioned at a ratio of 500 L/min. for 30 min, and airborne particles were collected by a filter. The substance caught on the filter was dissolved in a solvent and was analyzed by HPLC.

Result:
The workplace exposure level near the following machinery was determined as follows

Dryer :	less than 0.01 mg/m^3 (analytical limit)
Hopper:	less than 0.01 mg/m^3 (analytical limit)
Packing machine:	0.38 mg/m^3

Reference: Sumitomo Chemical (1997)

(b) At an user's facility:
Exposure is possible during charging the substance from packaging into a molding machine; a worker may be exposed to dust for half an hour a day. The worker is recommended (by the MSDS) to put on protective gear such as mask, rubber gloves and goggles to prevent exposure. Spill is collected and burnt.

(B) Potential environmental exposure:

(a) At a production site:

Source: Media of release: Process waste water
Quantities per media:. Estimated max. 0.073 kg/year in a production site in Japan (1998), in which ca. 800 t/year of the chemical substance was produced. (estimated by Sumitomo)

Remarks: Data used for the estimation:

Waste water released:	3670 m3/year
Water solubility:	0.02 mg/L
Dilution factor at a municipal facility:	0.1

Reference: Sumitomo Chemical (1999c)

(b) At an user's facility:
No substantial exposure is probable. Water is not used for cleaning of molding machine. Spill is collected and burnt. (estimated by Sumitomo)

Reference: Sumitomo Chemical (1999c)

1.10 ADDITIONAL REMARKS

A. Options for disposal:

Incineration; release to sewage system for waste water treatment.

B. Other remarks

None.

2. PHYSICAL-CHEMICAL DATA

*2.1 MELTING POINT

Value:	130 - 131°C
Decomposition:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Sublimation:	Yes **[ ]** No **[X]** Ambiguous **[ ]**
Method:	Not disclosed
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Beilstein Database (2000).

***2.2 BOILING POINT**

Value:	187 °C
Pressure:	at 0.07 hPa
Decomposition:	Yes **[ ]** No **[ ]** Ambiguous **[X]**
Method:	Not disclosed
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Chemische Werke Lowi GmbH & Co. (IUCLID, 1999)

†2.3 DENSITY (relative density)

(a)

Type:	Bulk density **[X]**; Density **[ ]**; Relative Density **[ ]**
Value:	0.35 g/cm3.
Temperature:	20 °C
Method:	Not disclosed
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Remarks:	
Reference:	Chemische Werke Lowi GmbH & Co. (IUCLID, 1999)

(b)

Type:	Bulk density **[ ]**; Density **[X]**; Relative Density **[ ]**
Value:	1.08 g/cm3.
Temperature:	Not disclosed
Method:	Not specified
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	Crystal density
Reference:	Chetkina, L. A. et. al., (1984)

***2.4 VAPOUR PRESSURE** *(if more than one, identify the recommended value)*

(a)

Value:	4.7×10^{-11} Pa
Temperature:	
Method:	calculated **[X]**; measured **[ ]** Calculated with the estimated Henry's Law constant (7.9×10^{-12} atm-m^3/mol; Section 2.13, B) and the water solubility (5.9×10^{-5} mol/m^3; Section 2.6 (a))
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Sumitomo Chemical (2000a).

(b)

Value:	<0.1 hPa
Temperature:	20 °C
Method:	calculated **[ ]**; measured **[X]** Details not given
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Chemische Werke Lowi GmbH & Co. (IUCLID, 1999)

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

Log Pow:	6.25
Temperature:	not specified
Method:	not specified
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	

Reference: Chemicals Inspection and Testing Institute of Japan (1992).

***2.6 WATER SOLUBILITY**

A. Solubility

(a)

Value:	0.02 mg/L
Temperature:	Not specified
Description:	Miscible **[ ]**; Of very high solubility **[ ]**; Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble**[ ]**; Of low solubility**[ ]**; Of very low solubility **[** X **]**; Not soluble **[ ]**
Method:	See remarks.
GLP:	Yes **[ ]** No **[ ]** ? **[** X **]**
Remarks:	The value is converted to the Molar concentration to be 5.9 x 10^{-5} mol/m^3
Reference:	Chemicals Inspection and Testing Institute of Japan (1992).

(b)

Value:	<0.01 g/L
Temperature:	20 °C
Description:	Miscible **[ ]**; Of very high solubility **[ ]**; Of high solubility **[ ]**; Soluble **[ ]**; Slightly soluble **[ ]**; Of low solubility **[ ]**; Of very low solubility **[** X **]**; Not soluble **[ ]**
Method:	Details not given
GLP:	Yes **[ ]** No **[** X **]** ? **[ ]**
Remarks:	
Reference:	Chemische Werke Lowi GmbH & Co. (IUCLID, 1996)

B. pH Value, pKa Value

No data available

2.7 FLASH POINT *(liquids)***:**

Not applicable

2.8 AUTO FLAMMABILITY *(solid/gases)***:**

No data available

2.9 FLAMMABILITY

Results:	Extremely flammable **[ ]**; Extremely flammable - liquefied gas **[ ]**; Highly Flammable **[ ]**; Flammable **[ ]**; Non flammable **[X]**; Spontaneously flammable in air **[ ]**; Contact with water liberates highly flammable gases **[ ]**; Other **[ ]**
Method:	Not specified
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Chemische Werke Lowi GmbH & Co. (IUCLID, 1999)

2.10 EXPLOSIVE PROPERTIES

No data available

2.11 OXIDISING PROPERTIES

No data available

†2.12 OXIDATION: REDUCTION POTENTIAL

Value:	$E_{1/2}$ = 0.35 mV at pH 6.8
Method:	Polarography (1969)
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	
Reference:	Vodzinskii et. al.(1969).

2.13 ADDITIONAL DATA

A. Partition coefficient between soil/sediment and water (Kd)

Value:	1.5 x 10^5 (Koc)
Method:	Estimated according to the QSAR equation in the EU-TGD.
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Remarks:	The QSAR equation employed is for predominantly hydrophobics: log Koc = 0.81 x log P + 0.10 [European Commission (1996)].
Reference:	Sumitomo Chemical (2000b).

B. Other data

Results:	Henry's Law constant to be 7.9 x 10^{-12} atm-m^3/mol.
Remarks:	estimated value
Reference:	STN international (1999).

3. <u>ENVIRONMENTAL FATE AND PATHWAYS</u>

3.1 STABILITY

***3.1.1 PHOTODEGRADATION**

Type:	Air **[X]**; Water **[ ]**; Soil **[ ]**; Other **[ ]**
Light source:	Sunlight **[ ]**; Xenon lamp **[ ]**; Other **[ ]**
Light spectrum:	
Relative intensity:	
Spectrum of substance:	
Concentration of Substance:	
Temperature:	
Direct photolysis:	
Half life:	
Degradation:	
Quantum yield:	
Indirect Photolysis:	
Type of sensitizer:	OH
Concentration of sensitizer:	1.5 x 10^6 OH/cm^3
Rate constant (radical):	40.8578 x 10^{-12} cm^3/molecule-sec
Degradation:	50% after 3.141 hours
Method:	calculated **[X]**; measured **[ ]**
GLP:	Yes **[ ]** No **[X]** ? **[ ]**

Test substance: purity: unknown
Remarks: Estimated by using AOPWIN (ver.1.80), SRC-AOP for Microsoft Windows.
Reference: Sumitomo Chemical (2000c).

***3.1.2 STABILITY IN WATER**

No data available

Considering the low water solubility of the substance, hydrolysis would be of minor importance in distribution behavior in the environment.

3.1.3 STABILITY IN SOIL

No data available

***3.2 MONITORING DATA (ENVIRONMENTAL)**

No data available

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

***3.3.1 TRANSPORT**

No data available

***3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)**

Media: Air-biota **[ ]**; Air-biota-sediment-soil-water **[X]**; 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) **[ ]**; Other (measurement)**[ ]**
Results:

Compartment	Release 100% to air	Release 100% to water	Release 100% to soil
Air	0.0 %	0.0 %	0.0 %
Water	0.0 %	6.9 %	0.0 %
Soil	99.8 %	0.0 %	99.9 %
Sediment	0.1 %	93.1 %	0.1 %

Remarks: See Appendix I for details.
Reference: Sumitomo Chemical (2000d).

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

Results:
Remarks:
Reference:

***3.5 BIODEGRADATION**

Type: aerobic **[X]**; anaerobic **[ ]**

Inoculum:	adapted **[ ]**; non-adapted **[X]**; standardized activated sludge, 30 mg/L as suspended solid
Concentration of the chemical: 100mg/L related to COD **[ ]**; DOC **[ ]**; test substance **[X]**	
Medium:	water **[X];** water-sediment **[ ];** soil **[ ];** sewage treatment **[ ]**
Degradation:	0 % after 28 days (based on BOD) 1% after 28 days (based on HPLC analysis for the parent)
Results:	readily biodeg. **[ ];** inherently biodeg. **[ ]**; under test condition no biodegradation observed **[** X **]**, other **[ ]**
Kinetic	(e.g. Zahn-Wellens-Test) . . . . . % in . . . . . (time)
Method:	MITI (I) method (1974), corresponding to the OECD 301C (1981)..
GLP:	Yes **[X]** No **[ ]** ? **[ ]**
Test substance:	purity: Not specified
Remarks:	The chemical is not readily biodegradable.
Reference:	Chemicals Inspection and Testing Institute of Japan (1992).

3.6 BOD_5, COD OR RATIO BOD_5/COD

No data available

3.7 BIOACCUMULATION

Species:	Carp(*Cyprinus carpio*)
Exposure period:	8 weeks
Temperature:	25 °C
Concentration:	1.0 mg/L and 0.1 mg/L
BCF:	23-37 (1.0 mg/L) 60-125 (0.1 mg/L)
Elimination:	Yes **[ ]** No **[X]** ? **[ ]**
Method:	MITI method (1974), corresponding to the previous OECD 305C (1981)..
Type of test:	calculated **[ ]**; measured **[** X **]** static **[ ]**; semi-static **[ ]**; flow-through **[** X **]**; other *(e.g. field test)* **[ ]**
GLP:	Yes **[** X **]** No **[ ]** ? **[ ]**
Test substance:	purity: Not specified
Remarks:	The average lipid content of carp was 4.7%. The stock solution for exposure was prepared with castor oil (HCO-40) according to the guideline.
Reference:	Chemicals Inspection and Testing Institute of Japan (1992).

3.8 ADDITIONAL REMARKS

A. Sewage treatment

No data available

B. Other information

None

4. ECOTOXICITY

*4.1 ACUTE/PROLONGED TOXICITY TO FISH

(a)

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

Exposure period: 96 hrs
Results: LC_{50} (24h) >5.0 mg/l (nominal concentration)
LC_{50} (48h) >5.0 mg/l (nominal concentration)
LC_{50} (72h) >5.0 mg/l (nominal concentration)
LC_{50} (96h) >5.0 mg/l (nominal concentration)
NOEC = 5.0 mg/l (nominal concentration)
LOEC >5.0 mg/l (nominal concentration)
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: OECD TG 203 (1992)
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: purchased from Merck (Lot No. S19983), purity: 97.5%
Remarks: Due to low water solubility of the test chemical, homogenous solution could be attained only at (and less than) the nominal concentration of 5.0 mg/L with the maximum allowable dispersant concentration of 100 mg/L (DMF/Castor oil (HCO-40) = 3/1, w/w). The actual concentrations (measure by GC-MS) at the initiation and 48 hrs of exposure were 4.0 mg/L and 4.3 mg/L, respectively. No toxic effect was observed during the study.
Reference: Environment Agency of Japan (1999a).

(b)
Type of test: static **[ ]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**
open-system **[ ]**; closed-system **[ ]**
The test system employed was static or semi-static (not specified)
Species: Medaka (*Oryzias latipes*)
Exposure period: 48 hrs
Results: LC_{50} (48h) >500 mg/l
Analytical monitoring: Yes **[ ]** No **[ ]** ? **[X]**
Method: MITI method (JIS K 0102-1986, 71)
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: purity: Not specified
Remarks: Even at this stringently dispersed concentration of 500 mg/L, the mortality observed during the study was less than 50%.
Reference: Chemicals Inspection and Testing Institute of Japan (1992).

(c)
Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**
open-system **[ ]**; closed-system **[ ]**
Species: Fathead minnow (*Pimephales promelas*)
Exposure period: 96 hrs
Results: LC_{50} (96hr) = 0.31 mg/l
Analytical monitoring: Yes **[ ]** No **[X]** ? **[ ]**
Method: EPA-660/3-75-009 (1975)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance:purity: Not specified
Remarks: Not assignable due to lack of essential information (e.g. purity, composition, additives) on the test substance SANTOWHITE PC. No analytical monitoring was either conducted in the study.
Reference: ABC Lab. (1983).

(d)
Type of test: static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**
open-system **[ ]**; closed-system **[ ]**
Species: Goldorfe
Exposure period: 48 hrs

Results:	LC_{50} (48h) = 50 mg/l
Analytical monitoring:	Yes **[ ]** No **[ ]** ? **[X]**
Method:	Not specified
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance:purity:	Not specified
Remarks:	Not assignable due to lack of essential data (e.g. about compliance or non-compliance on GLP, analytical monitoring and test method employed).
Reference:	IUCLID (1996).

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

*A. Daphnia

Type of test:	static **[X]**; semi-static **[ ]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[ ]**
Species:	*Daphnia magna*
Exposure period:	48 hrs
Results:	EC_{50} (24h) >4.8 mg/l (Measured concentration) EC_{50} (48h) >4.8 mg/l (Measured concentration) NOEC = 0.74 mg/l (Measured concentration)
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**
Method:	OECD TG 202 (1984)
GLP:	Yes **[** X **]** No **[ ]** ? **[ ]**
Test substance:	Lot No. S19983 (purchased from Merck) , purity: 97.5%
Remarks:	Because of the low water solubility of the substance, homogenous solution could be attained only at (and less than) the nominal concentration of 5 mg/L with the maximum allowable dispersant concentration of 100 mg/L (DMF/Castor oil (HCO-40) = 3/1, w/w). Twenty daphnids (4 replicates; 5 organisms per replicate) each were exposed to the test substance at the actual concentrations (measured by GC-MS) of 0.40, 0.74, 1.1, 2.3 and 4.8 mg/L. Solvent control (DMF/Castor oil (HCO-40) = 3/1, w/w) was also set, and no effect (i.e. immobilization of organisms) was observed during the study.
Reference:	Environment Agency of Japan (1999b).

C. Other aquatic organisms

No data available

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

Species:	*Selenastrum capricornutum* (ATCC22662 strain)	
Endpoint:	Biomass **[X]**; Growth rate **[** X **]**; Other **[ ]**	
Exposure period:	72 hrs	
Results:	Biomass	EC_{50} (0-72 h) > 5.0 mg/L (Nominal concentration) NOEC (0-72 h) = 0.63 mg/L (Nominal concentration)
	Growth rate	EC_{50} (24-48 h) > 5.0 mg/L (Nominal concentration) NOEC (24-48 h) = 2.5 mg/L.(Nominal concentration) EC_{50} (24-72 h) > 5.0 mg/L (Nominal concentration) NOEC (24-72 hrs) = 1.3 mg/L.(Nominal concentration) LOEC = ... mg/l
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**	
Method:	OECD TG 201 (1984)	

	open-system **[ ]**; closed-system **[ ]**
GLP:	Yes **[** X **]** No **[ ]** ? **[ ]**
Test substance:	Lot No. S19983 (purchased from Merck) , purity: 97.5%
Remarks:	Static system. Triplicates to each level. Because of the low water solubility of the substance, homogenous solution could be attained only at (and less than) the nominal concentration of 5 mg/L with the maximum allowable dispersant concentration of 100uL/L (DMF/Castor oil HCO-40 = 3/1, w/w). Thus, the nominal concentrations were set at 0.63, 1.3, 2.5 and 5.0 mg/L, and the actual concentrations (measured by GC-MS) were in the range of 70-96 % to the nominal ones during exposure.
Reference:	Environment Agency of Japan (1999c).

4.4 TOXICITY TO BACTERIA

No data available

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH

No data available

(*)4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATE

Type of test:	static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other *(e.g. field test)* **[ ]**; open-system **[ ]**; closed-system **[ ]**	
Species:	*Daphnia magna*	
Endpoint:	Mortality **[** X **]**; Reproduction rate **[** X **]**; Other **[ ]**	
Exposure period:	21 days	
Results:	Mortality	LC_{50} (21 d) = 1.0 mg/L (Measured concentration)
	Reproduction	EC_{50} (21 d) = 1.1 mg/L (Measured concentration)
		NOEC = 0.34 mg/L (Measured concentration)
		LOEC = 0.89 mg/L (Measured concentration)
Analytical monitoring:	Yes **[X]** No **[ ]** ? **[ ]**	
Method:	OECD TG 211 (1997), revised version of OECD TG 202 (1984)	
GLP:	Yes **[X]** No **[ ]** ? **[ ]**	
Test substance:	Lot No. S19983 (purchased from Merck), purity: 97.5%	
Remarks:	Ten daphnids (Ten replicates; One organism per replicate) each were exposed to the actual concentrations (measured by GC-MS) of 0.046, 0.12, 0.34, 0.89 and 1.9 mg/L. The solvent control (DMF/Castor oil (HCO-40) = 3/1, w/w, 50 μL/L) was also set, and no mortality as well as no significant toxic effect were observed during the study. The 95% confidence interval of the LC_{50} was from 0.71 to 1.4 mg/L.	
Reference:	Environment Agency of Japan (1999d).	

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No data available

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

No data available

4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)

No data available

4.7 BIOTRANSFORMATION AND KINETICS

No data available

4.9 ADDITIONAL REMARKS

None

5. TOXICITY

*5.1 ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY

(a)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat (SD) male/female
Value:	>5000 mg/kg b.w.
Discriminating dose:	1000, 2500, 5000 mg/kg
Method:	Unknown
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Test substance :	Commercial grade (purity: Unknown)
Remarks:	No toxic symptom observed
Reference:	Sumitomo Chemical Co. (1977a)

(b)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat (strain and sex: Unknown)
Value:	>10000 mg/kg b.w.
Discriminating dose:	Unknown
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	
Reference:	Bayer AG (1988) American Cyanamid Corporation (1988)
Note:	Data from IUCLID (1996): original report: Not available.

(c)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat (Wistar) male/female
Value:	>5000 mg/kg b.w.
Discriminating dose:	Unknown
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Commercial grade (purity: Unknown)
Remarks:	
Reference:	Takagi et al. (1994)

(d)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[ ]**; LDL_0 **[X]**; Other **[ ]**
Species/strain:	Rat (strain and sex: Unknown)
Value:	1000 mg/kg b.w.
Discriminating dose:	Unknown
Method:	Unknown
GLP:	Yes **[ ]** No **[**X**]** ? **[ ]**
Test substance:	Grade and purity: Unknown
Remarks:	
Reference:	Anon. (1973a)
Note:	Data from RTECS (2000): original report: Not available.

(e)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rat (strain and sex: Unknown)
Value:	5000 mg/kg b.w.
Discriminating dose:	Unknown
Method:	Unknown
GLP:	Yes **[ ]** No **[**X**]** ? **[ ]**
Test substance:	Grade and purity: Unknown
Remarks:	
Reference:	Stasenkova et al. (1977)
Note:	Data cited by Takagi et al (1994); original report: Not available.

(f)

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	mouse (strain and sex: Unknown)
Value:	11000 mg/kg b.w.
Discriminating dose:	Unknown
Method:	Unknown
GLP:	Yes **[ ]** No **[**X**]** ? **[ ]**
Test substance:	Grade and purity: Unknown
Remarks:	
Reference:	Stasenkova et al. (1977)
Note:	Data cited by Takagi et al (1994); original report: Not available.

5.1.2 ACUTE INHALATION TOXICITY

Type:	LC_0 **[X]**; LC_{100} **[ ]**; LC_{50} **[ ]**; LCL_0 **[ ]**; Other **[ ]**
Species/strain:	Other (species: Unknown)
Exposure time:	4 hours
Value:	100 mg/m^3
Method:	Unknown
GLP:	Yes **[ ]** No **[X]** ? **[ ]**
Test substance :	Commercial grade (purity: Unknown)
Remarks:	No toxic symptom observed
Reference:	Bayer AG (published year: Unknown)
Note:	Data from IUCLID (1996); original report: Not available.

5.1.3 ACUTE DERMAL TOXICITY

Type:	LD_0 **[ ]**; LD_{100} **[ ]**; LD_{50} **[X]**; LDL_0 **[ ]**; Other **[ ]**
Species/strain:	Rabbit (strain and sex: Unknown)
Value:	>10000 mg/kg b.w.

Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	
Reference:	American Cyanamid Corporation (1988)
Note:	Data from IUCLID (1996); original report: Not available.

5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

No data available

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

Species/strain:	Rabbit (strain and sex: Unknown)
Results:	Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification:	Highly corrosive (causes severe burns) **[ ]**; Corrosive (causes burns) **[ ]**; Irritating **[ ]**; Not irritating **[X]**
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	
Reference:	American Cyanamid (1988)
Note:	Data from IUCLID (1996); original report: Not available.

5.2.2 EYE IRRITATION/CORROSION

(a)

Species/strain:	Rabbit (strain and sex: Unknown)
Results:	Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification:	Irritating **[ ]**; Not irritating **[X]**; Risk of serious damage to eyes **[ ]**
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	
Reference:	American Cyanamid Corporation (1988)
Note:	Data from IUCLID (1996); original report: Not available.

(b)

Species/strain:	Rabbit (strain and sex: Unknown)
Results:	Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[X]**; Slightly irritating **[ ]**; Not irritating **[ ]**
Classification:	Unknown
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	Moderate irritating was observed at 24 hr after 100 mg/eye application.
Reference:	Anon (1986b)
Note:	Data from RTECS(2000); original report: Not available.

5.3 SKIN SENSITISATION

(a)

Type:	Human maximization test
Species/strain:	Human
Results:	Sensitizing **[ ]**; Not sensitizing **[X]**; Ambiguous **[ ]**
Classification:	Not sensitizing
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	The experiment was carried out for the evaluation on skin sensitization in human. The substance (25% concentration) was applied to the back of 25 adult men in a course of five 48-hour exposures with 1-day withdrawal. The substance was also applied to the same site with 5% sodium lauryl sulfate for 1-day. Two weeks after the last application, a challenge patch test was carried out with 0.5 g (10% concentration) of the substance at a naive site under an occlusive dressing. The substance was not irritating or allergic.
Reference:	American Cyanamid (1980)
Note:	The results are based on the abstract in HSDB or TOXLINE. Original report could not be obtained because of wrong citation.

(b)

Type:	Allergic and irritant Patch-Test
Species/strain:	human
Results:	Sensitizing **[ ]**; Not sensitizing **[X]**; Ambiguous **[ ]**
Classification:	Not sensitizing
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	Patch tests were performed using 1 % concentration of the substance applied to 13 persons with rubber product sensitivity. No one showed positive reaction after 48hr occlusive patch.
Reference:	Kanto et al. (1985)

(c)

Type:	Allergic and irritant Patch-Test
Species/strain:	human
Results:	Sensitizing **[ ]**; Not sensitizing **[X]**; Ambiguous **[ ]**
Classification:	Not sensitizing
Method:	Unknown
GLP:	Yes **[ ]** No **[ ]** ? **[X]**
Test substance :	Grade and purity: Unknown
Remarks:	Patch tests were performed using 0.1, 1, 10 % concentration of the substance applied to16 persons with rubber product sensitivity. No one showed positive reaction after 48hr occlusive patch.
Reference:	Kanto et al. (1999)

*5.4 REPEATED DOSE TOXICITY

(a)

Test Substance	6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol Produced by Ouchi Shinko Chemical. purity: Unknown
Method	
Method:	Unknown

Test type: Chronic toxicity study
GLP: No
Year: 1994
Species: Rat
Strains: Wistar
Route of Administration: Oral (by feeding)
Duration of test: 18 months
Doses: 100, 300, 1000 ppm (in diet)
Sex: Male/Female
Exposure period: 18 months
Frequency of treatment: Daily
Control group and treatment: Basal diet (no treatment)
Post exposure observed period: No
Statistical analysis: Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data
Test condition: Age at study initiation was 5 week old (393±21g for male, 230±15g for female). Number of animals per sex per dose was 30, of which 5 animals were sacrificed at 6 month and another 5 animals at 12 month for hematological and serum biochemical examinations). The animals were given diet of pellets mixing this substance.General condition was observed daily. Body weight and food consumption were determined monthly. Hematological and serum biochemical examination were performed for 5 animals/sex/dose group at 6, 12 and 18 month. Organ weights were measured in brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands. Histopathological examinations were carried out in brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands, salivary glands, esophagus, stomach, small intestine, pancreas, urinary bladder, seminal vesicles, epididymis, ischiac nerve, uterus, prostate, mesenteric lymph nodes, thymus, spinal cord, skeletal muscle, born marrow.

Results

NOAEL: Male: 300 ppm (12.7 mg/kg/day), Female: 300 ppm (15.1 mg/kg/day)
LOAEL: Male: 1000 ppm (42.3 mg/kg/day), Female: 1000 ppm (54.2 mg/kg/day)
Toxic effects:

Male
<u>At 1000 ppm:</u>
Suppression of body weight gain from the month 6. Increase or increase-tendency in relative liver weight. Decrease in absolute and relative testis weight. Atrophy of testicular tubules. Spermatogenic arrest. Epididymis hypospermia.

Female
<u>At 1000 ppm:</u>
Suppression of body weight gain from the month 1. Increase or increase-tendency in relative liver weight.

Remarks: Mean efficiency of feed utilization was dose-dependently decreased in both sexes.

Conclusions: Toxic effects in this study were suppression of body weight gain, increase in liver weight, decrease in testis weight, and histopathological lesions in the testis and the epididymis. The NOAELs are 12.7 mg/kg/day (300 ppm) for male and 15.1 g/kg/day (300 ppm) for female.

Data Quality: Valid. (limitation: the study was conducted without GLP)

Reference: Takagi et al., National Institute of Health Science, Japan (1994)

(b)

Test Substance 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
Produced by Sumitomo Chemical, Lot No.: 710140, purity 98.2 %, Kept at room temperature until use.

Method

Method: OECD TG 421
Test type: OECD Preliminary Reproduction Toxicity Screening Test
GLP: Yes
Year: 1999
Species: Rat
Strains: Crj:CD (SD)
Route of Administration: Oral (by gavage)
Duration of test: 53days
Doses: 12.5, 50, 200, 800 mg/kg/day (in 5% gum arabic)
Sex: Male/Female
Exposure period: Male; 50-52 days
Female; 40-48 days (from 14 days before mating to the day 3 of lactation)
Frequency of treatment: Daily
Control group and treatment: Concurrent vehicle
Post exposure observed period: No
Statistical analysis Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data
Test condition: Age at study initiation was 10 week old (332-383 g for male, 206-238 g for female). Number of animals per sex per dose was 12. Five percent gum Arabic was used as a vehicle. Functional observation was not performed because the test was conducted by the TG adopted in 1990.

Results

NOAEL: Male: 12.5 mg/kg/day, Female: 50 mg/kg/day
LOAEL: Male: 50 mg/kg/day, Female: 200 mg/kg/day
Toxic effects:

Male
<u>At 50 mg/kg/day:</u>
Giant cell formation in the testis. Decrease in sperm motility ratio and number of sperms in the cauda epididymis. Increase in abnormal sperm ratio.
<u>At 200 mg/kg/day:</u>
Atrophy of the testis and the epididymis. Decrease in the absolute and relative testis and epididymis weights. Atrophy of seminiferous tubules. Degeneration of seminiferous tubules. Decrease in number of sperm in the cauda epididymis. Giant cell formation.
<u>At 800 mg/kg/day:</u>
Transient decrease in food consumption. Atrophy of the testis, the epididymides and the seminal vesicle. Decrease in the absolute and relative testis and epididymis weights. Atrophy of seminiferous tubules in the testis. Observation of no motile sperm. Increase tendency in number of abnormal sperm. Decrease in number of sperm in the cauda epididymis.

Female
<u>At 200 mg/kg/day:</u>
Suppression of body weight gain during the lactation period. Lower food consumption during pre-mating, pregnancy and lactation periods.
<u>At 800 mg/kg/day:</u>
Suppression of body weight gain during the pregnancy and the lactation periods. Lower food consumption during pre-mating, pregnancy and lactation periods.

Remarks:

Conclusions: Toxic effects in this study are suppression of body weight gain, low food consumption, and histopathological lesions in the testis and the epididymis. The NOAELs are 12.5 mg/kg/day for male and 50 mg/kg/day for female.

Data Quality: Valid without restriction

Reference: Ministry of Health and Welfare: Japan, Toxicity Testing Report of Environmental Chemicals 7, 423-437 (1999)

(c)

Test Substance 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
Produced by Sumitomo Chemical, Lot No.: 40401, purity: > 98 %, Kept at room temperature until use.

Method

Method: TG for 28-day repeat dose toxicity testing of chemicals (Japan)
Test type: 28-day Repeat Dose Toxicity Test
GLP: Yes
Year: 1996
Species: Rat
Strains: Crj:CD (SD)
Route of Administration: Oral (by gavage)
Duration of test: 43days
Doses: 50, 200, 800 mg/kg/day (in 5% gum arabic)
Sex: Male/Female
Exposure period: 28 days
Frequency of treatment: Daily
Control group and treatment: Concurrent vehicle
Post exposure observed period: 14 day (for 800 mg/kg/day group)
Statistical analysis Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data
Test condition: Age at study initiation was 6 week old (209-235 g for male, 142-164 g for female). Number of animals per sex per dose was 6 for the groups at 50, 200 mg/kg/day and 12 at 0, 800 mg/kg/day. Five percent gum Arabic was used as a vehicle.

Results

NOAEL: Male: Not determined, Female: 50 mg/kg/day
LOAEL: Male: 50 mg/kg/day, Female: 200 mg/kg/day
Toxic effects:

Male
<u>At 50 mg/kg/day:</u>
Prolongation of PT and APTT. Increase in liver weight. Degeneration of step 19 spermatids.
<u>At 200 mg/kg/day:</u>
Prolongation of PT and APTT. Increase in liver weight. Mild centrilobular hepatocyte hypertrophy. Sperm retention and degeneration of step 19 spermatids. Vacuolation of Sertoli cells in the testis.
<u>At 800 mg/kg/day:</u>
Prolongation of PT and APTT. Increase in liver weight. Mild centrilobular hepatocyte hypertrophy. Sperm retention and degeneration of step 19 spermatids. Vacuolation of Sertoli cells in the testis.

Female
<u>At 200 mg/kg/day:</u>
Prolongation of PT and APTT. Increase in liver weight. Mild centrilobular hepatocyte hypertrophy. Increase in adrenal weight.
<u>At 800 mg/kg/day:</u>

Prolongation of PT and APTT. Increase in liver weight. Mild centrilobular hepatocyte hypertrophy. Increase in adrenal weight.

Remarks: The toxic effects disappeared or showed a tendency for recovery trend 14 days after the substance withdrawal except the histopathological changes of the testis. In the testis of 800 mg/kg/day recovery group, more sever changes such as giant cell formation and decrease in germ cells were observed.

Conclusions: Toxic effects in this study are prolongation of PT and APTT, increase in liver and adrenal weights, and histopathological lesions in the liver and the testis.

The NOAELs are less than 50 mg/kg/day for both sexes.

Data Quality: Valid without restriction

Reference: Ministry of Health and Welfare: Japan, Toxicity Testing Report of Environmental Chemicals 4, 409-430 (1996)

(d)

Species/strain: Rat (Wistar)

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

Route of Administration: Oral (by feeding)

Exposure period: 12 weeks

Frequency of treatment: Daily

Post exposure observation period: No

Dose: 1200, 6000, 30000 ppm

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

NOAEL Not determined

LOAEL: 1200 ppm (ca. 60 mg/kg)

Results:

Male

<u>At 1200 ppm</u>

Histopathological changes in the testis.

<u>At 6000 ppm</u>

Histopathological changes in the testis. Decrease in testis weight. Suppression of body weight gain. Death. Toxicity to liver, thymus and bone marrow.

<u>At 30000 ppm</u>

Histopathological changes in the testis. Decrease in testis weight. Suppression of body weight gain. Death. Toxicity to liver, thymus and bone marrow.

Female

<u>At 6000 ppm</u>

Histopathological changes in the ovary and the uterus. Decrease in ovary weight. Suppression of body weight gain. Death. Toxicity to liver, thymus and bone marrow.

<u>At 30000 ppm</u>

Histopathological changes in the ovary and the uterus. Decrease in ovary weight. Suppression of body weight gain. Death. Toxicity to liver, thymus and bone marrow.

Method: Dose-finding test for chronic toxicity study

Test condition: Number of animals per sex per dose was 10.

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

Test substance : Commercial grade (purity: Unknown)

Reference: Takagi et al, National Institute of Health Science, Japan (1994)

(e)
Species/strain: Rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral (by feeding)
Exposure period: 90 days
Frequency of treatment: Daily
Post exposure observation period: Unknown
Dose: 330, 1000, 3000 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOAEL: 330 ppm (ca. 16.5 mg/kg) for male, 3000 ppm (ca. 150 mg/kg) for female
LOAEL: 1000 ppm ca. 50 mg/kg) for male
Results:
Male:
At 1000 ppm
Increase in liver weight. Histpathological changes in the testis.
At 3000 ppm
Increase in liver weight. Histpathological changes in the testis.
Lower body weight. Decrease in kidney weight.
Method: Unknown
Test condition: Number of animals per sex per dose was 25.
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Grade and purity: Unknown
Remarks: One male and one female died at the end of experiment.
Reference: American Cyanamid Company (1965)
Note: Data from IUCLID (1996); original report: Not available.

(f)
Species/strain: Dog (Beagle)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral (by feeding)
Exposure period: 90 days
Frequency of treatment: Daily
Post exposure observation period: Unknown
Dose: 330, 1000, 3000 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOAEL: 330 ppm (ca. 11 mg/kg.)
LOAEL: 1000 ppm (ca. 33 mg/kg)
Results: Blood serum protein, glucose, urea nitrogen were normal. Prothrombine value was within control level. Pathological changes or statistical difference in organ weight were not observed.
At 1000 ppm
ALP activity was affected.
At 3000 ppm
ALP activity was affected.
Method: Unknown
Test condition: Number of animals per sex per dose was 1.
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Grade and purity: Unknown
Reference: American Cyanamid Company (1965)
Note: Data from IUCLID (1996); original report: Not available.

(g)
Species/strain: Rat (SD)

Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral (by feeding)
Exposure period: 1 week
Frequency of treatment: Daily
Post exposure observation period: No
Dose: 3860 ppm (1.135 mmol %)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOAEL: Not determined
LOAEL: Not determined
Results: A study on hepatic lipids with male rats fed at 3860 ppm (1.135 mmol %) for 1 week. The decrease in triglyceride (liver) and plasma total cholesterol were observed.
Method: Other
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Commercial grade (purity: Unknown)
Reference: Takahashi et al. (1981a)

(h)
Species/strain: Rat (SD)
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral (by feeding)
Exposure period: 1 week
Frequency of treatment: Daily
Post exposure observation period: No
Dose: 3860 ppm
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**;
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOAEL: Not determined
LOAEL: Not determined
Results: The prothrombin and kaolin-partially thromboplastin time (PTT) indexes decreased to 32% and 37% of control's, respectively. The substance was reported to cause death due to hemorrhage at high dosage or at the condition with Vitamin K deficient diet.
Method: Other
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Commercial grade (purity: Unknown)
Reference: Takahashi et al. (1981b)

(i)
Species/strain: . Rat
Sex: Female [ ]; Male [ ]; Male/Female [ ]; No data [X]
Route of Administration: Oral.
Exposure period: 10 month
Frequency of treatment: Daily
Post exposure observation period: .Unknown
Dose: 50 mg/kg/day
Control group: Yes [ ]; No [ ]; No data [X];
Concurrent no treatment [ ]; Concurrent vehicle [ ]; Historical [ ]
NOAEL: Not determined
LOAEL: Not determined
Results: Administration at 50 mg/kg/day for 10 month induced functional changes in the nervous system and the liver, and morphological changes in the testis.
Method: Unknown

GLP: Yes [ ] No [ ] ? [X]
Test substance : Grade and purity: Unknown
Reference: Stasenkova et al. (1977)

(j)
Species/strain: . Mouse
Sex: Female [ ]; Male [ ]; Male/Female [ ]; No data [X]
Route of Administration: Oral.
Exposure period: 10 month
Frequency of treatment: Daily
Post exposure observation period: .Unknown
Dose: 50 mg/kg/day
Control group: Yes [ ]; No [ ]; No data [X];
Concurrent no treatment [ ]; Concurrent vehicle [ ]; Historical [ ]
NOAEL: Not determined
LOAEL: Not determined
Results: Administration at 50 mg/kg/day for 10 month induced functional changes in the nervous system and the liver, and morphological changes in the testis.
Method: Unknown
GLP: Yes [ ] No [ ] ? [X]
Test substance : Grade and purity: Unknown
Reference: Stasenkova et al. (1977)

*5.5 GENETIC TOXICITY IN VITRO

A. BACTERIAL TEST

(a)
Test Substance 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
Produced by Sumitomo Chemical, Lot No. 40401, Purity: >98%,
Kept at room temperature until use

Method
Method: Guideline for Screening Mutagenicity Testing of Chemicals (Japan) and OECD TG 471 and TG 472
Test type: Reverse mutation assay
System of testing: Bacteria
GLP: Yes
Year: 1996
Species/Strain: *Salmonella typhimurium* TA100, TA1535, TA98, TA1537
Escherichia coli WP2 *uvr*A
Metabolic activation: S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone
Concentration: -S9 mix: 0, 313, 625, 1250, 2500, 5000 μg/plate
+S9 mix: 0, 313, 625, 1250, 2500, 5000 μg/plate
Statistical methods: No statistic analysis
Test conditions: Number of replicates: 2
Plates/test: 3
Procedure: Pre-incubation
Solvent: Acetone
Positive controls: -S9 mix;2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2), Sodium azide (TA1535) and 9-Aminoacridine (TA1537)
+S9 mix; 2-Aminoanthracene (five strains)

Results

Cytotoxic concentration: Toxicity was not observed up to 5000 μg/plate in five strains with or without S9 mix.
Precipitation concentration: Precipitation was observed at more than 313 μg/plate without S9 mix. and at more than 625 μg/plate with S9 mix.

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

Remarks:
Conclusions: Bacterial gene mutation is negative with and without metabolic activation
Data Quality: Valid without restriction
Reference: Ministry of Health and Welfare: Japan, Toxicity Testing Report of Environmental Chemicals 4, 409-430 (1996)

(b)
Type: Bacterial reverse mutation assay
System of testing: *Salmonella typhimurium* TA100, TA98
Concentration: -S9 mix: 0, 10, 100 and 1000 μg/plate
+S9 mix: 0, 10, 100 and 1000 μg/plate
Metabolic activation: With [ ]; Without [ ]; With and Without [X]; No data [ ]
S9: Unknown
Results:
Cytotoxicity concentration: Unknown

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

Method: Other (Ames 1975)
GLP: Yes **[ ]** No **[**X**]** ? **[ ]**
Test substance : Grade and purity: Unknown
Remarks:
Reference: Sumitomo Chemical (1977b)

(c)
Type: Bacterial DNA damage test (Rec-assay)
System of testing: *Bacillus subtilis* H17, M45 strain
Concentration: -S9 mix: 0, 10, 100, 1000 μg/disk
Metabolic activation: With **[ ]**; Without **[X]**; With and Without **[ ]**; No data **[ ]**
Results:

Genotoxic effects:	+	?	-
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

Method: Other (Kada 1972)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance : Grade and purity: Unknown
Remarks:
Reference: Sumitomo Chemical (1977b)

(d)
Type: Bacterial reverse mutation assay
System of testing: *Salmonella typhimurium* TA100, TA98
Concentration: Unknown
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[X]**; No data **[ ]**
S9: Unknown
Results:
Cytotoxicity concentration: Unknown

Genotoxic effects:	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**

Without metabolic activation: **[ ] [ ] [X]**
Method: Unknown
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Commercial grade (purity: Unknown)
Remarks:
Reference: Yamaguchi, et al. (1991)

(e)
Type: Bacterial reverse mutation assay
System of testing: Unknown
Concentration: Unknown
Metabolic activation: With **[ ]**; Without **[ ]**; With and Without **[ ]**; No data **[X]**
S9: Unknown
Results:
Cytotoxicity concentration: Unknown
Genotoxic effects: + ? -
with or without S9 mix.: Unknown **[ ] [ ] [X]**
Method: Unknown
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Grade and purity: Unknown
Remarks: An interim report. Final results: Not available
Reference: RCC Umwelt AG (1986)
Note: Data from IUCLID (1996): original report: Not available.

B. NON-BACTERIAL IN VITRO TEST

Test Substance 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
Produced by Sumitomo Chemical, Lot No. 40401, Purity: >98%

Method
Method: Guideline for Screening Mutagenicity Testing of Chemicals (Japan) and OECD TG 473
Test type: Chromosomal aberration test
System of testing: Non bacteria
GLP: Yes
Year: 1996
System of testing: CHL/IU cell
Metabolic activation: S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone
Concentration: -S9mix (continuous treatment): 0, 0.0020, 0.0040, 0.0080 mg/ml
-S9mix (short term treatment): 0, 0.00050, 0.0010, 0.0020 mg/ml
+S9mix (short-term treatment): 0, 0.0075, 0.015, 0.030 mg/ml
Statistical methods: Fisher's exact analysis
Test conditions: For continuous treatment, cells were treated for 24 or 48 hrs without S9 mix. For short-term treatment, cells were treated for 6 hrs with and without S9 mix. and cultivated with fresh media for 18 hrs.
Plates/test: 2
Solvent: Acetone
Positive controls: Mitomycin C for continuous treatment.
Cyclophosphamide for short-term treatment.

Results
Cytotoxic concentration: The concentrations of 50% growth inhibition were as follows;
-S9mix (continuous treatment): 0.008 mg/ml
-S9mix (short-term treatment): 0.002 mg/ml
+S9mix (short-term treatment): 0.03 mg/ml
Genotoxic effects: clastogenicity polyploidy

		+	?	-	+	?	-
	With metabolic activation:	**[ ]**	**[ ]**	**[X]**	**[ ]**	**[ ]**	**[X]**
	Without metabolic activation:	**[ ]**	**[ ]**	**[X]**	**[ ]**	**[ ]**	**[X]**

Remarks: In the short-term treatment without S9 mix., the treatment at highest concentration of 0.0020 mg/ml was too toxic for chromosome analysis.

Conclusions: Chromosomal aberration in CHL/IU cells is negative with and without metabolic activation.

Data Quality: Valid without restriction

Reference: Ministry of Health and Welfare: Japan, Toxicity Testing Report of Environmental Chemicals 4, 409-430 (1996)

* 5.6 GENETIC TOXICITY IN VIVO

Type: Micronucleus assay
Species/strain: Mouse (strain: Unknown)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Route of Administration: Unknown
Exposure period: Unknown
Doses: Unknown
Results:
Effect on mitotic index or P/N ratio: Unknown
Unknown

Genotoxic effects:	+	?	-
Unknown	**[ ]**	**[ ]**	**[ ]**

Method: Unknown
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Grade and purity: Unknown
Remarks: An interim report. Results: Not available
Reference: RCC Umwelt (1986)
Note: Data from IUCLID (1996); original report: Not available.

5.7 CARCINOGENICITY

Test Substance 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
Produced by Ouchi Shinko Chemical. purity: Unknown

Method
Method: Unknown
Test type: Chronic toxicity study
GLP: No
Year: 1994
Species: Rat
Strains: Wistar
Route of Administration: Oral (by feeding)
Duration of test: 18 months
Doses: 100, 300, 1000 ppm (in diet)
Sex: Male/Female
Exposure period: 18 months
Frequency of treatment: Daily
Control group and treatment: Basal diet (no treatment)
Post exposure observed period: No
Statistical analysis Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data
Test condition: Age at study initiation was 5 week old (393±21g for male, 230±15g for female). Number of animals per sex per dose was 30, of which 5 animals were sacrificed at 6 month and another 5 animals at 12 month for

hematological and serum biochemical examinations). The animals were given diet of pellets mixing this substance. Organ weights were measured in brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands. Histopathological examinations were carried out in brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands, salivary glands, esophagus, stomach, small intestine, pancreas, urinary bladder, seminal vesicles, epididymis, ischiac nerve, uterus, prostate, mesenteric lymph nodes, thymus, spinal cord, skeletal muscle, born marrow.

Results

CARCINOGENICITY: No neoplastic lesion attributable to the substance was observed in any organs of either sex, however this study is not qualified to be regarded as a carcinogenicity study.Therefore, no conclusion could be reached on the carcinogenicity.

Toxic effects:

Male

At 1000 ppm:

Suppression of body weight gain from the month 6. Increase or increase-tendency in relative liver weight.

Female

At 1000 ppm:

Suppression of body weight gain from the month 1. Increase or increase-tendency in relative liver weight.

Remarks:

Conclusions: No tumors were observed in a 18-month chronic feeding study with rats up to 1, 000 ppm, however this study is not qualified to be regarded as a carcinogenicity study.
Therefore, no conclusion could be reached on the carcinogenicity.

Data Quality: Valid. (limitation: the study was not designed for carcinogenicity and carried out using relatively small numbers of animals without GLP)

Reference: Takagi et al., National Institute of Health Science, Japan (1994)

*5.8 TOXICITY TO REPRODUCTION

Test Substance 6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol
Produced by Sumitomo Chemical, Lot No.: 710140, purity 98.2 %, Kept at room temperature until use.

Method

Method: OECD TG 421
Test type: OECD Preliminary Reproduction Toxicity Screening Test
GLP: Yes
Year: 1999
Species: Rat
Strain: Crj:CD (SD)
Route of Administration: Oral (by gavage)
Doses: 12.5, 50, 200, 800 mg/kg/day (in 5% gum arabic)
Sex: Male/Female
Exposure period: Male; 50-52 days
Female; 40-48 days (from 14 days before mating to the day 3 of lactation)
Frequency of treatment: Daily
Control group and treatment: Concurrent vehicle
Frequency of treatment: Daily
Duration of test: 53 days
Premating exposure period for males: 14 days

Premating exposure period for females: 14 days
Statistical analysis Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data
Test condition: Age at study initiation was 10 week old (332-383 g for male, 206-238 g for female). Number of parents per sex per dose was 12. Five percent gum Arabic was used as a vehicle. Male/female per cage was 1/1, length of cohabitation was 14 days, and the proof of pregnancy was judged by formation of vaginal plug. Functional observation, measurement of anogenital distance and so on were not performed because the test was conducted by the TG adopted in 1990.

Results
NOAEL: 50 mg/kg/day for female reproductive toxicity
12.5 mg/kg/day for male reproductive toxicity
200 mg/kg/day for developmental toxicity

Toxic effects:

Maternal
At 200 mg/kg/day:
Decrease in number of corpora lutea, implantation scars and pups born.
At 800 mg/kg/day:
Decrease in number of corpora lutea, implantation scars and pups born. One dam was unable to deliver pups, one dam lost all the pups during the lactation period.

Pups
At 200 mg/kg/day:
Decrease in number of pups.
At 800 mg/kg/day:
Decrease in number of pups. Decrease in live birth index. Decrease in body weights of both sexes on the day 4 of lactation. Increase in number of stillbirths.

Remarks: External anomalies were not observed
Conclusions: Effects on female reproductive parameters are decrease in corpora lutea, implantation scars, pups born, and those on developmental parameters are low body weight gain of offspring and increased number of stillbirths.

The NOAEL for female reproductive toxicity is 50 mg/kg and that for developmental toxicity is200 mg/kg/day.

As for male reproductive toxicity, the NOAEL is 12.5 mg/kg/day based on testicular toxicity described in REPEATED DOSE TOXICITY (b).

Data Quality: Valid without restriction
Reference: Ministry of Health and Welfare: Japan, Toxicity Testing Report of Environmental Chemicals 7, 423-437 (1999)

*5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY

(a)
Species/strain: Rat (Wistar)
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral.
Duration of the test: Until the day 20 of pregnancy
Exposure period: The day 7-17 of pregnancy
Frequency of treatment: Daily
Doses: 93.5,187, 375 mg/kg
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**; olive oil
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**

TERATOGENICITY Not teratogenic
NOAEL: 93.5 mg/kg for maternal toxicity
187 mg/kg for foetal toxicity
Results:
Not teratogenic in external, visceral and skeletal observations
Effects on Dam
At 187 mg/kg
Diarrhea, Hair fluffing. Suppression of body weight gain and food consumption.
At 375 mg/kg
Diarrhea, Hair fluffing. Suppression of body weight gain and food consumption. Death (2 dams).
Effects on Fetus
At 375 mg/kg
Increase in fetal death rate.
Method: Unknown
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance : Grade and purity: Unknown
Remarks: Numbers of dams were 23 (0 mg/kg:), 20 (93.5 mg/kg), 20 (187 mg/kg) and 15 (375 mg/kg)
Reference: Tanaka et al. (1990)

(b)
Species/strain: Rat
Sex: Female **[X]**; Male **[ ]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Oral (by feeding)
Duration of the test: Until the day 22 of pregnancy
Exposure period: The day 0-22 of pregnancy
Frequency of treatment: Daily
Doses: Total 0.25 g/body
Control group: Yes [X]; No [ ]; No data [ ];
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOAEL: Not determined
Results: The implanted fetus were resorpted by 17.1% in comparison with 10.6% of the control's.
Method: Unknown
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance : Grade and purity: Unknown
Remarks:
Reference: Telford et al. (1962)

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities

No data available

B. Toxicodynamics, toxicokinetics

No data available

* 5.11 EXPERIENCE WITH HUMAN EXPOSURE

No data available

6. REFFERENCES

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Appendix I. Proofs of the calculations for theoretical distribution of 6,6-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol.

Substance: 6,6-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol

Scenario 1

Compartment	Emission rate [kg/h]	Concentration [g/m^3]	Amount [kg]	Percent [%]	Transformation rate	
					Reaction	Advection
Air	1000	4.3E-07	4.3E+03	0.0	9.4E+02	4.3E+01
Water	0	5.3E-04	1.1E+04	0.0	7.3E-08	1.1E+01
Soil	0	6.3E+01	1.0E+08	99.8	6.9E-04	
Sediment		1.4E+00	1.4E+05	0.1	9.8E-07	2.8E+00
		Total amount	1.0E+08			

Scenario 2

Compartment	Emission rate [kg/h]	Concentration [g/m^3]	Amount [kg]	Percent [%]	Transformastion rate	
					Reaction	Advection
Air	0	1.3E-14	1.3E-04	0.0	2.9E-05	1.3E-06
Water	1000	3.9E-02	7.9E+05	6.9	5.5E-06	7.9E+02
Soil	0	2.0E-06	3.1E+00	0.0	2.2E-11	
Sediment		1.1E+02	1.1E+07	93.1	7.3E-05	2.1E+02
		Total amount	1.1E+07			

Scenario 3

Compartment	Emission rate [kg/h]	Concentration [g/m^3]	Amount [kg]	Percent [%]	Transformation rate	
					Reaction	Advection
Air	0	9.3E-13	9.3E-03	0.0	2.1E-03	9.3E-05
Water	0	3.9E-02	7.9E+05	0.0	5.5E-06	7.9E+02
Soil	1000	5.8E+03	9.3E+09	99.9	6.5E-02	
Sediment		1.1E+02	1.1E+07	0.1	7.3E-05	2.1E+02
		Total amount	9.3E+09			

(Continued)

Appendix I. (Continued)

Physico-chemical parameter

Molecular weight		340.51	Measured
Melting point ［℃］		130	Measured
Vapor pressure [Pa]		4.7E-11	Estimated
Water solubility [g/m^3]		2.0E-02	Measured
log Kow		6.25	Measured
Half lives [h] (Note 1)	In air	3.141	Estimated
	In water	1E+11	Estimated
	In soil	1E+11	Estimated
	In sediment	1E+11	Estimated

Temperature [℃]	25

Environmental parameter

		Volume [m^3]	Depth [m]	Area [m^2]	Organic carbon content [－]	Lipid content [－]	Density [kg/m^3]	Residence Time [h]
Bulk air	Air	1.0E+13					1.2	100
	Particles	2.0E+03						
	Total	1.0E+13	1000	1E+10				
Bulk water	Water	2.0E+10					1000	1000
	Particles	1.0E+06			0.04		1500	
	Fish	2.0E+05				0.05	1000	
	Total	2.0E+10	10	2E+09				
Bulk soil	Air	3.2E+08					1.2	
	Water	4.8E+08					1000	
	Solid	8.0E+08			0.04		2400	
	Total	1.6E+09	0.2	8E+09				
Bulk Sediment	Water	8.0E+07					1000	
	Solid	2.0E+07			0.06		2400	50000
	Total	1.0E+08	0.05	2E+09				

Intermedia transport parameter ［m/h］

Air side air-water MTC	5	Soil air boundary layer MTC	5
Water side air-water MTC	0.05	Sediment-water MTC	1E-04
Rain rate	1E-04	Sediment deposition	5E-07
Aerosol deposition	6E-10	Sediment resuspension	2E-07
Soil air phase diffusion MTC	0.02	Soil water runoff	5E-05
Soil water phase diffusion MTC	1E-05	Soil solid runoff	1E-08

(Note 1) The half life in air is estimated by using AOPWIN (ver.1.80). See section 3.1.1.
Other half lives are estimated according to the method specified in the EU-TGD (European Commission, 1996).

ROBUST SUMMARY
for
6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol

PHYSICAL/CHEMICAL ELEMENTS

MELTING POINT

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
- Remarks:

METHOD

- **Method/guideline:** Not specified (using melting point measuring apparatus)
- **GLP:** No
- **Year:** ranging from 1941 to 1976
- **Remarks:**

RESULTS

- **Melting point value:** 130-131 °C
- **Decomposition:** No
- **Sublimation:** No
- **Remarks:** selected from seven available data appeared in a scientific documents, ranging from 129 -131.21 °C. studies .

CONCLUSIONS

Melting point is 130 - 131 °C.

DATA QUALITY

- **Reliabilities:** Not evaluated.
- **Remarks:** From 10 data cited in the Beilstein Data, two were neglected because the data sources were patent. From the remaining, data of the range within 1°C were chosen for consideration.

REFERENCES *(Free Text)*

Beilstein Database, 2000

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

BOILING POINT

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
- Remarks:

METHOD

- **Method:** Not specified
- **GLP:** Not specified
- **Year:** 1995 (published year)
- **Remarks:**

RESULTS

- **Boiling point value:** 187 °C
- **Pressure:** 0.07
- **Pressure unit:** hPa
- **Decomposition:** Information not available
- **Remarks:**

CONCLUSIONS

Boiling point is 187 °C at 0.07 hPa.

DATA QUALITY

- **Reliabilities:** Not able to evaluate.
- **Remarks:** No other data existed.

REFERENCES *(Free Text)*

IUCLID Data Set (1999); (reported by Chemische Werke Lowi GmbH & Co., Waldkraidung, Germany)

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

DENSITY (Crystal)

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
- Remarks:

METHOD

- **Method:** Not given in the Database.
- **GLP:** Not specified
- **Year:** 1984 (published year)
- **Remarks:**

RESULTS

- **Density of the crystal :** 1.08 g/cm^3
- **Method of crystallization:** Not given in the Database.
- **Temperature:** Not specified.
- **Remarks:**

CONCLUSIONS

The density of the crystal is 1.08 g/cm^3

DATA QUALITY

- **Reliabilities:** Not able to evaluate.
- **Remarks:** No other data existed.

REFERENCES *(Free Text)*

Data source: Beilstein Database, 2000
Original reference: Chetkina, L. A. et. al., J. Struct. Chem. (Engl. Trans.), 1984, 25, 935-939

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

Bulk density is 0.35 g/cm^3 (IUCLID 1999, Chemische Werke Lowi GmbH & Co., Waldkraidung, Germany)

VAPOUR PRESSURE

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** Calculation
- **GLP:** No
- **Year:** 2000
- **Remarks:**
  Calculated by multiplying the Henry's law constant (7.9 x 10^{-12} atm-m^3/mol) by the water solubility (5.9 x 10^{-5} mol/m^3), which are both considered reliable as indicated in other pages.

RESULTS

- **Vapour pressure value:** 4.7 x 10^{-11} Pa
- **Temperature:** Unknown
- **Decomposition:**
- **Remarks:**

CONCLUSION

Vapour pressure is 4.7 x 10^{-11} Pa

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**
  In order to obtain the definite vapour pressure (that is used in the fugacity calculation), this estimation is conducted. The value is considered as reliable because both the Henry's law constant and the water solubility employed are reliable, and because the estimated value is consistent with the measured one (<0.1 hPa, IUCLID (1999), Chemische Werke Lowi GmbH & Co., Wald Kraidung, Germany).

REFERENCES *(Free Text)*

- Unpublished data (calculated by Sumitomo Chemical Co., Ltd., Japan)

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

PARTITION COEFFICIENT

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- Remarks:

METHOD

- **Method:** Flask-shaking method, but detailed information is not specified.
- **GLP:** No
- **Year:** 1992 (published year)
- **Remarks:**

RESULTS

- **log Pow:** 6.25
- **Temperature:** Unknown
- **Remarks:**

CONCLUSION

The log Pow value determined for the substance is 6.25.

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**

Approved value by the Japanese government.
The value 6.25 is consistent with the value estimated by using CLOGP, ver.2.0.0b (i.e. 7.5), suggesting that the substance is highly hydrophobic.

REFERENCES (Free Text)

Chemicals Inspection and Testing Institute of Japan (1992), "Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan."

CLOGP calculation: Unpublished data (calculated by Sumitomo Chemical Co., Ltd., Japan).

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

WATER SOLUBILITY

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** Detailed information is not specified.
- **GLP:** Not specified
- **Year:** 1992 (published year)
- **Remarks:**

RESULTS

- **Value (mg/L):** 0.02 mg/L
- **Description of solubility:** Of very low solubility
- **pH value:**
- **pKa value:**
- **Temperature:** Unknown
- **Remarks:**

CONCLUSION

The solubility in water is very low.
The value 0.02 mg/L is converted to the molar concentration to be 5.9×10^{-5} mol/m^3.

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**

Approved value by the Japanese government.

REFERENCES (Free Text)

Chemicals Inspection and Testing Institute of Japan (1992), "Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan."

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

PARTITION COEFFICIENT BETWEEN SOIL/SEDIMENT AND WATER (KD)

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** Estimation by the method specified in the EU-TGD.
- **GLP:** No
- **Year:** 1999
- **Remarks:**
  The QSAR equation employed is for predominantly hydrophobics:
  log Koc = 0.81 x log Pow + 0.10 [European Commission (1996)].
  The log Pow value substituted is 6.25.

RESULTS

- **Koc:** 1.5×10^5
- **Temperature:**
- **Remarks:**

CONCLUSION

The Koc of the substance is 1.5×10^5.

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**

The value is estimated according to the authorized QSAR equation with the reliable log Pow value, and is consistent with the value estimated according to the SRC model (i.e. 59800).

REFERENCES (Free Text)

Unpublished data (calculated by Sumitomo Chemical Co., Ltd., Japan).

SRC model calculation: STN international (1999), HSDB (Hazardous Substances Data Bank).

European Commission (1996), "Technical guidance document in support of commission directive 93/67/EEC on risk assessment for new notified substances and commission regulation (EC) No 1488/94 on risk assessment for existing substances."

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

HENRY'S LAW CONSTANT

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** Estimation by the bond contribution method developed by Meylan and Howard (1991).
- **GLP:** No
- **Year:** 1991 (published year)
- **Remarks:**

RESULTS

- **Henry's Law Constant:** 7.9 x 10^{-12} atm-m^3/mol
- **Temperature:**
- **Remarks:**

CONCLUSION

The Henry's law constant of the substance is 7.9 x 10^{-12} atm-m^3/mol.

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**

The value is peer-reviewed by the National Library of Medicine's.

REFERENCES (Free Text)

STN international (1999), HSDB (Hazardous Substances Data Bank).

Meylan, W.M. and Howard, P.H. (1991), Environ. Toxicol. Chem. 10, 1283-93.

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

Oxidation/Reduction Potential

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
- Remarks: Source not disclosed.

METHOD

- **Method/guideline:** Anodic voltammetry (polarography)
- **Type (test type):** Half-wave potential as a function of pH
- **GLP:** No
- **Year:** 1969 (published year)
- **Remarks:** Solution of the test substance (not more than $1x10^{-4}$ mol/L) in universal buffer solution containing isopropanol (40% in concentration) was electrically oxidized at 20 °C at the polarization rate of 10 mV/sec. Half-wave potential was measured by use of a high-purity, fine-grain graphite electrode as the indicator electrode and a $Cd/CdSO_4$ comparison electrode. Measurements were carried out in 3 - 5 times.

RESULTS

- **Half-wave potential:** $E_{1/2}$ = 0.35 mV.
- **Temperature:** 20 °C.
- **Oxidization products:** not studied
- **Remarks:**

CONCLUSIONS

The half-wave potential ($E_{1/2}$) is 0.35 mV at 20 °C .

DATA QUALITY

- **Reliabilities:** key study
- **Remarks:** Well conducted study

REFERENCES (Free Text)

Vodzinskii, Yu. V., et. Al., J. Gen. Chem. USSR (Engl. Trans.), 1969, 39, 1168-1173.

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

ENVIRONMENTAL FATE AND PATHWAY

PHOTODEGRADATION (INDIRECT-IN AIR)

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** Estimation by the AOPWIN, ver.1.80, based on the Atkinson model recommended in the OECD Guidance.
- **GLP:** No
- **Year:** 2000
- **Type of Sensitizer:** OH
- **Concentration of Sensitizer:** 1.5 x 10^6 OH/cm^3
- **Remarks:**

RESULTS

- **Rate Constant (Radical):** 40.8578 x 10^{-12} cm^3/molecule-sec
- **Degradation:** 50% after 3.141 hours
- **Remarks:**

CONCLUSION

The substance in air is indirectly photodegradated with the half life of 3.141 hours.

DATA QUALITY

- **Reliability:** Reliable
- **Remarks:**

The value is estimated with the method recommended in the OECD Guidance.

REFERENCES (Free Text)

Unpublished data (calculated by Sumitomo Chemical Co., Ltd., Japan)

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Test (test type):** Calculation
- **Method:** Fugacity level III
- **Year:** 2000
- **Remarks:**

The parameter used are shown in the Appendix.

RESULTS

- **Media:** Air-Biota-Sediment-Soil-Water
- **Amount Percent:**
- **Remarks:**

Compartment	Amount %		
	Release 100% to air	Release 100% to water	Release 100% to soil
Air	0.0%	0.0%	0.0%
Water	0.0%	6.9%	0.0%
Soil	99.8%	0.0%	99.9%
Sediment	0.1%	93.1%	0.1%

CONCLUSION

If released to water, the majority of the substance shows a tendency to distribute into sediment phase.
Emission to other compartments will result in the distribution into soil compartment.

DATA QUALITY

- **Reliability**: Key study
- **Remarks**:

The model employed is developed by the Japanese government.

REFERENCES (Free Text)

Unpublished data (calculated by Sumitomo Chemical Co., Ltd., Japan)

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

Appendix: Parameters used in the Fugacity Calculations

Physico-chemical parameter

Molecular weight		340.51	Measured
Melting point［℃］		130	Measured
Vapor pressure [Pa]		4.7E-11	Estimated
Water solubility [g/m^3]		2.0E-02	Measured
log Kow		6.25	Measured
Half lives [h] (Note 1)	In air	3.141	Estimated
	In water	1E+11	Estimated
	In soil	1E+11	Estimated
	In sediment	1E+11	Estimated

Temperature [℃]	25

Environmental parameter

		Volume [m^3]	Depth [m]	Area [m^2]	Organic carbon content [－]	Lipid content [－]	Density [kg/m^3]	Residence Time [h]
Bulk air	Air	1.0E+13					1.2	100
	Particles	2.0E+03						
	Total	1.0E+13	1000	1E+10				
Bulk water	Water	2.0E+10					1000	1000
	Particles	1.0E+06			0.04		1500	
	Fish	2.0E+05				0.05	1000	
	Total	2.0E+10	10	2E+09				
Bulk soil	Air	3.2E+08					1.2	
	Water	4.8E+08					1000	
	Solid	8.0E+08			0.04		2400	
	Total	1.6E+09	0.2	8E+09				
Bulk Sediment	Water	8.0E+07					1000	
	Solid	2.0E+07			0.06		2400	50000
	Total	1.0E+08	0.05	2E+09				

Intermedia transport parameter

［m/h］

Air side air-water MTC	5	Soil air boundary layer MTC	5
Water side air-water MTC	0.05	Sediment-water MTC	1E-04
Rain rate	1E-04	Sediment deposition	5E-07
Aerosol deposition	6E-10	Sediment resuspension	2E-07
Soil air phase diffusion MTC	0.02	Soil water runoff	5E-05
Soil water phase diffusion MTC	1E-05	Soil solid runoff	1E-08

(Note 1) The half life in air is estimated by using AOPWIN (ver.1.80).
Other half lives are estimated according to the method specified in the EU-TGD (European Commission, 1996).

BIODEGRADATION

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** MITI (I) method (1974), equivalent to the OECD 301C (1981).
- **GLP:** Yes
- **Year:** 1992 (published year)
- **Contact Time:** 28 days
- **Inoculum:** Non adapted standardized activated sludge, prepared as specified in the OECD 301C.
- **Remarks:**

The concentration of the substance is 100 mg/L. The concentration of the inoculum is 30 mg/L, as suspended solid.

RESULTS

- **Degradation rate:** 0% after 28 days (based on BOD)
  1% after 28 days (based on HPLC analysis for the parent)
- **Degradability:** Not readily biodegradable
- **Kinetics:** Not specified
- **Metabolites (Degradation product):** Not specified
- **Remarks:**

CONCLUSION

The substance is not readily biodegradable.

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**

The data is approved by the Japanese government.

REFERENCES (Free Text)

Chemicals Inspection and Testing Institute of Japan (1992), "Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan."

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

BIOACCUMULATION

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** MITI method (1974), equivalent to the OECD 305C (1981).
- **GLP:** Yes
- **Year:** 1992 (published year)
- **Fish Species:** Carp (*Cyprinus carpio*)
- **Exposure Period:** 8 weeks
- **Temperature:** 25 ^{o}C
- **Concentrations:** 1.0 mg/L and 0.1 mg/L
- **Remarks:**

The stock solution for exposure was prepared with castor oil (HCO-40) according to the guideline. The exposure was conducted under flow-through conditions. No elimination experiment was conducted.

RESULTS

- **Bioconcentration factor:**

Exposure conc.	2 week	4 week	6 week	8 week
1.0 mg/L	37, 28	32, 31	24, 23	30, 23
0.1 mg/L	89, 120	77, 60	125, 121	108, 97

- **Kinetics:** Not conducted
- **Remarks:**

The average lipid content of carp was 4.7%.

The exposure concentrations measured were well maintained in the range specified in the guideline (i.e. ±20% of the nominal ones). Bioconcentration equilibrium was also well established for each exposure level as indicated in the above table.

The BCF values at 1.0 mg/L were significantly lower than those at 0.1 mg/L.

Since the nominal exposure concentrations of 0.10 and 1.0 mg/L are only 5 and 50 times higher than the water solubility (i.e. 0.02 mg/L), and since the caster oil (HCO-40) would make the substance bioavailable (i.e. solubilized) even at these relatively high nominal concentrations, the observed dependency of the apparent BCF values on exposure concentrations would not be attributable to the limited bioavailability of the substance.

As indicated in the Appendix, the substance would be difficult to be directly taken up through fish gill membrane due to the restricted permeability of the steric hindered molecule. Some fractions of the substance, on the other hand, would be adsorbed to the surface of fish body and/or other fractions adsorbed to feeds would be also orally taken up into fish body.

These somewhat complex mechanisms would finally result in the observed dependency of BCF values on exposure concentrations. Somewhat low BCF values of up to around 100 compared to those predicted by the hydrophobicity (i.e. logP = 6.25) should be also attributable to the steric hindrance of the molecule.

CONCLUSION

The BCF of the substance is 23-125.

DATA QUALITY

- **Reliability:** Key study
- **Remarks:**

The data is approved by the Japanese government.

REFERENCES (Free Text)

Chemicals Inspection and Testing Institute of Japan (1992), "Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan."

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

Appendix. Three dimensions of the boxed substance 6,6'-di-tert-Butyl-2,2'-methylene di-p-cresol

Opperhuizen et.al. (1985) investigated the bioconcentration of polychlorinated naphthalenes in guppy and suggested that an uptake limitation through fish gill membrane would be observed when two of the three dimensions of a boxed molecule are beyond 9.5 angstrom.

As shown below, three dimensions of the boxed substance estimated with Quanta (ver.4.0) are 14.0, 10.7 and 8.3 angstrom, suggesting the restricted membrane permeation of this substance.

1. Software: Quanta ver.4.0

2. Procedure:

(1) The conformation (i.e. the internal coordinate) of the substance was optimised by using the Molecular Dynamics (CHARMm program; the temperature employed for intermolecular vibration was up to 4000K).
(2) Then, the three dimensions of the optimised molecule are estimated by measuring interatomic distances and by taking the van der Waals volume of the atoms into account.

3. Results

Firstly, the procedure was validated with using the chemicals from Opperhuizen et.al. The second smallest dimension estimated for each molecule is indicated in Table 1(a). Although some overestimation tendencies are observed for all the chemicals (i.e. from 0 to 0.9 angstrom overestimated), the results seem to be comparable.

The optimised structure of the substance 6,6'-di-tert-Butyl-2,2'-methylene di-p-cresol is shown in Fig.1 and three dimensions of this optimised molecule are indicated in Table 1(b). As shown in this Table, the second smallest dimension was 10.7 angstrom, large enough to restrict the uptake through fish gill membrane.

4. Reference

Opperhuizen, A. et al. "Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals." Chemosphere, vol.14, No. 11/12, 1985, pp. 1871-1896.

Table 1. Results of estimations

Chemical	logBCF	LogP	Data from Opperhuizen et.al.	Our calculation with QUANTA
			2nd smallest dimension (Å)	2nd smallest dimension (Å)
(a)				
1,4-Dichloronaphthalene	3.4	4.9	8.6	9.4
1,8-Dichloronaphthalene	3.8	4.4	8.6	9.0
1,2,3,4-Tetrachloronaphthalene	4.5	5.9	9.1	9.6
Octachloronaphthalene	**0**	**8.4**	**9.8**	**10.5**
2,3,7,8-tetrachlorodibenzo-p-dioxin	>3	>6	7.6	7.6
Octachlorodibenzo-p-dioxin	**0**	**>6**	**9.8**	**10.4**
Hexachlorobenzene	>4	5.8	8.7	9.6
Hexabromobenzene	**0**	**>6**	**9.6**	**10.1**

Decachlorobiphenyl	>5	>6	8.7	9.6
Decabromobiphenyl	**0**	**>6**	**9.6**	**10.1**
(b)				
6,6'-di-tert-Butyl-2,2'-methylene di-p-cresol	**2.1**	**6.25**	**-**	**X=14.0 Y=10.7 Z=8.3**

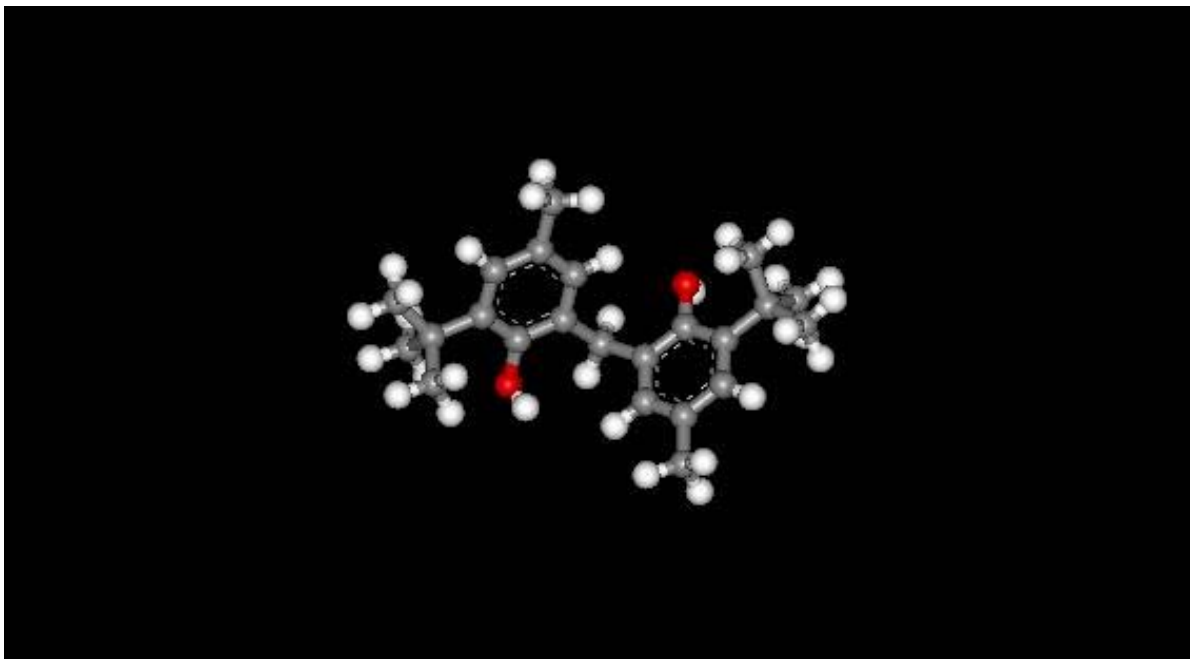

Fig.1 Optimised conformation of the substance 6,6'-di-tert-Butyl-2,2'-methylene di-p-cresol

ECOTOXICITY ELEMENTS

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

Purchased from Merck (lot No. S19983), purity 97.5%.

METHOD

- **Method:** According to the OECD guideline 203 (1992).
- **Exposure Period/Endpoint:** 96 hr, Mortality
- **Nominal Concentrations:** 5.0 mg/L, Solvent Control and Control
- **GLP:** Yes
- **Year:** 1998
- **Fish Species/Strain/Supplier:** Medaka (*Oryzias latipes*), Obtained from commercial hatcheries (Izumimoto hatcheries, Osaka prefecture).
- **Analytical Monitoring:** Yes. Exposure water sampled at the initiation and 48 hr of the exposure were extracted with hexane and analyzed for the substance by using GC-MS.
- **Statistical Methods:**

Due to low water solubility of the test substance, limit exposure experiment with only one nominal concentration was conducted. Thus, no statistical method for the determination of LC_{50} value was employed. A regression line for GC-MS analyses was derived by least square calculations. Numerical values were rounded according to the method specified in JIS.

- **Remarks:**

Test fish

Acclimated under test condition (water quality, temperature, etc.) for more than 12 days, and no mortality was observed for 7 days before the exposure. Fed daily with commercial fish feed (TetraMin staple food) and starved for 24 hr before the exposure.

The fish body weight and body length measured at the initiation of exposure were 0.17 g (0.16 - 0.20 g) and 2.2 cm (2.0 – 2.3 cm) on the average.

Test conditions

Test system: Semi-static (renewal rate was every 48 hr).

Dilution water: Dechlorinated tap water (pH 8.1, hardness 55.2 mg/L as $CaCO_3$).

Preparation of test solution for exposure: The substance was mixed with DMF/Castor oil HCO-40 (3/1(w/w); 20 times weight to the substance) and dissolved into dilution water to prepare the nominal concentration of 5.0 mg/L for exposure (No precipitations as well as emulsions were observed at this nominal concentration). Due to low water solubility of the substance, homogenous solution could be attained only at (and less than) this nominal concentration with the maximum allowable dispersant concentration of 100 mg/L.

Test volume for exposure: 5.0L (in aquarium: 5.0L all glass aquarium (inner size: 21 x 16 x 23 cm))

Number of replicate, fish per replicate: One replicate, ten fish per replicate.
Water chemistry during exposure: Measurements of temperature, pH and DO were conducted daily (including before and after renewal). The measured values were:
Test temperature: Ranged from 23.7 to 23.8 ^{o}C,
pH: Ranged from 7.5 to 7.8,
DO: Ranged from 5.8 to 8.3 mg/L (more than 60% of saturation).

Analytical methods
GC column: DB-1

The m/z for quantification: 177.

Pretreatment: Exposure water as well as control water each were extracted with the equivalent volume of hexane, and the hexane layer, thus obtained, was injected into GC-MS (directly or after concentration if necessary) for quantification.

Recovery ratios fortified at the concentrations of 0.050 mg/L and 5.0 mg/L were 83 % (82 – 84%) and 92 % (91 – 94%) on the average.

RESULTS
Measured Exposure Concentration:

Nominal Concentration (mg/L)	Measured value (mg/L)	
	At the initiation of exposure	After 48 hr of exposure
Control	< 0.01	<0.01
Solvent Control	<0.01	<0.01
5.0	4.0 (Note)	4.3 (Note)

(Note) The geometric mean value was 4.1 mg/L, corresponding to 83 % of the nominal concentration. Thus, the results (e.g. LC_{50} values) were expressed based on the nominal exposure concentrations.

- **LC_{50}:**

Exposure Period (hr)	LC_{50} values calculated (mg/L)
24	>5.0 (based on nominal concentration)
48	>5.0 (based on nominal concentration)
72	>5.0 (based on nominal concentration)
96	>5.0 (based on nominal concentration)

(Note) The geometric mean value was 4.1 mg/L, corresponding to 83 % of the nominal concentration. Thus, the results (e.g. LC_{50} values) were expressed based on the nominal exposure concentrations.

- **Statistical Results:** Not conducted
- **Remarks:**

- Biological observations/Abnormal responses: Visual inspections of test aquaria (i.e. Control, Solvent control and 5.0 mg/L of Nominal concentration) conducted every 24 hr revealed that no toxic symptoms in fish (e.g. abnormal respiration, abnormal swimming, inverted, etc.) were observed during the experiment.

- Cumulative mortality:

Nominal Concentrations (mg/L)	Cumulative Number of Dead (Percent Mortality)			
	24hr	48 hr	72hr	96 hr
Control	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)	0 (0)	0 (0)
5.0 mg/L	0 (0)	0 (0)	0 (0)	0 (0)

- Highest concentration in 0% mortality/Lowest concentration in 100% mortality:

Exposure Period (hr)	Highest concentration in 0% mortality	Lowest concentration in 100% mortality
24	5.0	>5.0
48	5.0	>5.0
72	5.0	>5.0
96	5.0	>5.0

Reference substance/Results: Copper sulfate (5 hydrate), lot No.PAH2068, Wako Pure Chemical Ind. The 96 hr-LC_{50} value determined was 4.0 mg/L.

Any observations: No precipitation as well as no milky cloud were observed in the test solutions during exposure.

CONCLUSION

Due to low water solubility of the substance, the limit experiment was conducted with the maximum dispersable concentration of 5.0 mg/L. The determined 96 hr-LC_{50} value was more than 5.0 mg/L based on the nominal concentration. No toxic symptoms were observed during the study. Experimental designs and results were well documented and prescribed conditions in the guideline were well satisfied.

DATA QUALITY

- **Reliability:** Reliable with no restriction
- **Remarks:**

The test is approved by the Japanese government.

Although the tested nominal concentration was relatively high compared to the water solubility of the substance (i.e. 0.02 mg/L), the reported result is considered to be quite valid because:
(1) The study was conducted according to the OECD guideline 203.
(2) No precipitations as well as no emulsions were observed by visual inspections at the nominal exposure concentration of 5.0 mg/L.
(3) No toxic symptoms in fish during 96 hrs of exposure can be well understood by taking the restricted uptake of the steric hindered molecule through fish gill membrane into account as suggested in the bioconcentration study.

REFERENCES (Free Text)

Environmental Agency of Japan (1999a), Acute toxicity of 2,2-Methylenebis (6-*tert*-butyl)-*p*-cresol to Medaka (*Oryzias latipes*), EFA98004.

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

ACUTE TOXICITY TO FISH

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

METHOD

- **Method:** Japanese Industrial Standard (JIS K 0102-1986-71), titled "Testing methods for industrial waste water".

- **Exposure Period/Endpoint:** 48 hr, Mortality

- **Nominal Concentrations:** Not specified

- **GLP:** Yes

- **Year:** 1992 (published year)

- **Fish Species/Strain/Supplier:** Medaka (*Oryzias latipes*), Obtained from Nakashima Fish Farm.

- **Analytical Monitoring:** Not specified

- **Statistical Methods:** Doudoroff or Probit method for estimating LC_{50} value

- **Remarks:**

Test fish
Acclimated under flow through conditions at 25 +/- 2 ^{o}C for about 28 days.

Test conditions
Test system: Static or Semi-static (renewal rate was every 8 to 16 hrs).

Dilution water: Underground water. The quality of water was confirmed to meet the ministerial ordinance of the Ministry of Health and Welfare (Aug. 31, 1978) in total hardness and evaporated residue. The other parameters were also confirmed to meet the water quality criteria for fisheries (1983).

Preparation of test solution for exposure: Not specified

Exposure aquarium: 4L round glass vessel

Number of replicate, fish per replicate: 1 replicate, 10 fish per replicate

Water chemistry during exposure: Not specified other than temperature (25 +/- 2 ^{o}C)

RESULTS

- **Measured Exposure Concentration:** Not specified
- **LC_{50}:** 48hr-LC_{50} > 500 mg/L
- **Statistical Results:** Not specified
- **Remarks:**

Reference substance / Results: $HgCl_2$. The response observed for the same lot of fish satisfied with the specified criteria.

CONCLUSION

The determined 48hr-LC_{50} value was more than 500 mg/L. This suggests that even at the stringently dispersed concentration of 500 mg/L, the mortality observed was less than 50%.

DATA QUALITY

- **Reliability:** Not assignable.
- **Remarks:**

The test is approved by the Japanese government.

REFERENCES (Free Text)

Chemicals Inspection and Testing Institute of Japan (1992), "Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan."

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

ACUTE TOXICITY TO AQUATIC INVERTEBRATES

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

Purchased from Merck (lot No. S19983), purity 97.5%.

METHOD

- **Method:** According to the OECD guideline 202 (1984).

- **Exposure Period/Endpoint:** 48 hr, Immobility

- **Nominal Concentrations:** 0.50, 0.90, 1.6, 2.8, 5.0 mg/L, Solvent Control and Control

- **GLP:** Yes

- **Year:** 1998

- **Test Organism Species/Strain/Supplier:** Water flea (*Daphnia magna*). Obtained from National Institute for Environmental Studies, and subcultured at the testing facility. Newly hatched juveniles were employed in the experiment.

- **Analytical Monitoring:** Yes. Exposure water sampled at the initiation and the termination (i.e. 48 hr) of the exposure were extracted with hexane and analyzed for the substance by using GC-MS.

- **Statistical Methods:**

Since the rate of immobility observed at the maximum concentration (i.e. 5.0 mg/L nominal) was less than 50%, no statistical method (e.g. Binominal, Moving average or Probit method) was employed for determining the EC_{50} value. A regression line for GC-MS analyses was derived by least square calculations. Numerical values were rounded according to the method specified in JIS.

- **Remarks:**

Test organisms

A matured female daphnia was selected from the subculture. On the next day, newly hatched juveniles were transferred into other culture vessels and further cultured for two to four weeks under the following conditions; temperature 20 +/- 1 ^{o}C, photoperiod 16hr light – 8hr dark, culture density 20 to 40 individuals per one liter of dilution water, feeding 0.1 to 0.2 mg carbon per day with *Chlorella vulgaris*. Mortality observed during this culture period was 1%. Newly hatched juveniles from, thus cultured, parents were used in the experiment.

Test conditions

Test system: Static

Dilution water: Dechlorinated tap water (pH 8.1, hardness 55.2 mg/L as $CaCO_3$).

Preparation of test solution for exposure: The substance was mixed with DMF/Castor oil HCO-40 (3/1(w/w)) and dissolved into dilution water to prepare the nominal concentrations described above (No precipitations as well as emulsions were observed in all the exposure levels). To set the dispersant concentrations equal (i.e. 100 mg/L) between the nominal concentrations, DMF/Castor oil HCO-40 solution was also used for the preparations. Due to low water solubility of the substance, homogenous solution could be attained only at (and less than) the nominal concentration of 5.0 mg/L with this maximum allowable dispersant concentration of 100 mg/L.

Test volume for exposure: 100 mL (in 100 mL beaker)

Number of replicate, individuals per replicate: Four replicates, Five individuals per replicate.

Water chemistry during exposure: Measurements of temperature, pH and DO were conducted at the initiation and the termination of exposure. The measured values were:

Test temperature: Ranged from 19.7 to 20.0 ^{o}C,
pH: Ranged from 8.1 to 8.2,
DO: Ranged from 8.2 to 8.6 mg/L (more than 60% of saturation).

Analytical methods
GC column: DB-1

The m/z for quantification: 177.

Pretreatment: Exposure water as well as control water each were extracted with the equivalent volume of hexane, and the hexane layer, thus obtained, was injected into GC-MS (directly or after concentration if necessary) for quantification.

Recovery ratios fortified at the concentrations of 0.050 mg/L and 5.0 mg/L were 83 % (82 – 84%) and 92 % (91 – 94%) on the average.

RESULTS

- **Measured Exposure Concentration:**

Nominal Concentration (mg/L)	Measured value (mg/L)			Percent to the nominal
	At the initiation	At the termination	Geometric mean	
Control	<0.01	<0.01	-	-
Solvent Control	<0.01	<0.01	-	-
0.50	0.38	0.42	0.40	80
0.90	0.74	0.74	0.74	82
1.6	1.4	0.92	1.1	71
2.8	2.2	2.5	2.3	84
5.0	4.8	4.8	4.8	96

(Note) The geometric mean values were corresponding to 71 to 96% of the nominal concentrations. Thus, the results (e.g. EC_{50} values) were expressed based on the geometric mean

values of the measured concentrations.

・**EC_{50} (Immobility):**

Exposure Period (hr)	EC_{50} values calculated (mg/L)	95% Confidence Limit	Statistical Method
24	>4.8 (based on measured concentration)	-	-
48	>4.8 (based on measured concentration)	-	-

・**Statistical Results:** Not conducted

・**Remarks:**

Cumulative number of immobilized daphnia:

Measured Concentrations (mg/L)	Cumulative Number of Immobilized Daphnia (Percent immobility)	
	24 hr	48 hr
Control	0 (0)	0 (0)
Solvent Control	0 (0)	0 (0)
0.40	0 (0)	0 (0)
0.74	0 (0)	0 (0)
1.1	0 (0)	1 (5)
2.3	0 (0)	1 (5)
4.8	0 (0)	7 (35)

- No Observed Effect Concentration (NOEC) /Lowest concentration in 100% immobility (based on the geometric means of the measured concentrations) :

Exposure Period (hr)	No Observed Effect Concentration (NOEC)	Lowest Concentration in 100% immobility
24	4.8	>4.8
48	0.74	>4.8

Reference substance/Results: Potassium dichromate, lot No.ACQ2610, Wako Pure Chemical Ind. The 48 hr-EC_{50} value determined was 0.54 mg/L.

Any observations: Not described

CONCLUSION

Due to low water solubility of the substance, maximum dispersable concentration was limited to be 5.0 mg/L (nominal). The determined 48 hr-EC_{50} value was more than 4.8 mg/L based on the geometric mean of the measured concentrations. The 48hr-NOEC was evaluated to be 0.74 mg/L. Experimental designs and results were well documented and prescribed conditions in the guideline were well satisfied.

DATA QUALITY

・**Reliability:** Reliable with no restriction

・**Remarks:**

The test is approved by the Japanese government.

Although the tested nominal concentrations were all above the water solubility of the substance,

the reported result is considered to be quite valid because:
(1) The study was conducted according to the OECD guideline 202.
(2) No precipitations as well as emulsions were observed by visual inspections for up to 5.0 mg/L of the nominal concentrations.
(3) Clear conc-effect relationships as well as progressivity of the effects were observed (i.e. suggesting the substance bioavailable).
(4) Somewhat low toxicity as compared to those predicted with its hydrophobicity (e.g. by using ECOSAR program) can be well understood by considering the restricted uptake of the steric hindered molecule as exemplified in the bioconcentration study.

• ***REFERENCES (Free Text)***

Environmental Agency of Japan (1999b), Acute Immobility Test of 2,2-Methylenebis (6-*tert*-butyl)-*p*-cresol to *Daphnia magna*, EDI98004.

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

TOXICITY TO AQUATIC PLANTS

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

Purchased from Merck (lot No. S19983), purity 97.5%.

METHOD

- **Method:** According to the OECD guideline 201 (1984).

- **Exposure Period/Endpoint:** 72 hr, Biomass and Growth rate

- **Nominal Concentrations:** 0.63, 1.3, 2.5, 5.0 mg/L, Solvent Control and Control

- **GLP:** Yes

- **Year:** 1998

- **Test Organism Species/Strain/Supplier:** Green algae (*Selenastrum capricornutum*), the strain ATCC22662 obtained from American Type Culture Collection, and subcultured at the testing facility.

- **Analytical Monitoring:** Yes. Exposure water sampled at the initiation and the termination (i.e. 72 hr) of the exposure were extracted with hexane and analyzed for the substance by using GC-MS.

- **Statistical Methods:**

Since the rates of inhibition observed at the maximum concentration (i.e. 5.0 mg/L nominal) was less than 50%, no statistical method for determining the EC_{50} values were employed.

For the determination of NOEC's, Bartlett and Dunnett methods were used. A regression line for GC-MS analyses was derived by least square calculations. Numerical values were rounded according to the method specified in JIS.

- **Remarks:**

Test organisms

Before starting exposure experiment, algae was cultured under test conditions for three days.

Test conditions

Test system: Static (with shaking)

Growth/Test medium: The medium specified in the OECD guideline

Preparation of test solution for exposure: The substance was mixed with DMF/Castor oil HCO-40 (3/1(w/w)) and dissolved into test medium to prepare the nominal concentrations described above (No precipitations as well as emulsions were observed in all the exposure levels). To set the

dispersant concentrations equal (i.e. 100 uL/L) between the nominal concentrations, DMF/Castor oil HCO-40 solution was also used for the preparations. Due to low water solubility of the substance, homogenous solution could be attained only at (and less than) the nominal concentration of 5.0 mg/L with the maximum allowable dispersant concentration of 100 uL/L.

Test volume for exposure: 100 mL (in 500 mL conical flask with poromeric silicone cap)

Number of replicate: Triplicate

Initial cell density: 1 x 10^4 cells/mL

Light conditions: 4000-5000 lx, continuous

Water chemistry during exposure: Temperature in the incubation chamber was daily measured. The pH values at the initiation and the termination of exposure were also measured. The measured values were:

Temperature: Ranged from 23.5 to 23.7 ^oC,
pH: Ranged from 7.4 to 7.7
Analytical methods
GC column: DB-1
The m/z for quantification: 177.

Pretreatment: After centrifugation to remove suspended algae, exposure water as well as control water each were extracted with the twofold volume of hexane, and the hexane layer, thus obtained, was concentrated and injected into GC-MS for quantification.
Recovery ratios fortified at the concentrations of 0.050 mg/L and 5.0 mg/L were 83 % (82 – 84%) and 92 % (91 – 94%) on the average.

RESULTS

・**Measured Exposure Concentration:**

Nominal Concentration (mg/L)	Measured value (mg/L)			
	At the initiation	% to nominal	At the termination	% to nominal
Control	<0.01	-	<0.01	-
Solvent Control	<0.01	-	<0.01	-
0.63	0.56	89	0.47	75
1.3	1.2	92	0.91	70
2.5	2.4	96	2.1	84
5.0	4.8	96	4.2	84

(Note) The measured concentrations at the initiation of exposure were corresponding to 89 to 96% of the nominal concentrations (i.e. less than 20% of deviation). Thus, the results (e.g. EC_{50} values) were expressed based on the nominal concentrations.

・**Validity of test:** The test was valid since the cell density in control increased by 278 times at the termination of experiment.

・**EC_{50}'s and NOEC's:**

Exposure Period (hr)	Biomass		Growth rate	
	EC_{50} (mg/L)	NOEC (mg/L)	EC_{50} (mg/L)	NOEC (mg/L)
0 – 72	>5.0	0.63	-	-
24 – 48	-	-	>5.0	2.5
24 – 72	-	-	>5.0	1.3

(NOTE) These values were based on the nominal concentrations).

• **Statistical Results:** The NOEC's described above were defined as the maximum nominal concentrations at which biomass and growth rate were not significantly different from the control (p = 5%).

• **Remarks:**

Cell density measured during the experiment:

Nominal Concentrations (mg/L)	Mean Cell Density (x10^4 cells/mL) +/- SD			
	0 hr	24 hr	48 hr	72 hr
Control	I. +/- 0	7.67 +/- 0.32	45.9 +/- 0.9	278 +/- 7
Solvent Control	II. +/- 0	7.81 +/- 0.21	44.1 +/- 1.7	280 +/- 26
0.63	III. +/- 0	7.27 +/- 0.56	42.9 +/- 1.2	271 +/- 3
1.3	IV. +/- 0	7.32 +/- 0.32	42.4 +/- 0.3	242 +/- 7
2.5	V. +/- 0	7.52 +/- 0.25	41.0 +/- 1.7	226 +/- 12
5.0	1.00 +/- 0	7.45 +/- 0.14	37.6 +/- 1.5	185 +/- 8

- Growth rate curve (Area under the curve and the slope evaluated)

Nominal Concentrations (mg/L)	0 – 72 hr		24 – 48 hr		24 – 72 hr	
	Mean Area	Inhibition %	Mean Rate	Inhibition %	Mean Rate	Inhibition %
Control	45569440	0	0.0745	0	0.0748	0
Solvent Control	45426080	0.3	0.0721	3.2	0.0745	0.4
0.63	43989280	3.5	0.0740	0.7	0.0754	-0.9
1.3	40351360	11.5	0.0732	1.7	0.0729	2.5
2.5	38121920	16.3	0.0706	5.3	0.0708	5.3
5.0	32365760	29.0	0.0674	9.5	0.0669	10.6

Reference substance/Results: Potassium dichromate, lot No.ACQ2610, Wako Pure Chemical Ind. The 72 hr-EC_{50} value (Biomass) determined was 0.44 mg/L.

Any observations: Not described

CONCLUSION

Due to low water solubility of the substance, maximum dispersable concentration was limited to be 5.0 mg/L (nominal). The determined 48 hr-EC_{50} value was more than 5.0 mg/L (for biomass and growth rate) based on the nominal concentrations. The NOEC value for biomass (0-72 hr) was evaluated to be 0.63 mg/L. The NOEC's for growth rate were 2.5 mg/L (24-48 hr) and 1.3 mg/L (24-72 hr). Experimental designs and results were well documented and prescribed conditions in the guideline were well satisfied.

DATA QUALITY

- **Reliability:** Reliable with no restriction
- **Remarks:**

The data is approved by the Japanese government.

Although the tested nominal concentrations were all above the water solubility of the substance, the reported result is considered to be quite valid because:
(1) The study was conducted according to the OECD guideline 201.
(2) No precipitations as well as emulsions were observed by visual inspections for up to 5.0 mg/L of the nominal concentrations.
(3) Clear conc-effect relationships as well as progressivity of the effects were observed (i.e. suggesting the substance bioavailable).
(4) Somewhat low toxicity as compared to those predicted with its hydrophobicity (e.g. by using ECOSAR program) can be well understood by considering the restricted uptake of the steric hindered molecule as exemplified in the bioconcentration study.

REFERENCES (Free Text)

Environmental Agency of Japan (1999c), Growth Inhibition Test of 2,2-Methylenebis (6-*tert*-butyl)-*p*-cresol to *Selenastrum capricornutum*, EAI98004.

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

TEST SUBSTANCE

- 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
- **Remarks:**

Purchased from Merck (lot No. S19983), purity 97.5%.

METHOD

- **Method:** According to the OECD guideline 211 (1997).

- **Exposure Period/Endpoint:** 21 days, Mortality and Reproduction rate

- **Nominal Concentrations:** 0.10, 0.22, 0.46, 1.0, 2.2 mg/L, Solvent Control and Control

- **GLP:** Yes

- **Year:** 1998

- **Test Organism Species/Strain/Supplier:** Water flea (*Daphnia magna*). Obtained from National Institute for Environmental Studies, and subcultured at the testing facility. Newly hatched juveniles were employed in the experiment.

- **Analytical Monitoring:** Yes. Newly prepared exposure water at Day 2, 9 and 16 as well as exposure water before renewal at Day 5 12 and 19 were extracted with hexane and analyzed for the substance by using GC-MS.

- **Statistical Methods:**

The LC_{50} value was calculated with the Probit method. The EC_{50} value for reproduction rate was calculated with the Logistic method. For the determinations of NOEC and LOEC, the Dunnett method was used. A regression line for GC-MS analyses was derived by least square calculations. Numerical values were rounded according to the method specified in JIS.

- **Remarks:**

Test organisms

A matured female daphnia was selected from the subculture. On the next day, newly hatched juveniles were transferred into other culture vessels and further cultured for more than two weeks under the following conditions; temperature 20 +/- 1 ^{o}C, photoperiod 16hr light – 8hr dark, culture density 20 individuals per one liter of dilution water, feeding 0.1 to 0.2 mg carbon per day with *Chlorella vulgaris.* Newly hatched juveniles from, thus cultured, parents (with 0% mortality during this culture period) were used in the experiment.

Test conditions

Test system: Semi-static (Tree renewals per week; on Mon., Wed. and Fri.)

Dilution water: Dechlorinated tap water (pH 8.1, hardness 55.2 mg/L as $CaCO_3$).

Preparation of test solution for exposure: The substance was mixed with DMF/Castor oil HCO-40

(3/1(w/w)) and dissolved into dilution water to prepare the nominal concentrations described above (No precipitations as well as emulsions were observed in all the exposure levels). To set the dispersant concentrations equal (i.e. 50 uL/L) between the nominal concentrations, DMF/Castor oil HCO-40 solution was also used for the preparations.

Test volume for exposure: 80 mL (in 100 mL beaker)

Number of replicate, individuals per replicate: Ten replicates, One daphnia per replicate.

Water chemistry during exposure: Measurements of temperature, pH, DO and total hardness were conducted at Day 0, 9 and 16 for newly prepared exposure solution and Day 2, 12 and 19 for exposure solution before renewal. The measured values were;

Test temperature: Ranged from 20.0 to 20.1 ^{o}C,
pH: Ranged from 8.0 to 8.3,
DO: Ranged from 7.6 to 8.7 mg/L (more than 60% of saturation).
Total hardness: Ranged from 60 to 85 mg/L (as $CaCO_3$).

Analytical methods
GC column: DB-1

The m/z for quantification: 177.

Pretreatment: Exposure water as well as control water each were extracted with the equivalent volume of hexane, and the hexane layer, thus obtained, was injected into GC-MS (directly or after concentration if necessary) for quantification.

Recovery ratios fortified at the concentrations of 0.050 mg/L and 5.0 mg/L were 83 % (82 – 84%) and 92 % (91 – 94%) on the average.

RESULTS

- **Measured Exposure Concentration (Note 1):**

Nominal Concentration (mg/L)	Measured value (mg/L) and [% to nominal]						Time-weighted Average (mg/L)
	Day 2	5	9	12	16	19	
Control	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-
Solvent Control	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-
0.10	0.077 [77]	0.028 [28]	0.071 [71]	<0.01 [<10]	0.078 [78]	<0.01 [<10]	0.046 (Note 2) [46]
0.22	0.19 [86]	0.13 [59]	0.17 [77]	0.059 [27]	0.21 [95]	0.025 [11]	0.12 [53]
0.46	0.44 [96]	0.37 [80]	0.39 [85]	0.24 [52]	0.51 [110]	0.17 [37]	0.34 [74]
1.0	0.92 [92]	1.2 [120]	0.86 [86]	0.92 [92]	1.2 [120]	0.41 [41]	0.89 [89]
2.2	2.0 [91]	2.1 [95]	1.7 [77]	2.0 [91]	-	-	1.9 (Note 3) [89]

(Note 1) The Day 2, 9 and 16 were for newly prepared exposure solution and Day 5, 12 and 19 were for the solution before renewal.

(Note 2) Geometric mean of Day 2 and 5.
(Note 3) Time weighted mean of Day 2, 5, 9 and 12.

・**LC_{50} and EC_{50} (Reproduction rate):**

Exposure Period (days)	values calculated (mg/L)	95% Confidence Limit	Statistical Method
21	LC_{50}=1.0 (based on measured concentration)	0.71 – 1.4	Probit
21	EC_{50}=1.1 (based on measured concentration)	-	Logistic

・**NOEC and LOEC (Reproduction rate):**
NOEC = 0.34 mg/L
LOEC = 0.89 mg/L

・**Statistical Results:** The NOEC described above was defined as the maximum concentration at which reproduction rate was not significantly different from the control.
The LOEC was the minimum concentration at which significant difference was observed.

・**Remarks:**
Cumulative number of dead daphnia and mortality:

Measured Concentrations (mg/L)	Day 21	
	Cumulative Number of Dead	Mortality (%)
Control	0	0
Solvent Control	0	0
0.046	0	0
0.12	0	0
0.34	0	0
0.89	3	30
1.9	10	100

Time of the first production of juveniles: Day 7 to 8

Mean cumulative numbers of juveniles produced per adult:

Measured Concentrations (mg/L)	Mean Cumulative No of Juvenile (Day 21)
Control	101.9
Solvent Control	143.9
0.046	137.7
0.12	140.7
0.34	117.5
0.89	59.1
1.9	0.0

Reference substance/Results: Potassium dichromate, lot No.ACQ2610, Wako Pure Chemical Ind. The 48 hr-EC_{50} (Immobility) value determined was 0.54 mg/L/

Any observations: Not described

CONCLUSION

The determined 21 day-LC_{50} and EC_{50} values were 1.0 mg/L and 1.1 mg/L based on the measured concentrations. The NOEC and LOEC for reproduction rate were evaluated to be 0.34 mg/L and 0.89 mg/L, respectively. Experimental designs and results were well documented and prescribed conditions in the guideline were well satisfied.

DATA QUALITY

- **Reliability:** Reliable with no restriction
- **Remarks:**

The data is approved by the Japanese government.

Although the tested nominal concentrations were all above the water solubility of the substance, the reported result is considered to be quite valid because:

(1) The study was conducted according to the OECD guideline 211.
(2) No precipitations as well as emulsions were observed by visual inspections for up to 2.2 mg/L of the nominal concentrations.
(3) Clear conc-effect relationships as well as progressivity of the effects (as compared to the acute study) were observed (i.e. suggesting the substance bioavailable).
(4) Somewhat low toxicity as compared to those predicted with its hydrophobicity (e.g. by using ECOSAR program) can be well understood by considering the restricted uptake of the steric hindered molecule as exemplified in the bioconcentration study.

REFERENCES (Free Text)

Environmental Agency of Japan (1999d), Reproduction Inhibition Test of 2,2-Methylenebis (6-*tert*-butyl)-*p*-cresol to *Daphnia magna*, EDR98004.

OTHER

- **Last changed:**
- **Order number for sorting**
- **Remarks:**

HEALTH ELEMENTS

REPEATED DOSE TOXICITY (a)

TEST SUBSTANCE

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
Remarks: Source: Ouchi Shinko Chemical. Purity: Unknown

METHOD

- **Method/guideline:** Unknown
- **Test type:** Chronic Toxicity study
- **GLP:** No
- **Year:** 1994 (published year)
- **Species:** Rat
- **Strain:** Wistar
- **Route of administration:** oral (by feeding)
- **Doses/concentration levels:** 0, 100, 300, 1000 ppm (in diet)
- **Sex:** Male & Female
- **Exposure period:** 18 months
- **Frequency of treatment:** Daily
- **Control group and treatment:** Basal diet (no treatment)
- **Post exposure observation period:** none
- **Duration of test:** 18 months
- **Statistical methods:** Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- ***Age at study initiation*:** 5 week old for both sexes
- ***Weight at study initiation*:** 393±21 g for male, 230±15 g for female
- ***No. of animals per sex per dose*:** 30 per sex per dose group (5 were sacrificed at 6 month and another 5 at 12 month for hematological and serum biochemical examinations).

Study Design:

- ***Vehicle*:** none (pellets of diet containing the substance)
- ***Satellite groups and reasons they were added*:** none
- ***Clinical observations performed and frequency*:**
  General condition was observed daily. Body weight and food consumption were determined monthly. Hematological and serum biochemical examination were performed for 5 animals/sex/dose group at 6, 12 and 18 month.
- ***Organs examined at necropsy:***
  Organ weight: brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands
  Microscopic: all the group/ brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands, salivary glands, esophagus, stomach, small intestine, pancreas, urinary bladder, seminal vesicles, epididymis, ischiac nerve, uterus, prostate, mesenteric lymph nodes, thymus, spinal cord, skeletal muscle, born marrow

RESULTS

- **NOAEL**
  Male: 300 ppm (12.7 mg/kg/day), Female: 300 ppm (15.1 mg/kg/day)

- **LOAEL**
  Male: 1000 ppm (42.3 mg/kg/day), Female: 1000 ppm (54.2 mg/kg/day)

REMARKS FIELD FOR RESULTS

– ***Body weight:*** Significant suppression of body weight gain was observed from the month 6 in the male group at 1000 ppm and from the month 1 in the female group at 1000 ppm.
– ***Food/water consumption:*** No significant effect was observed.

	Males				Females			
Diet level (ppm)	**0**	**100**	**300**	**1,000**	**0**	**100**	**300**	**1,000**
Final mean body weight (g)	545	528	520	498*	375	368	353	278*
Mean food intake (g/rat/day)	16.6	16.7	16.6	16.2	12.2	12.7	12.2	11.5

(significant: not specified the degree)*

– ***Haematology:***
In the hematological and serum biochemical analysis, several parameters demonstrated significant alternation. However, none appeared to be of biological significance, since they did not show the same tendency throughout the experimental period and/or the degrees of change were very small.
Mortality and time to death: Survival rates in all treated groups were comparable to those of control.
– ***Gross pathology incidence and severity:***

– ***Organ weight changes:***
Male: Increase in liver weight at 1000 ppm (absolute ($p<0.05$) and relative ($p<0.01$)).
Decrease in testis weight at 1000 ppm (absolute and relative) ($p<0.01$)
Female: Increase in liver weight at 1000 ppm (relative) ($p<0.01$).

	Males		Females	
Diet level (ppm)	**0**	**1,000**	**0**	**1,000**
Absolute weight				
Liver (g, Mean ± SD)	12.28 ± 0.93	14.19 ± 1.35*	7.60 ± 0.88	7.39 ± 0.83
Testis (g, Mean ± SD)	3.28 ± 0.48	0.82 ± 10.18**		
Relative weight				
Liver (g%, Mean ± SD)	2.37 ± 0.16	3.00 ± 0.13**	2.08 ± 0.15	2.79 ± 0.35**
Testis (g%, Mean ± SD)	0.63 ± 0.10	0.17 ± 0.05*		

*(* p <0.05, ** p <0.01)*

Histopathology *(incidence and severity)**:***

Male: Atrophy of testicular tubules and spermatogenic arrest and epididymis hypospermia were observed in the 1000 ppm group.

Female: No significant effect was observed.

Diet level (ppm)	**degree***	**0**	**100**	**300**	**1,000**
No. of animals		19	19	18	19
Testis, tubules					
Atrophy	±	0	1	0	0
	+	0	0	0	0
	++	2	0	0	0
	+++	0	3	1	19
Spermatogenic arrest					
	++	2	0	0	0
	+++	0	1	1	19
Epididymis					
Hypospermia	++	2	0	0	0
	+++	0	1	1	19

CONCLUSIONS

Toxic effects in this study are suppression of body weight gain, increase in liver weight, decrease in testis weight, and histopathological lesions in the testis and the epididymis.

The NOAELs are 12.7 mg/kg/day (300 ppm) for male and 15.1 mg/kg/day (300 ppm) for female.

DATA QUALITY

· **Reliabilities:** Valid. (limitation: the study was conducted without GLP)

Remarks field for Data Reliability

Well conducted study, carried out by National Institute of Health Science (Japan).

REFERENCES (Free Text)

Takagi et al.; Journal of Toxicological Science, Vol. 19, 77-89 (1994)

GENERAL REMARKS

REPEATED DOSE TOXICITY (b)

TEST SUBSTANCE

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
Remarks: Source: Sumitomo Chemical, Lot No.: 710140, Purity 98.2 %, Kept at room temperature until use

METHOD

- **Method/guideline:** OECD TG 421
- **Test type:** OECD Preliminary Reproduction Toxicity Screening Test
- **GLP:** Yes
- **Year:** 1999 (published year)
- **Species:** Rat
- **Strain:** Cij;CD (SD)
- **Route of administration:** oral (by gavage)
- **Doses/concentration levels:** 0, 12.5, 50, 200, 800 mg/kg/day (in 5% gum arabic)
- **Sex:** Male & Female
- **Exposure period:**
  Males; for 50-52 days from 14 days prior to mating
  Females; for 40-48 days from 14days prior to mating to the day 3 of lactation
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** none
- **Duration of test:** Male; for 51-53 days
  Female; for 41-49 days (until the day 4 of lactation)
- **Statistical methods:** Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- ***Age at study initiation*:** 10 week old for both sexes
- ***Weight at study initiation*:** 332-383 g for males, 206-238 g for females
- ***No. of animals per sex per dose*:** 12 per sex per dose group

Study Design:

- ***Vehicle*:** 5 % gum Arabic
- ***Satellite groups and reasons they were added*:** none
- ***Clinical observations performed and frequency*:**
  General condition was observed twice a day. Body weight and food/water consumption were determined twice a week.
- ***Organs examined at necropsy:***
  Organ weight: testis, epididymis, cauda epididymis,ovary
  Sperm examination: motility, viability, morphology
  Microscopic: control & all treated groups / testis, caput epididymis, control & 800 m/kg groups/ seminal vesicle, ovary

RESULTS

· **NOAEL**
Male: 12.5 mg/kg/day, Female: 50 mg/kg/day

· **LOAEL**
Male: 50 mg/kg/day (histopathological changes in testis),
Female: 200 mg/kg/day (suppression of body weight gain)

REMARKS FIELD FOR RESULTS

–***Body weight:*** Suppressions of body weight gain were observed during the lactation period in the 200mg/kg female and during the pregnancy & lactation period in the 800 mg/kg female.

Body weights in female

Female					
Dose level (mg/kg/day)	**0**	**12.5**	**50**	**200**	**800**
Body weight (g, Mean ± SD)	310.4 ± 12.3	3122 ± 18.9	310.7± 17.2	287.4 ± 13.3**	281.9 ± 22.9**

(p<0.05, ** p<0.01)*

–***Food/water consumption:*** Decrease in food consumption were observed during pre-mating, pregnancy and lactation period in the female groups at 200 mg/kg and higher dose groups.

(The decreased tendencies were described in the graphs in the report, however, the exact figures were not present. Statistical significant were; p<0.05 at 200 ppm and p<0.01 at 800 ppm on the day 4 of lactation)

–***Clinical signs*** *(description, severity, time of onset and duration)**:***
Transient salivation was observed in the 200 mg/kg and higher dose groups of both sexes.

–***Mortality and time to death:*** The death was not observed in any group.

–***Gross pathology incidence and severity:***
Male: Atrophy of testis and epididymis were observed in the 200 mg/kg and higher dose groups (200mg/kg; 4/12, 800m/kg; 12/12). (see *Histopathology*)
Female: No significant effect was observed.

–***Organ weight changes:***
Male: Decrease in absolute and relative testis and epididymis weights were observed in the 200 mg/kg and higher dose groups.
Female: No significant effect was observed.

Testis and epididymis weights in male

Male					
Dose level (mg/kg/day)	**0**	**12.5**	**50**	**200**	**800**
Testis					
absolute wt. (g, Mean ± SD)	3.550 ± 0.333	3.598 ± 0.320	3.558 ± 0.302	2.983 ± 0.767*	1.736 ± 0.263**
relative wt. (g%, Mean ± SD)	0.666 ± 0.082	0.674± 0.072	0.655 ± 0.046	0.557 ± 0.157*	0.338 ± 0.050**
Epididymis					
absolute wt. (g, Mean ± SD)	1.255 ± 0.143	1.343 ± 0.118	1.196 ± 0.113	1.108 ± 0.125*	0.924 ± 0.100**
relative wt. (g%, Mean ± SD)	0.235 ± 0.034	0.250± 0.024	0.220 ± 0.018	0.205 ± 0.027*	0.180 ± 0.020**

(p<0.05, ** p<0.01)*

–***Histopathology*** *(incidence and severity)**:***
Male*:* Giant cell formation was observed at 50 mg/kg group (2/12). Degeneration of seminiferous tubules at 200 ppm (1/12). Atrophy of seminiferous tubules and decrease in sperm were observed in the 200 mg/kg and higher dose groups.

Female*:* No significant effect was observed.

Histopathological changes in testis and epididymis male

Dose level (mg/kg/day)	0	12.5	50	200	800
Testis					
Atrophy, seminiferous tuble	0/12	0/12	0/12	6/12**	12/12**
Degeneration, seminiferous tuble	0/12	0/12	0/12	1/12	0/12
Decrease, sperm	0/12	0/12	0/12	1/12	0/12
Giant cell formation	0/12	0/12	2/12	2/12	0/12
Epididymis					
Decrease, sperm	0/12	0/12	0/12	9/12**	12/12**

*(* p<0.05, ** p<0.01)*

–**Sperm examination:**
Decrease in the number of total sperm, the rate of motile, viable sperm and increase in the rate of morphologically abnormal sperm were observed in the 50 mg/kg and higherdose groups. In the 800 mg/kg group, no motile sperm and morphologically abnormal sperm were observed in almost all sperm.

Sperm abnormality in male

Dose level (mg/kg/day)	0	12.5	50	200	800
Sperm motion parameters					
After 30 min. incubation					
Motility ratio (%)	71.96 ±9.69	4.92 ± 7.81	60.42± 10.26**	14.50 ± 21.75**	0.00 ± 0.00**
Curvilinear velocity (um/s)	348.95 ±20.87	369.08 ± 16.17*	364.94± 18.14	301.08 ± 104.59	-
Bear cross frequency (Hz)	30.64 ±1.77	30.16 ± 1.59	32.91± 1.70**	29.98 ± 10.51*	-
Morphology of sperm					
Abnormal ratio (%)	1.55 ±3.63	0.55 ± 0.55	8.11± 6.33**	56.33 ± 29.03**	-
Viabiity (%)	98.57 ±2.04	99.56 ± 0.47	89.19± 11.47**	71.68 ± 9.31**	-
Survivability (%)	83.29 ±6.87	86.44 ± 3.27	66.03± 17.79**	39.03 ± 15.16**	-
Number of sperms in left epididymis cauda ($x10^6$)	207.41 ±60.16	222.42 ± 49.26	128.00± 39.88**	60.73 ± 29.17*	-
Number of sperms/g (left	707.41±153.02	704.85 ± 154.64	503.29± 159.44**	238.88 ± 102.42**	-

*(*p <0.05, **p<0.01)*

CONCLUSIONS

Toxic effects in this study are suppression of body weight gain, low food consumption, histopathological lesions in the testis and the epididymis, and sperm abnormality.

The NOAELs are 12.5 mg/kg/day for male and 50 mg/kg/day for female.

DATA QUALITY

· **Reliabilities:** Valid without restriction

Remarks field for Data Reliability
Well conducted study, carried out by Nihon Bioresearch Inc. Hashima Laboratory (Japan)..

REFERENCES (Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals 7, 423-437 (1999)

GENERAL REMARKS

REPEATED DOSE TOXICITY (c)

TEST SUBSTANCE

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
Remarks: Source: Sumitomo Chemical, Lot No.: 40401, Purity >98 %, Kept at room temperature until use

METHOD

- **Method/guideline:** TG for 28-day repeat dose toxicity testing of chemicals (Japan)
- **Test type:** 28-day Repeat Dose Toxicity Test
- **GLP:** Yes
- **Year:** 1996 (published year)
- **Species:** Rat
- **Strain:** Cij;CD (SD)
- **Route of administration:** oral (by gavage)
- **Doses/concentration levels:** 0, 50, 200, 800 mg/kg/day (in 5% gum arabic)
- **Sex:** Male & Female
- **Exposure period:** 28 days
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** 14 days (for 800 mg/kg/day group)
- **Duration of test:** 43 days
- **Statistical methods:** Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- ***Age at study initiation*:** 6 week old for both sexes
- ***Weight at study initiation*:** 209-235 g for male, 142-164 g for female
- ***No. of animals per sex per dose*:** 6 per sex per dose for the group at 50, 200 mg/kg/day. 12 per sex per dose for the group at 0, 800 mg/kg/day.

Study Design:

- ***Vehicle*:** 5 % gum Arabic
- ***Satellite groups and reasons they were added*:** none
- ***Clinical observations performed and frequency*:**
  General condition was observed twice a day. Body weight and food/water consumption were determined twice a week.
  Hematological, blood chemical and urinary analysis were carried out at the day 28 (all groups) and at the day 43 (control and 800 mg/kg: recovery groups).
- ***Organs examined at necropsy:***
  organ weight: brain, heart, lungs, thymus, liver, kidney, spleen, adrenals, testes, ovary
  microscopic: control & all treated groups /liver, testis,
  control & 800 m/kg groups/ spleen, heart, kidney, adrenals

RESULTS

- **NOAEL**
  Male: Not determined
  Female: 50 mg/kg/day

- **LOAEL**
  Male: 50 mg/kg/day (histopathological changes in the testis),
  Female: 200 mg/kg/day (increase in liver weight, prolongation of PT and APTT)

REMARKS FIELD FOR RESULTS

–***Body weight:*** No significant effect was observed.

–***Food/water consumption:*** Transient decrease in food consumption (800mg/kg, male, early period) and transient increase in food consumption (800 mg/kg, male, late period & 50 mg/kg, male, late period) were observed

–***Clinical signs*** *(description, severity, time of onset and duration)**:***
No significant effect was observed.

–***Haematology*:**

Male: Prolongation of PT and APTT (more than 50 mg/kg). Decrease in hemoglobin at 50 mg/kg ($p<0.05$). Increase in platelet at 800 mg/kg ($p<0.05$). Decrease in MCV at 800 mg/kg of recovery group ($p<0.05$).

Female: Prolongation of PT and APTT (more than 200 mg/kg). Decrease in eosinophil at 800 mg/kg of recovery group ($p<0.05$).

	28 days dosing groups				14 days recovery groups	
Dose level (mg/kg/day)	**0**	**50**	**200**	**800**	**0**	**800**
No. of animals	6	6	6	6	6	6
male						
PT(sec)	15.9 ±3.6	19.6 ± 3.3	24.1 ±2.8**	31.9 ± 3.6**	14.1 ± 0.9	14.7 ± 3.1
APTT (sec)	21.6 ±2.4	24.4 ± 3.1	26.5 ±1.6**	30.5 ± 1.8**	21.2 ± 1.7	21.9 ± 3.0
Hemoglobin (g/dl)	15.0 ±0.3	14.3 ± 0.3*	14.5 ±0.4	14.5 ± 0.6	14.9 ± 0.6	14.6 ± 0.5
Platelet (10^4/ul)	101.8 ±7	104 ± 10.6	109.1 ±9.1	116.9 ± 8.4*	98.8 ± 11.3	106.9 ± 18.2
MCV (fl)	59 ±2	59 ± 2	59 ±3	59 ± 2	58 ± 2	56 ± 2*
female						
PT(sec)	10.7 ±0.6	10.8 ± 0.5	11.9 ±1.1	17.3 ± 2.3**	10.6 ± 0.4	10.5 ± 0.3
APTT (sec)	18 ±1.6	18.1 ± 1.4	20.9 ±1.76**	24 ± 1.2**	18.6 ± 2.4	17.2 ± 0.7
Eosinophil (%l)	0.2 ±0.4	0.7 ± 0.8	0.3 ±0.5	0.7 ± 0.8	0.0 ± 0.0	1.2 ± 1.5*

*(* $p<0.05$, ** $p<0.01$)*

–***Biochem:***

Males: Increases in total protein (more than 50 mg/kg). Increase in albumin at 800 mg/kg ($p<0.05$). These were considered not to be significant biologically.

	28 days dosing groups				14 days recovery groups	
Dose level (mg/kg/day)	**0**	**50**	**200**	**800**	**0**	**800**
No. of animals	6	6	6	6	6	6
T. protein (g/dl)	4.8 ±0.1	5.1 ± 0.2**	5.1 ±0.1**	5.1 ± 0.2**	5.0 ± 0.2	5.3 ± 0.3*
Albumin (g/dl)	3.5 ±0.1	3.6 ± 0.2	3.6 ±0.1	3.8 ± 0.1*	3.5 ± 0.1	3.7 ± 0.2

(p<0.05, ** p<0.01)*

–***Mortality and time to death:*** none

–***Gross pathology incidence and severity:*** No significant effect was observed.

–***Organ weight changes:***

Male: Increase in liver weight (more than 50 mg/kg).

Female: Increase in liver weight (more than 200 mg/kg). Increase in adrenal weight (more than 200 mg/kg).

	28 days dosing groups				14 days recovery groups	
Dose level (mg/kg/day)	**0**	**50**	**200**	**800**	**0**	**800**
No. of animals	6	6	6	6	6	6
male						
Liver (absolute: g)	10.31±0.68	11.76± 0.89	12.89±1.45**	13.43±1.78**	11.42± 0.63	13.16± 2.32
Liver (relative: %g)	2.89±0.15	3.27± 0.08**	3.43±0.19**	3.62±0.28**	2.68± 0.08	3.04± 0.24*
female						
Liver (absolute: g)	6.79±0.35	7.35±0.32	7.65±0.67*	8.83±0.43**	6.70±0.35	7.60± 0.39**
Liver (relative: %g)	3.02±0.13	3.26±0.16	3.61±0.23**	3.92±0.18**	2.72±0.09	3.12±0.20**
Adrenal (absolute: mg)	73.7±8.8	69.0±6.1	80.7±10.5	83.3±9.6	73.6±11.8	78.9± 4.8
Adrenal (relative: %mg)	32.8±3.9	30.6±2.7	38.1±4.0*	36.9±3.4	29.9±4.1	32.3±1.7

*(*p <0.05, **p <0.01)*

–***Histopathology*** *(incidence and severity)****:***

Male: Degeneration of step 19 spermatids (more than 50 mg/kg). Sperm retention and vacuolation of Sertoli cells in the testis (more than 200 mg/kg). Mild centrilobular hepatocyte hypertrophy (more than 200 mg/kg).

In the testis at 800 mg/kg/day (recovery group), more sever changes such as giant cell formation and decrease in germ cells were observed.

Female: Mild centrilobular hepatocyte hypertrophy (more than 200 mg/kg)

	28 days dosing groups				14 days recovery groups	
Dose level (mg/kg/day)	**0**	**50**	**200**	**800**	**0**	**800**
male						
Liver						
Hypertrophy centrilobular	0/6	0/6	1/6	1/6	0/6	0/6
Testis						
Vacuolation, Sertoli cells	0/6	0/6	6/6	6/6	0/6	5/6
Sperm retention	0/6	0/6	6/6	6/6	0/6	5/6
Degeneration, step 19 spermatids	0/6	3/6	6/6	6/6	0/6	5/6
Giant cell formation	0/6	0/6	0/6	0/6	0/6	4/6
Nuclear vacuolation, spermatids	0/6	0/6	0/6	0/6	0/6	4/6
Germ cells, decreased	0/6	0/6	0/6	0/6	0/6	2/6
female						
Liver						
Hypertrophy centrilobular	0/6	0/6	1/6	2/6	0/6	0/6

CONCLUSIONS

Toxic effects in this study are prolongation of PT and APTT, increase in liver and adrenal weights, histopathological lesions in the liver and the testis, and sperm abnormality.

The NOAELs are less than 50 mg/kg/day for male and 50 mg/kg/day for female.

DATA QUALITY

· **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Safety Assessment Laboratory, Panapharm Laboratories Co.. Ltd. (Japan).

REFERENCES (Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals 4, 409-430 (1996)

GENERAL REMARKS

TOXICITY TO REPRODUCTION/DEVELOPMENT

TEST SUBSTANCE

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
Remarks: Source: Sumitomo Chemical, Lot No.: 710140, Purity 98.2 %, Kept at room temperature until use

METHOD

- **Method/guideline:** OECD TG 421
- **Test type:** OECD Preliminary Reproduction Toxicity Screening Test
- **GLP:** Yes
- **Year:** 1999 (published year)
- **Species:** Rat
- **Strain:** Cij;CD (SD)
- **Route of administration:** oral (by gavage)
- **Doses/concentration levels:** 0, 12.5, 50, 200, 800 mg/kg/day (in 5% gum Arabic)
- **Sex:** Male & Female
- **Exposure period:** Male; for 50-52 days from 14 days prior to mating
  Female; for 40-48 days from 14days prior to mating to the day 3 of lactation
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Post exposure observation period:** none
- **Duration of test:** Male: for 51-53 days
  Female: for 41-49 days (until the day 4 of lactation)
- **Statistical methods:** Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- ***Age at study initiation*:** 10 week old for both sexes
- ***Weight at study initiation*:** 332-383 g for males, 206-238 g for females
- ***No. of animals per sex per dose*:** 12 per sex per dose group

Study Design:
The animals were sacrificed on the day 4 of lactation for females. Females with no delivery were killed on the day 25 of pregnancy.

- ***Vehicle*:** 5 % gum Arabic
- ***Satellite groups and reasons they were added*:** none
- ***Mating procedures*:** Male/female per cage; 1/1, length of cohabitation; at the most 14 days, until proof of pregnancy (formation of vaginal closing or sperm detection in vagina)
- ***Clinical observations performed and frequency*:**
  Parent: General appearance twice a day
  Foetus: General appearance once a day after birth
- ***Organs examined at necropsy*:**
  Parent: organ weight: testis, epididymis, cauda epididymis,ovary
  Sperm examination: motility, viability, morphology
  Microscopic: control & all treated groups / testis, caput epididymis,
  control & 800 m/kg groups/ seminal vesicle, ovary
  Foetal: full macroscopic examinations on all of pups
- ***Parameters assessed during study*:**
  Body weight (twice a week), food/water consumption (twice a week), No. of pairs with successful copulation, copulation index (No. of pairs with successful copulation/No. of pairs mated x 100), pairing days until copulation, No. of pregnant females, fertility index = (No. of pregnant animals/No. of pairs with successful copulation x 100), No. of corpora lutea, No. of implantation sites, implantation index (No. of implantation sites/No. of corpora lutea x 100), No. of living pregnant females, No. of pregnant females with parturition, gestation length, No. of pregnant females with live pups on day 0, gestation index (No. of females with live pups/No. of living pregnant females x 100), No. of pregnant females with live pups on day 4, delivery index (No. of pups born/No. of implantation sites x 100), No. of pups alive on day 0 of lactation, live birth index (No. of live pups on day 0/No. of pups born x 100), sex ratio (Total No. of male pups/Total No. of female pups), No. of pups alive on day 4 of lactation, viability index (No. of live pups on day 4/No. of live pups on day 0 x 100), body wt. of live pups (on day 0 and 4)

RESULTS

- **NOAEL: female reproductive toxicity:**
  50 mg/kg/day

- **NOAEL: male reproductive toxicity:**
  12.5 mg/kg/day

- **NOAEL: developmental toxicity:**
  200 mg/kg/day

- **Actual dose received by dose level by sex if available:**
  0, 12.5, 50, 200, 800 mg/kg/day for both sexes

- **Maternal data with dose level (with NOAEL value):**

No significant effect was observed in the 50 mg/kg and lower dose groups. Decrease in number

of corpora lutea, implantation scars and pups born were observed in the 200 mg/kg and higher dose groups. One dam was unable to deliver pups, and one dam lost all pups during the lactation in the 800 mg/kg group.

· **Pups data with dose level (with NOAEL value):**

No significant effect was observed in the 50 mg/kg and lower dose groups. Decrease in number of pups was observed in the 200 mg/kg and higher dose groups. Decrease in body weights of both sexes on the day 4 of lactation (male: $p<0.05$, female: tendency) and increase in number of stillbirths were noted in the 800 mg/kg group. The significant increase in body weight at 200 mg/kg was considered not to be significant biologically, because of not significant at 800 mg/kg.

Dose level (mg/kg/day)		**0**	**12.5**	**50**	**200**	**800**
No. of pairs mated		12	12	12	12	12
No. of pregnant females		12	12	12	12	12
Corpora lutea		16.4 ± 3.0	16.4 ± 2.6	16.3 ± 1.5	15.1 ± 1.4	14.1 ± 1.6*
Implantation scars		14.3 ± 3.0	14.7 ± 1.1	15.2 ± 1.3	13.5 ± 1.4	13.1 ± 1.5*
Pups born		13.5 ± 3.3	13.5 ± 1.0	14.8 ± 1.3	11.7 ± 1.4**	12.2 ± 1.8*
Delivery Index (%)		93.5 ± 8.9	92.2 ± 5.2	97.3 ± 3.3	87.2 ± 10.5*	92.8 ± 5.7
Live pups born		13.1 ± 3.2	13.3 ± 0.8	14.3 ±1.3	11.5 ± 1.0**	11.3 ± 4.1
Dead pups on day 0 of lactation		0.4 ± 0.7	0.2 ± 0.4	0.4 ± 0.5	0.2 ± 0.6	0.9 ± 2.7
Live birth index (%)		97.1 ± 4.6	98.9 ± 2.5	97.2 ± 3.5	98.8 ± 3.9	89.9 ± 3.0
Live pups on day 4 of lactation		13.1 ± 3.2	13.3 ± 0.8	14.3 ±1.4	11.4 ± 1.0**	12.4 ± 1.8
Body weight of live pups (g)						
on day 0	**Males**	6.78 ± 0.40	6.81 ± 0.23	6.90 ± 0.50	7.60 ± 0.52**	6.97 ± 0.71
	Females	6.43 ± 0.37	6.40 ± 0.17	6.53 ± 0.44	7.25 ± 0.49**	6.61 ± 0.66
on day 4	**Males**	11.02± 0.83	11.05 ± 0.77	10.58 ± 1.11	11.29 ± 1.14	9.68 ± 1.72*
	Females	10.33 ± 0.81	10.36 ± 0.67	10.20 ± 1.15	10.77 ± 1.04	9.30 ± 1.64

(p <0.05, ** p <0.01)*

REMARKS FIELD FOR RESULTS.

–***Mortality and day of death*:** The death was not observed in any group.
–***Body weight**:* Suppressions of body weight gain were observed during the lactation period in the 200mg/kg female and during the pregnancy & lactation period in the 800 mg/kg female.
–***Food/water consumption:*** Decrease in food consumption was observed during pre-mating, pregnancy and lactation period in the female at 200 mg/kg and higher doses.
–***Reproductive data*:** Decrease in implantation scars was considered to be the response followed by decrease in corpora lutea.
–***Pups data*:**
***Grossly visible abnormalities, external, soft tissue and skeletal abnormalities*:** no statistically significant effect was observed.

CONCLUSIONS

Effects on female reproductive parameters are decrease in corpora lutea, implantation scars, pups born, and those on developmental parameters are low body weight gain of offspring and increased number of stillbirths.

The NOAEL for female reproductive toxicity is 50 mg/kg and that for developmental toxicity is200 mg/kg/day.

As for male reproductive toxicity, the NOAEL is 12.5 mg/kg/day based on testicular toxicity described in REPEATED DOSE TOXICITY (b).

DATA QUALITY

· **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Nihon Bioresearch Inc. Hashima Laboratory (Japan).

REFERENCES (Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals 7, 423-437 (1999)

GENERAL REMARKS

DEVELOPMENTAL TOXICITY / TERATOGENICITY

TEST SUBSTANCE
6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
Remarks: Source: Ouchi Shinko Chemical., Lot No. 608008, Purity: Unknown

METHOD
- **Method/guideline:** Unknown
- **GLP:** No
- **Year:** 1990 (published year)
- **Species:** Rat
- **Strain:** Wistar
- **Route of administration:** oral (by gavage)
- **Doses/concentration levels:** 0, 93.5, 187, 375 mg/kg
  (volume of treatment : 5, 1.25, 2.5, 5 mL/kg, respectively)
- **Sex:** Female
- **Exposure period:** 7-17 day of pregnancy
- **Frequency of treatment:** Daily
- **Control group and treatment:** Concurrent vehicle
- **Duration of test**: 20 day of pregnancy
- **Statistical methods**: Chi square test, t- test or rank sum test

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:
- ***Age at study initiation*:** 12-13 weeks old for female, 14 weeks old for mating males
- ***No. of animals per sex per dose*:** 20-24 copulated females sex per dose group

Study Design:
- ***Vehicle*:** Olive oil
- ***Satellite groups and reasons they were added*:** none
- ***Clinical observations performed and frequency*:**
  General condition was observed daily.
- ***Mating procedures*:** male/female per cage. When signs of copulation, formation of vaginal plug or sperm detection in vagina is observed in the morning, the day is determined to be Day 0 of pregnancy.
- ***Parameters assessed during study:***
  maternal: body weight change, food consumption, mortality, No. of corpora lutea, No

of implants, No. of dams with dead implants only

fetal: No. of live fetus, sex ratio, body weight, No. of dead implants (early, late), No. of fetus with external, visceral or skeletal malformation, No. of fetus with skeletal variation, fetal ossification state

· ***Organs examined at necropsy:***

maternal: ovary(copora lutea), uterus

fetal: external observations on all fetus. One thirds are for visceral, the others are for skeletal observation.

RESULTS

Teratogenicity

Not teratogenic

NOAEL for developmental toxicity

NOAEL for maternal toxicity is 93.5 mg/kg/day.
NOAEL for fetal development is 187 mg/kg/day.

REMARKS FIELD FOR RESULTS

Maternal data :

· ***Clinical sings***

Suppression of body weight gain and food consumption, toxic signs of rough fur, soiled perineal region, and diarrhea were observed in 187 and 375 mg/kg groups

Mortality

In 375 mg/kg group, 2 dams were died with severe diarrhea.

Effects of the substance to pregnant rats

Dose (mg/kg)	**0**	**93.5**	**187**	**375**
No. of dams	24	20	20	22
No. of dead dams	0	0	0	2
mortality (%)	0	0	0	9.1

Fetal data :

· ***Fetal death***

A slight increase in fetal death was observed in 375 mg/kg group.

Effects of the substance to pregnant rats on fetal development

Dose (mg/kg)	**0**	**93.5**	**187**	**375**
No. of dams	24	20	20	20
No. of dead Implants	26	12	7	69
early	20	12	7	68
late	6	0	0	1
mortality (%)	8.6	4.1	2.4	28.4
No. of dams with dead implants only (%)	1(4.2)	0	0	5(25.0)

Fetal mortality (%) is given as the average of incidence in each litter

Teratogenicity data :

· ***External observations***

No significant effect was observed.

- ***Visceral observations***
  No significant effect was observed.

Effects on visceral development of the fetus

Dose (mg/kg)	**0**	**93.5**	**187**	**375**
No. of dams	23	20	20	15
No. of fetus examined	102	115	94	66
Dilatation of renal pelvis **(%)**	0	1(1.3)	0	2(3.1)

Occurrence rate (%) in parenthesis given as the average of incidence in each litter

- ***Skeletal observations***
  No significant effect was observed.

Effects on skeletal development of the fetus

Dose (mg/kg)	**0**	**93.5**	**187**	**375**
No. of dams	23	20	20	15
No. of fetus examined	171	173	170	114
No. of fetus with skeletal malformation	0	0	0	0
No. of fetus with skeletal variations				
Cervical ribs (%)	1(0.7)	0	3(1.6)	2(1.7)
Varied cervical centra (%)	1(0.7)	0	0	0
Varied thoracic centra (%)	1(0.7)	1(0.5)	1(0.5)	1(0.8)
Shortened 13th rib (%)	1(0.7)	0	0	0
Varied sternebrae (%)	62(35.9)	92(53.5)	91(52.9)	75(66.7)
Lumber ribs (5)	84(48.4)	90(53.6)	91(53.4)	89(77.4)
rudimentary	82(47.5)	81(48.2)	82(48.2)	86(74.7)
extra (14th rib)	2(0.9)	9(5.4)	9(5.2)	3(2.7)
27 presacral vertebrae	0	0	1(0.6)	0
Ossification state (average number)				
Metacarpari	7.9	7.8	7.9	7.7
Metarsi	8.0	8.0	8.0	8.0
Sarco-caudal vertebra	7.8	7.8	7.8	7.7

Occurrence rate (%) in parenthesis given as the average of incidence in each litter

CONCLUSIONS
Maternal toxic effects were suppression of body weight gain, food consumption and toxic signs such as diarrhea, and fetal toxic effect was increase in fetal death.

There is no teratogenic effect in external, visceral and skeletal observations.

The NOAELs are 375 mg/kg for maternal toxicity and 187 mg/kg/day for fetal development.

DATA QUALITY
Reliabilities: Valid. (limitation: the study was conducted without GLP)

Remarks field for Data Reliability

Well conducted study, carried out by National Institute of Health Science (Japan).

REFERENCES (Free Text)
Tanaka et. Al. (1990); Eisei Shikensho Hokoku, 108, 52-57.

GENERAL REMARKS

GENETIC TOXICITY IN VITRO (BACTERIAL TEST)

TEST SUBSTANCE

6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
Remarks: Source: Sumitomo Chemical, Lot No. 40401, Purity: >98%, Kept at room temperature until use

METHOD

- **Method/guideline**: OECD TG 471 and TG 472
- **Test type**: Reverse mutation assay
- **GLP**: Yes
- **Year**: 1996 (published year)
- **Species/Strain:** *Salmonella typhimurium* TA100, TA1535, TA98, TA1537
  Escherichia coli WP2 *uvr*A
- **Metabolic activation:** S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone
- **Statistical methods**: No statistic analysis

REMARKS FIELD FOR TEST CONDITIONS

Study Design:

- ***Concentration*:** -S9 mix: 0, 313, 625, 1250, 2500, 5000 μg /plate
  +S9 mix: 0, 313, 625, 1250, 2500, 5000 μg /plate
- ***Number of replicates*:** 2
- ***Plates/test*:** 3
- ***Procedure*:** Pre-incubation
- ***Solvent*:** Water
- ***Positive controls*:** -S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98, WP2), Sodium azide (TA1535) and 9-Aminoacridine (TA1537)
  +S9 mix; 2-Aminoanthracene (five strains)

RESULTS

- **Cytotoxic concentration:**

  Toxicity was not observed up to 5000 μg /plate in five strains with or without S9 mix.

- **Precipitation concentration:**

  Precipitation was observed at more than 313 μg /plate without S9 mix. and at more than 625 μg /plate with S9 mix.

- **Genotoxic effects:**

	+	?	-
With metabolic activation:	**[ ]**	**[ ]**	**[X]**
Without metabolic activation:	**[ ]**	**[ ]**	**[X]**

REMARKS FIELD FOR RESULTS.

CONCLUSIONS

Bacterial gene mutation is negative with and without metabolic activation

DATA QUALITY

· **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan).

REFERENCES (Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals 4, 409-430 (1996)

GENERAL REMARKS

GENETIC TOXICITY IN VITRO (NON-BACTERIAL IN VITRO TEST)

TEST SUBSTANCE

· 6,6'-di-*tert*-butyl-2,2'-methylenedi-*p*-cresol (CAS No. 119-47-1)
· Remarks: Source: Source: Sumitomo Chemical, Lot No. 40401, Purity: >98%

METHOD

· **Method/guideline**: OECD TG 473

· **Test type**: Chromosomal aberration test

· **GLP***:* Yes

· **Year**: 1996 (published year)

· **Species/Strain:** CHL/IU cell

· **Metabolic activation:** S9 from rat liver, induced with phenobarbital and 5,6-benzoflavone

· **Statistical methods**: Fisher's exact analysis

REMARKS FIELD FOR TEST CONDITIONS

Study Design:
For continuous treatment, cells were treated for 24 or 48 hrs without S9 mix. For short-term treatment, cells were treated for 6 hrs with and without S9 mix. and cultivated with fresh media for 18 hrs.

· ***Concentration*:** -S9 mix (continuous treatment): 0, 0.0020, 0.0040, 0.0080 mg/ml
-S9 mix (short-term treatment): 0, 0.00050, 0.0010, 0.0020 mg/ml
+S9 mix (short-term treatment): 0, 0.0075, 0.015, 0.030 mg/ml
· ***Plates/test*:** 2
· ***Solvent*:** Distilled water
· ***Positive controls*:** Mitomycin C for continuous treatment
Cyclophosphamide for short-term treatment

RESULTS

· **Cytotoxic concentration:**

The concentrations of 50% growth inhibition were as follows:
-S9mix (continuous treatment): 0.008 mg/ml
-S9mix (short-term treatment): 0.002 mg/ml
+S9mix (short-term treatment): 0.03 mg/ml

· **Genotoxic effects:**

	clastogenicity			polyploidy		
	+	?	-	+	?	-
– With metabolic activation:	**[ ]**	**[ ]**	**[X]**	**[ ]**	**[ ]**	**[X]**

– Without metabolic activation: **[ ] [ ] [X] [ ] [ ] [X]**

REMARKS FIELD FOR RESULTS.

Structural chromosomal aberrations and polyploidy were not induced up to a maximum concentration of 0.008 mg/ml on continuous treatment, and 0.03 or 0.001 mg/ml on short-term treatment with and without an exogenous metabolic activation system, respectively.

CONCLUSIONS

Chromosomal aberration in CHL/IU cells is negative with and without metabolic activation.

DATA QUALITY

· **Reliabilities:** Valid without restriction

Remarks field for Data Reliability

Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan).

REFERENCES (Free Text)

Ministry of Health and Welfare: Japan, Toxicity Testing Reports of Environmental Chemicals 4, 409-430 (1996)

GENERAL REMARKS

CARCINOGENICITY

TEST SUBSTANCE

6,6'-di-*tert*-Butyl-2,2'-methylenedi-*p*-cresol (CAS No 119-47-1)
Remarks: Source: Ouchi Shinko Chemical. Purity: Unknown

METHOD

- **Method/guideline:** Unknown
- **Test type:** Chronic Toxicity study
- **GLP:** No
- **Year:** 1994 (published year)
- **Species:** Rat
- **Strain:** Wistar
- **Route of administration:** oral (by feeding)
- **Doses/concentration levels:** 0, 100, 300, 1000 ppm (in diet)
- **Sex:** Male & Female
- **Exposure period:** 18 months
- **Frequency of treatment:** Daily
- **Control group and treatment:** Basal diet (no treatment)
- **Post exposure observation period:** none
- **Duration of test:** 18 months
- **Statistical methods:** Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

REMARKS FIELD FOR TEST CONDITIONS

Test Subjects:

- ***Age at study initiation*:** 5 week old for both sexes
- ***Weight at study initiation*:** 393±21 g for male, 230±15 g for female
- ***No. of animals per sex per dose*:** 30 per sex per dose group (5 were sacrificed at 6 month and another 5 at 12 month for hematological and serum biochemical examinations).

Study Design:

- ***Vehicle*:** none (pellets of diet containing the substance)
- ***Satellite groups and reasons they were added*:** none
- ***Organs examined at necropsy:***
  Organ weight: brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands
  Microscopic: all the group/ brain, heart, lungs, liver, kidney, spleen, adrenals, testes, ovaries, pituitary and thyroid glands, salivary glands, esophagus, stomach, small intestine, pancreas, urinary bladder, seminal vesicles, epididymis, ischiac nerve, uterus, prostate, mesenteric lymph nodes, thymus, spinal cord, skeletal muscle, born marrow

RESULTS

- **MTD**
  Suppression of body weight gain, increase in liver weight were observed at the highest dose of

1000 ppm in both sexes.

· **Carcinogenicity**
No neoplastic lesion attributable to the substance was observed in any organs of either sex. however this study is not qualified to be regarded as a carcinogenicity study. Therefore, no conclusion could be reached on the carcinogenicity.

REMARKS FIELD FOR RESULTS

– ***Body weight:*** Significant suppression of body weight gain was observed from the month 6 in the male group at 1000 ppm and from the month 1 in the female group at 1000 ppm.

	Males				Females			
Diet level (ppm)	**0**	**100**	**300**	**1,000**	**0**	**100**	**300**	**1,000**
Final mean body weight (g)	545	528	520	498*	375	368	353	278*

(significant: not specified the degree)*

– ***Organ weight changes:***
Male: Increase in liver weight at 1000 ppm (absolute ($p<0.05$) and relative ($p<0.01$)).
Decrease in testis weight at 1000 ppm (absolute and relative) ($p<0.01$)
Female: Increase in liver weight at 1000 ppm (relative) ($p<0.01$).

	Males		Females	
Diet level (ppm)	**0**	**1,000**	**0**	**1,000**
Absolute weight				
Liver (g, Mean ± SD)	12.28 ± 0.93	14.19 ± 1.35*	7.60 ± 0.88	7.39 ± 0.83
Testis (g, Mean ± SD)	3.28 ± 0.48	0.82 ± 10.18**		
Relative weight				
Liver (g%, Mean ± SD)	2.37 ± 0.16	3.00 ± 0.13**	2.08 ± 0.15	2.79 ± 0.35**
Testis (g%, Mean ± SD)	0.63 ± 0.10	0.17 ± 0.05*		

*(* $p<0.05$, ** $p<0.01$)*

Histopathology** (neoplasm)**:
It is described that "no neoplastic lesions which could be attributed to this compound were observed in any organs of either sex". No other information is available.

CONCLUSIONS

No tumors were observed in a 18-month chronic feeding study with rats up to 1, 000 ppm, however this study is not qualified to be regarded as a carcinogenicity study. Therefore, no conclusion could be reached on the carcinogenicity.

DATA QUALITY

· **Reliabilities:** Valid (limitation: the study was not designed for carcinogenicity and carried out using relatively small numbers of animals without GLP)

Remarks field for Data Reliability
Well conducted study, carried out by National Institute of Health Science (Japan).

REFERENCES (Free Text)

Takagi et al.; Journal of Toxicological Science, Vol. 19, 77-89 (1994)

GENERAL REMARKS