FOREWORD INTRODUCTION

O-TOLUENESULFONAMIDE

CAS N°: 88-19-7

SIDS Initial Assessment Report for 14th SIAM

(Paris, March 26-28, 2002)

Chemical Name: o-Toluenesulfonamide

CAS No: 88-19-7

Sponsor Country: Japan

National SIDS Contact Point in Sponsor Country:

Mr. Yasuhisa Kawamura,
Ministry of Foreign Affairs, Japan

HISTORY:

The original draft documents were prepared by Japanese government.

Tests:

No testing ( )

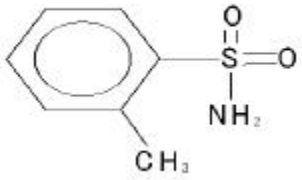
Testing (x) Vapor pressure, log P_{ow}, Water solubility, Hydrolysis and Photolysis, Biodegradation, Environmental fate, Acute toxicity to fish, daphnia and algae, Chronic toxicity to daphnia, Acute toxicity, Combined repeated and reproductive/developmental toxicity, Ames test and Chromosomal aberration test

COMMENTS:

Deadline for circulation: February 1, 2002

Date of Circulation: February 1, 2002

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	88-19-7
Chemical Name	*o*-Toluenesulfonamide
Structural Formula	

RECOMMENDATIONS

The chemical is currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

o-Toluenesulfonamide orally administered was rapidly eliminated mostly to urine in rats. In human subjects it was excreted to urine more slowly than that in rats. The main metabolites were 2-sulfamoyl-benzyl alcohol and its sulphate and glucuronic acid conjugates in both rats and humans. Saccharin was also detected as a metabolite in urine especially in humans.

The oral LD_{50} value for rats was greater than 2,000 mg/kg b.w. in males and between 1,000 and 2,000 mg/kg b.w. in females [OECD TG 401]. Sedation, passivity and catalepsy appeared even at the lowest dose of 700 mg/kg b.w. It is reported that this chemical was moderately irritating to eyes in rabbits but the reliability of the study is uncertain. There is no available information on skin irritation and sensitization.

In accordance with an OECD combined repeated dose and reproductive/developmental toxicity screening test [TG 422], o-toluenesulfonamide was given to male and female SD rats by gavage at 0, 20, 100, 500 mg/kg b.w./day for at least 38 days. Three females died and two females were sacrificed in moribund condition during the pre-mating period at 500 mg/kg b.w. Decreased locomotor activity and appearance of prone position and salivation were observed in both sexes at 100 and 500 mg/kg b.w. In the same groups, low body weights were recorded in both sexes. In histopathological examinations, hypertrophy of the centrilobular hepatocytes with the cytoplasm having a ground glass appearance was observed in both sexes at 100 and 500 mg/kg b.w. in a dose-dependent manner. In addition, the incidence of fibrosis and cellular infiltration of the pericardium, and fibrosis and cellular infiltration of the capsule and atrophy of the thymus were significantly increased in females of at 500 mg/kg b.w.. In the kidneys, eosinophilic body was observed in males of all treated groups, maybe due to the complex accumulation of this chemical with the male rat specific protein, alpha-2u-globulin. Based on clinical signs and hepatic change, the NOAEL for repeated dose toxicity is considered to be 20 mg/kg b.w./day for both sexes.

Regarding genotoxicity, a bacterial test [OECD TG 471] and a chromosomal aberration test [OECD TG 473] *in vitro* were negative with and without metabolic activation. One mammalian spot test in mice demonstrated inconclusive results and two micronucleus tests *in vivo* in mice (gavage and i.p.) showed negative results. However the experimental condition of all these studies are not sufficiently reported. Therefore, the genotoxic potential of this chemical *in vivo* is inconclusive.

In a two generation lifetime feeding study, male and female SD rats were given o-toluenesulfonamide in the diets at 0, 2.5, 25 and 250 mg/kg b.w./day. No increase in any tumour incidence was noted in all dose groups of both generations. Two 2-year oral rat studies also demonstrated no carcinogenicity of this chemical. Only one lifetime feeding study showed low incidence of urinary bladder tumors of rats but the reliability of this study is uncertain

because of poor reporting A cell transformation assay using mammalian cultured cells showed negative results. Based on a weight of evidence approach, the available data indicates that this chemical is not carcinogenic.

In an OECD combined repeated dose and reproductive/developmental toxicity screening test [TG 422], a significant reduction in body weights of pups was observed on days 0 and 4 in both sexes of rats at 500 mg/kg b.w. In a two generation lifetime feeding study, decrease of litter size and pup body weight was observed at 250 mg/kg b.w./day. Based on an overall evaluation of both results, the appropriate NOAEL for reproductive/developmental toxicity is considered to be 100 mg/kg b.w./day .

Environment

o-Toluenesulfonamide is soluble in water (1.6 g/L at 25 °C) and has a low vapor pressure (6.6 x 10^{-5} Pa at 25 °C) Its log Kow is 0.84. *o*-Toluenesulfonamide is not readily biodegradable (OECD TG301C: 0 % by BOD after 14 days), but its experimental BCF of less than 2.6 (OECD TG 305) suggests that this chemical does not bioaccumulate in aquatic organisms. Hydrolysis is not expected to occur. If released to the atmosphere, this chemical mainly exists in the particulate phase according to its low vapor pressure. Particulate phase of *o*-Toluenesulfonamide may be physically removed from the air by dry and wet deposition.

Acute toxicty of o-Toluenesulfonamide has been tested in three aquatic species of three trophic levels. For algae (*Selenastrum capricornutum*) a ErC50 of 170 mg/L (OECD TG 201, growth rate for 24-48hr and also 24-72 h) and a 72hEbC50 of 57 mg/L (OECD TG201, biomass) were determined. For daphnids (*Daphnia magna*) a 48 h EC50 of 210 mg/L (OECD TG 202 part 1), and for fish (*Oryzias latipes*) a 96 h LC50 of >100 mg/L (OECD TG 203) were reported.

Two chronic toxicity values, for alga (*Selenastrum capricornutum*) and daphnids (*Daphnia magna*) were available. For algae, a 72 h NOEC on growth inhibition of 7.7 mg/L(OECD TG 201, based on growth rate), and for daphnids, a 21 d NOEC of 49 mg/L(OECD TG 202, reproduction) were reported.

Exposure

o-Toluenesulfonamide was mainly produced as a chemical intermediate for the production of saccharin in the past, but now saccharin is normally manufactured without using this chemical although minor amounts are still used for this purpose. A mixture of *o*-Toluenesulfonamide with the *p*-isomer is used as a plasticizer for hot-melt adhesives, a chemical intermediate for fluorescent pigments and a chemical intermediate for plasticizer resins. The production volume of this chemical in Japan was about 50 tonnes in 2000.

NATURE OF FURTHER WORK RECOMMENDED

This chemical is not a candidate for further work because all SIDS endpoints are sufficient.

FULL SIDS SUMMARY

CAS NO: 88-19-7		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point		Unknown	157.2 °C
2.2	Boiling Point		OECD TG 103	> 270 °C
2.3	Density		JIS K7112	1.461 g/cm^3 at 25°C
2.4	Vapour Pressure		OECD TG 104	6.6 x 10^{-5} Pa at 25 °C
2.5	Partition Coefficient (Log P_{ow})		measured	0.84
2.6 A.	Water Solubility		OECD TG 105	1.6 g/L at 25 °C
B.	pH		-	-
	pKa		OECD TG 112	10.18
2.12	Oxidation: Reduction Potential		-	None
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation		Calculated	$T_{1/2}$ = 315 hours (indirect)
3.1.2	Stability in Water		OECD TG 111	Stable at pH 4, 7 and 9 at 50 °C
3.2	Monitoring Data		-	No data is available.
3.3	Transport and Distribution		Calculated (Level III Fugacity Model)	(Release 100 % to air) Air 0.0 % Water 41.6 % Soil 58.2 % Sediment 0.2 % (Release 100 % to water) Air 0.0 % Water 99.6 % Soil 0.0 % Sediment 0.4 % (Release 100 % to soil) Air 0.0 % Water 36.3 % Soil 63.5 % Sediment 0.2 %
3.5	Biodegradation		OECD TG 301C	Not readily biodegradable
3.7	Bioaccumulation		OECD TG 305	BCF= 0.4 – 0.9 (3 mg/L) BCF < 2.6 (0.3 mg/L)
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	96hLC50>100 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 202	48hEC50 = 210 mg/L 48hEC0 = 95 mg/L
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selenastrum capricornutum*	OECD TG 201	ErC50 (24-48 hr) = 170 NOEC = 79 mg/L ErC50 (24-72 hr) = 170 NOEC = 7.7 mg/L 72hEbC50 = 57 mg/L 72hNOEbC = 7.7 mg/L
4.5.2	Chronic Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 211	21d LC50 >100 mg/L 21d EC50 = 79 mg/L 21d NOEC = 49 mg/L
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rats	OECD TG 401	LD_{50} = >2,000 mg/kg b.w. for male LD_{50} = 1,000 ~ 2,000 mg/kg b.w. for female
5.1.2	Acute Inhalation Toxicity			No study
5.1.3	Acute Dermal Toxicity			No study
5.2.1	Skin Irritation	Rabbit	Other	Moderate irritat ing (low reliability)
5.2.2	Eye Irritation			No study
5.3	Sensitization			No study
5.4	Repeated Dose Toxicity	Rat	OECD TG 422	NOAEL = 20 mg/kg b.w./day

5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	*S.typhimurium, E. coli*	OEC D TG 471	- (With metabolic activation) - (Without metabolic activation)
B.	Non-Bacterial In Vitro Test (Chromosomal aberrations)	CHL cells	OECD TG 473	- (With metabolic activation) - (Without metabolic activation)
5.6	Genetic Toxicity In Vivo	Mouse	Other (Micronucleus, by gavage)	Negative
		Mouse	Other (Micronucleus, i.p.)	Negative
		Mouse	Other (Spot test)	Inconclusive
5.7	Carcinogenicity	Rat	Other (Feeding, Two generation)	No tumours
		Rat	Other (Feeding)	Low incidence of urinary bladder tumours (low reliability)
5.8	Toxicity to Reproduction	Rat	OECD TG 422	NOAEL = 100 mg/kg b.w./day
		Rat	Other (Two generation)	NOAEL = 25 mg/kg b.w./day LOAEL = 250 mg/kg b.w./day
5.9	Developmental Toxicity/ Teratogenicity	Rat	OECD TG 422	NOAEL = 100 mg/kg b.w./day
		Rat	Other (Two generation)	NOAEL = 25 mg/kg b.w./day LOAEL = 250 mg/kg b.w./day
5.11	Experience with Human Exposure			No study

SIDS INITIAL ASSESSMENT REPORT

1. IDENTITY

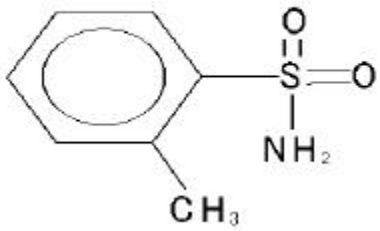
Chemical Name: *o*-Toluenesulfonamide

Synonyms: *o*- Methylbenzensulfonamide
2-Methylbenzensulfonamide
Toluene-2-sulfonamide

CAS Number: 88-19-7

Empirical Formula: $C_7H_9NO_2S$

Structure:



General Substance Information

Substance type: organic

Physical status: solid

Purity: 99.9 % w/w

Physical and chemical properties

o-Toluenesulfonamide is white powder or crystal which is soluble in water (1.6 g/L at 25 °C). Other physical-chemical properties are shown in Table 1.

Table 1: Physical-chemical properties

	Protocol	Results
Melting Point	Unknown	157.2 °C
Boiling Point	OECD TG 103	> 270 °C
Density	JIS K7112	1.461 g/cm^3 at 20 °C
Partition Coefficient (Log P_{ow})	Calculation	0.84
Vapor Pressure	OECD TG 104	6.6 x 10^{-5} Pa at 25 °C
Partition Coefficient (Log P_{ow})	Unknown	0.84
Water Solubility	OECD TG 105	1.6 g/L at 25°C
pKa	OECD TG 112	10.18

2. GENERAL INFORMATION ON EXPOSURE

2.1. Production and import

o-Toluenesulfonamide was mainly produced as a chemical intermediate for the production of Saccharin in the past, but now Saccharin is normally manufactured without using this chemical although minor amounts are still used for this purpose. The production volume of this chemical in Japan was about 50 tonnes in 2000, and the imported volume into Japan is about 30 tonnes in 2000. The worldwide production volume of this chemical is not known.

2.2 Use Pattern

o-Toluenesulfonamide is mainly used as an additive in the chemical industry. Minor amounts are used as a chemical intermediate for the production of Saccharin. A mixture of *o*-Toluenesulfonamide with the *p*-isomer is used as a plasticizer for hot-melt adhesives, a chemical intermediate for fluorescent pigments and a chemical intermediate for plasticizer resins.

2.3 Occupational Exposure

The occupational exposure routes are inhalation of dust, since this chemical is solid and the vapor pressure of this chemical is 6.6×10^{-5} Pa at 25 °C, and dermal contact. This chemical is produced in a closed system in Japan. Exposure to this chemical may occur in the packing process to paper bags. Workers operate the packing machine, but adjust the packing weight manually, so inhalation and dermal contact may occur. Measured data of airborne concentration of this chemical was not available. The estimated exposure concentration for this operation using the EASE model was 0.5-5 mg/m^3, as non-fibrous dust, assuming dry manipulation without local exhaust ventilation, and ready aggregation. The duration of bag packing is one hour per day, and the EHE_{inh} for a worker operating paper bag packing was 0.09 mg/kg·bw/day. Estimated dermal exposure was 0.1-1 mg/cm^2/day, assuming non-dispersive use and direct handling with intermittent contact level. The EHE_{der} through both hands for the same worker is thereby 1.9 mg/kg·bw/day. The workers wear safety goggles, protective gloves, and dust masks to prevent accidental dermal contact and inhalation. No occupational exposure standard value was located.

2.4 Other information

None.

3. ENVIRONMENT

3.1 Environmental Fate and Pathways

o-Toluenesulfonamide is soluble in water (1.6 g/L at 25°C) and has a low vapor pressure (6.6 x 10^{-5} Pa at 25°C). Partition co-efficient (P_{ow}) is 0.84. *o*-Toluenesulfonamide is not readily biodegradable (OECD TG301C: 0 % by BOD after 14 days), but its experimental BCF values (OECD TG 305: < 2.6) suggests that this chemical does not bioaccumulate in aquatic organisms. Hydrolysis is not expected to occur (OECD TG 117: stable at pH 4, 7 and 9 at 50 °C for five days). If released to the atmosphere, this chemical mainly exist in the particulate phase according to its low vapor pressure. Vapor phase *o*-Toluenesulfonamide may be physically removed from the air by dry and wet deposition.

The potential environmental distribution of *o*-Toluenesulfonamide obtained from a generic level III fugacity model under three emission scenarios is shown in Table 2. The results show that if *o*-Toluenesulfonamide is released to the air compartment, this chemical has a tendency to move to the water and soil compartments. If released to the water compartment, this chemical stays in this compartment, and if release to the soil compartment, about 40 % of *o*-Toluenesulfonamide moves to the water compartment.

Table 2: Environmental distribution of *o*-Toluenesulfonamide using a generic level III fugacity modelunder three emission scenarios

Compartment	Release: 100 % to air	Release: 100 % to water	Release: 100 % to soil
Air	0.0 %	0.0 %	0.0 %
Water	41.6 %	99.6 %	36.3 %
Soil	58.2 %	0.0 %	63.5 %
Sediment	0.2 %	0.4 %	0.2 %

The pKa of the substance being 10.18, the substance will mainly be present in a protonated form under environmental conditions. Some interaction with the mineral fraction of soil and sediment is possible. In the above results with the fugacity model, the fractions in soil and sediment could be underestimated.

3.2 Toxicity to Aquatic Organisms

o-Toluenesulfonamide has been tested in three aquatic species of three trophic levels. Results are summarized in Table 3. On acute toxicity, for algae (*Selenastrum capricornutum*) a ErC50 of 170 mg/L (OECD TG 201, grow th rate for 24-48hr and also 24-72 h) and a 72hEbC50 of 57 mg/L (OECD TG201, biomass) were determined. For daphnids (*Daphnia magna*) a EC50 of 210 mg/L (OECD TG 202 part 1, 48 h), and for fish (*Oryzias latipes*) a LC50 of >100 mg/L(OECD TG 203, 96 h) were reported, however the test using fish was conducted as a limit test in which one concentration of 100 mg/L and control was examined(Japan EA, 2000). The lowest acute toxicity result for this substance was reported on algal inhibition.
Two chronic toxicity values, for alga (*Selenastrum capricornutum*) and daphnid (*Daphnia magna*) were available. For alga, a 72 h NOEC on growth inhibition of 7.7 mg/L(OECD TG 201, based on growth rate), and for daphnids a 21 d NOEC on reproduction of 49 mg/L(OECD TG 202, reproduction) were reported. All the values shown here were obtained from the tests conducted under GLP and well documented (Japan EA, 2000).

Table 3: Summary of effects of o-Toluenesulfonamide on aquatic organisms

Organisms	Test duration	Result (mg/L)	Reference
Aquatic plants, e.g. algae			
Green alga (*Selenastrum capricornutum*)	72 h (op,s)	Growth rate ErC50(24-48hr) = 170 NOEC = 79 mg/L ErC50(24-72hr) = 170 NOEC = 7.7 mg/L Biomass EC_{50} (72hr) = 57 (mc) NOEC (72hr) = 7.7 (mc)	Japan EA: 2000
Invertebrates			
Water flea (*Daphnia magna*)	48 h (s)	EC_{50} (Imm) = 210 (mc)	Japan EA: 2000
	48 h (s)	EC_0 (Imm) = 95 (mc)	
	21 d (ss)	LC_{50} >100 (mc)	Japan EA: 2000
	21 d (ss)	EC_{50} (Rep) = 79 (mc)	
	21 d (ss)	NOEC (Rep) = 49 (mc)	
Fish			
Medaka (*Oryzias latipes*)	96 h (ss)	LC_{50} > 100(nc)	Japan EA: 2000
	96 h (ss)	LC_0 = 100(nc)	

cl = closed system, op = open system, s = static, ss = semi-static, nc = nominal mc = measured concentration
Bms = biomass Imm = immobilization Rep = reproduction

3.3 Toxicity to Terrestrial Organisms

There is no available information.

3.4 Other

There is no available information.

3.5 Initial Assessment for the Environment

o-Toluenesulfonamide is not readily biodegradable (OECD TG301C: 0 % by BOD after 14 days), but its experimental BCF values (OECD TG 305: < 2.6) suggests that this chemical does not bioaccumulate in aquatic organisms. Hydrolysis is not expected to occur (OECD TG 117: stable at pH 4, 7 and 9 at 50 °C for five days). If released to the atmosphere, this chemical mainly exists in the particulate phase according to its low vapor pressure. Vapor phase *o*-Toluenesulfonamide may be physically removed from the air by dry and wet deposition. A generic level III fugacity model shows that if *o*-Toluenesulfonamide is released to the air compartment, this chemical has a tendency to move to the water and soil compartments. If released to the water compartment, this chemical stays in

this compartment, and if release to the soil compartment, about 40 % of *o*-Toluenesulfonamide moves to the water compartment.

Algae are the most sensitive organisms among three trophic levels based on both acute and chronic toxicity results reported for the algae *Selenastrum capricornutum* (72 h EC50 of 57 mg/L and 72 h NOEC of 7.7 mg/L on growth inhibition, respectively).

The predicted no effect concentration (PNEC) of 0.077 mg/L for the aquatic organisms is calculated from the 72 h NOEC for *Selenastrum capricornutum* using an assessment factor of 100, because only two chronic data (*Daphnia* and *Selenastrum*) are available.

4. HUMAN HEALTH HAZARD

4.1 EFFECTS ON HUMAN HEALTH

a) Toxicokinetics and metabolism

Two animal studies and one human study are available.

In the first study by Minegishi et al. (1972), after a single dose of 300 mg/kg b.w. of [^{35}S] o-toluenesulfonamide to male Wistar rats, the radioactivity was recovered about 85 % in urine and about 10 % in feces mostly within 48 hr. The urine was analysed after treatment with conc. HCl. Approximately 50 % of metabolites excreted in urine were o-sulfamoylbenzoic acid, while the compound excreted in feces was unchanged.
[Methyl-^{14}C] o-Toluenesulfonamide was administered at a single dose by gavage to female Wistar rats and urine was analysed after treatment without conc. HCl [Renwick et al.: 1978].
At 20 mg/kg b.w., this chemical was rapidly eliminated (92 % within 24 hr) and most of the radioactivity (79 %) was recovered in the urine with little in the faeces. At larger doses (125 and 200 mg/kg b.w.), this chemical was eliminated more slowly (70 and 43% respectively in 24 hr) but the final radioactivity ratio in urine and faeces was hardly changed. In contrast to the above-mentioned study by Minegishi et al. (1972), the main metabolites in urine were 2-sulfamoylbenzyl alcohol and its sulphate and glucuronic acid conjugates (80 %) at 200 mg/kg. Other metabolites found in the urine were including saccharin (3 %), 2-sulfamoylbenzoic acid (2%) and N-acethyltoluene sulfonamide (6%) together with the unchanged compound (5%).
In human subjects orally given 0.2 mg/kg b.w. [methyl-^{14}C] o-toluenesulfonamide, it was eliminated more slowly than in rats, with about 56 % recovery in the urine in 24 hr and 86 % in 48 hr [Renwick et al.: 1978]. Negligible amounts of the radioactivity (less than 1%) appeared in the faeces. The main metabolites in urine were 2-sulfamoylbenzyl alcohol and its sulphate and glucuronic acid conjugates (35 %) and saccharin (35%). This result suggests species differences in the metabolism of o-toluenesulfonamide.

b) Acute toxicity

Three acute toxicity studies were reported for rats as shown in Table 4. The study by MHW (1999) was identified as a key study because it was well conducted and used a current protocol (OECD TG 401) in compliance with GLP [MHW, Japan: 1999]. As for the other two, no details were reported.

In the study by MHW , SD rats (5 animals/sex/group) were administered by gavage with o-toluenesulfonamide at a single dose of 0, 700, 1,000, 1,400 and 2,000 mg/kg b.w. Three females given 1,400 mg/kg b.w. and two females given 2,000 mg/kg b.w. were found dead but there were no death in males of all treated groups. Sedation, passivity, catalepsy and prostration appeared even at the lowest dose of 700 mg/kg b.w. Lateral position, abnormal breathing and hypothermia were also found in both sexes of the higher dosing groups. At autopsy, reddish discoloration of the lung, pale colored areas of the gastric mucosa and cysts in the renal cortex were observed in dead animals. The LD_{50} values were greater than 2,000 mg/kg b.w. for males and between 1,000 and 2,000 mg/kg b.w. for females.

Table 4: Acute toxicity of o-toluenesulfonamide in rats

Route	Animals	Type	Values	References
Oral	Rat	LD_{50}	> 2,000 mg/kg b.w. for males	MHW, Japan: 1999
			1,000 ~ 2,000 mg/kg b.w. for females	MHW, Japan: 1999
	Rat	LD_{50}	ca. 2,000 mg/kg b.w.	Schmähl: 1978
	Rat	LD_{50}	4,870 mg/kg b.w.	Marhold et al.: 1986

Human data

There is no available information on humans.

Conclusions:

The oral LD_{50} value in rats was greater than 2,000 mg/kg b.w. for males and between 1,000 and 2,000 mg/kg b.w. for females.

c) Repeated dose toxicity

Only one study [MHW, Japan: 1999] is available for repeated dose toxicity. This study was identified as a key study because it was well conducted and used OECD TG 422 in compliance with GLP. In addition, although there are two 2-year carcinogenicity studies and one lifetime feeding study (See the section e) Carcinogenicity), the data quality was not high enough to be used for the assessment of the repeated dose toxicity because these studies were not conducted according to standard test guideline or GLP and only histopathology examinations were reported. Details of the study by MHW (1999) are as follows.

SD rats received o-toluenesulfonamide by gavage at doses of 0, 20, 100 and 500 mg/kg b.w./day according to an OECD combined repeated dose and reproductive/developmental toxicity screening test. Males were dosed for 42 days and females were dosed from 14 days before mating, throughout pregnancy to day 3 of lactation. Haematological and blood chemical examination was conducted only for males.

Three females died and two females were sacrificed in moribund condition during the pre-mating period at 500 mg/kg b.w., but no males were found dead or moribund in any group. Decreased locomotor activity and appearance of prone position and salivation were observed in both sexes at 100 and 500 mg/kg b.w. In these groups, significantly low body weights were recorded in both sexes. Blood chemical examination in males showed an increase in total cholesterol at 100 mg/kg b.w. or more, and a decrease in glucose and triglyceride at 500 mg/kg b.w. Relative liver weight of males at 500 mg/kg b.w. and of females at 100 and 500 mg/kg b.w., and relative kidney weight of males at 100 and 500 mg/kg b.w. and of females at 500 mg/kg b.w. were significantly increased. In histopathological examinations, hypertrophy of the centrilobular hepatocytes with the cytoplasm having a ground glass appearance was observed in both sexes at 100 and 500 mg/kg b.w. In the kidneys, increased incidence and severity of eosinophilic body was observed in males of all treated groups. In addition, the incidence of fibrosis and cellular infiltration of the pericardium, and fibrosis and cellular infiltration of the capsule and atrophy of the thymus were significantly increased in females at 500 mg/kg b.w.. Renal change observed in male rats may be due to the complex accumulation of this chemical with the male rat specific protein, alpha-2u-globulin, although no direct evidence is given. Therefore, the NOAEL for repeated dose toxicity is considered to be 20 mg/kg b.w./day for both sexes, based on clinical signs and hepatic change.

There is no available information on humans.

Conclusions:
In an oral repeated dose study in rats, the major effects of this chemical were clinical signs such as decreased locomotor activity and prone position, and histopathological change in liver. The NOAEL is considered to be 20 mg/kg b.w./day for both sexes.

d) Genotoxicity

Table 5: Summary of genotoxicity assays

Type of test	Test system	Doses	Result	Reference
Bacterial test				
Ames test *(reverse mutation)*	*S. typhimurium* (TA98, TA100, TA1535, TA1537) *E. coli* (WP2 *uvr* A)	Up to 5,000 ug/plate	**Negative** (+ & - MA*)	MHW, Japan: 1999
Ames test *(reverse mutation)*	*S. typhimurium* (TA1535, TA100, TA98, TA1537)	Up to 1,000 ug/plate	**Negative** (+ & - MA)	Stolts et al.: 1977
Ames test *(reverse mutation)*	*S. typhimurium* (TA1530, TA1535, TA1537, TA1538, TA100, TA98)	Up to 4 x 10^{-2} mol/plate	**Negative** (+ & - MA)	Poncelet et al.: 1979
Ames test *(reverse mutation)*	*S. typhimurium* (TA1535, TA100, TA1538, TA98, TA1537)	Up to 18,000 ug/plate	**Weakly positive: TA98** (+ MA) **Negative: TA98** (- MA) **and other strains** (+ & -MA)	Eckhardt et al.: 1980
Ames test *(reverse mutation)*	*S. typhimurium* (TA98, TA 100, TA 1535, TA1537)	Up to 18,000 ug/plate	**Negative** (+ MA)	Herbold: 1981
Ames test *(reverse mutation)*	*S. typhimurium* (TA1535, TA1538, TA98, TA100)	Up to 2,500 ug/plate	**Negative** (+ MA)	Ashby et al.: 1978
Gene mutation assay	*Saccharomyces cerevisiae* (D4)	Up to 1,000 ug/plate	**Negative** (+ & - MA)	Jagannath & Brusick: 1978
Non-bacterial in vitro test				
Ouabain-resistant mutation assay	Human RSa cells	Up to 1,800 ug/mL	**Negative** (- MA)	Suzuki & Suzuki: 1988
Chromosomal aberration test	CHL cells	Up to 3,000 ug/mL	**Negative** (+ & - MA)	MHW, Japan: 1999
Chromosomal aberration test	CHO-K1 cells	Up to 400 ug/mL	**Negative** (- MA)	Masubuchi et al.: 1978
In vivo test				
Mammalian spot test	Mouse	1,000 mg/kg b.w. (oral)	**Inconclusive**	Fahrig: 1982
Micronucleus assay	Mouse	1,026 mg/kg b.w. (by gavage)	**Negative**	Eckhardt et al.: 1980
Micronucleus assay	Mouse	Up to 1,026 mg/kg b.w. (i.p.)	**Negative**	Eckhardt et al.: 1980

*MA: Metabolic activation

Bacterial tests

Seven studies were reported as shown in Table 5. Among them, only one study [Eckhardt et al.: 1980] showed weakly positive result because this chemical induced a 2-3-fold increase of mutation in TA98 with an exogenous metabolic activation system. However, this change was not accompanied by a dose-response relationship. Another study conducted under the same experimental conditions was not able to confirm this weakly positive result and the previous observation was assumed to be variations within the spontaneous response range of TA98 [Herbold et al.: 1982]. The study by MHW (1999) was identified as a key study because all experimental conditions were sufficient such as strains, testing concentrations, with and without metabolic activation, test guideline (Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Test Guidelines 471) and GLP. The result of the study was negative in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538, and *Escherichia coli* WP2 *uvr*A at up to 5,000 ug/plate with or without an exogenous metabolic activation system.

Non-bacterial in vitro tests

Four studies were reported as non-bacterial *in vitro* tests as shown in Table 5, and all of them showed negative results. One ouabain-resistant mutation assay [Suzuki & Suzuki: 1988] and one chromosome aberration test [Masubuchi et al.: 1978] were conducted only without metabolic activation. The chromosomal aberration test by MHW (1999) conducted in cultured Chinese hamster lung (CHL/IU) cells according to Japanese test guideline equivalent to OECD TG 473 under GLP, was identified as a key study because all experimental conditions and reporting were sufficient. This chemical did not induce structural chromosomal aberrations and/or polyploidy at up to 3,000 ug/ml with or without an exogenous metabolic activation system. Cytotoxicity was observed at 2,250 ug/ml and more for continuous treatment without S9 mix but not up to 3,000 ug/ml for short-term treatment with and without S9 mix. The maximum concentration of 3,000 ug/ml is above the established maximum concentration of 10 mM as recommended in the OECD TG.

In vivo test

One mammalian spot test [Fahrig: 1982] and two micronucleus assays [Eckhardt et al.: 1980] were reported as shown in Table 5. Because these experiments were not conducted according to standard test guidelines or GLP, none of them were considered as key studies.

In a mammalian spot test [Fahrig: 1982], 80 pregnant mice (embryo are heterozygous for 4 different recessive coat-color genes) were orally given 1,000 mg/kg b.w. at the 10th day post-conception. 30~50 mice had litters surviving for 2 weeks and the color spots in about 200 offsprings were analyzed. Triplicate experiments were performed and at least 1 offspring with a colour spot was observed in each experiment, but only one case did lead to a significant result (1/285 offspring with gray spots and 3/285 with light-brown spots, compared with 0/182 with spots in the control group). Based on these results, the conclusion of the genotoxic potential is considered to be inconclusive. Furthermore the reliability of this study is limited because of no information on the positive control and historical control data in this report.

Micronucleus tests were conducted in NMRI mice, which were given o-toluenesulfonamide twice at a 24 hr interval, intraperitoneally (up to 1,026 mg/kg b.w.) or by gavage (1,026 mg/kg b.w.) [Eckhardt et al.: 1980]. As a result, this chemical did not increase the rate of micronuclei in bone marrow erythrocytes. However there are several insufficient experimental conditions such as no rationale for the choice of the highest dose, only single time point for examination, and so on. Therefore, the reliability of these data are limited.

Conclusions:

A bacterial test and a chromosomal aberration test *in vitro* were negative with and without metabolic activation system. One mammalian spot test in mice demonstrated inconclusive results and two micronucleus tests *in vivo* in mice (gavage and i.p.) showed negative results. However the experimental condition of all these studies are not sufficiently reported. Therefore, the genotoxic potential of this chemical *in vivo* is inconclusive.

e) Carcinogenicity

Four carcinogenic studies were reported in rats, as shown in Table 6. The incidence of urinary bladder tumour was slightly increased in one lifetime feeding study [Schmähl: 1978], while no carcinogenic effects were observed in the other three studies. Hooson et al. (1980) reported two 2-year studies, in which female Wistar rats were given this chemical at 0 or 0.1 % (70 mg/kg b.w./day) in the drinking water, or 0 or 79 mg/kg b.w./day in the diet. These 2-year studies were conducted in only females and in only one dosed group. Arnold et al. (1980) conducted a two generation lifetime feeding study, which is identified as a key study because this study was well conducted and reported. Details of the studies by Schmähl (1978) and by Arnold et al. (1980) are as follows.

In the study by Schmähl (1978), o-toluenesulfonamide was fed to male and female SD rats at 0, 20 and 200 mg/kg b.w. for lifetime. A treatment-related effect on the survival time was not observed. In the urinary bladder, carcinoma (1/76 animals at 200 mg/kg b.w.) and papillomas (3/75 at 20 mg/kg b.w.and 4/76 at 200 mg/kg b.w.) were observed but no tumours occurred in the control group (71 animals). However, there was no sufficient information on historical data, the purity of the test chemical, statistical analysis, the sexes of animals with bladder tumours and the presence or absence of bladder parasites. Based on this lack of information and the low incidence of bladder tumour, the reliability of the carcinogenic potential of this chemical is considered to be low.

In a two generation lifetime feeding study, SD rats (32 days old) were given o-toluenesulfonamide in the diet at 0 (control), 2.5, 25, 250 mg/kg b.w./day (50 animals/sex/group) or 250 mg/kg b.w./day with 1 % NH_4Cl in the drinking water (40 males and 38 females) [Arnold et al.: 1980]. Rats were exposed to this chemical from 90 days before mating in the first generation (for 142 weeks) and after weaning in the second generation (for 127 weeks).

The animals were free of urinary bladder parasites. There were no treatment-related effects associated with longevity. Urinary bladder tumours, all of which were benign, were observed in one male each of the 0, 2.5 and 250 mg/kg b.w. group and in one female of the 2.5 mg/kg b.w. group in the first generation, and 2 females of the 2.5 mg/kg b.w. group in the second generation. However, the incidence was neither statistically significant nor dose-related. No other dose-related tumours were observed in any groups.

Table 6: Summary of carcinogenic studies for o-toluenesulfonamide

Animals (the number)	Exposure periods	Exposure routes	Doses (mg/kg b.w./day	Results	Reference
SD Rat Male & female (71-76/group)	Lifetime	Oral (feed)	0, 20, 200	Urinary bladder tumours	Schmähl: 1978
SD Rat Male & female (50/ group)	Lifetime (two generations)	Oral (feed)	0, 2.5, 25, 250	No change in tumour incidence	Arnold et al.: 1980
Wistar Rat Female (63/group)	2 years	Oral (drinking water)	0, 70	No change in tumour incidence	Hooson et al.: 1980
Wistar Rat Female (50/group)	2 years	Oral (feed)	0, 79	No change in tumour incidence	Hooson et al.: 1980

There is no information available on humans.

Conclusions:

In a two generation lifetime feeding study, no increase in any tumour incidence was noted in rats of both generations at up to 250 mg/kg b.w./day. Two 2-year oral rat studies also demonstrated no carcinogenicity of this chemical. Only one lifetime feeding study showed a low incidence of urinary bladder tumors in rats but the reliability of this study is uncertain because of poor reporting. A cell transformation assay using mammalian cultured cells showed negative results. Based on a weight of evidence approach, the available data indicates that this chemical is not carcinogenic.

f) Reproduction/developmental toxicity

Two studies on reproductive/developmental toxicity are available. The study by MHW (1999) was identified as a key study because it was well conducted according to OECD TG 422 (combined repeated dose and reproductive/ developmental toxicity screening test) under GLP. Arnold et al. (1980) conducted a two generation lifetime feeding study. Although the detailed analysis of reproductive parameters such as ovulation, implantation, delivery and lactation was not conducted, this study was considered as useful information for the assessment. Details of both studies are as follows.

o-Toluenesulfonamide was administered to SD rats by gavage at doses of 0, 20, 100 and 500 mg/kg b.w. from 14 days before mating to 14 days after mating in males and from 14 days before mating to day 3 of lactation in females [MHW, Japan: 1999].

Three females died and two females were sacrificed in moribund condition during the pre-mating period at 500 mg/kg b.w. Clinical signs such as decrease in locomotor activity and appearance of prone position and salivation, and significant lowering of body weights were observed in both sexes at 100 and 500 mg/kg b.w. In the 500 mg/kg b.w. group, the delivery index, number of pups alive on day 0 of lactation and birth index were slightly decreased but not statistically

significant. There was no effect of the test compound on the ovulation, copulation, fertility, delivery and lactation at dose levels up to 100 mg/kg b.w.
Significant reduction in body weights of pups was observed on days 0 and 4 in both sexes at 500 mg/kg b.w. The NOAEL was considered to be 100 mg/kg b.w./day for both reproductive and developmental toxicity.

In a two generation lifetime feeding study, SD rats were given o-toluenesulfonamide in the diet at 0 (control), 2.5, 25 or 250 mg/kg b.w./day [Arnold et al.: 1980]. At 90 days after onset of the administration, the rats were mated on a one-to-one basis in the same group and their pups after weaning were also given this chemical at the same dose as their parents.

The time-to-death for parental animals was not affected by treatment. The average body weight for parental animals were significantly lower in the 250 mg/kg group than that in the control group, but there is no information on the period showing this result. There was a statistically significant decrease in the litter size at postpartum day 1 and 4 in the 250 mg/kg b.w. group. When the average pup body weight at day 4 after birth was adjusted for litter size, there was a significantly lower average body weight at 250 mg/kg b.w./day. The NOAEL for reproductive/developmental toxicity was considered to be 25 mg/kg b.w./day.

There is no information available on humans.

Conclusions:

Lower litter size and pup body weight were found in an OECD combined study and a two generation lifetime study. Based on an overall evaluation of both results, the appropriate NOAEL for reproductive/developmental toxicity is considered to be 100 mg/kg b.w./day.

g) Other human health related information

Irritation and sensitisation

- Moderate irritating to eyes in rabbits at 100 mg/24 hr was reported [Marhold et al.: 1986]. But the reliability of this study is uncertain.
- There is no data available on skin irritation and sensitization.

Conclusions:
It is reported that this chemical was moderately irritating to eyes in rabbits but the reliability of the study is uncertain. There is no information available on skin irritation and sensitization.

Mutation study in insects

Three sex-linked recessive lethal assays were reported as shown in Table 7. One sex-linked recessive lethal mutation assay [Eckhardt et al.: 1980] was identified as a key study. In this study, this chemical was fed to male *Drosophila melanogaster* (sample size: about 4000 X-chromosomes) at 2.5 mM for 3 days and the treated males were mated individually with three virgin females. The frequency of sex-linked recessive lethal mutations was significantly increased only in 1 of 3 broods (0.60 % compared with 0.27 % in the control), showing effects on mature Drosophila sperm.

Table 7: Summary of insect mutation test

Type of test	Test system	Doses	Result	Reference
Sex-linked recessive lethal assay	*Drosophila melanogaster* (fruit fly)	2.5 mM in 5 % sucrose solution (oral feed)	**Weakly positive**	Eckhardt et al.: 1980
Sex-linked recessive lethal assay	*Drosophila melanogaster* (fruit fly)	0.2 ul (5mM in 0.7 % saline; abdominal injection)	**Negative**	Kramers: 1977
Sex-linked recessive lethal assay	*Drosophila melanogaster* (fruit fly)	5 mM in 5 % sucrose solution (oral feed)	**Negative**	Kramers: 1977

Conclusions:

Sex-linked recessive lethal assay in *Drosophila melanogaster* indicated weakly positive result.

h) Information on related chemicals

Structurally related chemicals

p-Toluenesulfonamide (CAS No.: 70-55-3)

Oral LD_{50} value in rats is more than 2,000 mg/kg b.w. for both sexes [MHW, Japan: 1994]. For repeated oral dose toxicity in rats at least for 38 days, salivation and urinary bladder changes were observed even at the lowest dose of 120 mg/kg b.w. [MHW, Japan: 1994]. This chemical was not mutagenic in bacteria with and without an exogenous metabolic activation system at up to 5000 ug/plate [MHW, Japan: 1994]. No chromosomal aberrations or polyploidy were induced in CHL cells up to 1.7 mg/ml with metabolic activation and 1.3 mg/ml without metabolic activation [MHW, Japan: 1994]. There are no available data on carcinogenicity. For reproductive/developmental toxicity, the abnormality of delivery state were observed accompanied with decrease in live birth index and pup weight at birth, and the NOAEL was 300 mg/kg b.w./day [MHW, Japan: 1994].

m-Toluenesulfonamide

There is no available information on m-toluenesulfonamide.

Metabolite especially in humans

Saccharin (81-07-2)

Oral LD_{50} value is 17,000 mg/kg in mouse [Expeam Experientia, Switzerland: 1979]. Hasegawa & Cohen (1986) showed that administration of saccharin at 2500 mg/kg b.w./day for 10 weeks does not produce effects on urinary bladder in rats. Saccharin was not mutagenic to bacteria [Ishidate et al.: 1984] but induced aneuploidy in yeast [Parry et al.: 1981]. Saccharin was not genotoxic in human or rodent cells *in vitro* [Ashby & Ishidate: 1986, Saxholm et al.: 1979, Traul et al.: 1981, Brogger et al.: 1979]. It also weakly induced DNA single-strand breaks in rat hepatocyte cultures [Sina et al.: 1983]. Saccharin did not produce tumours in male and female mice in multigenerational feeding study at 0, 0.2 or 0.5 % [Kroes et al., 1977]. Saccharin, generally as the sodium salt, has been tested for developmental and reproductive toxicity in mice, rats, hamsters and rabbits [Arnold et al.: 1983]. The effects have generally been limited to reductions in body weights at high dietary concentrations (5 - 7 %).

The International Agency for Research on Cancer (IARC) had evaluated in 1999 that saccharin and its salts are not classifiable as to their carcinogenicity to humans (Group 3) [IARC: 1999].
In making its evaluation, the Working Group concluded that sodium saccharin produces urothelial bladder tumours in rats by a non-DNA-reactive mechanism that involves the formation of a urinary calcium phosphate-containing precipitate, cytotoxicity and enhanced cell proliferation. This mechanism is not relevant to humans because of critical interspecies differences in urine composition.

4.2 Initial Assessment for Human Health

o-Toluenesulfonamide orally administered was rapidly eliminated mostly to urine in rats. In human subjects it was excreted to urine more slowly than that in rats. The main metabolites were 2-sulfamoyl-benzyl alcohol and its sulphate and glucuronic acid conjugates in both rats and humans. Saccharin was also detected as a metabolite in urine especially in humans.

The oral LD_{50} value for rats was greater than 2,000 mg/kg b.w. in males and between 1,000 and 2,000 mg/kg b.w. in females [OECD TG 401]. Sedation, passivity and catalepsy appeared even at the lowest dose of 700 mg/kg b.w. It is reported that this chemical was moderately irritating to eyes in rabbits but the reliability of the study is uncertain. There is no available information on skin irritation and sensitization.

In accordance with an OECD combined repeated dose and reproductive/developmental toxicity screening test [TG 422], o-toluenesulfonamide was given to male and female SD rats by gavage at 0, 20, 100, 500 mg/kg b.w./day for at least 38 days. Three females died and two females were sacrificed in moribund condition during the pre-mating period at 500 mg/kg b.w. Decreased locomotor activity and appearance of prone position and salivation were observed in both sexes at 100 and 500 mg/kg b.w. In the same groups, low body weights were recorded in both sexes. In histopathological examinations, hypertrophy of the centrilobular hepatocytes with the cytoplasm having a ground glass appearance was observed in both sexes at 100 and 500 mg/kg b.w. in a dose-dependent manner. In addition, the incidence of fibrosis and cellular infiltration of the pericardium, and fibrosis and cellular infiltration of the capsule and atrophy of the thymus were significantly increased in females at 500 mg/kg b.w.. In the kidneys, eosinophilic body was observed in males of all treated groups, maybe due to the complex accumulation of this chemical with the male rat specific protein, alpha-2u-globulin. Based on clinical signs and hepatic change, the NOAEL for repeated dose toxicity is considered to be 20 mg/kg b.w./day for both sexes.

Regarding genotoxicity, a bacterial test [OECD TG 471] and a chromosomal aberration test [OECD TG 473] *in vitro* were negative with and without metabolic activation. One mammalian spot test in mice demonstrated inconclusive results and two micronucleus tests *in vivo* in mice (gavage and i.p.) showed negative results. However the experimental condition of all these studies are not sufficiently reported. Therefore, the genotoxic potential of this chemical *in vivo* is inconclusive.

In a two generation lifetime feeding study, male and female SD rats were given o-toluenesulfonamide in the diets at 0, 2.5, 25 and 250 mg/kg b.w./day. No increase in any tumour incidence was noted in all dose groups of both generations. Two 2-year oral rat studies also demonstrated no carcinogenicity of this chemical. Only one lifetime feeding study showed low incidence of urinary bladder tumors of rats but the reliability of this study is uncertain because of poor reporting. A cell transformation assay using mammalian cultured cells showed negative results. Based on a weight of evidence approach, the available data indicates that this chemical is not carcinogenic.

In an OECD combined repeated dose and reproductive/developmental toxicity screening test [TG 422], significant reduction in body weights of pups was observed on days 0 and 4 in both sexes of rats at 500 mg/kg b.w. In a two generation lifetime feeding study, decrease of litter size and pup body weight was observed at 250 mg/kg b.w./day. Based on an overall evaluation of both results, the appropriate NOAEL for reproductive/developmental toxicity is considered to be 100 mg/kg b.w./day.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Physical/chemical property, production, use and distribution

o-Toluenesulfonamide was mainly produced as a chemical intermediate for the production of Saccharin in the past, but now Saccharin is normally manufactured without using this chemical although minor amounts are still used for this purpose. A mixture of *o*-Toluenesulfonamide with the *p*-isomer is used as a plasticizer for hot-melt adhesives, a chemical intermediate for fluorescent pigments and a chemical intermediate for plasticizer resins. The production volume of this chemical in Japan was about 50 tonnes in 2002, and the imported volume into Japan was about 30 tonnes in 2000.

If released to the atmosphere, this chemical mainly exists in the particulate phase according to its low vapor pressure. Vapor phase *o*-Toluenesulfonamide may be physically removed from the air by dry and wet deposition. A generic level III fugacity model shows that if *o*-Toluenesulfonamide is released to the air compartment, this chemical has a tendency to move to the water and soil compartments. If released to the water compartment, this chemical stays in this compartment, and if released to the soil compartment, about 40 % of *o*-Toluenesulfonamide moves to the water compartment.

Environment

o-Toluenesulfonamide is soluble in water (1.6 g/L at 25°C) and has a low vapor pressure (6.6×10^{-5} Pa at 25°C). Its partition co-efficient (P_{ow}) is 0.84. *o*-Toluenesulfonamide is not readily biodegradable (OECD TG301C: 0 % by BOD after 14 days), but its experimental BCF values (OECD TG 305: < 2.6) suggests that this chemical does not bioaccumulate in aquatic organisms. Hydrolysis is not expected to occur (OECD TG 117: stable at pH 4, 7 and 9 at 50 °C for five days).

Acute toxicty of *o*-Toluenesulfonamide has been tested in three aquatic species of three trophic levels. For algae (*Selenastrum capricornutum*) a EC50 of 170 mg/L (OECD TG 201, growth rate 24-48hr and also 24-72h) and a 72h EbC50 of 57 mg/L (OECD TG201, biomass) were determined For daphnids (*Daphnia magna*) a 48 h EC50 of 210 mg/L (OECD TG 202 part 1), and for fish (*Oryzias latipes*) a 96 h LC50 of >100 mg/L (OECD TG 203) were reported.

Two chronic toxicity values, for algae (*Selenastrum capricornutum*) and daphnids (*Daphnia magna*) are available. For algae a 72 h NOEC on growth inhibition of 7.7 mg/L (OECD TG 201, based on growth rate) and for daphnids, a 21 d NOEC of 49 mg/L(OECD TG 202, reproduction) were reported.

Human health

The oral LD_{50} value was greater than 1,000 mg/kg b.w. in rats. This chemical seems moderately irritating to eyes in rabbits. There is no information available on skin irritation and sensitization. The NOAEL for repeated dose toxicity was considered to be 20 mg/kg b.w./day, based on clinical signs and hepatic change. Genotoxicity tests in bacteria and mammalian cells *in vitro* were negative. However *in vivo* tests demonstrated inconclusive results. Based on a weight of evidence approach, the available data indicates that this chemical is not carcinogenic. As for reproductive/developmental toxicity, the appropriate NOAEL for reproductive/developmental toxicity is considered to be 100 mg/kg b.w./day, based on an overall evaluation of two studies.

5.2 Recommendations

This chemical is currently of low priority for further work.

6. References

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SIDS Dossier

o-Toluenesulfonamide

CAS No. 88-19-7

Existing Chemical	:	ID: 88-19-7
CAS No.	:	88-19-7
Producer Related Part		
Sponcor country	:	Japan
Creation date	:	18.12.2000
	:	16.01.2002
Printing date		
Revision date	:	
Date of last Update	:	16.01.2002
Number of Pages	:	6939
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD and Company Information

1.0.2 Location of Production Site

1.0.3 Identity of Recipients

1.1 General Substance Information

Substance type : organic
Physical status : solid
Purity : % w/w
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
11.01.2002

1.1.0 Details on Template

1.1.1 Spectra

1.2 Synonyms

2-Methylbenzensulfonamide
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
11.01.2002
o-Methylbenzensulfonamide
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
11.01.2002
Toluene-2-sulfonamide
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
11.01.2002

1.3 Impurities

1.4 Additives

1.5 Quantity

1.6.1 Labelling

1.6.2 Classification

1.7 Use Pattern

1.7.1 Technology Production/Use

1.8 Occupational Exposure Limit Values

1.9 Source of Exposure

1.10.1 Recommendations/Precautionary Measures

1.10.2 Emergency Measures

1.11 Packaging

1.12 Possib. of Rendering Subst. Harmless

1.13 Statements Concerning Waste

1.14.1 Water Pollution

1.14.2 Major Accident Hazards

1.14.3 Air Pollution

1.15 Additional Remarks

1.16 Last Literature Search

1.17 Reviews

1.18 Listings e.g. Chemical Inventories

2.1 Melting Point

Value	:	= 157.2 °C
Sublimation	:	
Method	:	other: Unknown
Year	:	
GLP	:	no data
Test substance	:	no data
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	Melting point is 99.9 %.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
11.01.2002		

2.2 Boiling Point

Value	:	> 270 °C at
Decomposition	:	yes
Method	:	OECD Guideline 103 "Boiling Point/boiling Range"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Tokyo Kasei Kogyo Co., Ltd. Purity: 99.9 %
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	This chemical is decomposed at 270 C. Boiling point of this chemical is more than 270 C.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
11.01.2002		

2.3 Density

Type	:	density
Value	:	= 1.461 g/cm3 at 25° C
Method	:	other: JIS K7112
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Tokyo Kasei Kogyo Co., Ltd. Purity: 99.9 %
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	Densitiy is 1.461 g/cm3.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
13.01.2002		

2.3.1 Granulometry

2.4 Vapour Pressure

Value	:	= .00000066 hPa at 25° C
Decomposition	:	no
Method		OECD Guideline 104 "Vapour Pressure Curve"
Year	:	1999
GLP	:	yes

Test substance	:	other TS: Tokyo Kasei Kogyo Co., Ltd. Purity: 99.9 %
Decomposition	:	no
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion		Vapor pressure is 0.000066 Pa at 25 C.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
11.01.2002		

2.5 Partition Coefficient

Log pow	:	= .84 at ° C
Method		other (measured)
Year	:	
GLP	:	no data
Test substance	:	
Test Condition	:	determination at pH 2
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
11.01.2002		(19)

2.6.1 Water Solubility

Value	:	= 1.6 g/l at 25 ° C
Qualitative	:	soluble (1000-10000 mg/L)
Pka	:	10.18 at 25 ° C
PH	:	at and ° C
Method	:	OECD Guideline 105 "Water Solubility"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Tokyo Kasei Kogyo Co., Ltd. Purity: 99.9 %
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	Water solubility is 1.6 g/L at 25 C.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
11.01.2002		

2.6.2 Surface Tension

2.7 Flash Point

2.8 Auto Flammability

2.9 FLAmmability

2.10 Explosive Properties

2.11 Oxidizing Properties

2.12 Additional Remarks

3.1.1 Photodegradation

Type	:	air
Light source	:	
Light spect.	:	nm
Rel. intensity	:	based on Intensity of Sunlight
Indirect photolysis		
Sensitizer	:	OH
Conc. of sens.	:	500000 molecule/cm3
Rate constant	:	= .000000000001224 cm3/(molecule*sec)
Degradation	:	= 50 % after 315 hour(s)
Deg. Product	:	
Method	:	other (calculated): Calculated by AOP Win (Syracuse Research Corporation)
Year	:	2002
GLP	:	no data
Test substance	:	
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	The half life time of this substance by the reaction with OH radicals in air is 315 hours.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
11.01.2002		

3.1.2 Stability in water

Type	:	abiotic
t1/2 pH4	:	at degree C
t1/2 pH7	:	at degree C
t1/2 pH9	:	at degree C
Deg. Product	:	no
Method	:	OECD Guideline 111 "Hydrolysis as a Function of pH"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Tokyo Kasei Kogyo Co., Ltd. Purity: 99.9 %
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion	:	This chemical is stable at pH 4, 7 and 9 at 50 C for five days.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
11.01.2002		

3.1.3 Stability in soil

3.2 Monitoring data

3.3.1 Transport between environmental compartments

Type	:	fugacity model level III
Media	:	
Air (level I)	:	
Water (level I)	:	
Soil (level I)	:	
Biota (level II / III)	:	
Soil (level II / III)	:	

Method :
Year : 2002
Method : Paramters used in this calculation are as follows;
Molecular weight: 171.22
Melting pint: 157.2 C
Vapor pressure: 6.6E-5 Pa
Water solubility: 1.6 g/L
Log Pow: 0.84
Half-life time in air: 315 hours
Half-life time in water: 240,000 hours
Half-life time in soil: 240,000 hours
Half-life time in sediment: 720,000 hours
Result : Estimated Distribution under three emission scenarios

Compartment	Release 100% to Air	Release 100% to Water	Release 100% to Soil
Air	0.0 %	0.0 %	0.0 %
Water	41.6 %	99.6 %	36.3 %
Soil	58.2 %	0.0 %	63.5 %
Sediment	0.2 %	0.4 %	0.2 %

Source : Chemicals Evaluation and Research Institute (CERI) Tokyo
Conclusion : If this substance is released to the air compartment, this chemmical has a tendency to move to the water and soil compartments. If released to the water comparment, this chemical stays in this compartment, and if released to the soil compartment, about 40 % of this chemical moves to the water compartment.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
13.01.2002

3.3.2 Distribution

3.4 Mode of degradation in actual use

3.5 Biodegradation

Type : aerobic
Inoculum : activated sludge
Concentration : 300mg/l related to Test substance
related to
Contact time : 14 day
Degradation : = 0 % after 14 day
Result : under test conditions no biodegradation observed
Deg. Product : no
Method : OECD Guideline 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year : 1975
GLP : yes
Test substance : other TS: Purity: 99.7 %
Result : Biodegradability of this substance
0 % by BOD after 14 days
0 % by TOC after 14 days
Source : Chemicals Evaluation and Research Institute (CERI) Tokyo

Conclusion	:	This substance is not readily biodegradable.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
11.01.2002		

3.6 Bod5, Cod or Bod5/Cod ratio

3.7 Bioaccumulation

Species	:	*Cyprinus carpio* (Fish, fresh water)
Exposure period	:	42 day at 25 degree C
Concentration	:	
Elimination	:	no data
Method	:	OECD Guideline 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year	:	1975
GLP	:	yes
Test substance	:	other TS: Purity: 99.7
Result	:	Bioconcentration factors (BCF) are as follows BCF = 0.4 - 0.9 when the test concentration is 3 mg/L BCF = less than 2.6 when the test concentration is 0.3 mg/L
Source	:	Chemicals Evaluation and Research Institute (CERI) Tokyo
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
13.01.2002		

3.8 Additional remarks

4.1 Acute/prolonged toxicity to fish

Type	:	semistatic
Species	:	*Oryzias latipes* (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	yes
LC50	:	> 100
Method	:	OECD Guideline 203 "Fish, Acute Toxicity Test"
Year	:	2000
GLP	:	yes
Test substance	:	other TS: Kanto Kagaku., Purity; => 90%, Lot No.904S4056
Method	:	-Test Organisms: a) Size (length and weight): BL: 2.0(1.8-2.1) cm, n=10 BW: 0.12(0.082-0.14) grams, n=10 b) Age: Not described c) Supplier/Source: Izumimoto Yosyokujyo (fish farm) d) Any pretreatment: None, any treatment was done during acclimization and the mortality was not observed in a week just before the testing. -Test Conditions: a) Dilution Water Source: Dechlorinated tap water b) Dilution Water Chemistry: 55.6mg/L(as CaCO3), pH:7.7 Alkalinity: 37.5mg/L, COD: 0.2 mg/L. c) Exposure Vessel Type: 5 L test solution in a 5 L Glass Beaker d) Nominal Concentrations: Control and 100mg/L; e) Vehicle/Solvent and Concentrations: Not used f) Stock Solutions Preparations and Stability: Not described g) Number of Replicates: 1 h) Fish per Replicates: 10 i) Renewal Rate of Test Water: Every 48 hours j) Water Temperature: 23.6 - 23.9ºC k) Light Condition: 16:8 h ours, light-darkness cycle (room light) l) Feeding: No - Analytical Procedure: The test substance was measured by using HPLC -Statistical Method: a) Data Analysis: No data analysis for this was a limit test. b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): geometiroc mean was used
Result	:	- Measured Concentrations: The tested concentrations were measured at 0 hour and 48 hours later (before exchange of test solution) . The tested concentration at 48 hours later was showed as 100 % of nominal concentration.

Nominal concentration mg/L	Measured concentration, mg/L		Mean measured concentration mg/L
	0 Hour (new)	48 Hours (old)	
Control	<1	<1	-
100	97	110	100

- Water chemistry in test (pH and DO): pH 7.5 - 8.0, DO 5.1 - 8.1 mg/L

-Effect Data(mortality): 96hr LC50 > 100 mg/L

- Cumulative Mortality: No death occurred to the tested fish during the test

period.

Nominal Concentration mg/L	Mean Measured Concentration mg/L	Cumulative Mortality (Percent Mortality) 24hr	48hr	72hr	96hr
Control	-	0 (0)	0 (0)	0 (0)	0 (0)
100	100	0 (0)	0 (0)	0 (0)	0 (0)

-Other Effect:No toxicological symptom was observed during the test period.

Nominal Concentration mg/L	Mean Measured Concentration mg/L	Symptoms (Symptom -number of fish) 24hr	48hr	72hr	96hr
Control	-	Normal	Normal	Normal	Normal
100	100	Normal	Normal	Normal	Normal

- Calculation of toxic values: Based on the nominal concentrations, because the tested concentration at 48 hours later was showed as 100 % of nominal concentration.

Source : EA, Japan (2000),Environmental Agency Japan.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
16.01.2002 (3)

4.2 Acute toxicity to aquatic invertebrates

Type : static
Species : *Daphnia magna* (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Analytical monitoring : yes
NOEC :
EC0 : = 95
EC50 = 210
Method : OECD Guideline 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year : 2000
GLP : yes
Test substance : other TS: Kanto Kagaku., Purity; => 90%, Lot No.904S4056
Method : -Test Organisms:
a) Age: < 24 h old
b) Supplier/Source: National Institute for Environmental Studies (JAPAN)
- Test Conditions:
a) Dilution Water Source: Dechlorinated tap water
b) Dilution Water Chemistry (pH, EC, Total hardness ,Alkalinity, etc.): pH 7. 7, EC 171 micro S/cm, Hardness 55.6 mg/L(as CaCO3), Alkalinity 37.5 mg/L
c) Exposure Vessel Type: 100 mL test solution in a 100 mLGlass Beaker
d) Nominal Concentrations: control, 32, 56, 100, 180 and 320 mg/L
e) Vehicle/Solvent and Concentrations: Not used
f) Stock Solutions Preparations and Stability: Not described
g) Number of Replicates: 4
h) Individuals per Replicates: 5
i) Renewal Rate of Test Water: None

j) Water Temperature: 19.6 ºC
k) Light Condition: 16:8 hours, light-darkness cycle (room light)
l) Feeding: No

- Analytical Procedure: The test substance was measured by using HPLC

- Statistical Method:
a) Data Analysis: Doudoroff Method for EC50
b) Method of Calculating Mean Measured Concentrations (i.e.arithmetic mean, geometric mean, etc.): Geometric Mean

Result : - Measured Concentrations : The tested concentrations were measured at 0 hour and 48 hours later. The tested concentration at 48 hours later was showed as 92 - 130 % of nominal concentration.

Nominal concentration mg/L	Measured concentration, mg/L 0 Hour (new)	48 Hours (old)	Mean* measured concentration mg/L
Control	<1	<1	-
32	45	39	42
56	57	63	60
100	95	96	95
180	160	170	160
320	340	350	340

*Geometric Mean

- Water chemistry in test (pH and DO): pH 8.1 - 8.2, DO 8.3 - 8.7 mg/L

-Effect Data(immobilization):48hr EC50 = 210 mg/L 48hr EC0 = 95 mg/L (the highest concentration of no effects were observed)

- Cumulative number of Immobilized Daphnia:

Measured concentration mg/L	Number of Immobilized Daphnia (percent immobility) 24 hour	48 hour
Control	0(0)	0(0)
42	0(0)	0(0)
60	0(0)	0(0)
95	0(0)	0(0)
160	1(5)	4(20)
340	20(100)	20(100)

- Calculation of toxic values: Based on the measured concentrations, because the tested concentration at 48 hours was showed as 92 - 130 % of nominal concentration.

Source : EA, Japan (2000),Environmental Agency Japan.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
16.01.2002 (3)

4.3 Toxicity to aquatic plants e.g. algae

Species : *Selenastrum capricornutum* (Algae)

Endpoint : Biomass
Exposure period : 72 hour(s)
Unit : mg/l
Analytical monitoring : Yes
NOEC : = 7.7
EC50 : = 57
Method : OECD Guideline 201 "Algae, Growth Inhibition Test"
Year : 2002
GLP : yes
Test substance : other TS: Kanto Kagaku., Purity; => 90%, Lot No.904S4056
Method : - Test Organisms:
a) Method of Cultivation: OECD medium to culture
b) Stain Number: ATCC22662
c) Supplier/Source: American Type Culture Collection

- Test Conditions:
a) Medium: OECD medium
b) Exposure Vessel Type: 100 mL Medium in a 500 mL Erlenmeyer Flask with silicon cap
c) Nominal Concentrations: control, 3.2, 10, 32, 100 and 320 mg/L.
d) Vehicle/Solvent and Concentrations: Not used.
e) Stock Solutions Preparations and Stability: Not described
f) Number of Replicates: 3
g) Initial Cell Number: 10,000 cells/mL
h) Water Temperature: 23.4 - 23.6 ºC
i) Light Condition: 4,000 - 4,900lux, continues
j)Shaking: 100 rpm

- Analytical Procedure: The test substance was measured by using HPLC
- Statistical Method: a) Data Analysis: Probit Method for EC50, Dunnett Method for NOEC
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Arithmetic mean was used for estimating for ECs and NOECs

Remark : NOEC was determined based on growth inhibition.
Result : - Measured Concentrations : The tested concentrations were measured at 0 hour and 72 hours later. The tested concentration at 72 hours later was showed as 56 - 80 % of nominal concentration.

Nominal concentration mg/L	Measured concentration, mg/L		Percent of nominal	
	0 Hour	72 Hours	0 Hour	72 Hours
Control	<1	<1	-	-
3.2	2.4	1.8	75	56
10	7.4	7.9	74	79
32	27	23	84	72
100	78	80	78	80
320	260	240	82	75

-Water chemistry in test (pH and DO): pH 7.3 - 7.6
-Effect: based on biomass(area method)
EbC50(0-72hr) = 57 mg/L (95% C. I.: 50 - 56)
NOEC = 7.7 mg/L rate method
-Effect data : based on growth rate
ErC50(24-48hr) = 170 mg/L (95% C. I.: 150 - 210)
NOEC = 79 mg/L
ErC50(24-72hr) = 170 mg/L (95% C. I.: 150 - 200)
NOEC = 7.7 mg/L

- Mean Cell Concentration of each Flask

Mean Measured Concentration mg/L	Cell Density (x 10,000 cells/mL) 0 hr (SD)	24 hr (SD)	48 hr (SD)	72 hr (SD)
Control	1.00(0)	8.16(0.26)	59.0(1.6)	285(14)
2.2	1.00(0)	7.54(0.47)	54.2(3.8)	287(20)
7.7	1.00(0)	8.09(0.30)	54.1(6.9)	257(18)
25	1.00(0)	6.94(0.35)	43.2(1.9)	197(11)
79	1.00(0)	6.20(0.26)	39.3(1.8)	157(11)
250	1.00(0)	2.30(0.08)	3.63(0.2	5.34(0.60)

SD: Standard deviation

- Growth Curves: Log phase during the test period

Source : EA, Japan (2000),Environmental Agency Japan.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
16.01.2002 (3)

4.4 Toxicity to microorganisms e.g. bacteria

4.5.1 Chronic toxicity to fish

4.5.2 Chronic toxicity to aquatic invertebrates

Species : *Daphnia magna* (Crustacea)
Endpoint : reproduction rate
Exposure period : 21 day
Unit : mg/l
Analytical monitoring : yes
NOEC : = 49
LCEC : = 100
EC50 : = 79
LC50 : > 100
Method : other: OECD TG 211 (revised edition of No.202)
Year : 2000
GLP : yes
Test substance : other TS: Kanto Kagaku., Purity; => 90%, Lot No.904S4056
Method : - Test Organisms:
a) Age: Not described
b) Supplier/Source: National Institute for Environmental Studies (JAPAN)

- Test Conditions:
a) Dilution Water Source: Dechlorinated tap water was used
b) Dilution Water Chemistry (pH, EC, Total hardness ,Alkalinity, etc.): pH 7. 7 EC 171 micro S/cm Hardness 55.6 mg/L(as CaCO3) Alkalinity 37.5 mg/L, Sodium 14.9 mg/L, Potassium 3.76 mg/L. COD 0.2 mg/L.
c) Exposure Vessel Type: 80 mL test solution in a 100 mL Glass Beaker
d) Nominal Concentrations: control, 10, 22, 46 and 1.00 mg/L.
e) Vehicle/Solvent and Concentrations: Not used
f) Stock Solutions Preparations and Stability: Not described
g) Number of Replicates: 10
h) Individuals per Replicates: 1

i) Renewal Rate of Test Water: 3 times per a week
j) Water Temperature: 19.6 - 20.1 ºC
k) Light Condition: 16:8 hours, light-darkness (room light)
l) Feeding: Not described

- Statistical Method:
a) Data Analysis: for LC50; Not described, Dunnett test for NOEC
b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Time-weighted mean

Remark : NOEC was determined based on the cumulative number of juveniles produced per adult alive for 21 days.

Result : - Effect: reproduction
- Measured Concentrations (as mg/L):

Nominal Concentration (mg/L)	Measured Concentration (mg/L) (percent of nominal)					
	2 day new	5 day old	9 day new	12 day old	16 day new	19 day old
Control	<0.07	<0.07	<0.07	<0.07	<0.07	<0.07
10	11(110)	9.8(98)	8.0(80)	12(120)	11(110)	9.5(95)
22	26(120)	22(100)	19(86)	23(100)	26(120)	21(95)
46	61(130)	48(100)	40(87)	52(110)	50(110)	43(93)
100	120(120)	100(100)	83(83)	100(100)	110(120)	95(95)

Nominal Concentration (mg/L)	Time-weighted mean during 21 days (mg/L) (percent of nominal)
Control	----
10	10(100)
22	23(100)
46	49(110)
100	100(100)

- Water chemistry in test (pH and DO): pH 7.9 - 8.2, DO 6.6 - 8.5 mg/L, Total hardness (as CaCO3): 55 - 80 mg/L

-Effect Data(reproduction):21days LC50 >100 mg/L21days EC50 = 79 mg/L21days NOEC = 49 mg/L21days LOEC = 100 mg/L - Cumulative Number of Died Parental Daphnids:

Measured Concentration (mg/L)	Cumulative Number of Died Parental Daphnids (days)										
	0	1	2	3	4	5	6	7	8	9	10
Control	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0

Measured Concentration (mg/L)	Cumulative Number of Died Parental Daphnids (days) 11	12	13	14	15	16	17	18	19	20	21
Control	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0

- Time (days) of the First Brood Production: Mean;Control (7 days), 10 mg/L (7 days), 23 mg/L (7 days), 49 mg/L (7days) and 100 mg/L (7 days)

- Mean Cumulative Number of Offsprings Produced per Adult: 21days; Control (112.9), 10 mg/L (140.6), 23 mg/L (140.8), 49 mg/L (122.5) and 100 mg/L (27.9)

- Calculation of toxic values: Based on the measured concentrations, because some test concenrations are showed as 120 % of nominal concentration.

Source : EA, Japan (2000),Environmental Agency Japan.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
16.01.2002 (3)

4.6.1 Toxicity to soil dwelling organisms

4.6.2 Toxicity to terrestrial plants

4.63 Toxicity to other Non-Mamm. terrestrial species

4.7 Biological effects monitoring

4.8 Biotransformation and kinetics

4.9 Additional remarks

5.1.1 Acute oral toxicity

Type	:	LD50
Species	:	rat
Strain	:	Crj: CD(SD)
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other: 0.5 w/v% CMC sodium solution
Method	:	OECD Guideline 401 "Acute Oral Toxicity"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Source: Tokyo Kasei Kogyo Co., Ltd., Lot No. GC01, Purity: 99 %, kept cool and dark until use
Result	:	LD50; Male > 2,000 mg/kg bw, female 1,000 ~ 2,000 mg/kg bw No. of deaths at each dose level; Dose Male Female 700 0/5 0/5 1000 0/5 0/5 1400 0/5 3/5 2000 0/5 2/5 Clinical signs; Sedation and prostration were observed from just after administration in all animals of treated groups. Passivity also appeared with time even at the lowest dose. In male, lateral position, abnormal breathing, staggering gait, listless and hypothermia were observed at 1,000 mg/kg bw and more. At 2,000 mg/kg bw, some males showed catalepsy. In female, catalepsy was observed even at 700 mg/kg bw and lateral position, abnormal breathing and hypothermia at the higher groups. Body weight; The tendency of the depression of body weight gain was shown on the second day in the groups of males given 1000 mg/kg or more and females given all doses. At autopsy; Reddish discoloration of the lung, pale colored areas of the gastric mucosa and cysts in the renal cortex were observed in dead animals. No abnormal findings were noticed in scheduled sacrificed animals.
Test condition	:	- Age at study initiation: 5 weeks old for males and females - Doses/concentration levels: 700, 1000, 1400, 2000 mg/kg - Post dose observation period: 14 days - No. of animals per sex per dose: 5 - Route of administration: Oral (gavage)
Conclusion	:	The LD50 values were more than 1000 mg/kg for females and more than 2000 mg/kg for males.
Reliability	:	(1) valid without restriction Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan).
Flag	:	Critical study for SIDS endpoint
25.12.2001		(12)

Type	:	LD50
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Value	:	ca. 2000 mg/kg bw
Method	:	other: no data
Year	:	
GLP	:	no data
Test substance	:	no data

Source	:	IARC (1987)
20.11.2001		(16)

Type	:	LD50
Species	:	rat
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Value	:	= 4870 mg/kg bw
Method	:	
Year	:	
GLP	:	no data
Test substance	:	no data
Source	:	RTECS Number: XT4900000
17.09.2001		(10)

5.1.2 Acute inhalation toxicity

5.1.3 Acute dermal toxicity

5.1.4 Acute toxicity, other routes

5.2.1 skin irritation

5.2.2 Eye irritation

Species	:	rabbit
Concentration	:	
Dose	:	100 mg
Exposure Time	:	24 hour(s)
Comment	:	
Number of animals	:	
Result	:	moderately irritating
EC classification	:	
Method	:	other: no data
Year	:	1986
GLP	:	no data
Test substance	:	no data
Source	:	RTECS
03.10.2001		(10)

5.3 Sensitization

5.4 Repeated dose toxicity

Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	Males; for 42 days Females; from 14 days before mating to day 3 of lactation
Frequency of treatment	:	Once daily
Post obs. period	:	1 day

Doses	:	0(vehicle), 20, 100, 500 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 20 mg/kg bw
Method	:	OECD combined study TG422
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Source: Tokyo Kasei Kogyo Co., Ltd., Lot No. GC01, Purity: 99 %, kept at room temperature until use
Result	:	NOAEL 20 mg/kg/day for both sexes

1) Mortality, moribund sacrifice and clinical signs

Males. There were no death or moribund sacrifice in any dose group. As changes in clinical signs, animals dosed 100mg/kg or more showed decrease in locomotor activity and prone position after initiation of administration, and salivation was observed from the mid period of administration. All these signs, however, disappeared by the next morning. Moreover, as changes unrelated to the test compound, loss of fur was observed in the control and 500 mg/kg groups. No abnormality was seen in the 20 mg/kg group.

Females. Three animals died on Day 3 of administration and two each animals were sacrificed in moribund condition on Days 3 and 5 of administration at 500 mg/kg. All these animals had shown decrease in locomotor activity and prone position at the first administration, but the signs disappeared by the next day. As the administration was repeated, however, the recovery of signs became worse, and animals found dead or sacrificed in moribund condition after showing sedation, reddish tear, dyspnea, lacrimation and chromaturia. Among survivors animals, signs such as decrease in locomotor activity and prone position, which were seen in the animals found dead or sacrificed in moribund condition, were observed after administration at 100 mg/kg or more. In the 100 mg/kg group, prone position was observed sporadically and all the animals made a recovery by the next day. In the 500 mg/kg group, however, there appeared cases showing waddling gait or extension of extremities and signs such as sedation, subnormal body temperature, reddish tear and brown urine, which were seen in the animals died or sacrificed in moribund condition. At the beginning of administration, there were cases in which the signs appearing after administration of the day before persisted, such as decrease of locomotor activity and prone position. Salivation began to be seen transiently mainly after administration and appeared also in the 100 mg/kg group at the time when the serious signs as above were not observed in the 500 mg/kg group any more. Loss of fur, which was unrelated to the test compound, was observed in the 100 mg/kg group. No abnormal finding of clinical signs was noted at 20 mg/kg.

2) Body weight and food consumption

Males. Significant decrease, when compared with the control values, was observed for body weight of Days 8-22 of administration and for food consumption of Days 1-8 and Days 8-15 of administration in the 100 mg/kg group, and significant decrease was also observed for body weight of Days 8-42 of administration and for food consumption of Days 1-8 of administration in the 500 mg/kg group ($p<0.01$, $p<0.05$). In the 20 mg/kg group, there was no significant difference from the control value either for the body weight or for the food consumption.

Body weight of male rats

Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Days of adminisration				
1	333.1±7.4	333.7±8.2	337.1±8.3	333.7±7.8
8	370.5±13.3	366.1±11.7	354.2±13.8**	348.9±14.8**
15	405.2±19.0	402.3±16.6	383.6±18.3*	380.2±21.0**
22	430.5±22.1	429.4±19.3	408.8±22.0*	405.6±23.6*
29	456.0±29.7	451.9±21.4	435.1±27.7	426.9±25.2*
36	483.8±33.0	478.1±24.0	460.1±28.8	449.4±26.3**
42	503.4±35.9	497.1±25.8	479.6±32.4	467.4±26.9*

*: significant difference from control, p<0.05
**: significant difference from control, p<0.01

Females. At the beginning of administration before mating, significantly lower values, when compared with the control values, were recorded for the body weight on Day 8 of administration and for the food consumption on Days 1-8 of administration at 500 mg/kg (p<0.01, p<0.05). During the gestation and lactation periods, the body weight gain at 100 mg/kg or more was suppressed, with the body weight on Day 7of gestation and on Day 4 of lactation being significantlylower than the control values (p<0.01, p<0.05). In the 500 mg/kg group, the food consumption was also significantly decreased, when compared with the control group, on Days 0-7 of gestation (p<0.05). In the 20 mg/kg group, no significant difference was noted for any period.

Body weight of female rats

Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Days of adminisration				
1	216.3±6.2	216.4±5.5	216.3±5.8	216.1±6.3
8	232.9±7.9	232.2±7.7	226.5±9.1	215.4±17.1*
15	251.3±15.6	250.9±12.9	241.9±8.1	234.3±17.2
Days of pregnancy				
0	255.2±15.9	256.6±13.1	246.5±9.3	237.2±16.3
7	303.6±19.4	300.6±12.5	282.2±10.3*	278.4±2.35*
14	341.1±26.3	340.9±18.2	319.5±12.1	315.5±26.2
20	414.5±35.2	409.9±35.3	389.1±18.7	375.9±40.0
Days of lactation				
0	313.1±18.7	303.7±21.3	296.2±23.2	284.9±23.7
4	344.1±19.7	337.0±19.6	317.3±24.8*	299.8±17.0**

*: significant difference from control, p<0.05
**: significant difference from control, p<0.01

3) Urinalysis

In the 20 and 100 mg/kg groups, there were 2 and 1 cases of occult blood positive, respectively. These cases were judged to be unrelated to the administration of testcompound, however, since there were no positive cases both in the control and 500 mg/kg group. For other parameters, no animal in any dose group showed abnormality.

4) Examinations at necropsy

A. Males

(1) Hematology

When compared with the control group, significantly higher values were obtained for MCH in all the groups given o-toluenesulfonamide and for MCHC in the groups given 100 mg/kg or more (p<0.05, p<0.01). In addition, the platelet count at 500 mg/kg was significantly higher than that of the control group (p<0.05). The clotting time w as significantlyshortened at 100 mg/kg or more when compared to the control (p<0.05), because some control animals showed a relatively prolonged PT. For APTT, there was no significant difference when compared with the control group. There were no effects of

test compound for any of the remaining parameters and for the differential white blood cell count.

Hematological findings of male rats

Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
MCH(pg)	17.9±1.4	18.7±0.4*	18.9±0.4**	18.9±0.4**
MCHC(%)	32.9±0.5	33.3±0.6	33.9±0.7**	34.4±0.6**
Platelet count ($x 10^4/mm^3$)	96.5±7.8	97.0±7.9	99.6±5.9	104.7±6.8*
PT (sec)	29.9±8.8	31.1±7.2	22.7±5.2*	21.6±5.8*

*: significant difference from control, p<0.05
**: significant difference from control, p<0.01

(2) Blood chemistry

ALP values were significantly lower than the control values in all the treatment groups (p<0.01). Total cholesterol level was significantly increased at 100 mg/kg or more when compared to the control (p<0.05, p<0.01). In the 500 mg/kg group, total protein and Gamma-GTP activity showed, though being a minor difference, a significant increase when compared to the control (p<0.05) and glucose level, triglyceride level and A/G ratio showed significantly lower values (p<0.05, p<0.01). For the remaining parameters, there was no significant difference between each treatment group and the control group.

Blood chemical findings of male rats

Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Total protein (g/dL)	5.4±0.2	5.3±0.3	5.4±0.2	5.6±0.2*
A/G	1.29±0.14	1.19±0.13	1.26±0.13	1.15±0.10*
Glucose (mg/dL)	144±10	142±13	135±11	128±14**
Total cholesterol (mg/dL)	37±6	39±8	49±13*	66±16**
Triglyceride (mg/dL)	45±15	35±14	39±13	27±11**
ALP(U/L)	261±35	218±40**	216±34**	199±31**
Gamma-GTP (U/L)	0±0	0±0	0±0	0±1*

*: significant difference from control, p<0.05
**: significant difference from control, p<0.01

(3) Necropsy

Changes associated with the administration of the test compound were as follows: liver, darkness at 100 mg/kg or more and swelling at 500 mg/kg; kidney, swelling and darkness at 100 mg/kg or more.

(4) Organ weight

For the body weight at necropsy, significant lower values than in the control were obtained at 500 mg/kg (p<0.01). When viewed by organ, the liver and kidney weights at 500 mg/kg showed a significant increase when compared with the control (p<0.05, p<0.01). The liver and kidney weight ratio to the body weight was significantly increased at 500 mg/kg and 100 mg/kg or more, respectively. The adrenal weight ratio to the body weight was significantly increased at 100 mg/kg or more (p<0.05, p<0.01). At 100 mg/kg or more, although there were some significant differences in the organ/body weight ratio for the heart and testis (p<0.05, p<0.01), their absolute weight did not show any dose-dependent changes. At 20 mg/kg, no significant difference was noted for the organ weight or organ/body weight ratio.

Absolute and relative organ weights of male rats

Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Terminal body weight(g)	469.6±33.8	464.0±24.8	443.9±30.7	432.7±26.4**
Heart(g)	1.33±0.13a	1.35±0.13	1.35±0.09	1.31±0.12
	0.28±0.02b	0.29±0.02	0.31±0.02	0.30±0.02*
Liver(g)	12.27±1.49	12.30±1.15	12.40±1.25	14.37±1.18**
	2.61±0.20	2.65±0.14	2.79±0.16	3.32±0.17**
Kidneys(g)	3.15±0.24	3.24±0.18	3.27±0.24	3.54±0.23**
	0.67±0.05	0.70±0.03	0.74±0.07**	0.82±0.06**
Adrenal glands(mg)	54.3±9.1	58.5±11.1	59.4±8.7	62.2±4.8
	11.6±1.8	12.6±2.0	13.4±1.6*	14.4±1.1**
Testes(g)	3.03±0.75	3.40±0.20	3.36±0.26	3.35±0.21
	0.65±0.16	0.74±0.06	0.76±0.08*	0.78±0.05**

a: absolute weight
b: relative weight
*: significant difference from control, $p<0.05$
**: significant difference from control, $p<0.01$

(5) Histopathology

There were dose-related findings in the liver and kidney. There were no abnormal changes in the brain, thymus, heart, adrenal gland and femoral bone marrow.

(Liver)

At 100 mg/kg or more, hypertrophy of the centrilobular hepatocytes with the cytoplasm having an ground glass appearance was observed in a dose-dependent manner ($p<0.01$).

Hypertrophy, hepatocyte, centrilobular, ground glass appearance

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No.of Animals	[13]	[13]	[13]	[13]
Total	0	0	11**##	13**##
±	0	0	8	7
+	0	0	3	6

±: very slight, +: slight, ++: moderate, +++: severe,
**: significant difference from control, $p<0.01$ (Mann-Whitney U test), ##: significant difference from control, $p<0.01$ (Fisher exact test)

(Kidney)

Although eosinophilic bodies were observed in all the groups including the control, the incidence and severity were significantly higher in the treatment groups than in the control ($p<0.01$). Although the kidney having eosinophilic bodies was PAS -stained, no positive reaction was obtained.

B. Females

(1) Necropsy

All the animals found dead during the course of the study showed retention of pleural effusion and redness or dark-reddish area in the lung, which was accompanied by incomplete involution, edema and adhesion to the diaphragm with white materials attached. Moreover, these animals showed whitish cortico-medullar border in the kidney, opaque left eyeball, swelling of the liver, pale discoloration of the glandular stomach mucosa, yellowish area in the glandular stomach mucosa, reduced size and pale discoloration of the spleen. Animals sacrificed in moribund condition, all showed retention of pleural effusion, pulmonary edema and redness or reddish area, with the lung

adhered to the costal pleura and diaphragm in some cases. Other than these, changes such as redness or pale discoloration of the cortico-medullar border in the kidney,opacity of the thymus, pale discoloration of the liver, reduced size and pale discoloration of the spleen, and yellowish area in the glandular stomach mucosa were observed. For animals died or sacrificed in a moribund condition, the esophagus which had been removed, and stored together with the abnormal lung was examined for the second time after histopathological examinations. As a result, perforation or reddish area was observed under the adventitia along the esophagus. Animals examined by periodic necropsy showed the following changes which were related to administration of test compound: the liver swelled at 100 mg/kg or more and darkness at 500 mg/kg; the lung adhered to the costal pleura and pale-discolored spots or areas at 100 mg/kg or more, adhered to the diaphragm and dark red spots or areas at 500 mg/kg. Moreover, the lung of the 100 mg/kg group adhered to costal pleura also showed attachment of yellowish white mass at the adhesion site.

(2) Organ weight

The body weights at necropsy of animals receiving 100 mg/kg or more were significantly lower than the control value ($p<0.05$, $p<0.01$). Although the absolute weight of each organ did not show any significant difference between each treatment group and the control, the organ/body weight ratio increased significantly with increasing tendency of the absolute weight for the liver at 100 mg/kg or more ($p<0.01$) and for the kidney ($p<0.05$) and adrenal gland ($p<0.01$) at 500 mg/kg. For the brain at 500 mg/kg, although the ratio to body weight was significantly higher ($p<0.05$) than in the control group, its absolute weight was not increased.

Absolute and relative organ weights of female rats

Dose	0 mg/kg	20 mg/kg	100 mg/kg	500 mg/kg
Terminal body weight(g)	344.1±19.7	337.0±19.6	317.6±24.8*	299.8±17.0**
Brain(g)	1.94±0.08a	1.84±0.07	1.87±0.07	1.86±0.10
	0.56±0.03b	0.55±0.03	0.59±0.05	0.62±0.04*
Liver(g)	13.57±0.99	13.96±1.39	14.20±1.71	15.12±1.54
	3.95±0.30	4.14±0.31	4.46±0.34**	5.05±0.40**
Kidneys(g)	2.10±0.20	2.08±0.23	2.19±0.15	2.19±0.28
	0.61±0.06	0.62±0.05	0.70±0.10	0.73±0.08*
Adrenal glands(mg)	74.6±10.8	79.6±9.3	75.2±8.0	85.5±16.4
	21.7±3.1	23.7±3.5	23.8±3.0	28.6±5.7**

a: absolute weight
b: relative weight
*: significant difference from control, $p<0.05$
**: significant difference from control, $p<0.01$

(3) Histopathology

For the liver, kidney, spleen, heart, thymus and urinary bladder, the following findings were noted. There were no abnormal findings, however, for the brain, adrenal gland, femoral bone marrow and the ovary of sterile females. Although the organs with abnormality observed at necropsy were examined, no abnormal change was detected. Moreover, after histopathological examinations, some animals (1 and 3 animals at 100 and 500 mg/kg, respectively) including one animal having a perforation were subjected to the second histopathological examinations, which resulted in additional findings in the esophagus, heart, thymus and lung. In the animals subjected to the second examinations, however, the pancreas (one each animal of the control and 100 mg/kg groups) and thyroid (1 and 3 animals at 100 and 500 mg/kg, respectively) showed no abnormal change.

(Liver)

Hypertrophy of centrilobular hepatocytes with cytoplasm of ground glass

appearance was observed in a dose-dependent manner in the groups receiving 100 mg/kg or more ($p<0.01$). At 500 mg/kg, this finding was noted in all the animals subjected to scheduled sacrifice but not noted in the animals necropsied in the mid-course of study (cases of death or moribund sacrifice).

Hypertrophy, hepatocyte, centrilobular, g round glass appearance

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	10**##	8**##
±	0	0	8	0
+	0	0	2	8

±: very slight, +: slight, ++: moderate, +++: severe, **: significant difference from control, $p<0.01$ (Mann-Whitney U test), ##: significant difference from control, $p<0.01$ (Fisher exact test)

(Kidneys)

Since vacuolation probably due to fat deposition was observed on the proximal uriniferous tubules of four animals in the 500 mg/kg group, these animals were subjected to the oil red O-staining by using one control animal as the control; the vacuoles proved to be fatty degeneration. Its incidence was significantly higher than in the control group ($p<0.05$). This change was observed mainly in the animals examined by mid-course necropsy and for animals examined by scheduled necropsy, there was no significant difference from control animals.

Degeneration, fatty, proximal tubule in kidney (females)

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	0	1
±	0	0	0	1
Necropsy for dead and sacrificed animals				
No. of Animals	[0]	[0]	[0]	[5]
Total				3
+				1
++				2

(Spleen)

At 500 mg/kg, the white and red pulps were found to be atrophied, with the incidence being significantly higher than the control group ($p<0.05$). Atrophy of the white pulp was observed mainly in animals examined by the mid-course necropsy. Atrophy of the red pulp was not observed in animals of scheduled necropsy but observed in all the animals of mid-course necropsy. When its incidence in animals of scheduled necropsy was compared to that in the control group, therefore, significant difference was not detected any more.

Atrophy, white pulp in spleen (female)

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	0	1
±	0	0	0	1
Necropsy for dead and sacrificed animals				
No. of Animals	[0]	[0]	[0]	[5]
Total				4
±				1
+				1
++				2

Atrophy, white pulp in spleen (female)

Dose (mg/kg)	0	20	100	500
No. of Animals	[0]	[0]	[0]	[5]
Total				5
±				1
+				4

(Heart)

At 500 mg/kg, fibrosis and cellular infiltration of the epicardium were noted in the animals subjected to scheduled necropsy. Cellular infiltration into the epicardium was observed also in the animals subjected to the second histopathological examination at mid-course necropsy. The incidence was significantly high, when compared to the control, both when only animals of scheduled necropsy were analyzed and when all the animals of the 500 mg/kg group were analyzed ($p<0.05$).

Fibrosis, epicardium (females)

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	0	4#
+	0	0	0	1
++	0	0	0	3

Cellular infiltration, epicardium (females)

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	0	4#
+	0	0	0	1
++	0	0	0	3
Necropsy for dead and sacrificed animals				
No. of Animals	[0]	[0]	[0]	[5]
Total				1
+				1

±: very slight, +: slight, ++: moderate, +++: severe, #: significant difference from control, $p<0.05$ (Fisher exact test)

(Thymus)

At 500 mg/kg, atrophy of the thymus was observed in animals examined by mid-course necropsy and periodic necropsy. Its incidence was significantly ($p<0.05$, $p<0.01$) higher than in control animals both when only animals of scheduled necropsy were analyzed and when all the animals of the 500 mg/kg group were analyzed. At 100 mg/kg or more, fibrosis, edema and cellular infiltration of the capsule as well as exudate containing neutrophils on the capsule were observed. The incidence of these findings at 500 mg/kg was compared with that in the control group. In the case when all the animals were analyzed, significantly ($p<0.05$, $p<0.01$) higher values were obtained for edema and cellular infiltration of the capsule, and when the animals subjected to scheduled necropsy were analyzed, significantly ($p<0.05$) higher values were obtained for fibrosis and cellular infiltration. In the 100 mg/kg group, there was no significant difference from the control group.

Atrophy of thymus (females)

Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	0	3#

±	0	0	0	1
+	0	0	0	1
++	0	0	0	1
Necropsy for dead and sacrificed animals				
No. of Animals	[0]	[0]	[0]	[5]
Total				3
++				2
+++				1

Fibrosis, capsule (females)				
Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No. of Animals	[13]	[13]	[13]	[8]
Total	0	0	1	3#
±	0	0	0	1
+	0	0	0	2

Cellular infiltration, capsule (females)				
Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No.of Animals	[13]	[13]	[13]	[8]
Total	0	0	1	3#
±	0	0	0	3
+	0	0	1	0
Necropsy for dead and sacrificed animals				
No.of Animals	[0]	[0]	[0]	[5]
Total				3
±				2
+				1

Edema, capsule (females)				
Dose (mg/kg)	0	20	100	500
Periodic necropsy				
No.of Animals	[13]	[13]	[13]	[8]
Total	0	0	1	4
+	0	0	1	3
++	0	0	0	1
Necropsy for dead and sacrificed animals				
No.of Animals	[0]	[0]	[0]	[5]
Total				3
+				2
++				1

±: very slight, +: slight, ++: moderate, +++: severe, #: significant difference from control, $p<0.05$ (Fisher exact test)

(Organs showing abnormal changes at necropsy)
At 500 mg/kg, very slight to slight focal hemorrhage and infiltration of neutrophils and lymphocytes in the lung were observed both in animals of mid-course necropsy and those ofkill on schedule. In the cases of mid-course necropsy, very slight to moderate edema and infiltration of macrophages as well as exudate containing neutrophils on the pleura were noted. There were also the cases in which foreign materials were mixed with the exudate. In animals of kill on schedule at 100 and 500 mg/kg, very slight to slight aggregation of foamy cells as well as slight to moderate fibrosis of the pleura was observed. In animals of mid-course necropsy at 100 and 500 mg/kg, very slight to moderate cases of cellular infiltration into the pleura were observed. Other than these cases, moderate foreign granuloma was observed on the pleura at 100 mg/kg. Of two animals of mid-course necropsy subjected to additional histopathological examinations, one animal showed in the

esophagus slight perforation, degeneration of the mucosa and muscular layers and infiltration of neutrophils, and moderate hemorrhages under the adventitia of esophagus and in the pleura. For the other animal, although histopathological examinations did not reveal any abnormal change in the site with miniscule perforation noted macroscopically, exudate containing neutrophils as well as foreign matters was noted on the pleura. Moreover, slight cellular infiltration was observed on the pleural plane of the diaphragm, accompanied by slight exudate containing neutrophils.

Test condition : 1) Test Subjects:
Age at study initiation: 8 weeks old for males and females
Weight at study initiation: 333.1±7.4 g for males, 216.3 ± 6.2 g for females
No. of animals per sex per dose: 13
Haematological and blood chemical examination was conducted only for males.

Conclusion : In oral repeated dose study in rats, major effects were histopathological change in liver and clinical signs such as decreased locomotor activity and prone position. In addition, effects were also observed on kidneys, heart, spleen and thymus. The kidney change observed in male rats is considered due to the accumulation of the male rat specific protein complex, alpha-2u-globulin. Therefore, the NOAEL is considered to be 20 mg/kg/day for both sexes.

Reliability : (1) valid without restriction
Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan).

Flag : Critical study for SIDS endpoint

08.01.2002 (12)

5.5 Genetic toxicity 'in vitro'

Type : Bacterial reverse mutation assay

System of testing : *Salmonella typhimurium* TA100, TA1535, TA98, TA1537, *Escherichia coli* WP2 uvrA

Concentration : 0, 312.5, 625, 1250, 2500, 5000 ug/plate

Cytotoxic conc. : See Result

Metabolic activation : with and without

Result : negative

Method : OECD Guideline 471 "Genetic Toxicology: *Salmonella thyphimurium* Reverse Mutation Assay"

Year : 1999

GLP : yes

Test substance : other TS: Source: Tokyo Kasei Kogyo Co., Ltd., Lot No. GC01, Purity: 99 %

Result : This chemical was not mutagenic in *Salmonella typhimurium* TA100, TA1535, TA98, TA1537 and *Escherichia coli* WP2 uvrA, with or without an exogenous metabolic activation system. No toxicity was observed up to a concentration of 5000 ug/plate, with or without metabolic activation.

Test condition : Procedures: Pre-incubation method
Solvent: DMSO
Positive controls : -S9 mix; 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (TA100, TA98, WP2 uvrA), Sodium azide (TA1535) and 9-Aminoacridine (TA1537)
+S9 mix; 2-Aminoanthracene(all strains)
Doses : -S9 mix; 0, 312.5, 625, 1250, 2500, 5000 ug/plate
+S9 mix; 0, 312.5, 625, 1250, 2500, 5000 ug/plate
S9 : Rat liver, induced with phenobarbital and 5,6-benzoflavone

Plates/test : 3
Number of replicates : 2

Reliability : (1) valid without restriction
Well conducted study, carried out by Research Institute for Animal Science in Biochemistry and Toxicology (Japan).

Flag : Critical study for SIDS endpoint

25.12.2001 (12)

Type : Ames test
System of testing : *Salmonella typhimurium* TA1535, TA100, TA98, TA1537
Concentration : 0, 50, 100, 500, 1000 ug/plate
Cytotoxic conc. :
Metabolic activation : with and without
Result : negative
Method : other: Ames et al. (1975)
Year : 1977
GLP : no data
Test substance : other TS: Source; Monsanto (USA), Purity; greater than 99.9 %, Lot no. 2021
Test condition : Solvent : DMSO
Plates/test : 2
Number of replicates : 2
S9: rat liver, induced by Aroclor 1254
Positive control: 2-Naphthylamine, Benzo(a)pyrene, Emodin

08.01.2002 (17)

Type : Ames test
System of testing : *Salmonella typhimurium* TA1530, TA1535, TA1537, TA1538, TA100, TA98
Concentration : up to 4x10-2 mol/plate
Cytotoxic conc. : No cytotoxic effects were observed.
Metabolic activation : with and without
Result : negative
Method : other: Plate incorporation test
Year : 1979
GLP : no data
Test substance : other TS: University of Louvain, Belgium, Purity; no data
Test condition : Plate/test: 2
Solvent: DMSO
S9: rat liver, induced by Aroclor 1254

08.01.2002 (14)

Type : Ames test
System of testing : *Salmonella typhimurium* TA1535, TA100, TA1538, TA98 and TA1537
Concentration : 0, 270, 1200, 2400, 3600, 4800, 7200, 9600, 12000, 14400, 18000 ug/plate
Cytotoxic conc. : No data
Metabolic activation : with and without
Result : positive
Method : other: standard incorporation assay
Year : 1980
GLP : no data
Test substance : other TS: Source; W. Priem & Co., Purity; less than 1 % p-toluenesulfonamide and no further impurities
Result : o-Toluenesulfonamide did not produce any mutagenic effect without metabolic activation. Likewise, in the presence of S9-mix on Vogel-Bonner-E medium, this chemical was not mutagenic in all strains. However, on ZLM-medium in the presence of S-9 mix, this chemical reproducibly induced a 2-3-fold increase over the spontaneous revertant frequency in the strain TA 98 (shown in the flowing table). Omission of NADPH from the S9-mix abolished these effects.

	His+-Revertants in strain TA 98/plate			
Medium	ZLM S9	ZLM + S9	VB - S9	VB + S9
	o-Toluenesulfonamide (ug/plate)			
0	10	14	19	47
270	14	20	25	48
1200	12	23	24	25
2400	13	23	24	45
3600		31a	16	45
4800	9	31a	17	45
7200		31a	16	44
9600	12		14	51
12000		36a	16	44
14400	9	36a	18	32
18000	5	27		34
Positive control				
Benz(a)pyrene (ug/plate)				
5		170		562

Figures represent the rounded average of three or four plates.
ZLM: ZLM-medium
VB: Vogel-Bonner-E medium
a: p =< 1 %

Test condition : Test was performed routinely using the standard plate incorporation assay. In addition to the standard Vogal-Bonner E- medium, another minimal medium (ZLM-medium) was used.
Solvent: DMSO
Positive control: Benz(a)pyrene (5 ug/plate)

08.01.2002 (4)

Type : Ames test
System of testing : *Salmonella typhimurium* TA98, TA100, TA1535, TA1537
Concentration : up to 18000 ug/plate
Cytotoxic conc. :
Metabolic activation : with
Result : negative
Method :
Year :
GLP : no data
Test substance :
Result : This chemical showed negative results on all strains in this test condition. The result in TA 98 with S9 was as follows.

	His+-Revertants in strain TA 98/plate		
Dose(ug/plate)	Medium		
	Vogal-Bonner E medium		ZLM
Batch	No. 1	No.2	No.3
0	35.5	35.5	39.8
3550	36.0	28.8	-
3600	-	-	30.3
4800	-	-	30.5
5333	35.5	28.8	-
7200	-	-	32.3
8000	26.0	29.0	-
9600	-	-	9.0
12000	20.3	6.8	4.0
14400	-	-	4.8
18000	4.3	5.0	6.5
Positive control			

Trypaflavine
200 327.5 - 1280.8

Test condition : The test was performed with 4 plates per dose or control. Besides the standard Vogel-Bonner agar, ZLM agar was used in the assay on S. typhimurium TA 98. All groups were tested with S9 mix. Additionally, at least the highest dose was also tested without S9 mix (no more details).
S9: Rat liver, induced by Aroclor 1254
Positive control: Trypaflavine (200 ug/plate)
Plate/test: 4

08.01.2002 (6)

Type : Ames test
System of testing : *Salmonella typhimurium* TA1535, TA1538, TA98, TA100
Concentration : 4, 20, 100, 500, 2500 ug/plate
Cytotoxic conc. :
Metabolic activation : with
Result : negative
Method : other: Ames et al. (1975)
Year : 1978
GLP : no data
Test substance : other TS: Source; ICI Organic Division, Purity: no data
Test condition : Solvent : DMSO
Plates/test : 2
S9: rat liver, induced by Aroclor 1254
Positive control: 2-nitrofluorene and 2-(1-chloro-2-isopropylaminoethyl)naphthalene (20, 100 and 500 ug/plate)

08.01.2002 (2)

Type : other: Gene mutation assay
System of testing : *Saccharomyces cerevisiae* (D4)
Concentration : Up to 1000 ug/plate
Cytotoxic conc. :
Metabolic activation : with and without
Result : negative
Method :
Year : 1978
GLP : no data
Test substance :

08.01.2002 (8)

Type : other: Ouabain-resistant mutation assay
System of testing : Human RSa cells
Concentration : 0, 900, 1800 ug/mL
Cytotoxic conc. : No observed
Metabolic activation : without
Result :
Method :
Year : 1988
GLP : no data
Test substance : other TS: Source; Wako Pure Chem. Ind. Ltd.
Test condition : Solvent: DMSO
No. of experiment: 2

08.01.2002 (18)

Type : Chromosomal aberration test
System of testing : Type of cell used : Chinese hamster lung (CHL) cells
Concentration : -S9 mix (24 and 48 hr continuous treatment); 0, 375, 750, 1500, 2250, 3000 ug/mL
-S9 mix (6 hr short-term treatment); 0, 375, 750, 1500, 3000 ug/mL
+S9 mix (6 hr short-term treatment); 0, 375, 750, 1500, 3000 ug/mL

Cytotoxic conc.	:	2250 ug/ml for continuous treatment For short-term treatment, cytotoxicity was not observed.
Metabolic activation	:	with and without
Result	:	negative
Method	:	OECD Guideline 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Source: Tokyo Kasei Kogyo Co., Ltd., Lot No. GC01, Purity: 99 %
Method	:	Guidelines for Screening Mutagenicity Testing of Chemicals (Japan) and OECD Te st Guideline 473 Solvent: DMSO Positive controls: -S9 mix, 1-methyl-3-nitro-1-nitrosoguanidine +S9 mix, Benzo[a]pyrene S9 : Rat liver, induced with phenobarbital and 5,6-benzoflavone Plates/test : 2
Result	:	This chemical did not induce structural chromosomal aberrations and/or polyploidy in CHL cells, with or without an exogenous metabolic activation system.
Remarks	:	3000 ug/ml was used as maximum concentration. This concentration is above 10 mM, which is established as the maximum concentration in OECD TG.
Reliability	:	(1) valid without restriction Well conducted study, carried out by Research Institute for Animal Science in Biochemistry and Toxicology (Japan).
Flag	:	Critical study for SIDS endpoint
08.01.2002		(12)

Type	:	Chromosomal aberration test
System of testing	:	CHO K1 cells
Concentration	:	0, 0.9, 14, 200, 400 ug/mL
Cytotoxic conc.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	
Year	:	1978
GLP	:	no data
Test substance	:	
Test condition	:	Solvent: DMSO Negative control: untreated Positive control: saccharin-Na
08.01.2002		(11)

5.6 Genetic toxicity 'in vivo'

Type	:	Mammalian spot test
Species	:	mouse
Sex	:	female
Strain	:	
Route of admin.	:	oral feed
Exposure period	:	See Test Condition
Doses	:	1000 mg/kg
Result	:	ambiguous

Method : Other
Year : 1982
GLP : no
Test substance : No data
Result : 3 experiments have been performed. Although at least 1 animal with color spot could be observed, only 1 experiment did lead to a significant result. According to this finding, a clear classification of o-toluenesulfonamide as to be mutagenic or non-mutagenic is not possible.

The effect of o-toluenesulfonamide in the mammalian spot test

	Offsprings survived	Offsprings with spots white*	white-gray*	light-gray**	gray**	light-gray**
Control	182	-	-	-	-	-
o-Toluenesulfonamide						
1000 mg/kg	183	-	-	-	1	-
1000 mg/kg	285	-	1	-	1	3a
1000 mg/kg	171	1	-	1	-	-

*: questionable genetic relevance
**: genetic relevance
a: 1 animal with 3 spots

Test condition : Embryos of the cross C57B1/6JHan x T stock which are heterozygous for 4 different recessive coat-color genes were treated in utero during the 10th day post-conception, when about 200 pigment precursor cells are available. The application of o-toluenesulfonamide was peroral in tragacanth mucilage. The control animals received tragacanth mucilage. About 80 pregnant mice per experiment were treated and 30~50 mice had litters surviving for 2 weeks and the color spots in about 200 offsprings were analyzed.
Reliability : (2) valid with restriction
This study was not conducted according to test guideline or under GLP. The positive control was not included in this study and there were no historical control data.
Flag : Critical study for SIDS endpoint
11.01.2002 (5)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : NMRI
Route of admin. : gavage
Exposure period : See Test Condition
Doses : 1,026 mg/kg
Result : negative
Method : other: The method of Schmid
Year : 1980
GLP : no
Test substance : other TS: Source; W. Priem & Co., Purity; less than 1 % PTS and no further impurities
Result : o-Toluenesulfonamide did not increase significantly the rate of micronuclei.

Results of micronucleus tests on mouse bone marrow

Compound	Doses (mg/kg)	Micronucleated Erythrocytes (0/00) (average of 4 mice)
o-Toluenesulfonamide (3% gum arabic)		
	o.p. 2 x 1026	2.1

	Accumulated controls	
	i.p., p.o.	1.6
	Positive control: Cyclophosphamide (Hanks' solution)	
	i.p. 1 x 112	57.4a
	i.p. 1 x 56	26.3a
	i.p. 1 x 28	10.3a

a: p < 1 %

Test condition : Four mice weighing about 30 g for each group were used. o-Toluenesulfonamide in 3 % gum arabic were administered by gavage. They were given twice at 24 h apart. 6 h after the second dose the animal were killed, and bone marrow smears were then prepared. They were stained with May-Gruenwald and Giemsa stains. 1000 polychromatic erythrocytes were analysed for each animal on cooled slides. For calculating the significance of Kastenbaum and Bowman were used.
There is no information as to the setting reason of the highest dose or the toxicity revealed in the o-toluenesulfonamide administrated group.

Reliability : (2) valid with restriction
This study was not conducted according to test guideline or under GLP. Furthermore, there are several insufficient experimental conditions such as no reason of the highest dose, only single time point for examination, and so on.

Flag : Critical study for SIDS endpoint
08.01.2002 (4)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : NMRI
Route of admin. : i.p.
Exposure period : See Test Condition
Doses : 171, 342, 685, 1,026 mg/kg
Result : negative
Method : other: The method of Schmid
Year : 1980
GLP : no
Test substance : other TS: Source; W. Priem & Co., Purity; less than 1 %p-toluenesulfonamide and no further impurities

Result : o-Toluenesulfonamide did not increase significantly the rate of micronuclei.

Results of micronucleus tests on mouse bone marrow

Compound	Doses (mg/kg)	Micronucleated Erythrocytes (0/00) (average of 4 mice)
o-Toluenesulfonamide (3% gum arabic)		
	i.p. 2 x 1026	2.7
	i.p. 2 x 685	2.1
	i.p. 2 x 342	1.1
	i.p. 2 x 171	2.7
Accumulated controls		
	i.p., p.o.	1.6
Positive control: Cyclophosphamide (in Hanks' solution)		
	i.p. 1 x 112	57.4a
	i.p. 1 x 56	26.3a
	i.p. 1 x 28	10.3a

a: p < 1 %

Test condition : Four mice weighing about 30 g for each group were used. o-Toluenesulfonamide in 3 % gum arabic were administered i.p. . They were given twice at 24 h apart. 6 h after the second dose the animal were killed, and bone marrow smears were then prepared. They were stained with

May-Gruenwald and Giemsa stains. 1000 polychromatic erythrocytes were analysed for each animal on cooled slides. For caluculating the significance of Kastenbaum and Bowman were used.
There is no information as to the setting reason of the highest dose or the toxicity revealed in the o-toluenesulfonamide administrated group.

Reliability : (2) valid with restriction
This study was not conducted according to test guideline or GLP. Furthermore, there are several insufficient experimental conditions such as no reason of the highest dose, only single time point for examination, and so on.

Flag : Critical study for SIDS endpoint
08.01.2002 (4)

5.7 Carcinogenity

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	over the whole lifetime from 3 months old
Frequency of treatment	:	daily
Post. obs. period	:	None
Doses	:	0, 20, 200 mg/kg bw
Result	:	See below
Control group	:	yes, concurrent vehicle
Method	:	other
Year	:	1978
GLP	:	no
Test substance	:	no data
Remark	:	There was no sufficient information on data on historical control, the purity of the test chemical, statistical analysis, the sexes of animals with bladder tumours and the presence or absence of bladder parasites.
Result	:	Treatment-related effect on survival time was not observed. In all groups, a large number of animals had died of lymphosarcomas. Lymphosarcomas developed in 7/71 at controls, 10/75 at 20 mg/kg and 10/76 at 200 mg/kg practically endemically between the 300th and 400th day of life, an observation which had not been made with other animals of the same species in former experiments. The incidence was practically identical in all three groups. The total incidences of malignant tumours were not different in treated group compared with controls. 3/76 leukosis occurred at the high dose and 5/75 at the low dose, compared with 0/71 in controls. However, the dose-dependency in the two treated group can not be recognized. In high-dose animals, 1/76 carcinoma and 4/76 papillomas of the bladder were found after 759, 861, 877, 878 and 996 days, respectively; in low-dose rats, 3/75 papillomas of the bladder occurred after 539, 766 and 873 days. No bladder tumours occurred in 71 controls.
Test condition	:	o-Toluenesulfonamide was mixed with the Altromine food. The initial number of animals amounted to 76 in all groups. After their deaths, they were dissected thoroughly and examined histologically if suspected macroscopical findings were observed. In every case, the bladder was examined histologically.
Reliability	:	The reliability of this study is uncertain. This study was not conducted according to test guideline or GLP. Furthermore, there was no sufficient information on historical data, the purity of the test chemical, statistical analysis, the sexes of animals with

bladder tumours and the presence or absence of bladder parasites.

Flag : Critical study for SIDS endpoint
08.01.2002 (16)

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	First generation: 142 weeks from 32 days of age Second generation: 127 week from 21 days of age
Frequency of treatment	:	Daily
Post. obs. period	:	None
Doses	:	0, 2.5, 25, 250 mg/kg/day, or 250 mg/kg/day with 1% NH4Cl in drinking water
Result	:	negative
Control group	:	yes, concurrent vehicle
Method	:	other: no data
Year	:	1980
GLP	:	no
Test substance	:	other TS: Source; Monsanto, Purity; less than 100 ppm impurity
Result	:	Statistical analysis of urinary pH values for male rats during the 18th month on test revealed that only the urine from the group of males receiving o-toluenesulfonamide at 250 mg/kg with NH4Cl in the drinking water significantly different when compared to males in other treatment groups; it was more acidic. The effect of dietary treatment upon urinary pH values of female rats was not as extensively studied except for control where no statistically significant differences were observed. 2. The growth curve for the F0 and F1 males and females receiving o-toluenesulfonamide at 250 mg/kg or 250 mg/kg with 1% NH4Cl in the drinking water were significantly different ($p < 0.05$) from control animals. 3. The F0 generation animals were kept on test for 142 weeks, at which time less than 3 % of the initial animals were alive. The F1 portion of the study was terminated after 127 weeks; when approximately 20 % of the initial animals were still alive. The time-to-death was normally distributed in the F0 generation and was not affected by treatment. In the F1 generation, no significant increase in the probability of dying by time t as a result of exposure to o-toluenesulfonamide was observed for either sex. 4. For the seven hematological parameters (number of erythrocytes, hematocrit, hemoglobin, mean corpuscular volume, total number of leukocytes, neutrophils and lymphocytes), there were no patterns suggesting an effect due to treatment, sex, generation, or variable measured. 5. There were no significant increased in the incidence of tumours. The incidence of bladder tumors in both generations is shown in Table below. The bladder tumors were found at the apex or funds of the bladder, except for one F1 female from the 2.5 mg/kg group which had a benign papilloma arising from the base of the bladder. They arose from a narrow pedicle which measured approximately 1 mm in diameter and 2 mm in length. The benign tumors were primarily soft, papillary, edematous, masses which were not particularly hyperemic or hemorrhagic. The benign papillomata in F0 animals consisted primarily of somewhat edematous, vascular, stroma covered by a layer of transitional epithelium that appeared normal while those in F1 animals were covered by a thick hyperplastic epithelium. Incidence of bladder tumors for rats fed diets containing o-toluenesulfonamide

	F0 generation		F1 generation	
	Benign	Malignant	Benign	Malignant
Males				
Control	1	0	0	0
o-Toluenesulfonamide				
2.5 mg/kg	1	0	0	0
25 mg/kg	0	0	0	0
250 mg/kg	1	0	0	0
o-Toluenesulfonamide with 1 % NH4Cl in drinking water				
250 mg/kg	0	0	0	0
Females				
Control	0	0	0	0
o-Toluenesulfonamide				
2.5 mg/kg	1	0	2	0
25 mg/kg	0	0	0	0
250 mg/kg	0	0	0	0
o-Toluenesulfonamide with 1 % NH4Cl in drinking water				
250 mg/kg	0	0	0	0

6. Some non-neoplastic lesion showed singnificant dose-response as shown in following table.

Non-neoplastic lesion with significant dose response* in F0 male rats					
Dose (mg/kg)	0	2.5	25	250	250 with NH4Cl
No. of Animals	49	49	50	50	39
Lung					
-Chronic respiratory disease-slight	13	17	26	20	28ab
Liver					
-Peliosis	4	9	18	13	8a

Non-neoplastic lesion with significant dose response in F0 female rats					
Dose (mg/kg)	0	2.5	25	250	250 with NH4Cl
No. of Animals	50	50	50	50	38
Spleen					
-Dense hematosiderosis	16	12	16	26	13
Kidneys					
-Pelvic subepithelial telangiectasia	1	3	2	9	2

Non-neoplastic lesion with significant dose response in F1 male rats					
Dose (mg/kg)	0	2.5	25	250	250 with NH4Cl
No. of Animals	50	50	50	50	49
Liver					
-Peliosis	10	9	5	20	14
Spleen					
-Dense hematosiderosis	5	2	7	13	17a
Pancreas					
-Focal chronic pancreatitis-slight	19	18	16	27	22
Non-neoplastic lesion with significant dose response in F1 female rats					
Dose (mg/kg)	0	2.5	25	250	250 with NH4Cl
No. of Animals	50	50	50	50	50
Liver					

-Centrilobular basophil chromogenesis					
	-	1	1	7	13a
-Peliosis	1	3	9	10	6a
Spleen					
-Dense hematosiderosis					
	4	18	7	20	25a

*: by Bartholomew's test
a: Significantly different from controls ($p<0.05$)
b: Significantly different from 250 mg o-toluenesulfonamide group ($p<0.05$)

Test condition : 1. No. of animals;
a) 0, 2.5, 25, 250 mg/kg/day: 50 males and 50 females per group
b) 250 mg/kg/day o-Toluenesulfonamide with 1% NH_4Cl in drinking water: 40 males and 38 females
2. After 3 months on test, the F0 rats were mated on a one-to-one basis; all litters were culled to 8 pups (4 males and 4 females) 4 days post partum in a random manner.
3. The pups were weaned onto their parents' diet, and 50 males and 50 females from each group were randomly selected to constitute the second generation (F1).
4. The two generations remained on test for 127 (F1) and 142(F0) weeks.
5. Histological examination was conducted for all organs including the bladder and tumors, all grossly abnormal areas of dermal, supportive, or skeletal tissues.
6. The animals were free of bladder parasites.
7. Statistical methods: Bonferrini's or t-tests for continuous data and Fisher's test for quantal data

Reliability : (2) valid with restriction
This study was not conducted according to test guideline or GLP.

Flag : Critical study for SIDS endpoint
15.01.2002 (1)

Species : Rat
Sex : Female
Strain : Wistar
Route of admin. : drinking water
Exposure period : 2 years
Frequency of treatment : Daily
Post. obs. period : None
Doses : See Test condition
Result : Negative
Control group : Yes
Method : other: No data
Year : 1980
GLP : no data
Test substance : other TS: Source; Monsanto, Purity; 1 peak on GLP analysis
Result : Although there was a tendency for rats in all groups treated with MNU to die earlier than rats not receiving MNU, no significant differences in mortality were seen in all groups. The behavior and appearance of rats in all groups were normal. Rats in groups A and B weighed less than their relevant controls at the start of this study and the differences had increased by 9 weeks. The reduced body-weight gains persisted throughout the experiment.

Administration of o-toluenesulfonamide was not associated with changes in urinary pH crystalluria or calculus formation, had no effect on the histology of normal bladder, and did not increase the incidence of bladder hyperplasia or neoplasia elicited by pretreatment with MNU. No other treatment-related

effects were observed in other organs.

Test condition : 1. No. of animals; 63 per group
2. Five groups were established as follows:
Group A were given o-toluenesulfonamide at the level of maximum solubility in tap water (0.1 %) which provided a daily intake of 70 mg/kg.
Group B were instilled intravesically via urethral catheter with 0.15 ml of a freshly prepared saturated solution of N -methyl -N-nitrosourea (MNU) for 2 weeks before administration of o-toluenesulfonamide (70 mg/kg)
Group C were pretreated with MNU as Group B and then received o-toluenesulfonamide at desired level of 0.08 mg/kg/day, the intake equivalent to the amount of o-toluenesulfonamide in the contaminated saccharin.
Group D served as untreated controls.
Group E were pretreated with MNU as Group B but then received no chemical. This group was served the MNU-treated control.

3. 4 rats from each group were killed at 4, 26 and 52 weeks for interium pathological examination. Animals that died during the study were autopsied, unless this was precluded by advanced autolysis or cannibalism. Those found in extremis, or surviving to 102 weeks were killed by exsanguination from the abdominal aorta under barbiturate anaesthesia. The kidneys were weighed, and fixed in 10 % buffered formalin. The presence of any macroscopic calculi in the urinary tract was noted. The bladders were gently distended by infection of fixative, opened and examined for gross lesions. All other tissues appearing abnormal at post mortem were preserved in formalin. After fixation, the bladders were cut transversely and the left longitudinally. All tissues were embedded in paraffin wax, sectioned and stained with haematoxylin and eosin.

4. Statistical methods: Student's t-tests for continuous data and Fisher's test or Chi square test for quantal data

08.01.2002 (7)

Species : rat
Sex : female
Strain : Wistar
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatment : daily
Post. obs. period : None
Doses : 0, 79 mg/kg/day
Result : negative
Control group : yes
Method : other: no data
Year : 1980
GLP : no data
Test substance : other TS: Source; Monsanto, Purity; 1 peak on GLP analysis
Result : Although there was a tendency for rats in all groups treated with MNU to die earlier than rats not receiving MNU, no significant differences in mortality were seen in all goups. The behavior and appearance of rats in all groups were normal.

Administration of o-toluenesulfonamide was not associated with changes in urinary pH crystalluria or calculus formation, had no effect on the histology

of normal bladder, and did not increase the incidence of bladder hyperplasia or neoplasia elicited by pretreatment with MNU. No other treatment-related effects were observed in other organs.

Test condition : 1. No. of animals; 50 per group
2. Three groups were established as follows:
Group A were fed o-toluenesulfonamide at a desired level of 70 mg/kg (Actual daily intake: 79 mg/kg).
Group B were instilled intravesically via urethral catheter with 0.15 ml of a freshly prepared saturated solution of N-methyl-N-nitrosourea (MNU) for 8 days before administration of o-toluenesulfonamide
Group C served as untreated controls.
3. Animals that died during the study were autopsied, unless this was precluded by advanced autolysis or cannibalism. Those found in extremis, or surviving to 102 weeks were killed by exsanguination from the abdominal aorta under barbiturate anaesthesia. The kidneys were weighed, and fixed in 10 % buffered formalin. The presence of any macroscopic calculi in the urinary tract was noted. The bladders were gently distended by infection of fixative, opened and examined for gross lesions. All other tissues appearing abnormal at post mortem were preserved in formalin. After fixation, the bladders were cut transversely and the left longitudinally. All tissues were embedded in paraffin wax, sectioned and stained with haematoxylin and eosin.
4. Statistical methods: Student's t-tests for continuous data and Fisher's test or Chi square test for quantal data

08.01.2002 (7)

5.8 Toxicity to reproduction

Type	:	One generation study
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	Males; for 42 days Females; from 14 days before mating to day 3 of lactation
Frequency of treatment	:	Once daily
Premating exposure period		
Male	:	14 days
Female	:	14 days
Duration of test	:	Males; 42 days Females; at least 38 days
Doses	:	0(vehicle), 20, 100, 500 mg/kg/day
Control group	:	yes, concurrent vehicle
Method	:	OECD combined repeated dose and reproductive/developmental toxicity screening test
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Source: Tokyo Kasei Kogyo Co., Ltd., Lot No. GC01, Purity: 99 %, kept at room temperature until use
Result	:	NOAEL 100 mg/kg/day for both reproductive and developmental toxicity 1) Parental toxicity Three females died and two females were sacrificed in moribund condition

during pre-mating period at 500 mg/kg b.w. Decrease in locomotor activity, prone position and salivation were observed in both sexes of 100 and 500 mg/kg b.w. In these groups, significantly low body weights were recorded in both sexes.

2) Findings relative to reproduction

A. Reproductive performance
At 500 mg/kg, since the number of females decreased due to death or moribund sacrifice, some males were allowed to mate with untreated females. The copulation index, precoital days, the frequency of estrous cycle during this period and the gestation index were comparable for mated pairs within the 500 mg/kg group and for the mated pairs with untreated females. These indices did not show any significant difference between the control and each treatment group.

B. Delivery and lactation
There was no abnormal finding for delivery and lactation in any of the groups.

C. Number of corpora lutea and im plantation sites and implantation index
For the number of corpora lutea, implantation sites and implantation index, there was no significant difference between the control and each treatment group.

D. Gestation index and duration of gestation
For delivery rate and duration of gestation, there was no significant difference between the control and each treatment group.

3) Findings of offspring

A. Clinical signs and viability
There was no significant difference between the control and each treatment group for the number of pups delivered, delivery index and 4-day viability index of pups. For sex ratio, there was no significant difference between the control and each treatment group. At 500 mg/kg, although there was a tendency in which the delivery index, number of pups alive on Day 0 of lactation and birth index of live pups slightly decreased, there was no significant difference from the control values. At 100 mg/kg or lower, the results were comparable to the control group.

B. Body weight
The body weight of male and female pups at 500 mg/kg were significantly lower than that of controls both on Day 0 and Day 4 of lactation ($p<0.05$, $p<0.01$). There was no significant difference from the control value both for males and females at 100 m g/kg or lower.

Dose level (mg/kg)

	0	20	100	500
Day 0 of lactation				
Pup weight in grams				
Male	7.4±1.0(10)	7.1±0.7(12)	6.8±0.8(12)	6.2±0.7*(7)
Female	7.0±0.7(10)	6.7±0.7(12)	6.4±0.6(12)	5.8±0.7**(7)

C. Morphology
External examination of pups on Day 0 of lactation and necropsy on Day 4 of lactation did not reveal any abnormal changes in any of the treatment groups. There was not abnormality detected by necropsy of dead pups.

Test condition : 1) Test Subjects:

Age at study initiation: 8 weeks old for males and females
Weight at study initiation: 333.1±7.4 g for males, 216.3 ± 6.2 g for females
2)No. of animals: 13 per sex per dose group
3)Statistical methods: Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

Reliability : (1) valid without restriction
Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan).

Flag : Critical study for SIDS endpoint

25.12.2001 (12)

Type : One generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : Oral-feed
Exposure period : First generation: 142 weeks from 32 days of age
Second generation: 127 week from 21 days of age
Frequency of treatment : Daily
Premating exposure period
Male : 90 days
Female : 90 days
Duration of test : See "Test condition"
Doses : 0, 2.5, 25, 250 mg/kg/day or 250 mg/kg/day with 1% NH4Cl in drinking water
Control group : yes, concurrent vehicle
Method : Other
Year : 1980
GLP : No
Test substance : other TS: Source; Monsanto, Purity; less than 100 ppm impurity
Result : NOAEL 25 mg/kg/day for both reproductive and developmental toxicity

The time-to-death for the F0 generation was normally distributed and was not affected by treatment. In the F1 generation, no significant increase in the probability of dying by time t as a result of exposure to o-toluenesulfonamide was observed for either sex. The average observed body weights for the F0 and F1 males and females surviving 80 weeks or more on test was significantly lower at 250 mg/kg o-toluenesulfonamide and 250 mg/kg o-toluenesulfonamide with 1 % NH4Cl in the drinking water, compared with control group (no information on the period showing these results).

No significant differences from the control group were observed for any of the reproductive parameters (no detail information). However, there was a statistically significant decrease in the litter size at postpartum day 1 and 4 in the 250 mg o-toluenesulfonamide group. When the average pup body weight at day 4 after birth was adjusted for litter size, there was a significantly lower average body weight in the 250 mg o-toluenesulfonamide group.

Dose (mg/kg)	0	2.5	25	250	250 with NH4Cl
No. of litters	41	40	41	49	33
Average litter size (day 1)	10.80	11.78	10.56	9.38a	9.70
Average litter size (day 4)	10.46	11.08	10.22	9.04a	9.12
Average body weight (day 4) (g)	11.56	11.07	11.00	9.26b	9.49b

a: statistically significant ($p<0.05$)

b: statistically significant ($p<0.05$) when the average pup weight was statistically adjusted to an average litter size of 11 pups based on an analysis of covariance with the covariate litter size.

Test condition : No. of animals;
a) 0, 2.5, 25, 250 mg/kg/day: 50 males and 50 females per group
b) 250 mg/kg/day o-Toluenesulfonamide with 1% NH4Cl in drinking water: 40 males and 38 females

After 3 months on test, the F0 rats were mated on a one-to-one basis; all litters were culled to 8 pups (4 males and 4 females) 4 days post partum in a random manner. The pups were weaned onto their parents' diet, and 50 males and 50 females from each group were randomly selected to constitute the second generation (F1). The two generations remained on test for 127 (F1) and 142(F0) weeks.

Reliability : (1) valid without restriction
Well conducted study, carried out by Hatano Research Institute, Food and Drug Safety Center (Japan).

Flag : Critical study for SIDS endpoint

25.12.2001 (1)

5.9 Developmental toxicity/teratogenicity

See the section 5.8 "Toxicity to reproduction"

5.10 Other relevant information

Type : Excretion
Remark : After oral administration of o-toluene (methyl-14C) sulfonamide an average of 94 % of the radioactivity was recovered, mostly in the urine and with little (5-7 %) in the faeces within 7 days. The urine contained about 78 % of the radioactivity at all dose levels, but the rate of eliminations decreased with increase in dose. Thus, the radioactivity in urine within 24 hr decreased (79, 58 and 36 % of dose), while the elimination from 24 hr to 48 hr after dosing increase (7, 14 and 33 % of dose) with increase in dose (20, 125, 200 mg/kg, respectively). However, the overall distribution of the radioactivity between urine and faeces was unchanged.
Test condition : Female Wistar rats were given o-toluene (methyl-14C) sulfonamide by gavage at 20, 125 or 200 mg/kg.

25.12.2001 (15)

Type : Excretion
Remark : At 0.2 mg/kg, the recovery was com plete with nearly all the radioactivity in the urine and little (< 1%) in the faeces within 4 days. 56 % of the radioactivity was recovered in the first day and 30 % in the second. At 0.4 mg/kg, the urinary elimination was 43 % within 24 hr and 70 % within 48 hr. In faeces, the radioactivity was not determined.
Test condition : Human subjects were orally given a single dose of o-toluene (methyl-14C) sulfonamide (0.2 mg/kg for one man and one woman, and 1.4 mg/kg for one man).

25.09.2001 (15)

Type : Metabolism
Remark : The main metabolites of o-toluenesulfonamide were 2-sulfamoyl-benzyl alcohol and its sulfate and glucuronic acid conjugates (80 % of 14C in the

urine of rats and 35 % in man) and saccharin (35% in man and 3 % in the rat). Other metabolites found in the urine were 2-sulfamoylbenzoic acid (2% in the rat and 4% in man) and N-acethyltoluene--sulfonamide (6% in rat and 2% in man) together with unchanged compound (5% in rat and 3% in man).

Test condition : o-Toluene (35S) sulfonamide was administered by gavage in a single dose of 0.4 mg/kg to human subjects or in a single dose of 200 mg/kg to female Wistar rats

25.12.2001 (15)

Type : other: Excretion and metabolism
Remark : After a single dose of o-toluene (35S) sulfonamide, the recovery was about 65 %, 83 % and 85 % in urine within 24, 48, and 96 hr, respectively, and about 10 % in feces within 96 hr. About 50 % of metabolites excreted in urine were o-sulfamoylbenzoic acids, while unchanged compound was excreted in feces.
Test condition : o-Toluene (35S) sulfonamide dissolved in water was administered in a single dose of 300 mg/kg by gavage to male Wistar rats weighing about 300 g.

25.12.2001 (13)

Type : other: Cell transformation assay
System of testing : BHK cells
Concentration : 0.025, 0.25, 2.5, 25, 250, 2500 ug/ml
Cytotoxic conc. : 2500 ug/ml
Metabolic activation : without
Result : negative
Method :
Year : 1978
GLP : no data
Test substance :
Test condition : Positive control: 2-Acetylaminofluorene (250 ug/ml)
Plate/test: 2

08.01.2002 (2)

Type : Sex-linked recessive lethal mutations test
Species :
Sex : male
Strain : other: *Drosophila melanogaster* (fruit fly)
Route of admin. : oral feed
Exposure period :
Doses : 2.5 mM in 5 % sucrose solution
Result : positive
Method :
Year : 1980
GLP : no data
Test substance : other TS: Source; W. Priem & Co., Purity; less than 1 % p-toluenesulfonamide and no further impurities
Result : o-Toluenesulfonamide induced recessive lethal mutations only in brood 1 (p: less than 1 %) at concentrations of 2.5 mM. In brood 3, they showed a sterilizing effect.

Recessive lethal mutations per X-chromosomes

Conc.(mM)	Brood1 (%)	Brood2 (%)	Brood3 (%)
Control			
0	19/7130 (0.27)	8/5525 (0.14)	19/4871 (0.39)
o-Toluenesulfonamide			
2.5	26/4310 (0.60)b	7/2800 (0.25)	10/1386 (0.72)

b: p =< 1%

Test condition : Test compounds were dissolved in 5 % sucrose solution containing 2 %

DMSO if necessary. The test solutions were fed to 1-2-day-old Berlin K males for 3 days, the doses were chosen to allow survival of at least 75% of the animals.
The treated males were mated individually to three Basc virgin females. A mating scheme, consisting of three successive broods (each lasting 3 days) was used. At the end of each period the treated male was transferred to a new vial and remated to three virgin females.

Flag : Critical study for SIDS endpoint
08.01.2002 (4)

Type : Sex-linked recessive lethal mutations test
Species :
Sex : male
Strain : other: *Drosophila melanogaster* (fruit fly)
Route of admin. : other: abdominal injection
Exposure period : 1-3 days
Doses : about 0.2 ul (5 mM in 0.7% saline)
Result : negative
Method :
Year : 1977
GLP : no data
Test substance : other TS: Source; Pfalz & Bauer, Purity; unknown
Result : o-Toluenesulfonamide did not show a mutagenic response.

Induction of sex-linked recessive lethal mutations

Conc. (mM)	Brood1 (%)	Brood2 (%)	Brood3 (%)
Control			
0	4/3136 (0.13)	7/2997 (0.23)	4/3118 (0.13)
o-Toluenesulfonamide			
5	2/774 (0.26)	1/684 (0.15)	0/780 (0.0)
5	2/969 (0.21)	3/808 (0.37)	3/983 (0.31)

Test condition : 1.Solvent; DMSO was used as an auxiliary solvent, at a final concentration of 2 %
2.Age of males at the start; 1 day
3.Age of females at mating; 2-6 days (occasionally 1 day)
4.Toxicity, fertility; Not noticeably affected
5.Brooding scheme; Treated males were mated with fresh virgins for three consecutive periods of at most 3 days: 4 virgins for each mating: males mated individually.
5.Scoring criteria in F2; When less than 4 round-eyed males were present in an F2 vial, re-tests were made to confirm possible (semi)-lethality.
6.Culturing temp.; 25 C
08.01.2002 (9)

Type : Sex-linked recessive lethal mutations test
Species :
Sex :
Strain : other: *Drosophila melanogaster* (fruit fly)
Route of admin. : oral feed
Exposure period : 3 days
Doses : 5 mM in 5 % sucrose solution
Result : negative
Method :
Year : 1977
GLP : no data
Test substance : other TS: Source; Pfalz & Bauer, Purity; unknown
Result : o-Toluenesulfonamide did not show a mutagenic response.

Induction of sex-linked recessive lethal mutations

Conc. (mM)	Brood1 (%)	Brood2 (%)	Brood3 (%)
Control	4/3136 (0.13)	7/2997 (0.23)	4/3118 (0.13)
o-Toluenesulfonamide			
5	1/532 (0.19)	0/576 (0.0)	0/56 (0.0)

Test condition : 1.Solvent; DMSO was used as an auxiliary solvent, at a final concentration of 2 %
2.Age of males at the start; 1 day
3.Age of females at mating; 2-6 days (occasionally 1 day)
4.Toxicity, fertility; Not noticeably affected
5.Brooding scheme; Treated males were mated with fresh virgins for three consecutive periods of at most 3 days: 4 virgins for each mating: males mated individually.
5.Scoring criteria in F2; When less than 4 round-eyed males were present in an F2 vial, re-tests were made to confirm possible (semi)-lethality.
6.Culturing temp.; 25 ºC

08.01.2002 (9)

5.11 Experience with human exposure

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