FOREWORD INTRODUCTION

1,3-Bis(aminomethyl)benzene (CAS No.: 1477-55-0)

SIDS Initial Assessment Report

FOR

13TH SIAM

(Bern, Switzerland, 6-9 November 2001)

Chemical Name: 1,3-Bis(aminomethyl)benzene

CAS No.: 1477-55-0

Sponsor Country: Japan

National SIDS Contact Point in Sponsor Country:
Mr. Yasuhisa Kawamura,
Ministry of Foreign Affairs, Japan

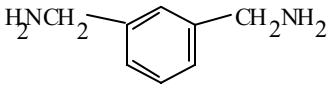
History:

Testing: **No testing** **( )**

Testing **( )**

Comments: ICCA Initiative work led by Mitsubishi Gas Chemical Company, Inc.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	1477-55-0
Chemical Name	1,3-bis(aminomethyl)benzene
Structural Formula	

RECOMMENDATIONS

The chemical is currently of low priority for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

There is no information on toxicokinetics. The toxicity of this chemical is entirely consistent with its corrosiveness at the site of first contact.

Oral LD_{50} of rats was 1090 mg/kg for males and 980 mg/kg for females [OECD TG 401]. The oral LD_{50} of mice was 1180 mg/kg [OECD TG 401]. The inhalation LC_{50} (4h) of rats was 0.8 mg/L for females but it was presumed to be more than 1.42 mg/L for males. The toxicity via oral administration and inhalation was tissue damage in the digestive and respiratory organs, respectively, which are the first contact sites. The chemical is corrosive to rat and mouse skin and a sensitiser in the guinea pig maximisation test.

In the 28-day repeated dose toxicity study [OECD TG 407], the chemical was given to rats by gavage at doses of 0, 10, 40, 150 and 600 mg/kg b.w/day. One male and four females died, and salivation, low locomotor activity and piloerection were noted in the 600 mg/kg group. Furthermore, ulceration, acanthosis with hyperkeratosis and submucosal inflammation were observed in the forestomach. No adverse effects were observed in the 150 mg/kg and the lower dose groups.

A reproductive /developmental toxicity screening test [OECD TG 421] of rats by gavage at 50, 150 and 450 mg/kg b.w/day for at least 41 days resulted in death in one male in the 150 mg/kg group, and three males and one female in the 450 mg/kg group. In almost all 450 mg/kg animals, the same histopathological changes as the above 28-day study were observed in the forestomach. No adverse effects were found at 50 mg/kg b.w/day.

Based on this information, the NOAEL for repeated dose toxicity is considered to be 50 mg/kg b.w/day.

In the above reproductive/developmental toxicity screening test [OECD TG 421] the substance was administered from 14 days before mating to 20 days after mating in males and to day 3 of lactation in females. No adverse effects were observed in terms of copulation, fertility, delivery and nursing of parents, and the viability, body weight and morphology of offsprings. The NOAEL for reproductive/developmental toxicity (F1 offspring) was 450 mg/kg b.w/day.

The chemical was not mutagenic in bacteria [OECD TG 471 & 472]. It induced neither chromosomal aberrations in mammalian cells *in vitro* [OECD TG 473] nor micronuclei in mouse bone marrow *in vivo* [OECD TG 474].

In clinical observation of workers during the manufacturing process, the chemical appears to act as a gastrointestinal irritant. It has also been shown to cause contact sensitisation reactions in workers at concentrations equal to and below 0.1 mg/m^3 (the occupational threshold limit value in the US).

Environment

The chemical has a log Pow value of 0.18 at 25 °C, a vapour pressure of 0.04 hPa at 25 °C, and a water solubility of > 100 000 mg/L. Fugacity model Mackay level III calculations suggest that the majority of the chemical would distribute to soil if released to soil and/or air compartment(s), and water if released to aquatic compartment.

The chemical is not readily biodegradable (49% after 28 d) or inherently biodegradable (BOD = 22%, TOC = 6% and analysis in HPLC = 21%) and it does not hydrolyse (half-life >1 y at 25 ^{o}C). However, the chemical does not bioaccumulate (BCF < 2.7 at 0.2 mg/L). The chemical will react with carbon dioxide to form the carbamate acid, and will undergo indirect photo-oxidation with hydroxy radicals ($T_{½}$ 5.39 h), and will therefore not persist in the atmosphere.

Acute toxicity data were available for three kinds of fish (Medaka, 96hLC_{50} = 87.6 mg/L; Golden orfe, 96hLC_{50} = 75 mg/L and Rainbow trout, 96hLC_{50} >100 mg/L). In *Daphnia magna*, acute toxicity values of 48hEC_{50} = 15.2 mg/L and 48hEC_{50} = 16 mg/L were reported. The chronic toxicity data for *Daphnia magna* were 6.77 mg/L EC_{50} (21d, reproduction inhibition) and 4.7 mg/L NOEC (21d, reproduction inhibition). The parental toxicity for *Daphnia magna* was 8.4 mg/L 21dLC_{50}. The results in algae were E_bC_{50} = 12 mg/L and NOEC = 6.25 mg/L (*Scenedesumus subspicatus*) and E_bC_{50} = 20.3 mg/L and NOEC (0 to 72 h) = 10.5 mg/L (*Selenastrum capricornutum*).

The predicted no effect concentration (PNEC) of 0.047 mg/L is estimated from the lowest chronic value (NOEC of 4.7mg/L, *D. magna* reproduction), by applying an assessment factor of 100 because two chronic studies are available (that is, in algae and daphnia).

Exposure

Production of the chemical in Japan is ca. 13 000 t/y (1999 – 2000). The chemical is an intermediate in the production of epoxy curing agents, polyamides and polyurethanes. Due to the chemical binding processes that occur during curing, finished products do not contain the chemical. The substance is also not present in the industrial intermediates used in the production of polyamides and polyurethanes, but a few percent is present in the epoxy curing agent. The great majority of the epoxy curing agent is assumed to be used by industrial or professional users. Greater than 99.9% of the substance is used in three categories: polyamide (major), epoxy curing agent, and polyurethane production.

Based on the chemical nature, physico-chemical properties and the annual production amount, a Mackay level III fugacity model calculation shows that the chemical would distribute mainly into water. However, the use as an intermediate indicates that most of the chemical will be consumed in the reaction process. Environmental exposure from manufacture is considered to be negligible, because aqueous waste from plant cleaning is sent to a waste-water treatment plant before release and exhaust gases are sent for incineration.

The manufacture of epoxy resins and other compounds are conducted in closed systems. Occupational exposure limit values are set world-wide as 0.1 mg/m^3 15 min STEL. In a model workshop system, MXDA airborne concentrations varied from 0.064 to 0.229 mg/m^3 without ventilation and 0.018 to 0.051 mg/m^3 with ventilation. The EASE model gave a dermal exposure (non-dispersive use, indirect handling) of much less than 0.1 mg/cm^2/day. Personal protective equipment (vapour masks, goggles, overalls, gloves) is worn during operations such as drum filling. For inhalation exposure, the expected human exposure (inhalation) would be EHEinh =0.0073 mg/kg/day on the highest vapour concentration of 0.051 mg/m^3 in the model workshop system. If absorption occurred through hands and forearms, the calculated EHEder would be 0.03 mg/kg/day.

NATURE OF FURTHER WORK RECOMMENDED

The substance is not a priority for further work in relation to the use of the substance as an intermediate in a closed system.

FULL SIDS SUMMARY

CAS NO: 1477-55-0		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point		Unknown method	14.1°C
2.2	Boiling Point		Other	273°C (at 1013 hPa)
2.3	Density (Relative)		Other: pycnometer	1.052 at 20 °C
2.4	Vapour Pressure		Other (measured) Other (calculated)	20 hPa at 145°C. 0.04 hPa at 25°C
2.5	Partition Coefficient (Log Pow)		OECD TG 107	0.18 at 25 °C
2.6 A.	Water Solubility		OECD TG 105	> 100000 m g/L at 25 °C
B.	pH			No data available
2.7	Flash Point		Other (open cup)	134°C
2.9	Flammability		Other: Flash point determination	Air: The substance is not flammable in air
2.10	Explosive Properties		Other: Prediction	Predicted not to have explosive properties
2.11	Oxidising Properties		Other: Prediction	Predicted to have no oxidising properties
2.12	Dissociation Constant		OECD TG 112	pK_a ca. 9.19 at 25°C
2.14	Viscosity		Other	6.8 cP at 20 °C
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation (Indirect)		EUSES v. 1 AOPWIN rate constant	$T_{1/2}$ = 5.39 h (OH radical)
3.1.2	Stability in Water		OECD TG 111	Stable at pH 4,7, 9 at 50°C
3.3	Transport and Distribution		Calculated (Fugacity Model Mackay Level III)	(100% to air) Air 0.215% Water 38.576% Soil 61.121% Sediment 0.088% (100% to water) Air $4x10^{-5}$% Water 99.760% Soil 0.012% Sediment 0.228% (100% to soil) Air 0.005% Water 36.138% Soil 63.775% Sediment 0.082%
3.5.	Biodegradation		OECD TG 301B OECD TG 302C	Not readily biodegradable (49%) Not inherently biodegradable (Analysis by HPLC: 21%, BOD 22%, TOC = 6%).
3.7	Bioaccumulation	*Cyprinus carpio*	OECD TG 305	The substance does not bioaccumulate BCF 1: < 0.3 (2 mg/L) BCF 2: < 2.7 (0.2 mg/L)

ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	*Oryzias latipes*	OECD TG 203	LC_0 (96 h): 56 mg/L LC_{50} (96 h): 87.6 mg/L LC_{100} (96 h): > 100 mg/L
		Golden Orfe	OECD TG 203	LC_{50} (96 h) = 75 mg/L
		Rainbow Trout	OECD TG 203	LC_{50} (96 h) > 100 mg/L
4.2	Acute Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 202	EC_{50} (24 h) = 35.1 mg/L EC_{50} (48 h) = 15.2 mg/L EC_{100} (24 h) = 50.0 mg/L EC_{100} (48 h) = 28 mg/L NOEC(24 h) = 16.0 mg/L NOEC(48 h) = 8.9 mg/L
		Daphnia magna	OECD TG 202	EC_{50} (48 h) = 16 mg/L
4.3	Toxicity to Aquatic Plants (Algae)	Selenastrum *capricornutum*	OECD TG 201	EbC_{50} (0-72 h) = 20.3 mg/L $NOEC_b$ (0-72 h) = 10.5 mg/L ErC_{50} (24-72 h) = 33 mg/L NOECr (24-72 h) = 22.9 mg/L
		Scenedesmus subspicatus		EbC_{50} (72 h) = 12 mg/L ErC_{50} (0-24 h) = 14 mg/L
4.5.2	Chronic Toxicity to Aquatic Invertebrates	*Daphnia magna*	OECD TG 211	EC_{50} (21 d) = 6.77 mg/L (Reproduction) NOEC (21 d) = 4.7 mg/L (Reproduction) LOEC (21 d) = 15 mg/L (Parental daphnia) LC_{50} (21 d) = 8.4 mg/L (Parental daphnia)
TOXICOLOGY				
5.1.1	Acute Oral Toxicity	Rat/Mouse	OECD TG 401	Rat: LD_{50} = 1090 mg/kg (1.04 mL/kg) (male) LD_{50} = 980 mg/kg (0.94 mL/kg) (female) Mice: LD_{50} = 1180 mg/kg (male)
5.1.2	Acute Inhalation Toxicity	Rat	Directive 84/449/EEC, B2	LC_{50} (4 h, female) = 0.8 mg/L of air LC_{50} (4h, male) > 1.42 mg/L of air
5.2.1	Skin Irritation	Rat/Mouse	Equivalent to Directive 84/449/EEC B4, except for test species	Corrosive
5.3	Skin Sensitisation	Guinea Pig	Directive 92/69/EEC, B6 (Maximisation test)	Sensitising (7/10)
5.4	Repeated Dose Toxicity (Oral)	Rat	Japanese TG	NOAEL (28 day) = 150 mg/kg/day
5.5	Genetic Toxicity *In Vitro*			
A.	Bacterial Test (Gene mutation)	S. typhimurium, E. coli	OECD TG 471 OECD TG 472	Negative (with metabolic activation) Negative (without metabolic activation)
B.	Non-Bacterial *In Vitro* Test (Chromosomal aberrations)	CHL/IU cells	Japanese TG and OECD TG 473	Negative (with metabolic activation) Negative (without metabolic activation)
C.	Non-Bacterial *In Vitro* Test (Chromosomal aberrations)	CHO cells	Directive 84/449/EEC, B10	Negative (With metabolic activation) Negative (Without metabolic activation)
5.6	Genetic Toxicity *In Vivo* (Oral)	Mouse	67/548/EEC, Method B12 and OECD TG 474	Negative
5.8	Reproduction/Developmental Toxicity	Rat	OECD TG 421	NOAEL Parental = 50 mg/kg/day (male) NOAEL Parental = 150 mg/kg/day (female) NOAEL Fl Offspring =450 mg/kg/day

SIDS INITIAL ASSESSMENT REPORT (SIAR)

1. IDENTITY

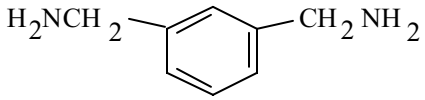
IUPAC Name: 1,3-bis(aminomethyl)benzene

Trade Name: MXDA

CAS Number: 1477-55-0

Molecular formula: $C_8H_{12}N_2$

Structural formula:



Molecular weight 136.22

Synonym: 1,3-benzenedimethanamine
1,3-bis-aminomethylbenzene
m-phenylenebis(methylamine)
m-xylene-alpha,alpha'-diamine
m-xylylenediamine
MXDA

Purity: >99% w/w

Impurities included: 3-methylbenzylamine, 3,5-dimethylbenzyl alcohol, 3,4-dimethylbenzyl alcohol, and 2,4-dimethylbenzyl alcohol. All impurities at ca. 0.08% or less.

Physical and chemical properties:

Melting point (Freezing point)	14.1 °C
Boiling point	273 °C at 1013 hPa
Density[3]	1.052 at 20 °C
Vapour pressure[4]	20 hPa at 145 °C 0.04 hPa at 25 °C
Partition coefficient	Log Pow 0.18 at 25 °C
Solubility	Water > 100 g/L Soluble in ether and benzene. Insoluble in n-hexane, cyclohexane and iso-octane
pK_a	9.19 at 25 °C
Hydrolysis	$T_{½}$ > 1 year at 25 °C (at pH 4, 7, and 9)
Flash point	134 °C (open cup)

2. GENERAL INFORMATION ON EXPOSURE

- The production volume of the chemical in Japan is ca. 13 000 t/y (1999 – 2000). Japan is the sole producer of this chemical. The chemical is an organic liquid made by the ammoxidation of m-xylene, followed by hydrogenation.
- The chemical is an intermediate in the production of epoxy curing agents, polyamides, polyurethanes, lacquers and plastics.
- The main source of pollution is emission in the place of use.

2.1 ENVIRONMENTAL FATE

- Fugacity Model Mackay level III calculations[5] suggest that the majority of the chemical would distribute to soil if released to soil and/or air compartment(s), and to water if released to the aquatic compartment.

Table 1. Fugacity Model Mackay Level III results

Release	Distribution (%)			
	Air	Water	Soil	Sediment
100% to air	0.215	38.576	61.121	0.088
100% to water	$4x10^{-5}$	99.760	0.012	0.228
100% to soil	0.005	36.138	63.775	0.082

- The chemical is ionised at environmental pHs and will have a positive charge. Cations are able to strongly adsorb to soil surfaces and, therefore, have a low mobility.
- Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 5.39 hours (calculated from EUSES v1.00 using the AOPWIN rate constant). The chemical is also expected to react with carbon dioxide to form the carbamate acid, and will therefore not persist in the atmosphere.
- The chemical is not readily biodegradable (49%, OECD 301B) nor inherently biodegradable (MITI II, corresponding to OECD 302C: BOD = 22%, TOC = 6% and analysis by HPLC = 21%).[6]
- Abiotic degradation by hydrolysis does not occur at pHs 4, 7 and 9. However, the bioaccumulation potential is low (BCF < 0.3 and < 2.7 at 2 and 0.2 mg/L, respectively, OECD TG305.[7]

2.2 HUMAN EXPOSURE

2.2.1 OCCUPATIONAL EXPOSURE

- The EASE model gives an expected human exposure of 2.8-16.7 mg/m^3 for a low-vapour-pressure liquid, local exhaust ventilation and non-dispersive use. However, where better data are available they should be used. For skin exposure, as the chemical is corrosive and a skin sensitiser, direct handling is unlikely, and EASE suggests that where there is indirect handling expected human exposure (dermal) is much less than 0.1 $mg/cm^2/day$. If absorption occurred

through hands and forearms, the calculated EHEder would be less than 0.03 mg/kg/day, but risk reduction measures make the expected human exposure (dermal) much less.

- In a model workshop system,[8] airborne concentrations of the chemical varied from 0.064 to 0.229 mg/m^3 in the absence of ventilation, and 0.018 to 0.051 mg/m^3 when ventilation was provided. As LEV is provided, the expected human exposure (inhalation) would be EHEinh =0.0073 mg/kg/day using the highest vapour concentration of 0.051 mg/m^3 in the model workshop system.
- Inhalation exposure limit values are set world-wide as 0.1 mg/m^{3,} 15 min STEL.

2.2.2 CONSUMER EXPOSURE

- No consumer exposure is expected as the chemical is transformed during use.
  The polyamide derived from MXDA and adipic acid is applied to engineering plastics for use in automobiles and food packaging films (CAS 25718-70-1, FDA approval: 21CFR section 177.1500). MXDA could not be detected in this polyamide. Epoxy curing agents based on MXDA are easily reacted with epoxy resins to be applied in paints, coatings and adhesives, making the three-dimensional cured structure. There is little remaining MXDA in them.

2.2.3 INDIRECT EXPOSURE VIA THE ENVIRONMENT

- Exposure via this route is unlikely. The chemical is not readily biodegradable, but it is not bioaccumulative. Manufacture occurs in an enclosed system and waste-water from plant cleaning is treated by activated sludge before discharge to municipal drains. Other waste will mainly be incinerated.
- Environmental exposure from the manufacturing plant:
  The chemical is present in the water system within the manufacturing plant. As most of this water is used in the incineration plant to control the temperature, most of the chemical is incinerated. The remaining volume of chemical, a few kilogrammes per hour, is discharged into an on-site waste-water treatment plant. After treatment the effluent is discharged into the municipal sewer. However, the concentration of the chemical in the effluent is below the limit of detection.

  The activated sludge from the water treatment unit is incinerated. There is no detectable emission of the chemical from the incineration plant.

  Environmental exposure from consumer use:
  Greater than 99.9% of the chemical is used as an intermediate in closed systems for the production of epoxy curing agents, polyamides (major application) and polyurethanes, and as such there will be very little exposure to the environment. No chemical is detected in the end consumer-products (polyamides, engineering plastics; epoxy curing agents, adhesives; polyurethanes, coatings, sealants and adhesives). Also, no chemical is detected in the industrial intermediates for these products, apart from a few percent in the epoxy resin hardeners. The great majority of these hardeners are assumed to be used by industrial or professional users.

  From the above, there is no emission of the chemical into the aqueous or terrestrial compartments. The chemical is of low volatility, so that release to the air compartment is also expected to be very low.

3. HUMAN HEALTH HAZARDS

3.1 EFFECTS ON HUMAN HEALTH

3.1.1 Toxicokinetics and toxicodynamics

No information is available on toxicokinetics. The chemical is alkaline in solution and the corrosive nature of the administered material is responsible for the lesions seen in the toxicity studies.

3.1.2 ACUTE TOXICITY

Available data are summarised in Table 2.

Table 2. Acute toxicity in experimental animals

Route	Species (sex)	Value	Reference
Oral	Rat (male)	LD_{50} 1090 mg/kg	(10)
	Rat (female)	LD_{50} 980 mg/kg	
	Mouse (male)	LD_{50} 1180 mg/kg	(10)
Inhalation	Rat (male)	LC_{50}(4h) > 1.42 mg/l (*)	(11)
	Rat (female)	LC_{50}(4h) 0.8 mg/L	

(*) Mortality observed: Death 1/5 at dose group of 0.74 mg/L, while no death at dose groups of control, 0.34 mg/L and 1.42mg/L.

Clinical observations in the acute oral toxicity study in rats continued for 14 days after oral dosing,and included decline of spontaneous movement some hours after dosing, followed by blepharoptosis, diarrhoea, and reddish brown 'vomit', and ‘tear flow’. Symptoms disappeared in surviving animals 3 to 7 days later. Animals that died showed signs of ataxia and laboured breathing.

Following inhalation, animals were observed for 21 days. Clinical signs during exposure were consistent with the inhalation of an irritant aerosol. Post-exposure clinical signs included brown staining and wet fur around the snout, lethargy, peripheral vasodilation, laboured breathing and death. Exaggerated respiratory movements and noisy respiration were evident for most of the observation period.

3.1.2.1 HUMAN DATA

Based on clinical observation of workers, the chemical acts as a gastrointestinal irritant[9]. This effect was ascribed to its caustic nature. The chemical was also reported as a potent dermatologic sensitiser of workers in plastic manufacturing.[4]

3.1.3 REPEATED DOSE TOXICITY[13]

There is one repeated dose oral toxicity study, conducted with rats. Further data on the effects of repeated oral doses of the chemical are reported as part of a reproduction/developmental toxicity study.

Table 3. Repeated dose toxicity studies

Species	Dose	NOEL (NOAEL)	Principal toxic effect	Reference
Rat	0, 10, 40, 150, 600 mg/kg b.w./day, by gavage for 28 days	150 mg/kg b.w./day	Ulceration of the gastric membranes	(13)
Rat	0, 50, 150, 450 mg/kg b.w./day by gavage for 41 to 48 days	Males: 50 mg/kg b.w./day; Females: 150 mg/kg b.w./day	Ulceration of the stomach	(14)

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In the 28 day study, conducted according to Japanese guidelines (equivalent to OECD TG 407), six animals of each sex were dosed orally, by gavage, once daily. Extra groups at 0 and 600 mg/kg b.w./day were sacrificed 14 days after the last dose to assess recovery.

One male and four females in the 600 mg/kg b.w./day dose group died between days 15 to 19. There were no other deaths. Clinical signs in the highest dose group included increased salivation, decreased movement, and piloerection. Males in the highest dose group showed a reduction in bodyweight gain during treatment, but recovered during the recovery period.

Increases in urinary protein, neutrophil count, prothrombin time and inorganic phosphorus were noted in males, as were serum triglycerides in females in the highest dose group. Decreases in haemoglobin concentration, haematocrit and activated partial thromboplastin time were also observed in males in the highest dose group of 600 mg/kg b.w./day.

Treatment-related changes occurred in the stomach, adrenals, and bone marrow. As would be predicted from the corrosive nature of the chemical and the method of administration, deep ulcers were observed in the anterior gastric mucous membrane of all animals given the highest dose. Pathological findings included ulceration, acanthosis and hyperkeratosis in the non-glandular mucosa of the stomach, caecal dilatation and hypertrophy of the adrenal cortex in the highest dose group. Abnormalities were also detected in the adrenals, including vacuolation of the cortex, too. Six animals in the highest dose group also showed a slight increase in granulocytic haematopoietic cells in the bone marrow. These effects disappeared or were substantially reduced in the recovery period. Based on these results, the site of primary damage was the stomach mucous membrane.

As result, the NOAEL (oral, rat, 28 day) was 150 mg/kg b.w./day.

3.1.4 GENETIC TOXICITY

3.1.4.1 GENOTOXICITY IN VITRO

Table 4. Genotoxicity studies.

Type of test	Test system	Dose	Result	Reference
Bacterial test (reverse mutation)	*S. typhimurium* TA 98, TA100, TA 1535, TA 1537; *E. coli* WP2uvrA	Six doses between 0-5 mg/plate	Negative, with and without metabolising system	(15)
In vitro mammalian	CHL/IU cells	80-330 ug/ml (-S9), 120-470 ug/ml (+S9)	Negative with and without metabolising	(16)

cytogenetics assay			system, but insufficient cells analysed in top group because of cytotoxicity	
	CHO cells	75-450 ug/ml (-S9), 200-800 ug/ml (+S9)	Negative with and without metabolic activation	(17)
Micronucleus test	Mouse	750 mg/kg b.w. by intubation	Negative	(18)

The chemical has been tested for reverse mutation in *Salmonella typhimurium* and *Escherichia coli* with and without exogenous metabolic activation according to standard Japanese test methods in full compliance with OECD TG 471 and 472.[15] The highest dose level is the maximum level required by the protocol in the absence of precipitation and cytotoxicity. Positive controls [2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide, sodium azide, 9- aminoacridine and 2-aminoanthracene] gave the expected response. The tests were negative, both in the presence and absence of a metabolising system. [15]

An *in vitro* mammalian cytogenetics study[15] was conducted in accordance with standard Japanese guidelines similar to OECD TG 473. The highest dose level was cytotoxic. Positive controls [mitomycin C, cyclophosphamide] gave the expected response (chromosomal aberrations). In the top dose group insufficient cells were analysed because of cytotoxicity.
The chemical was not clastogenic under the conditions of the assay.

A second *in vitro* mammalian cytogenetics assay was conducted in CHO cells[17] in accordance with OECD TG 473. Dose levels were 75 to 800 ug/ml, both in the presence and absence of a metabolic activation system. The maximum dose without cytotoxicity was 330 ug/ml. Positive controls (ethylmethane sulphonate and cyclophosphamide) gave the expected results. Although there was a statistically significant increase in chromosomal aberrations in the presence of S9 there was no dose-response relationship and the increase was not biologically significant. The chemical was not clastogenic under the conditions of the test.

3.1.4.2 GENOTOXICITY IN VIVO[18]

A mouse micronucleus assay (OECD TG 474) was undertaken. The animals received 750 mg/kg b.w. orally, by intubation. Higher doses resulted in significant mortality. Bone marrow smears were obtained from animals sacrificed at 24, 48 or 72 hours. The positive control (cyclophosphamide) gave the expected result. The chemical was negative in the test.

3.1.5 Carcinogenicity

There is no information available.

3.1.6 REPRODUCTION/DEVELOPMENTAL TOXICITY[14]

A reproduction/developmental toxicity screening test (OECD TG 421) was performed with rats. Both males and females (12 plus 12 per dose group, 96 in total) received the chemical orally, by gavage for 41 to 48 days. One male in the 150 mg/kg b.w./day group died on Day 38, and three males in the 450 mg/kg b.w./day group died on days 31, 38, and 39. One female in the 450 mg/kg b.w./day group died on day two of pregnancy. Bodyweight gain in test animals was lower than in the controls. On pathological examination, various changes in the stomach, such as ulceration of the fore-stomach and squamous epithelial hyperplasia associated with hyperkeratosis, were seen in

males and females of the 450 mg/kg group. The NOAEL was 50 mg/kg b.w./day for males and 150 mg/kg b.w./day for females.

Test chemical administration had no influence on mating ability, fertility, duration of oestrus or duration of pregnancy and parturition. Bodyweight gain of offspring was not affected by the test chemical and no abnormalities were seen on external examination at birth. Only sporadic differences were observed at necropsy. The NOAEL for reproductive/developmental toxicity (F1 offspring) was therefore 450 mg/kg b.w./day.

3.1.7 Other health information

Skin irritation was investigated in an apparently well-conducted and reported study,[19] conducted before Good Laboratory Practice was compulsory. Both rats and mice (three male and three female for each species) were exposed to the chemical for up to four hours at a dose rate of 1 ml/kg b.w./day spread over an area of 1 cm^2, although the site of application was not covered with an occlusive dressing. In the rat, subcutaneous haemorrhage occurred within three minutes, necrosis was evident after five minutes, and the test site was highly necrotic by 60 minutes. In the mouse, no effects were immediately apparent after application of the test chemical, but necrosis was evident after 10 to 20 minutes.

The chemical was a sensitiser when tested in a guinea pig maximisation test.[12] Dose levels of 2 %v/v and 1 %v/v in water were selected for the challenge from the induction studies. The chemical produced a 7/10 sensitisation rate and induced erythema (grade 2) and oedema (grade 1) at 24 hours. At 48 hours oedema and erythema were both grade 1, but desquamation and scabbing, along with other skin irritation reactions, were observed.

3.2 INITIAL ASSESSMENT FOR HUMAN HEALTH

The initial assessment for human health can be found in Section 5.1.2.2, Human Health Hazards.

4. HAZARDS TO THE ENVIRONMENT

4.1 AQUATIC EFFECTS

In the following table the results from acute and chronic tests with aquatic organisms are presented.

Table 5. Acute and chronic studies in aquatic organisms

Organism	Test duration	Result (mg/L)	Reference
fish			
Medaka (Oryzias latipes)	96 hours (ss)	LC_{50} (96 h) = 87.6 mg/L	(20)
Golden Orfe	96 hours (ss)	LC_{50} (96 h) = 75 mg/L	(20)
Rainbow trout	96 hours (s)	LC_{50} (96 h) > 100 mg/L	(20)
Invertebrates	48 hours (s)	EC_{50} (immobilisation) = 15.2 mg/L	(21)
Water Flea	48 hours (s)	EC_{50} (immobilisation) = 16 mg/L	(21)
(*Daphnia magna*)	21 days (ss)	EC_{50} (reproduction) = 6.77 mg/L LC_{50} (parent) = 8.4 mg/L NOEC (reproduction) = 4.7 mg/L	(22)
Green algae			
Selenastrum capricornutum	72 hours (s)	E_bC_{50} (biomass, 0 to 72 h) = 20.3 mg/L $NOEC_b$ (0 to 72 h) = 10.5 mg/L E_rC_{50} (growth rate, 24 to 72 h) = 33.3 mg/L $NOEC_r$ (24 to 72 h) = 22.9 mg/L	(23)
Scenedesmus subspicatus	72 hours (s)	E_bC_{50} (biomass, 72 h) = 12 mg/L E_rC_{50} (growth rate, 0 to 24 h) = 14 mg/L $NOEC_b$ (0 to 72 h) = 6.25 mg/L	(23)

(s): Static conditions
(ss): Semi-static conditions

4.1.1 TERRESTRIAL EFFECTS

There is no available information.

4.3 OTHER ENVIRONMENTAL EFFECTS

There is no available information.

4.4 INITIAL ASSESSMENT FOR THE ENVIRONMENT

The chemical is not readily biodegradable (49%, OECD 301B) or inherently biodegradable (MITI II, corresponding to OECD 302C: BOD = 22%, TOC = 6% and analysis in HPLC = 21%). It does not bioaccumulate (BCF < 0.3 and < 2.7 at 2 and 0.2 mg/L, respectively).

Acute toxicity data were available for three kinds of fish (Medaka, $96hLC_{50}$ = 87.6 mg/L; Golden orfe, $96hLC_{50}$ = 75 mg/L and Rainbow trout, $96hLC_{50}$ >100 mg/L). In *Daphnia magna*, acute toxicity values of $48hEC_{50}$ = 15.2 mg/L and $48hEC_{50}$ = 16 mg/L were reported. The chronic

toxicity data for *Daphnia magna* were 6.77 mg/L EC_{50} (21d, reproduction inhibition) and 4.7 mg/L NOEC (21d, reproduction inhibition). The parential toxicity for *Daphnia magna* was 8.4 mg/L 21d LC_{50}. The results in algae were E_bC_{50} = 12 mg/L and $NOEC_b$ (0 to 72 h) = 6.25 mg/L (*Scenedesumus subspicatus*) and E_bC50 = 20.3 mg/L and $NOEC_b$(0 to 72h)=10.5 mg/L (*Selenastrum capricornutum*).

The predicted no effect concentration (PNEC) of 0.047 mg/L is estimated from the lowest chronic value (NOEC of 4.7mg/L, *D.magna* reproduction), by applying an assessment factor of 100 because two chronic studies are available (that is, in algae and daphnia).

5 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

5.11 PHYSICAL/CHEMICAL PROPERTY, PRODUCTION, USE AND DISTRIBUTION

The production volume of the chemical in Japan is ca. 13 000 t/y (1999 – 2000). The chemical is an intermediate in the production of epoxy curing agents, polyamides and polyurethanes. There are several grades and types of epoxy resin. Due to the chemical binding processes that occur during curing, finished products do not contain the chemical. Hence consumers will not be exposed to the chemical.

5.1.2 HUMAN HEALTH

5.1.2.1 EXPOSURE

The manufacture of resins and other compounds using MXDA as intermediates are conducted in closed systems, and therefore worker exposure is minimal. Occupational exposure limit values are set world-wide as 0.1 mg/m^3 (15 min STEL).

In a model workshop system,[8] airborne concentrations of the chemical varied from 0.064 to 0.229 mg/m^3 in the absence of ventilation, and 0.018 to 0.051 mg/m^3 when ventilation was provided.
The EASE model gave a vapour concentration (LEV, non-dispersive use, low tendency to become airborne) as 2.8 to 16.7 mg/m^3 and a dermal adsorption rate (non-dispersive use, indirect handling) as less than 0.1 mg/cm^2/day. For inhalation exposure, the expected human exposure (inhalation) would be EHEinh =0.0073 mg/kg/day based on the highest vapour concentration of 0.051 mg/m^3 in the model workshop system.

If absorption occurred through hands and forearms, the calculated EHEder would be 0.03 mg/kg/day. As the chemical is corrosive and a skin sensitiser, risk reduction measures make the expected human exposure (dermal) much less than this estimation.

5.1.2.2 HUMAN HEALTH HAZARDS

There is no information on toxicokinetics. The toxicity of this chemical is entirely consistent with its corrosiveness at the site of first contact.

The oral LD_{50} of rats was 1090 mg/kg for males and 980 mg/kg for females [OECD TG 401] and the inhalation LC_{50} (4 h) was 0.8 mg/L for female rats. The toxicity via oral administration and inhalation was tissue damage in the digestive and respiratory organs, respectively, which are the first contact sites. The chemical is corrosive to rat and mouse skin and a sensitiser in the guinea pig maximisation test.

In a 28-day repeated dose toxicity study [OECD TG 407], the chemical was given to rats by gavage at doses of 0, 10, 40, 150 and 600 mg/kg b.w./day. One male and four females died, and salivation, low locomotor activity and piloerection were noted in the 600 mg/kg group. Furthermore, ulceration, acanthosis with hyperkeratosis and submucosal inflammation were observed in the forestomach. No adverse effects were observed in 150 mg/kg and the lower dose groups.
On the other hand, a reproductive/developmental toxicity screening test [OECD TG 421] with rats by gavage at 50, 150 and 450 mg/kg b.w./day for at least 41 days resulted in death in one male in the 150 mg/kg group and to three males and one female in the 450 mg/kg group.

In almost all 450 mg/kg animals, the same histopathological changes as in the above described 28-day study were observed in the forestomach. No adverse effects were found at 50 mg/kg/ b.w./day. Based on these information, the NOAEL for repeated dose toxicity is considered to be 50 mg/kg b.w./day.

In the above described reproductive/developmental toxicity screening test [OECD TG 421] the substance was administered from 14 days before mating to 20 days after mating in males and to day 3 of lactation in females. No adverse effects were observed in terms of copulation, fertility, delivery and nursing of parents, and the viability, body weight and morphology of offsprings. The NOAEL for reproductive/developmental toxicity was 450 mg/kg b.w./day.

The chemical is not mutagenic in bacteria [OECD TG 471 & 472]. It induced neither chromosomal aberration in mammalian cells *in vitro* [OECD TG 473] nor micronuclei in mouse bone marrow *in vivo* [OECD TG 474].

Based on clinical observations of workers during the manufacturing process, the chemical appears to act as a gastrointestinal irritant. It has been also shown to cause contact sensitisation reactions in workers at concentrations equal to and below 0.1 mg/m^3 (Threshold limit value of US in occupational place).

5.1.3 ENVIRONMENT

The chemical has a log Pow value of 0.18 at 25 °C, a vapour pressure of 0.04 hPa at 25 °C, and a water solubility of > 100 000 mg/L. Fugacity model Mackay level III calculations suggest that the majority of the chemical would distribute to soil if released to soil and/or air compartment(s), and water if released to the aquatic compartment. The chemical is ionised at environmental pHs and will have a positive charge. Cations are able to strongly adsorb to soil surfaces and, therefore, have a low mobility. The chemical is not readily biodegradable (BOD 22%), and it does not hydrolyse (half life >1 y at 25 °C). The substance appears significantly degradable in a standard ready biodegradability test. While this is not supported by the results of a MITI II inherent biodegradability test, it does nevertheless suggest that degradation may occur in waste-water treatment, and possibly also the environment. The chemical does not bioaccumulate (BCF < 2.7 at 0.2 mg/L). Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 5.39 hours. The chemical will also react with carbon dioxide to form the carbamate acid and will therefore not persist in the atmosphere.

Acute toxicity data were available for three kinds of fish (Medaka, LC_{50} = 87.6 mg/L; Golden orfe, LC_{50} = 75 mg/L and Rainbow trout, LC_{50} >100 mg/L). In *Daphnia magna*, acute toxicity values of 48hEC_{50} = 15.2 mg/L and 48hEC_{50} = 16 mg/L are reported. The chronic toxicity data for *Daphnia magna* were 8.4 mg/L EC_{50} (21d, reproduction) and 4.7 mg/L NOEC (21d, reproduction). The results in algae were ErC_{50} = 12 mg/L and $NOEC_b$ (0 to 72 h) = 10.5 mg/L.

The predicted no effect concentration (PNEC) of 0.047 mg/L is estimated from the lowest chronic value (NOEC of 4.7mg/L, *D. magna* reproduction), by applying an assessment factor of 100 because two chronic studies are available (that is, in algae and daphnia).

5.2 RECOMMENDATIONS

The chemical is currently of low priority for further work.

6. REFERENCES

(1) Manufacturers safety data sheet, 1997, Mitsubishi Gas Chemical Company

(2) HEDSET submission 1995

(3) Specific gravity data from the test material supplier was converted from d20/20 by JS. Report No.: K2249 (1987)

(4) Value of 15 mmHg in Sigma-Aldrich Library of Regulatory and Safety Data was converted into the unit of hPa. Calculated value at 25 °C is 0.04 hPa Supplements to the Sixth Edition, Documentation of the Threshold Limit Values and Biological Exposure Indices, Sixth Edition, 1991, ACGIH Worldwide, Cincinnati, Ohio, USA.

(5) Level III model for Fugacity, Trent University, Canada

(6) Mead C (1998) Meta-Xylenediamine: Assessment of ready biodegradability; CO_2 evolution test, SafePharm Laboratories, Project No: 930/019. (unpublished). METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan).

(7) METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan). Test No.: 80245

(8) Determination of m-xylylene diamine concentration in air of a model workroom. Toray Research Center Inc. 1991 (Japan) (unpublished).

(9) Supplements to the Sixth Edition, Documentation of the Threshold Limit Values and Biological Exposure Indices, Sixth Edition, 1991, ACGIH Worldwide, Cincinnati, Ohio, USA.

(10) Acute oral toxicity test of meta-xylenediamine & 1,3-bis aminomethyl cyclohexane Mitsubishi Gas Chemical Company, October 8, 1980 (unpublished).

(11) Jackson GC, Molloy GK, Hardy CJ, Gregson RL, Offer JM, Gopinath C (1993) MXDA: Acute inhalation toxicity in rats 4-hour exposure. Huntingdon Research Centre Ltd., Report No.: MGC 2/921548 (unpublished).

(12) Allen DJ (1998) MXDA: Magnusson & Kligman maximisation study in the guinea pig, SafePharm Laboratories, UK, Project No: 930/039 (unpublished).

(13) MHW, Japan (1996), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (387 – 394).

(14) MHW, Japan(2001), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (971 – 984).

(15) MHW, Japan(1996), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (395 – 398).

(16) MHW, Japan(1996), Ministry of Health and Welfare, Toxicity Testing Reports of EnvironmentalChemicals, Vol.3 (399-401).

(17) Enninga IC, (1989) Evaluation of the ability of Metaxylenediamine to induce chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells (including multiple fixation times), RCC Notox B.V. Report No. 017324

(18) Enninga IC, (1990) Micronucleus test in bone marrow cells of the mouse with metaxylenediamine, RCC Notox B.V. Report No. 017313

(19) MXDA: a skin corrosive test of metaxylene diamine founded on the Standards of the United Nations. Niigata Laboratories, Mitsubishi Gas Chemical Company (1986) (unpublished).

(20) MOE, Japan (2000) Ministry of Environment : unpublished report of Acute toxicity study to Medaka (*Oryzias latipes*) on 1,3-benzenedimethanamine P. Wetton, Meta-Xylenediamine: acute toxicity to Golden Orfe (*Leuciscus Idus*), SafePharm Laboratories, UK, report No: 930/018 (1988) (unpublished). P Wetton, Acute toxicity to Rainbow Trout, SafePharm Laboratories, UK, report 930/003 (1995) (unpublished).

(21) MOE, Japan (2000) Ministry of Environment : unpublished report of Acute immobilisation test to *Daphnia magna* on 1,3-benzenedimethanamine. Wetton P, Acute toxicity to *Daphnia Magna*, SafePharm Laboratories, report 930/002 (1995) (unpublished).

(22) MOE, Japan (2000) Ministry of Environment : unpublished report of A reproduction-inhibition study to *Daphnia magna* on 1,3-benzenedimethanamine.

(23) MOE, Japan (2000) Ministry of Environment : unpublished report of A growth–inhibition study to algae (*Selenastrum capricornutum*) on 1,3-benzenedimethanamine. C Mead, Algal inhibition test, SafePharm Laboratories, report 930/001 (1995) (unpublished).

SIDS Dossier

Existing Chemical	:	ID: 1477-55-0
Memo	:	ICCA HPV 2001
CAS No.	:	1477-55-0
EINECS Name	:	m-phenylenebis(methylamine)
EC No.	:	216-032-5
Synonym	:	1,3-Benzenedimethanamine, MXDA
Molecular Formula	:	C8H12N2

Producer related part

Company	:	Safepharm Laboratories
Creation date	:	26.02.2001

Substance related part

Company	:	Safepharm Laboratories
Creation date	:	26.02.2001
Status	:	
Memo	:	MXDA: ICCA2001
Printing date	:	28.06.2001
Revision date	:	
Date of last update	:	28.06.2001
Number of pages	:	93
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
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 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
Name	:	MITSUBISHI GAS CHEMICAL COMPANY IN C.
Contact person	:	Mr Seki
Street	:	2-5-2, Marunouchi, Chiyoda-ku
Town	:	100-8324 Tokyo
Country	:	Japan

27.02.2001

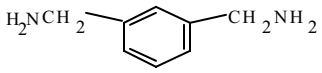
1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

Name	:	Ministry of Foreign Affairs, Japan

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	1,3-bis(aminomethyl)benzene
Smiles Code	:	H_2NCH_2-C_6H_4-CH_2NH_2
Molecular formula	:	$C_8H_{12}N_2$
Molecular weight	:	136.22



27.06.2001

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organic
Physical status	:	liquid
Purity	:	>= 99 % w/w
Colour	:	colourless
Odour	:	None stated

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

1,3-benzenedimethanamine
27.02.2001

1,3-bis(aminomethyl)benzene
27.02.2001

1,3-bis-aminomethylbenzene (Czech name)
27.02.2001

m-phenylenebis(methylamine)
27.02.2001

m-xylene-alpha,alpha'-diamine (ACGIH and OSHA)

27.02.2001

m-xylylendiamine (Czech name)
27.02.2001

m-xylylenediamine
27.02.2001

Methylamine, m-phenylenebis
27.02.2001

MXDA
27.02.2001

1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No : 100-81-2
EC-No : 202-890-8
EINECS-Name : 3-methylbenzylamine
Value : ca. .001 % w/w
27.02.2001

Purity : typical for marketed substance
CAS-No : 27129-87-9
EC-No : 248-241-2
EINECS-Name : 3,5-dimethylbenzyl alcohol
Value : ca. .07 % w/w
27.02.2001

Purity : typical for marketed substance
CAS-No : 6966-10-5
EC-No : 230-175-0
EINECS-Name : 3,4-dimethylbenzyl alcohol
Value : ca. .05 % w/w
27.02.2001

Purity : typical for marketed substance
CAS-No : 16308-92-2
EC-No : 240-393-8
EINECS-Name : 2,4-dimethylbenzyl alcohol
Value : ca. .08 % w/w
27.02.2001

Purity : typical for marketed substance
CAS-No : 7732-18-5
EC-No : 231-791-2
EINECS-Name : Water
Value : ca. .02 % w/w
03.05.2001

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : ca. 13000 - tonnes manufactured per annum
03.05.2001

1.6.1 LABELLING

Labelling : as in Directive 67/548/EEC
Specific limits : no
Symbols : C
R-Phrases : (20) Harmful by inhalation
(22) Harmful if swallowed
(34) Causes burns
(43) May cause sensitisation by skin contact
S-Phrases : (24) Avoid contact with skin
(26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(28) After contact with skin, wash immediately with plenty of…(to be specified by the manufacturer
(36/37/39) Wear suitable protective clothing and eye/face protection
(45) In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Labelling : Provisionally by manufacturer / importer
Specific limits : no
Symbols : C
R-Phrases : (22) Harmful if swallowed
(34) Causes burns
S-Phrases : (7) Keep container tightly closed
(24/25) Avoid contact with skin and eyes
(26) In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
(36/39) Wear suitable protective clothing and eye/face protection
Country : EU
Remark : TLV 0.1mmg/m3 (ACGIH, USA)
27.02.2001

1.6.2 CLASSIFICATION

Classified : as in Directive 67/548/EEC
Class of danger : corrosive
R-Phrases : (20) Harmful by inhalation
(22) Harmful if swallowed
(34) Causes burns
(43) May cause sensitisation by skin contact
Country : EU
27.02.2001

1.6.3 PACKAGING

Memo : Transport code: UN No. 2735; Class 8 (Corrosive material); Packing group 2

27.02.2001

Memo : Can: 18 kg net. Drum can: 200 kg net. ISO container: 20 000 kg net.
27.02.2001

1.7 USE PATTERN

Type of use	:	industrial
Category	:	Chemical industry: used in synthesis
27.02.2001		
Type of use	:	type
Category	:	Use resulting in inclusion into or onto matrix
Remark	:	The substance is an intermediate used in the production of epoxy curing agents, polyamides, and polyurethanes.

27.02.2001 (13)

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit	:	MAC (NL)
Limit value	:	.1 mg/m3
Remark	:	Ceiling limit = 0.1 mg/m3. Defined Oct 1997 Other Exposure Limits: Australia: TWA = 1 mg/m3 Jan 1993 Belgium: STEL= 0.1 mg/m3 Jan 1993 Denmark: TWA = 0.02ppm (0.1mg/m3) Skin Jan 1993 Finland: TWA = 0.1 mg/m3 Skin Jan 1993 France: STEL = 0.1 mg/m3 Jan 1993 Switzerland: TWA = 0.1 mg/m3 Skin Jan 1993

27.02.2001

Type of limit	:	TLV (USA)
Limit value	:	.1 mg/m3
Remark	:	TVL-C: Should not be exceeded in any part of the work place. Reference: ACGIH-Threshold limit values (1992-1993)

27.02.2001

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

Remark	:	The substance is not classified as being in one of the categories of substances which are covered by Directive

82/502/EEC (The Seveso Directive).

19.03.2001

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type	:	EINECS
Additional information	:	EINECS number: 2160325

27.02.2001

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure	:	Human: exposure through intended use
Exposure to the	:	Substance
Remark	:	The main source of pollution is believed to be emission in the place of use, for example civil engineering and construction, where the substance is used as an epoxy curing agent. Production process: The substance is made by ammoxidation of m-xylene, followed by hydogenation. Exposure to the substance is considered to be minimal. In clinical observation of workers during the manufacturing process, the substance appears to act as a gastrointestinal irritant. (Morgan, J.F. (24/04/1973) Letter to TLV Committee from E.I. du Pont de Nemours & Co., Haskell Laboratory for Toxicology and Industrial Medicine, Wilmington, DE.) In a model workshop system, airborne concentrations varied from 0.064 to 0.229 mg/m^3 without ventilation, and 0.018 to 0.051 mg/m^3 with ventilation. Reference: Determination of m-xylylenediamine concentration in the air of a model workroom, Toray Research Center Inc. (1991) Manufacturers report. The substance is an intermediate in the production of epoxy curing agents, polyamides and polyurethanes. Using the EASE model for predicting worker exposure: For dermal exposure to a liquid which is included onto a matrix and non-dispersive use for incidental handling, the exposure level is predicted to be 0 – 0.1 mg/cm2/day. The substance has a saturated vapour concentration which is lower than the Threshold Limit Value, therefore, inhalation exposure should be addressed: The pattern of control is not considered to be full containment, with a low tendancy to become airborne and LEV is used, inhalation exposure to the vapour is predicted to be 0.5 – 3.0 ppm (2.8 – 16.7 mg/m3)

27.06.2001 (10)

Source of exposure	:	Environment: exposure from production
Exposure to the	:	Substance

27.06.2001

Source of exposure	:	Environment: exposure from production
Exposure to the	:	Degradation/Transformation Products
Remark	:	The substance will ionise in water at environmental pHs

27.06.2001

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search	:	External
Chapters covered	:	3, 4, 5
Date of search	:	04.11.1999
28.02.2001		

1.13 REVIEWS

Memo	:	RTECS: PF8970000
Remark	:	Contents of RTECS review:

International Occupational Exposure Values:
Values given in Section 1.8.1

General information:
Synonyms
Structure
Classification

Chemical and Physical properties:
Colourless liquid with faint ammoniacal odour
Specific gravity, boiling point, freezing point, vapour pressure, flash point, solubility, decomposition products.

Sources of occupational exposure.

Toxicity studies:
Acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, skin irritation, skin sensitisation.
In-vivo mouse micronucleus, In-vivo chromosome aberration, In-vitro chromosome aberration.

Human studies:
Worker observations: irritation and sensitisation.

Recommendations for occupational exposure.

09.03.2001

2.1 MELTING POINT

Value	:	= 14.1 °C
Method	:	other
Year	:	1994
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	Freezing point determination of a liquid. METHOD FOLLOWED: Unknown method GLP: No GLP specification given
Reliability	:	(2) valid with restrictions Test data derived from biodegradability test data from MITI evaluation of existing chemicals. Secondary literature review: RTECS Review.
Flag	:	Critical study for SIDS endpoint

27.06.2001 (29)

2.2 BOILING POINT

Value	:	= 273 °C at 1013 hPa
Decomposition	:	ambiguous
Method	:	other
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	RESULTS: HEDSET: ca. 274 deg C (no decomposition) SDS: 273 deg C RTECS: > 200 deg C (decomposition from 250 deg C)
Reliability	:	(2) valid with restrictions Secondary literature review
Flag	:	Critical study for SIDS endpoint

27.06.2001 (20)

2.3 DENSITY

Type	:	relative density
Value	:	= 1.052 at 20 °C
Method	:	other: pycnometer
Year	:	1994
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(2) valid with restrictions Secondary literature review

27.06.2001 (26)

2.3.1 GRANULOMETRY

Method	:	Method not applicable to liquids.

28.02.2001

2.4 VAPOUR PRESSURE

Value	:	20 hPa at 145 °C
Method	:	other (measured)
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(2) valid with restrictions Secondary literature review
Flag	:	Critical study for SIDS endpoint
27.06.2001		(25)

Value	:	ca. 4 hPa at 121 °C
Method	:	other (measured): no information available
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(4) not assignable Secondary literature review
02.05.2001		(27)

Value	:	.01 hPa at 25 °C
Method	:	other (calculated)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	MPBPWIN v 1.31, for Windows. Computer estimation
Result	:	A prediction of vapour pressure at 145°C is 16.2 mmHg (2159 hPa). This results correlates with the literature figure given. No validation against known substances of similar structure was performed. The programme uses boiling point and structure to predict the vapour pressure. Therefore, a computer estimate of the vapour pressure at 25°C (0.00783 mm Hg = 0.01 hPa) can be considered to be scientifically valid. Further calculations have estimated the vapour pressure at 25°C to be 0.04 hPa. (Reference: Documentation of the Threshold Limit values and Biological Exposure Indices, Supplements to the Sixth Edition 1991)
Reliability	:	(2) valid with restrictions The calculation method has been proved to be unreliable for certain substances. This result should be viewed with discretion.
Flag	:	Critical study for SIDS endpoint
09.05.2001		(22)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	ca. .18 at 25 °C
pH value	:	ca. 10.3
Method	:	OECD Guideline 107 „Partition Coefficient (n-octanol/water), Flask-shaking Method“
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	Guideline study: Shake Flask Method No deviations noted

Test condition	:	Test substance concentration: 6.63 mg Rotation and time duration of shaking: 20 times/min for 5 mins Number of Replica: 2 Analysis: HPLC
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint
30.04.2001		(28)

Partition coefficient	:	octanol-water
Log pow	:	ca. .15 at °C
Method	:	other (calculated): KOWWIN v 1.65
Year	:	2001
GLP	:	no
Reliability	:	(2) valid with restrictions The calculated value accords with the experimental value. No validation against known substances of similar structure was performed.
09.05.2001		(22)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	> 100000 mg/L at 25 °C
Method	:	OECD Guideline 105
Year	:	1999
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(1) valid without restriction Guideline study: Flask method and visual inspection
Flag	:	Critical study for SIDS endpoint
14.05.2001		(28)

Solubility in	:	Water
Value	:	ca. 6.6 g/L at °C
Method	:	other: calculation method (WSKOW v. 1.36)
Year	:	2000
GLP	:	no
Reliability	:	(4) not assignable The calculation method has been proved to be unreliable for certain substances. No validation against known substances of similar structure was performed.
30.04.2001		(22)

Result	:	SOLUBLE: Reported soluble in water, ether, benzene. No specific result given INSOLUBLE: n-hexane, cyclohexane, isoctane
Reliability	:	(4) not assignable Secondary literature review: manufacturer data
30.04.2001		(19)
		(20)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value	:	= 134 °C
Type	:	open cup
Method	:	other: no information available
Year	:	1994
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	RESULTS: HEDSET: = 134 deg C SDS: 139 deg C RTECS: > 112 deg C (Closed cup)
Reliability	:	(4) not assignable Secondary literature review
09.05.2001		(7)

Value	:	248 °C
Method	:	other: method not detailed
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(2) valid with restrictions Secondary literature review
09.05.2001		(6)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

Result	:	non flammable
Method	:	other: Flash point determination
Year	:	1994
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	The substance is a liquid, therefore a flash point determination is applicable for determination of flammability.
Result	:	RESULT: Air: The substance is not flammable in air Contact with water and pyrophoric properties of solids and liquids: Based upon the known chemical and physical properties o f the substance and its chemical structure, negative results are predicted for both these flammability tests. Secondary literature review: manufacturer data
01.03.2001		(19)

2.10 EXPLOSIVE PROPERTIES

Result	:	not explosive
Method	:	other: prediction.
Method	:	METHOD: Prediction based upon structural considerations and knowledge

from manufacture and use.
Result : The substance is predicted not to have explosive properties.
Reliability : (4) not assignable
Secondary literature review: manufacturer data
01.03.2001 (19)

2.11 OXIDIZING PROPERTIES

Result : no oxidizing properties
Method : other: prediction.
Method : METHOD: Current EU methods for determination of oxidising properties are not applicable to liquids.
Prediction based upon structural considerations and knowledge from manufacture and use.
Result : The substance is predicted not to have oxidising properties.
Reliability : (4) not assignable
Secondary literature review: manufacturer data
01.03.2001 (17)

2.12 ASSOCIATION CONSTANT

Acid-base constant : pKa ca. 9.19 at 25°C
Method : OECD Guideline 112
Year : 1999
GLP : no data
Test substance : as prescribed by 1.1 – 1.4
Method : Dissociation constant in water: Titration method
Result : Measured results: 9.22, 9.18 and 9.16.
The substance contains two groups which can dissociate. Only one pKa value was measured. This may possibly be due to the two pKa values being so close that only one measurement was recorded.
Reliability : (1) valid without restriction
Guideline study
30.04.2001 (28)

2.13 VISCOSITY

Test type : other: no information available
Test procedure : No information available
Value : = 6.8 - mPa s (dynamic):centipoise at 20 °C
Year : 1995
GLP : no data
Test substance : as prescribed by 1.1 – 1.4
Reliability : (4) not assignable
Secondary literature review: manufacturer data
09.03.2001 (19)

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Relative intensity	:	based on intensity of sunlight
Indirect Photolysis:		
Halflife t1/2	:	ca. 5.4 hour(s)
Type of reactant	:	OH
Concentration	:	5×10^{11} molecules / m^3 in atmosphere
Method	:	other (calculated using EUSES v1.00)
Year	:	2001
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(2) valid with restrictions Basic data given, comparable to guidelines/standards.

28.06.2001 (9)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	> 1 year at °C
t1/2 pH7	:	> 1 year at °C
t1/2 pH9	:	> 1 year at °C
Method	:	OECD Guideline 111 "Hydrolysis as a Function of pH"
Year	:	1999
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Although the substance does not hydrolyse, it will ionise at environmental pHs
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

26.06.2001 (28)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III

Table 1: Summary of emissions for release to separate compartments

	1000 kg/h emission to these compartments separately		
	Air	**Water**	**Soil**
In air	0.215%	0.00004%	0.005%
In water	38.576%	99.76%	36.138%
In soil	61.121%	0.012%	63.775%
In sediment	0.088%	0.228%	0.082%

Method : Other: Fugacity Model Level III, Trent University
Year : 2001
Method : The Level III model devloped by Trent University, Canada is a refinement of EQC. FUGMOD, EQC and the Level III program are all based upon the same set of models except the default environments differ slightly. More environmental parameters can be controlled in Level III, it was therefore possible to create the EQC default environment within Level III.
Result :

Table 2: Level III model values of environmental fate

Air	Amount	kg	0.255
	Fraction	%	0.0013
	Concentration	ng/m^3	0.0000717
Water	Amount	kg	19519
	Fraction	%	96.0411
	Concentration	ng/L	60.8
Soil	Amount	kg	743
	Fraction	%	3.6541
	Concentration	ng/g	0.00127
Sediment	Amount	kg	61.7
	Fraction	%	0.3035
	Concentration	ng/g	0.0444

Test condition : Amount of chemical: 13000000 kg
Reliability : (1) valid without restriction
Reliable model
Flag : Critical study for SIDS endpoint
28.06.2001 (40)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, non-adapted
Concentration : 17.1 mg/L related to Test substance
Degradation : = 49 (±) % after 28 day(s)
Result : under test conditions no biodegradation observed
Method : OECD 301B and 92/69/EEC Method C.4-C
Year : 1998
Reliability : (1) valid without restriction
Basic data given, comparable to guidelines/standards
Flag : Critical study for SIDS endpoint
02.08.2001 (32)
Type : aerobic
Inoculum : activated sludge, non-adapted
Concentration : 100 mg/L related to Test substance
Degradation : = .4 (±) % after 14 day(s)
Result : under test conditions no biodegradation observed
Method : OECD 301C

Year	:	1993
Reliability	:	(4) not assignable Secondary literature review: manufacturer data
30.04.2001		(18)

Type	:	Aerobic, inherent biodegradation
Result	:	under test conditions no biodegradation observed
Method	:	other: OECD 302C (Modified MITI (2))
Year	:	1983
GLP	:	yes
Test substance	:	As prescribed by 1.1 – 1.4
Result	:	BOD = 22% TOC = 6% Specific compound analysis: 21%
Test substance	:	Batch no: K-245
Conclusion	:	The substance is not readily biodegradable
Reliability	:	(1) valid without restriction Basic data given, comparable to guidelines/standards.
Flag	:	Critical study for SIDS endpoint
27.06.2001		(8)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5		
Method	:	other: no information available
Year	:	1984
Concentration	:	2 g/L related to Test substance
BOD5	:	= 2220 mg/L
GLP	:	no data
COD		
Method	:	other: no information available
Year	:	1984
COD	:	= 25 mg/g substance
GLP	:	no data
Reliability	:	(4) not assignable Secondary literature review
26.06.2001		(13)

3.7 BIOACCUMULATION

Species	:	*Cyprinus carpio* (Fish, fresh water)
Exposure period	:	42 day(s) at °C
Method	:	OECD Guideline 305
Year	:	1984
GLP	:	no data
Test substance	:	As prescribed by 1.1 – 1.4
Result	:	BCF 1: < 0.3 BCF 2: < 2.7
Test condition	:	DILUTION WATER: Flow rate of test water: 582 L/day TEST SYSTEM: Concentrations: 1st dose level = 2 mg/L (ppm), 2nd dose level = 0.2 mg/L (ppm).
Test substance	:	Batch no: K-245
Conclusion	:	The substance does not bioaccumulate

Reliability : (1) valid without restriction
Basic data given, comparable to guidelines/standards.

26.06.2001 (30)

3.8 ADDITIONAL REMARKS

Remark : The substance contains two amine groups which at environmental pHs will ionise to form ammonium groups gaining two positive charges. Cations are able to strongly adsorb to soil surfaces and, therefore, have a low mobility.

27.02.2001

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	*Oryzias latipes* (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/L
LC0	:	= 56
LC50	:	= 87.6
LC100	:	> 100
Analytical monitoring	:	yes
Method	:	OECD Guideline 203 "Fish, Acute Toxicity Test"
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	METHOD FOLLOWED: OECD Guideline 203 (1992) DEVIATIONS FROM GUIDELINE: The pH values in the 18.0 mg/L concentration or higher dose groups were 8.6 to 9.4 which are considered to be slightly high. This was considered to be due to the slightly alkaline nature of the test substance. The study is considered not to be affected by this deviation. GLP: No GLP data but expected to be according to GLP if carried out in accordance with OECD Guidelines. STASTICAL METHOD: Data Analysis: Binomial method for LC50 and 95% confidence limits Method of Calculating Mean Measured Concentrations: Geometric mean ANALYTICAL METHOD: HPLC: Column: Inertsil ODS-3V, 5um, 4.6 x 150 mm Mobile phase: Acetonitrile 10%, 0.1% Phosphoric acid + 10 mM sodium 1-pentanesulfonate 90%. Flow rate: 1 ml/min UV detector: 210 nm Volume: 20 ul Temperature: 40°C
Test condition	:	TEST ORGANISMS: Wild caught: None Age/size/weight/loading: 10 fish per dose level Average length = 1.88 cm (1.61 - 2.22 cm) Average weight = 0.116g (0.074 - 0.189g) STABILITY OF THE TEST CHEMICAL SOLUTIONS: The substance was stable within the conditions of the test. All test concentrations were therefore based on nominal test substance concentrations. REFERENCE SUBSTANCE: None DILUTION WATER: Alkalinity: 48 mg/L Hardness: 60 mg/L pH: 7.6 (22°C) Oxygen content: Conductance: 170 uS/cm Flow rate of test water: 582 L/day TEST SYSTEM: Concentrations: Control, 10.0, 18.0, 32.0, 56.0, 100 mg/L Dosing rate: continuous

Renewal of test solution: Every 24 hours
Exposure vessel type: 5.0 litre tank.
Number of replicates, fish per replicate: 1 vessel per concentration, 10 fish per vessel
Test temperature: 24 +/- 1°C
Dissolved oxygen: 5.1 - 8.4 mg/L
pH: 6.9 - 9.4
Intensity of irradiation: 1000 lux
Photoperiod: 16 hours
DURATION OF TEST: 96 hours
TEST PARAMETER: Mortality
SAMPLING: Every 24 hours
MONITORING OF TEST SUBSTANCE CONCENTRATION:
HPLC: HPLC method as shown in Method freetext.

Result : 56 > LC 50 > 100 mg/L

EXPOSED:
Abnormal swimming was observed in the 100 mg/L group. No abnormal symptoms were seen in the control group during the exposure period.
Other effects: None noted.

CONTROL:
Number of adverse effects: None

Table 1. Measured Concentration of the Test Substance in Test Water (Semi-Static Condition)

Nominal Concentration mg/L	Measured Concentration, mg/L (Percent of Nominal)		Mean[a] Measured Concentration mg/L
	0 Hr (new)	24 Hr (old)	
Control	<0.02	<0.02	-
10.0	9.99	10.1	10.0
	(100)	(101)	(100)
18.0	18.4	18.3	18.3
	(102)	(102)	(102)
32.0	32.8	32.5	32.6
	(103)	(102)	(102)
56.0	58.2	58.0	58.1
	(104)	(104)	(104)
100	103	103	103
	(103)	(103)	(103)

a: geometric mean

new: freshly prepared test solutions
old: test solutions after 24 hours exposure

Table 2. Mortality of the Medaka (*Oryzias latipes*) Exposed to the Test Substance

Nominal Concentration mg/L	Mean[a] Measured Concentration mg/L	Cumulative Mortality (Percent Mortality)			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	-	0 (0)	0 (0)	0 (0)	0 (0)
10.0	10.0	0 (0)	0 (0)	0 (0)	0 (0)
18.0	18.3	0 (0)	0 (0)	0 (0)	0 (0)
32.0	32.6	0 (0)	0 (0)	0 (0)	0 (0)
56.0	58.1	0 (0)	0 (0)	0 (0)	0 (0)
100	103	0 (0)	6 (60)	7 (70)	7 (70)

a: geometric mean

Table 3. Observation of the Highest Concentration in 0% Mortality and the Lowest Concentration in 100 % Mortality

Exposure Period (Hours)	Highest Concentration in 0% Mortality (mg/L)	Lowest Concentration in 100% Mortality (mg/L)
24	>100	>100
48	56.0	>100
72	56.0	>100
96	56.0	>100

Table 4. Observed Toxicological Symptoms

Nominal Concentration	Mean[a] Measured Concentration	Symptoms (Symptom -number of fish)		
mg/L	mg/L	24 Hours	48 Hours	72 Hours
Control	-	N	N	N
10.0	10.0	N	N	N
18.0	18.3	N	N	N
32.0	32.6	N	N	N
56.0	58.1	N	N	N
100	103	AS-10	AS-4	AS-3

a: geometric mean

N: No toxicological symptom was observed
AS: abnormal swimming

Conclusion : The substance is considered to be harmful to fish, with an LC50 < 100 mg/L.
Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
26.06.2001 (4)

Type : semistatic
Species : *Leuciscus idus* (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/L
LC0 : = 56
LC50 : = 75
LC100 : = 100
Analytical monitoring : no
Method : OECD Guideline 203 "Fish, Acute Toxicity Test"
Year : 1998
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Result : Sub-lethal effects of exposure were observed at test concentrations of 200 and 180 mg/L. These responses were swimming at the surface, loss of equilibrium and presence of moribund fish
Conclusion : The substance is considered to be harmful to fish, with an LC50 < 100 mg/L.
Reliability : (2) valid with restrictions
Guideline study but has deviations from present test method (no analysis of test substance).
02.082001 (33)

Type : static
Species : *Oncorhynchus mykiss* (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/L
LC0 : > 100
LC50 : > 100
LC100 : > 100
Analytical monitoring : no
Method : OECD Guideline 203 „Fish, Acute Toxicity Test“
Year : 1995

GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Reliability	:	(2) valid with restrictions Guideline study but has deviations from present test method (no analysis of test substance).

02.082001 (35)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	*Daphnia magna* (*Crustacea*)
Exposure period	:	48 hour(s)
Unit	:	mg/L
EC0	:	= 8.9
EC50	:	= 15.2
EC100	:	= 28
Analytical monitoring	:	yes
Method	:	OECD Guideline 202
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	METHOD FOLLOWED: OECD Guideline 202 „ daphnia sp., Acute immobilisation Test and Reproduction Tes t“ (1984) DEVIATIONS FROM GUIDELINE: pH values for the 16.0, 28.0 and 50.0 mg/L concentration levels were 8.7 or greater which were considered to be slightly high. This was considered to be due to the slightly alkaline nature of the test material. GLP: GLP compliant by order of Japanese Environment Agency. STATISTICAL METHODS: EC50 (48 hours): Moving average ANALYTICAL METHOD: HPLC: Column: Inertsil ODS-3V, 5 um, 4.6 x 150 mm Mobile phase: 0.1% phosphoric acid, 10 mM sodium 1-pentanesulfonate = 10:90 Flow rate: 1.0 ml/min UV detector: 210 nm Sample volume: 20 ul Temperature: 40°C
Test condition	:	TEST ORGANISMS: Species: daphnia STOCK AND TEST SOLUTION AND THEIR PREPARATION: DILUTION WATER: Elendt M4 Medium used as dilution water TEST SYSTEM: Test type: static Concentrations: control, 5.0, 8.9, 16.0, 28.0, 50.0 mg/L Renewal of test solution: No renewal Exposure vessel type: 100 ml vessel Number of replicates, individuals per replicate: 4 replicates per dose level, 20 individuals per replicate. (5 per vessel) Test temperature: 20°C Dissolved oxygen: 8.3 – 8.9 mg/L pH: 7.6 – 9.3 Adjustment of pH:

Intensity of irradiation: 800 lux
Photoperiod: 16 hours
DURATION OF TEST:
TEST PARAMETER: immobilisation
SAMPLING: Every 24 hours.
MONITORING OF TEST SUBSTANCE CONCENTRATION:
HPLC: Method as shown in method freetext

Result : EC50 (24 hour) = 35.1 mg/L EC100 (24 hour) = 50.0 mg/L
EC0 (24 hour) = 16.0 mg/L
EC50 (48 hour): 95% confidence limits = 12.3 – 18.7 mg/L
EC100 (48 hour) = 28.0 mg/L

EXPOSED:
5% immobilisation was observed in the 5.0 mg/L group at the end of the exposure period, however, this was considered to be within the range of spontaneous occurrence since no immobilisation was seen in the 8.9 mg/L group.
CONTROL:
Number/percentage of animals showing adverse effects: None

Table 1. Measured Concentrations of the Test Substance in Test Water (Static Conditions)

Nominal Concentration (mg/L)	Measured concentration (mg/L)				Geometric Mean During 48 Hours (mg/L)
	0 Hour New	Percent of Nominal	48 Hours Old	Percent of Nominal	
Control	<0.02	-	<0.02	-	-
5.00	4.98	100	4.92	98	4.95
8.90	9.03	101	9.04	102	9.03
16.0	15.9	99	15.9	99	15.9
28.0	28.0	100	27.9	100	27.9
50.0	49.6	99	49.7	99	49.6

new: freshly prepared test solutions
old: test solutions after 48 Hours exposure

Table 2. The Numbers of Immobile daphnia (Percent Immobility)

Nominal Concentration (mg/L)	Cumulative Numbers of Immobilized daphnia *(Percent Immobility)*	
	24 Hours	48 Hours
Control	0 (0)	0 (0)
5.00	0 (0)	1 (5)
8.90	0 (0)	0 (0)
16.0	0 (0)	10 (50)
28.0	2 (10)	20 (100)
50.0	20 (100)	20 (100)

Table 3. Calculated EiC50 Values

Exposure Period (Hours)	EiC50 (mg/L)	95-Percent Confidence Limits (mg/L)	Statistical Method
24	35.1	28.0 ~ 50.0	Binomial
48	15.2	12.3 ~ 18.7	Moving average

Table 4. Highest Concentration Resulting in 0% Immobility and Lowest Concentration resulting in 100% Immobility

Exposure Period (Hours)	Highest Concentration Resulting in 0% Immobility (mg/L)	Lowest Concentration Resulting in 100% Immobility (mg/L)
24	16.0	50.0
48	8.90	28.0

Conclusion : The substance is harmful to aquatic organisms, with an LC50 < 100 mg/L, under the conditions of the test
Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
27.06.2001 (3)

Type : static
Species : *Daphnia magna* (*Crustacea*)
Exposure period : 48 hour(s)
Unit : mg/L
NOEC : = 10
EC50 : = 16
EC100 : = 19
Analytical monitoring : no
Method : OECD Guideline (1984)
Year : 1995
GLP : no
Test substance : as prescribed by 1.1 - 1.4
Reliability : (2) valid with restrictions
Guideline study with deviations from present method
27.06.2001 (36)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : *Selenastrum capricornutum* (Algae)
Endpoint : Biomass
Exposure period : 72 hour(s)
Unit : mg/L
NOEC : = 10.5
EC50 : = 20.3
Endpoint : Growth rate
Unit : mg/L
NOEC (24 - 72 h) : = 22.9
EC50 (24 – 72 h) : = 33.3
Analytical monitoring : yes
Method : OECD Guideline 201 "Algae, Growth Inhibition Test"
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Method : METHOD FOLLOWED: OECD Guideline 20 " Alga, Growth -inhibition Test" (1984)

DEVIATIONS FROM GUIDELINE: Concentration of test material after 72 hours was found to decrease with decreased initial test material

concentration. This was thought to be due to transfer of the test material into the algae. Test results were still based on nominal test concentrations.

GLP: GLP compliant by order of Japanese Environment Agency.

METHOD OF CALCULATION:

A = ((N1-N0)/ 2) x t + ((N1 + N2-2N0)/2) x (t2-t1) +...+
((Nn-1 + Nn-2N0)/2) x (tn-tn-1)
IA = (Ac-At)/Ac x 100
u = lnNn-lnN1/tn -t1

ANALYTICAL METHOD: HPLC:
Column: Inertsil ODS-3V, 5 um, 4.6 x 150 mm
Mobile phase: 0.1% phosphoric acid, 10 mM sodium 1-pentanesulfonate = 10:90
Flow rate: 1.0 ml/min
UV detector: 210 nm
Sample volume: 20 ul
Temperature: 40°C

Test condition : TEST ORGANISMS:
Species: *Selenastrum capricornutum*
Initial cell concentration: 1 x 10 E+04 *Selenastrum capricornutum* cells/ml
STABILITY OF THE TEST CHEMICAL SOLUTIONS: The substance appeared to be stable under the conditions of the test.
GROWTH/TEST MEDIUM CHEMISTRY:
EDTA: 0.1 mg/L Na2EDTA.2H2O
TEST SYSTEM:
Test type: static, shaking culture (100 rpm)
Concentrations: control, 1.00, 2.19, 4.78, 10.5, 22.9, 50.0 mg/L
Renewal of test solution: No renewal
Exposure vessel type: 100 ml vessel
Number of replicates: 3 vessels per concentration.
Concentrations: 1 x 10 E+06 cells per vessel
Test temperature: 23 +/- 2°C
Dissolved oxygen:
pH: 7.8 - 10.2
Intensity of irradiation: 4000 lux (within +/- 20% variation near liquid surface)
Photoperiod: Continuous
DURATION OF TEST: 72 hours
TEST PARAMETER: Growth inhibition
MONITORING OF TEST SUBSTANCE CONCENTRATION: HPLC

Result : EXPOSED:
ErC50 (24 - 48 h) = 32.1 mg/L
NOEC (24 - 48h) = 10.5 mg/L
ErC50 (24 - 72h) = 33.3 mg/L
NOEC (24 - 72h) = 22.9 mg/L
CONTROL:
Number/percentage of animals showing adverse effects: None

Table 1. Measured Concentrations of the Test Substance in Test Water

Nominal Concentration (mg/L)	Measured Concentration (mg/L)			
	0 Hour	Percent of Nominal	72 Hour	Percent of Nominal
Control	<0.02	-	<0.02	-
1.00	0.98	98	0.65	65
2.19	2.14	98	1.53	70
4.78	4.67	98	4.01	84
10.5	10.2	97	9.37	89
22.9	22.3	97	21.7	95
50.0	48.4	97	48.2	96

Table 2. Cell Densities of *Selenastrum Capricornutum* during the 72-Hour Exposure

Nominal Concentration, mg/L	Vessel No.	Cell Densities (cells/mL)			
		0 Hour	24 Hours	48 Hours	72 Hours
Control	1	10000	74600	426900	2019500
	2	10000	69100	403900	2049500
	3	10000	75000	387900	1809500
	Average	10000	72900	406200	1959500
	SD	0	3300	19600	130800
1.00	1	10000	75600	443900	2479500
	2	10000	69700	392900	2539500
	3	10000	78600	436900	2619500
	Average	10000	74600	424600	2546200
	SD	0	4500	27600	70200
2.19	1	10000	66200	366900	2259500
	2	10000	79700	425900	2529500
	3	10000	77800	484900	2779500
	Average	10000	74600	425900	2522800
	SD	0	7300	59000	260100
4.78	1	10000	70400	365900	2279500
	2	10000	70400	437900	2349500
	3	10000	81200	416900	2049500
	Average	10000	74000	406900	2226200
	SD	0	6200	37000	157000
10.5	1	10000	74400	391900	1799500
	2	10000	71900	449900	2069500
	3	10000	82100	504900	2029500
	Average	10000	76100	448900	1966200
	SD	0	5300	56500	145700
22.9	1	10000	35800	143900	767500
	2	10000	31400	155900	820500
	3	10000	39000	185900	907500
	Average	10000	35400	161900	831800
	SD	0	3800	21600	70700
50.0	1	10000	11100	10100	11300
	2	10000	10800	10400	10400
	3	10000	11500	10000	9800
	Average	10000	11100	10200	10500
	SD	0	400	200	800

SD = Standard Deviation

Table 3. Percent Growth Inhibition of *Selenastrum capricornutum*

Nominal Concentration (Measured Conc. at 0Hr)		Area under the growth curves		Growth Rate			
mg/L	No.	Area A (0-72h)	Inhibition (%)*1 IA (0-72h)	Rate : (24-48h)	Inhibition (%)*1 I m (24-48h)	Rate : (24-72h)	Inhibition (%)*1 I m (24-72h)
Control	1	35670000		0.0727		0.0687	
	2	35346000		0.0736		0.0706	
	3	32224000		0.0685		0.0663	
	Average	34413000	-	0.0716	-	0.0685	-
	SD	1903000		0.0027		0.0022	
1.00 (0.98)	1	41622000		0.0738		0.0727	
	2	40976000		0.0721		0.0749	
	3	43206000		0.0715		0.0730	
	Average	41935000	-21.9	0.0725	-1.3	0.0735	-7.3
	SD	1147000		0.0012		0.0012	
2.19 (2.14)	1	36908000		0.0714		0.0735	
	2	41888000		0.0698		0.0720	
	3	46259000		0.0762		0.0745	
	Average	41685000	-21.1	0.0725	-1.3	0.0733	-7.0
	SD	4679000		0.0033		0.0013	
4.78 (4.67)	1	37225000		0.0687		0.0724	
	2	39793000		0.0762		0.0731	
	3	35948000		0.0682		0.0673	
	Average	37655000	-9.4	0.0710	0.8	0.0709	-3.5
	SD	1958000		0.0045		0.0032	
10.5 (10.2)	1	32185000		0.0692		0.0664	
	2	36757000		0.0764		0.0700	
	3	37842000		0.0757		0.0668	
	Average	35595000	-3.4	0.0738	-3.1	0.0677	1.2
	SD	3002000		0.0040		0.0020	
22.9 (22.3)	1	12923000		0.0580		0.0639	
	2	13741000		0.0668		0.0680	
	3	15688000		0.0651		0.0656	
	Average	14117000	59.0**	0.0633	11.6*	0.0658	3.9
	SD	1420000		0.0047		0.0021	
50.0 (48.4)	1	44000		-0.0039		0.0004	
	2	34000		-0.0016		-0.0008	
	3	34000		-0.0058		-0.0033	
	Average	37000	99.9**	-0.0038	105.3**	-0.0012	101.8*
	SD	6000		0.0021		0.0019	

*1 Values are the percent inhibition relative to the control
SD Standard deviation
* Indicates a significant difference (p=0.05) from the control.
** Indicates a significant difference (p=0.01) from the control.

Table 4. Calculated EC50 and NOEC

Based on I_A (0-72h) value (Areas under growth curve)

EbC50 (0-72) (mg/L)	95-Percent Confidence Limits (mg/L)	NOECb (0-72) (mg/L)
20.3*1	-	10.5

Based on Im (24-48h) value (Growth rates)

ErC50 (24-48) (mg/L)	95-Percent Confidence Limits (mg/L)	NOECr (24-48) (mg/L)
32.1*2	-	10.5

Based on Im (24-72h) value (Growth rates)

ErC50 (24-72) (mg/L)	95-Percent Confidence Limits (mg/L)	NOECr (24-72) (mg/L)
33.3*2	-	22.9

The EC50 values and associated 95% confidence limits were determined by least squares linear regression analysis of the logarithm of nominal test concentration against percent growth inhibition relative to the control.

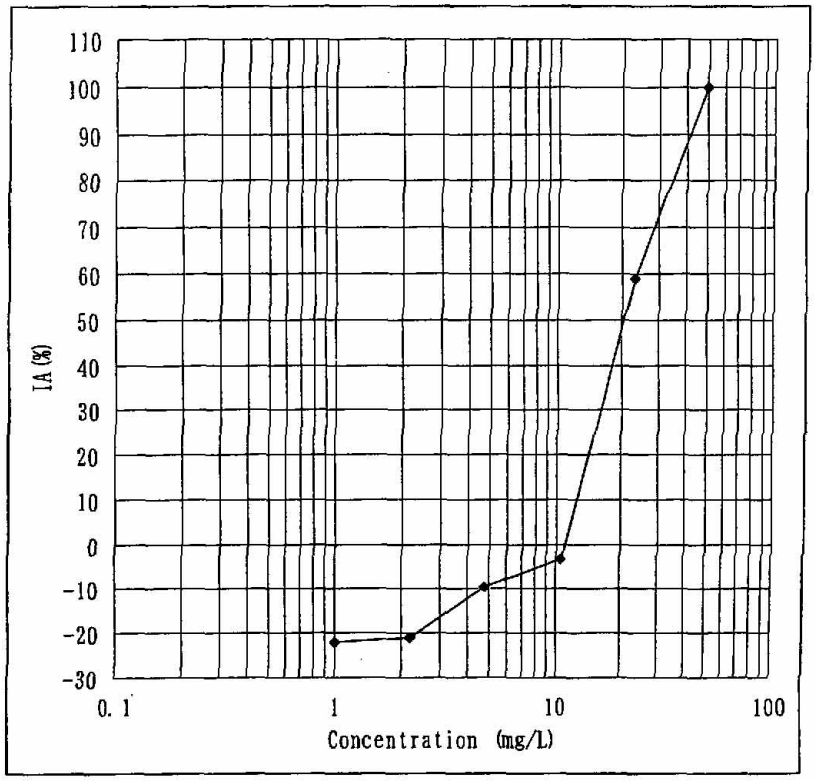
*1 using the concentrations of 10.5 and 22.9 mg/L in the regression analysis

*2 using the concentrations of 22.9 and 50.0 mg/L in the regression analysis

not calculated

The NOEC values were determined by an analysis of variance (ANOVA), Dunnett test, subsequent to Bartlett test for homogeneity of variances. Statistical analyses were performed using Yukms Statlight #4 software (Yukms Corp., Tokyo) and all tests of significance were at p=0.05, except Bartlett test, which was at p=0.01.

Figure 2 Concentration-Inhibition Curve Based on I_A Values Calculated from the Area under the Growth Curves



Conclusion	:	The substance has been shown to be harmful, EC50 < 100 mg/L, to aquatic organisms under the conditions of the test.
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint
27.06.2001		(1)
Species	:	*Scenedesmus subspicatus* (Algae)
Endpoint	:	growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/L
NOEC	:	= 6.25
EC50 (0-24 h)	:	= 14
Endpoint	:	Biomass
Exposure period	:	72 hour(s)
Unit	:	mg/L
NOEC	:	= 6.25
EC50	:	= 12
Analytical monitoring	:	no
Method	:	OECD Guideline 201 "Algae, Growth Inhibition Test"
Year	:	1996
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions Guideline study with deviations from present method
27.06.2001		(37)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	Aquatic
Species	:	*Pseudomonas putida*
Exposure period	:	16 hour(s)
Unit	:	mg/L
EC50	:	= 130
EC10	:	= 24
Analytical monitoring	:	no
Method	:	other: ISO 10712 and Bewertung Wassergefährdender Stoffe LTWS – Nr 10
Year	:	1998
GLP	:	yes
Test substance	:	as prescribed by 1.1 – 1.4
Conclusion	:	Corresponds to an evaluation number for the German Water Hazard Classification Scheme of 4.6
Reliability	:	(1) valid without restriction

02.08.2001 (34)

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(s)
Unit	:	mg/L
NOEC	:	= 4.7
LCEC	:	= 15
EC50	:	= 8.4
LC50	:	= 6.77
Analytical monitoring	:	yes
Method	:	other: OECD Guideline 211
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	METHOD FOLLOWED: OECD Guideline 211 „ *Daphnia magna*, Reproduction Test“ (1998) DEVIATIONS FROM GUIDELINE: None GLP: GLP compliant by order of Japanese Environment Agency. STATISTICAL METHODS: EC50 Immobility: Binomial EC50 Reproduction: Logit Comparison of mean numbers of juveniles produced per live adult after 21 days: Dunnett's multicomparison test ANALYTICAL METHOD: HPLC: Column: Inertsil ODS-3V, 5 um, 4.6 x 150 mm Mobile phase: 0.1% phosphoric acid, 10 mM sodium 1-pentanesulfonate = 10:90 Flow rate: 1.0 ml/min UV detector: 210 nm Sample volume: 20 ul Temperature: 40°C

Test condition : TEST ORGANISMS:
Species: daphnia
DILUTION WATER:
Elendt M4 Medium used as dilution water
Hardness: 195 – 225
TEST SYSTEM:
Test type: semi-static
Concentrations: control, 0.15, 0.47, 1.50, 4.70, 15.0 mg/L
Renewal of test solution: Every 24 hours
Exposure vessel type: 80 ml vessel
Number of replicates, individuals per replicate: 10
replicates per dose level, 1 individual per replicate.
Test temperature: 19.9 – 20 .4°C
Dissolved oxygen: 7.5 – 8.8 mg/L
pH: 7.4 – 8.8
Intensity of irradiation: 800 lux
Photoperiod: 16 hours
DURATION OF TEST:
TEST PARAMETER: Mortality and reproduction
SAMPLING: Every 24 hours.
MONITORING OF TEST SUBSTANCE CONCENTRATION: HPLC

Result : EXPOSED:
All parental daphnia in the highest concentration group died before reproduction occurred.
There were no dormant broods generated during the study.
CONTROL:
Number/percentage of animals showing adverse effects: None

Table 1. Calculated LC50 Values for Parental daphnia

Exposure Period (day)	LC50 *1 (mg/L)	95% Confidence Limits (mg/L)	Statistical Method
21	8.40	1.50 – 15.0	Binomial

*1: Based on the nominal concentration

Table 2 Calculated EC50 Values for Inhibition of Reproduction

Exposure Period (day)	EC50 *1 (mg/L)	95% Confidence Limits (mg/L)	Statistical Method
21	6.77	-- – –	Logit

*1: Based on the nominal concentration

--: Could not be determine

Table 3-1. Measured Concentrations of the Test Substance in Test water during a 21-day Exposure Period (Daphnia Reproduction Inhibition Test under the Semi-Static Test Conditions)

Nominal Concentration (mg/L)	Date	Measured Concentration (mg/L)						TWM*[1] (mg/L)	% of Nominal
		0 New	1 Old	7 New	8 Old	14 New	15 Old		
Control		<0.007	<0.007	<0.007	<0.007	<0.007	<0.007		
0.150		0.172	0.150	0.163	0.150	0.157	0.148	0.157	105
0.470		0.542	0.484	0.536	0.498	0.523	0.497	0.513	109
1.50		1.70	1.55	1.71	1.60	1.60	1.56	1.62	108
4.70		4.84	4.71	4.85	4.78	4.81	4.71	4.78	102
15.0		15.4	15.3	*	*	*	*	15.3	102

Table 3-2. Measured Concentrations as a Percentage of Nominal

Nominal Concentration (mg/L)	Date	Measured Concentration as a Percentage of Nominal					
		0 New	1 Old	7 New	8 Old	14 New	15 Old
0.150		115	100	109	100	105	99
0.470		115	103	114	106	111	106
1.50		113	103	114	107	107	104
4.70		103	100	103	102	102	100
15.0		103	102	*	*	*	*

New: Freshly prepared test solution
Old: Old test solution before renewal
*1: Time- weighted mean measured concentration during 21 days.
*: No measurement was made because all parental daphnia were dead.

	Concentration (mg/L)			% of Nominal		
	Min.		Max.	Min.		Max.
New	0.157	~	15.4	102	~	115
Old	0.148	~	15.3	99	~	107

Table 4-1. Cumulative Number of dead Parental Daphnia

Nominal Conc.	Days																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.150 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
0.470 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.50 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.70 mg/L	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
15.0 mg/L	0	1	1	5	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table 4-2. Mortality (%) of Parental Daphnia

Nominal Conc.	Days					
	1	2	4	7	14	21
Control	0	0	0	0	0	0
0.150 mg/L	0	0	0	0	10	10
0.470 mg/L	0	0	0	0	0	0
1.50 mg/L	0	0	0	0	0	0
4.70 mg/L	10	10	10	20	20	20
15.0 mg/L	10	10	90	100	100	100

Table 5. Cumulative Number of dead Parental daphnia

Nominal Conc.	Days																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.150 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
0.470 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1.50 mg/L	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4.70 mg/L	0	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
15.0 mg/L	0	1	1	5	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table 6. Mortality (%) of Parental daphnia

Nominal Conc.	Days					
	1	2	4	7	14	21
Control	0	0	0	0	0	0
0.150 mg/L	0	0	0	0	10	10
0.470 mg/L	0	0	0	0	0	0
1.50 mg/L	0	0	0	0	0	0
4.70 mg/L	10	10	10	20	20	20
15.0 mg/L	10	10	90	100	100	100

Table 7. Time (days) to First Brood Production

Vessel No.	Nominal Concentration, mg/L					
	Control	0.150	0.470	1.50	4.70	15.0
1	8	8	8	8	8	-
2	8	8	8	10	9	-
3	8	8	8	8	9	-
4	8	8	8	8	-	-
5	8	8	8	8	-	-
6	8	8	8	8	8	-
7	8	8	8	8	8	-
8	8	8	8	8	10	-
9	8	8	8	8	8	-
10	8	9	8	8	9	-
Min	8	8	8	8	8	-
Max	8	9	8	10	10	-

-: The parental daphnia was dead before first brood production.

Table 8. Mean Cumulative Numbers of Juveniles Produced per Adult Alive for 21 Days (EF1/P)

Nominal Conc.	Days															
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	0.0	0.0	11.4	11.5	11.5	34.9	38.5	38.5	58.5	68.8	68.8	73.6	90.5	90.5	90.5	107.5
0.150 mg/L	0.0	0.0	9.3	10.9	10.9	31.4	38.1	38.1	55.6	68.0	68.0	74.0	90.3	90.3	90.3	102.8
0.470 mg/L	0.0	0.0	11.2	12.3	14.0	39.7	46.8	46.8	62.8	75.5	75.5	80.4	100.4	100.4	100.4	116.9
1.50 mg/L	0.0	0.0	8.6	8.7	10.5	34.7	34.7	34.7	53.0	56.8	56.8	72.5	82.6	82.6	87.0	104.5
4.70 mg/L	0.0	0.0	6.5	10.8	12.6	33.8	36.9	40.6	54.4	70.3	74.6	80.6	97.8	97.8	103.8	118.8
15.0 mg/L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-: All parental daphnia were dead during a 21-days testing period.

Table 9: Cumulative Numbers of Juveniles Produced per Adult Alive for 21 Days in Each Test Vessels and Results of Statistical Comparison of the Mean Values (by Dunnett's Multicomparison Test)

Vessel No.	Nominal Concentration, mg/L					
	Control	0.150	0.470	1.50	4.70	15.0
1	123	99	103	89	118	D
2	109	118	121	86	113	D
3	106	D	99	96	118	D
4	111	119	139	117	D	D
5	109	112	103	95	D	D
6	113	116	124	109	132	D
7	119	88	116	109	135	D
8	100	128	133	101	103	D
9	110	58	115	122	116	D
10	75	87	116	121	115	D
Mean	107.5	102.8	116.9	104.5	118.8	0.0
S.D.	13.1	22.0	13.0	13.0	10.3	
Inhibition rate (%)		4.4	-8.7	2.8	-10.5	100.0
Significant difference		-	-	-	-	++

D: Were not included for calculation because the parental daphnia was dead during a 21-days testing period.
-: Indicates no significant difference.
*: Indicates a significantdifference (∀=0.05) from the control. (There was no sign in this test.)
**: Indicates a significant difference (∀=0.01) from the control. (There was no sign in this test.)
++: Statistical comparison test could not be performed for this concentration because adult alive after 21 days was none. However, we concluded that this concentration level showed adverse effect on daphnia reproduction.

No Observed Effect Concentration (NOEC): 4.70 mg/L
Lowest Observed Effect Concentration (LOEC) 15.0 mg/L

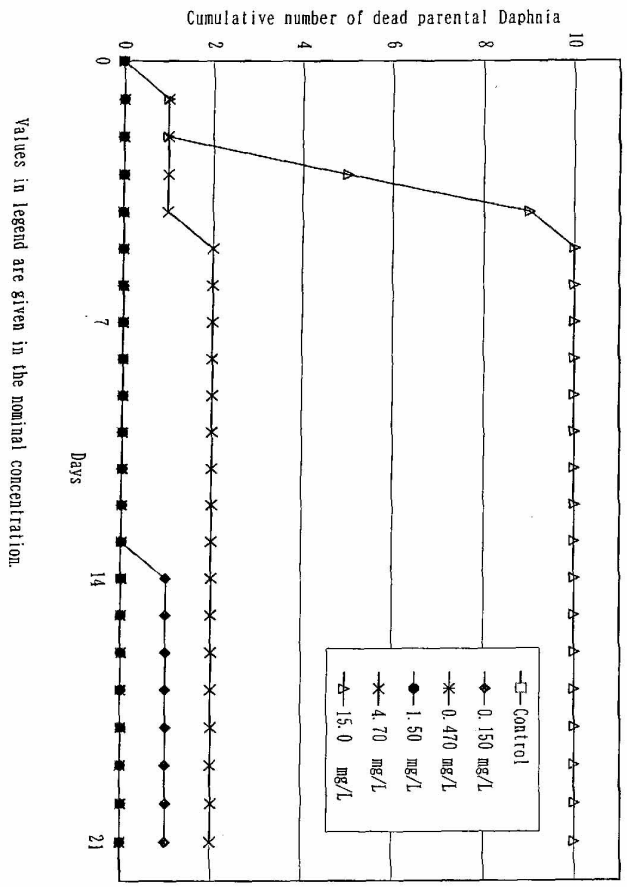
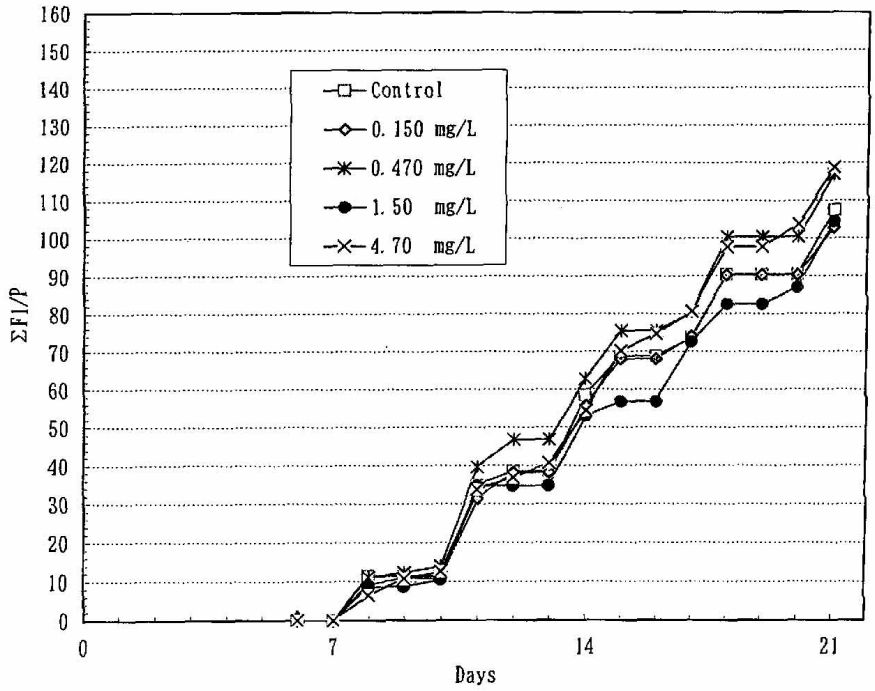


Figure 1 Cumulative Numbers of Dead Parental *Daphnia*

Values in legend are given in the nominal concentration.

Figure 2 Time Course of ΣF1/P for Each Concentration Level



Values in legend are given in the nominal concentration.

Conclusion	:	Chronic effects of toxicity were not observed at levels of 0.1 mg/L or less. The substance is not therefore harmful to aquatic organisms.
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint
27.06.2001		(2)

4.6.1TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARK

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1 ACUTE ORAL TOXICITY

Type	:	LD50
Method	:	OECD Guideline 401 „Acute Oral Toxicity“
Year	:	1980
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	METHOD FOLLOWED: Method in principle equivalent to Guideline OECD 401, except for particularities described in freetext TC. DEVIATIONS FROM GUIDELINE: LD50 values were calculated by the probit method from the number of animals that died during fourteen days after administration. GLP: GLP not compulsory at time study was performed. METHOD OF CALCULATION: Probit Method
Test condition	:	TEST METHOD: Two substances tested: MXDA and 1, 3-bis aminomethyl cyclohexane TEST ORGANISMS: Rat (conventional Wistar strain, male and female) Shizuoka Agricultural Cooperative Association for Laboratory Animals Age: 4 weeks old. Administration to animals at 5 weeks old and tamed for one week. Weight at study initiation: Not measured Controls: Plain diet NUMBER OF ANIMALS USED: 10 animals of each sex per dose level = 140 ADMINISTRATION: Feed withheld on the night prior to the test. Samples orally administered with no pre-treatment, using a metal canula. Feeding continued again 2-3 hours after dosing. Doses: MXDA undiluted (pH 8) Dose (ml/kg): Males; 0.721, 0.901, 1.121, 1.296, Control group (no administration) Females; 0.556, 0.659, 0.814, 0.941, 1.098, 1.235, 1.463, 1.818, Control group (no administration) Post dose observation period: 14 days EXAMINATION: Clinical observations, necropsy studies. Gross pathology appears to have been confined to intestinal tract.
Result	:	CLINICAL OBSERVATIONS: Decline of spontaneous movement was noted some hours after dosing and subsequently blephroptosis, diarrhea, and reddish brown vomit or flow of tears was observed. Symptoms disappeared 3-7 days later. Rats which died during the observation period presented ataxia and oppression in breathing before dying. AUTOPSY: For rats that died during the observation period, intense ulceration or necrosis was observed on the stomach or intestinal wall. Severer symptoms

were observed with increased dose levels.
For rats that were sacrificed after the observation period, the accretion of stomach with liver was observed in all dose groups, with increasing degree of accretion with higher dose levels.
No extreme changes were observed in any other organs.

TABLE 1: Acute oral toxicity, rat: Mortality data

Sex	Dose ml/kg	Number of days after dosing														Total mortality
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	
M	0.721	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1/10
	0.901	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1/10
	1.121	1	2	1	0	2	0	0	0	0	0	0	0	0	0	6/10
	1.296	6	0	2	2	0	0	0	0	0	0	0	0	0	0	10/10
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
F	0.556	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	0.659	2	1	0	0	0	0	0	0	0	0	0	0	0	0	3/10
	0.814	2	1	0	0	0	0	0	0	0	0	0	0	0	0	3/10
	0.941	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5/10
	1.098	5	0	0	0	0	0	0	0	0	0	0	0	0	0	5/10
	1.235	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9/10
	1.463	9	0	0	0	0	0	0	0	0	0	0	0	0	0	9/10
	1.818	10	0	0	0	0	0	0	0	0	0	0	0	0	0	10/10
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10

Test substance : PURITY: 99.9%
IMPURITY: As specified in 1.1 – 1.4
ANY OTHER INFORMATION:
Specific gravity: = 1.05

Conclusion : LD50:
Male: 1090 mg/kg (980-1190 mg/kg)
1.04 ml/kg (0.94-1.14 ml/kg)
Female: 980 mg/kg (870-1100 mg/kg)
0.94 ml/kg (0.83-1.05 ml/kg)
COMMENTS:
Accretion of the stomach with liver or intestinal canal is considered to occur in the course of recovery from injury.

Reliability : (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles.

Flag : Critical study for SIDS endpoint
25.06.2001 (5)

Type : LD50
Method : OECD Guideline 401 „Acute Oral Toxicity“
Year : 1980
GLP : no
Test substance : as prescribed by 1.1 – 1.4
Method : METHOD FOLLOWED:
Method in principle equivalent to Guideline OECD 401, except for particularities described in freetext TC.
DEVIATIONS FROM GUIDELINE:
LD50 values were calculated by the probit method from the number of animals that died during fourteen days after administration.
GLP:
GLP not compulsory at time study was performed.

METHOD OF CALCULATION:
Probit Method

Test condition : TEST METHOD: Two substances tested: MXDA and 1, 3-bis aminomethyl cyclohexane
TEST ORGANISMS:
Source:
Mice (SPF, ICR strain, male)
Shizuoka Agricultural Cooperative Association for Laboratory Animals
Age: 4 weeks old. Administration to animals at 5 weeks old and tamed for one week.
Weight at study initiation: Not measured
Controls: Plain diet
NUMBER OF ANIMALS USED:
10 animals of each sex per dose level = 70 (male only)
ADMINISTRATION:
Feed withheld on the night prior to the test.
Samples orally administered with no pre-treatment, using a metal canula.
Feeding continued again 2-3 hours after dosing.
Doses:
MXDA undiluted (pH 8)
MXDA: 10% dilution (pH 10)

10% dilution of MXDA
Dose (ml/kg): 5.96, 7.17, 8.77, 10.91, 13.06, 15.98
Extra group of 10 mice dosed with technically possible minimum quantity of sample (expected to be around the LD50 value of undiluted MXDA)
Post dose observation period: 14 days

EXAMINATION:
Clinical observations, necropsy studies. Gross pathology appears to have been confined to intestinal tract.

Result : CLINICAL OBSERVATIONS:
By some hours after dosing, sedative symptoms such as decline of spontaneous movement and blepharoptosis were observed and disappeared 3-5 days later.
The mice which succumbed during observation presented ataxia and oppression in breathing before their death.
Note: Toxicosis in mice dosed with undiluted MXDA was much severer than that of mice dosed with the dilution of the equivalent MXDA quantity.
AUTOPSY:
For mice that died during the observation period, intense ulceration was observed on the stomach and intestinal canal, and corrosive injuries noted on the abdominal organ adjacent to the above organs in mice at higher dose levels.
For mice that were sacrificed after the observation period, the accretion of the stomach with liver was observed in a few mice in the groups dosed with 7.17 and 8.77 ml/kg.
No extreme changes were observed in any other organs.

TABLE 1: Acute oral toxicity, mice: Mortality data

Dose ml/kg	Number of days after dosing														Total mortality	Comparison with undiluted MXDA
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
5.96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10	
7.17	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1/10	0.91 ml/kg
8.77	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1/10	6/10 mortality
10.91	0	2	2	0	0	0	0	0	0	0	0	0	0	0	4/10	
13.06	3	0	0	3	1	0	0	0	0	0	0	0	0	0	7/10	
15.98	10	0	0	0	0	0	0	0	0	0	0	0	0	0	10/10	

Test substance : PURITY: 99.9%
IMPURITY: As specified in 1.1 – 1.4
ANY OTHER INFORMATION:
Specific gravity: = 1.05

Conclusion : LD50:
10x dilution: 11.3 ml/kg (10.1-12.5 ml/kg)
Conversion to undiluted MXDA: 1180 mg/kg (1060-1310 mg/kg)

COMMENTS:
Accretion of the stomach with liver or intestinal canal is considered to occur in the course of recovery from injury.

Remark : The mice were tested using the 10 times diluted test substance. The effects of dilution were assessed by administering a single dose of undiluted test substance at the level expected to be around the LD_{50} (0.91 ml/kg), which gave 6/10 mortality. This was done because it is technically difficult to administer undiluted test material in such small quantities.

Reliability : (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles.

Flag : Critical study for SIDS endpoint

25.06.2001 (5)

Type : LD50
Method : Not specified
Species : rat
Result : 930 mg/kg
Test substance : as prescribed by 1.1 – 1.4

25.06.2001 (20)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC50
Value : > 1.42 mg/L (male), 0.8 mg/L (female)
Species : rat
Sex : male/female
Number of animals : 5/dose/sex
Vehicle : other: substance used as supplied
Doses : Control, 0.34, 0.74 and 1.42 mg/L
Exposure time : 4 hour(s)
Method : Directive 84/449/EEC, B.2 „Acute toxicity (inhalation)“
Year : 1993
GLP : yes
Test substance : as prescribed by 1.1 – 1.4
Method : METHOD FOLLOWED:
Study conducted in accordance with generally accepted scientific principles.

Method equivalent to: EPA TSCA 798.1150
OECD Guideline 403
EEC Method B2

STATISTICAL METHOD:
LC50 calculated by the log probit method of Miller and Tainter.
Standard error calculation: SE of LC50 = 2s/sq. root of 2N
Where:
2S = estimated increment in concentration of test substance between probits 4.0 and 6.0 corresponding to 16% and 84% mortality
N = total number of rats in groups with mortality between 6.7% and 93.3% (probits 3.5 - 6.5)

ANALYTICAL METHODS:
Concentration of MXDA in air: Nominal concentration calculated from amounts of MXDA dispersed and total volume of air supplied to exposure system. Air sample filtered through a weighed glass fibre filter (Whatman GF/A). Volume of air sample measured using a wet-type gas meter.
Particle size distribution: Marple cascade impactor.
Analysis for MXDA: Gas chromatography

Test condition : TEST ORGANISMS:
Source: Sprague-Dawley rat, Charles River UK Limited, Manston Road, Margate, Kent
Age: Male: 6 weeks
Female: 8 weeks
Weight at study initiation: ca. 200g
Number of animals: 20 male + 20 female, 5 animals of each sex per dose level including the control
Controls: No exposure.

ADMINISTRATION:
Type of exposure: Whole body
Concentrations:

Group	Amount in air (mg/L)	SD	Nominal (mg/L)
2	0.34	0.040	4.4
3	0.74	0.074	8.4
4	1.42	0.158	24.1

Measured concentrations were less than 9% of nominal. The low efficacy of the aerosolisation process was considered to be related to the cohesive nature of the test substance.
Particle size:

Group	MMAD(um)	sigma g	% respirable (6um)
2	2.2	1.44	99.9
3	2.3	1.68	96.6
4	2.0	1.42	99.8

There were no procedures to decrease the MMAD or to increase the number of particles in the sub-micron size range.
Type or preparation of particles: Generation by aerosol generator. Test substance supplied by syringe driven at constant rate by syringe pump. Compressed air supply was dried, filtered and oil-free.

EXAMINATIONS:
Clinical signs, bodyweight, food and water consumption, necropsy studies.

Result : LC50:
For male rats, the LC50 is considered to be more than 1.42 mg/L.
For female rats, the LC50 is 0.8 mg/L of air, SE = 0.49 mg/L.

MORTALITY:
In Group 2 (0.34 mg/L), 1 female rat was found dead on day 1 (am) of observation.
In Group 3 (0.74 mg/L), 1 male rat was found dead on Day 1 (pm) of observation. Two female rats were found dead on Day 3 (am) and 1 female rat was found dead on Day 11 (am) of the observation period.
In Group 4 (1.42 mg/L), 1 female rat was found dead on Day 1 (am), 1 female rat was found dead on Day 3 (am) and 1 female rat was found dead on Day 11 (am) of the observation period.

CLINICAL OBSERVATIONS:
During exposure: The signs seen during exposure were considered to be consistent with inhalation of an irritant aerosol and included partial closing of the eyes, salivation, exaggerated respiratory movements and test material on fur.
During the observation period: Signs evident in rats exposed to MXDA included gasping, brown staining and wet fur around the snout and jaws, lethargy, peripheral vasodilation, exaggerated respiratory movements, noisy respiration and death. Exaggerated respiratory movements and noisy respiration were evident for most of the observation period in the exposed rats. Lethargy was seen on Days 10 – 21 of the observation period in 1 or 2 female rats exposed at 1.42 mg/L (Group 4).
The majority of the surviving rats had recovered from the effects of exposure by the end of the observation period.
BODYWEIGHT:
There were moderate to marked decreases of bodyweight or reductions in the rate of bodyw eight gain for 2 – 3 days following exposure.
Subsequently, weight gain for rats that survived exposure to MXDA was similar to that of the control group.
FOOD CONSUMPTION:
Food consumption was generally reduced for up to 5 days following exposure to MXDA.
Group 4 female rats in particular showed variable food consumption during the observation period.
WATER CONSUMPTION:
Water consumption was generally reduced for up to 4 days following exposure to MXDA. In Group 3 and Group 4 females consumption was low throughout the observation period.
TERMINAL STUDIES:
Lungweight to bodyweight ratio: The lung weight to bodyweight ratios were higher than control values in a proportion of rats that died as a result of exposure to MXDA. The ratios were within normal limits for the control group rats and for the majority of the rats that survived exposure to MXDA.
One female rat in Group 4 (1.42 mg/L) had an abnormally high lung weight to bodyweight ratio.
Macroscopic findings:
The findings for rats that died as a result of exposure to MXDA were typified by congestion of the lungs. The stomachs of the decedents were found to be gas filled. Congestion of the lungs was found in 1 female rat surviving exposure at 1.42 mg/L (Group 4).
Microscopic findings:
a) Treatment related changes.
Varying degrees of acute inflammatory changes in the lungs comprising bronchiolitis, inflammation or alveolar macrophage aggregation at terminal bronchioles or, occasionally, acute exudative inflammation were seen in a number of rats from the high and intermediate exposure levels. Commonly, these changes were accompanied by congestion and/or intra-alveolar haemorrhage.
In addition, hyperplastic bronciolar epithelium was seen in one intermediate dose animal.

In a single (sporadic) animal from the low exposure level, areas of bronchioles were lined by a flattened, basophilic, possibly repairing/regenerating epithelium.
In the livers of a single intermediate and a single high exposure level animal there was a degree of centrilobular hepatocyte necrosis. In one additional female low exposure level animal, centrilobular sinusoidal dilatation and the presence of foamy sinusoidal cells were observed.

b) Incidental changes
No other histological changes were seen in the tissues examined.

Test substance : PURITY: 99.69%
IMPURITIES: As specified in 1.1 – 1.4
ANY OTHER INFORMATION:
Lot No.: 20217

Conclusion : Exposure of rats to MXDA at the high and intermediate dosage levels produced varying degrees of acute respiratory inflammatory changes. There was evidence of a similar change in a single animal only exposed at the low level.
In occasional animals at the high and intermediate exposure levels a centrilobular hepatocyte change, considered likely to be treatment-related, was noted.

Reliability : (1) valid without restriction
Guideline study.

Flag : Critical study for SIDS endpoint

25.06.2001 (16)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Method : Not specified
Species : rat
Result : 700 mg/L per hour
Test substance : as prescribed by 1.1 – 1.4
Reliability : (4) not assignable
Secondary literature review

25.06.2001 (20)

Type : LD50
Species : Rabbit
Value : > 2000 mg/kg bw
Method : Other
Year : 1975
GLP : No data
Test substance : as prescribed by 1.1 – 1.4
Remark : Draize method (aerosol generation system)
Source : Itochu Deutschland GmbH Dusseldorf

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rat
Concentration	:	undiluted
Exposure	:	Open
Exposure time	:	4 hour(s)
Number of animals	:	12
Result	:	corrosive
Classification	:	corrosive (causes burns)
Method	:	other: UN Transport Regulations
Year	:	1986
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	METHOD FOLLOWED: Test procedure for classification and labelling of dangerous substances for transport: Corrosivity. Method in principle equivalent to EEC Method B4 except for test species used. Test carried out in accordance with „the test methods and evaluation criteria in the evaluation of danger of unknown substances“ established by the Maritime Techniques Safety Bureau, Ministry of Transport (December 1985). DEVIATIONS FROM GUIDELINE: Deviations listed in freetext: Test Conditions. Deviations should not significally impact the interpretation of the data. GLP: GLP not compulsory at the time the studywas performed. METHOD OF CALCULATION: Corrosiveness of test substance evaluated based on dermal changes appearing at 3 minutes, 60 minutes and 4 hours after application in accordance with the criteria established by the International Maritime Organisation.
Test condition	:	TEST ORGANISMS: Source: Male/female Crj-Wistar rats, Japan Charles River Co., Ltd. Age: 5 weeks. Administration at 6 weeks. Weight at study initiation: Initial weight not given but stated to be almost the same by sex. Controls: No controls ADMINISTRATION: Area covered: 1 cm2/head Occulsion: No covering used. Vehicle: Substance applied as supplied. Total volume applied: 1ml/kg Removal of test substance: Substance not removed. EXAMINATIONS: Evaluation of severity of erythema, oedema, haemorrhage and necrosis were macroscopically observed whilst animals stood in fixation. Examination after 4 -hours: Skin incised and the cut surfaces and subcutaneous region in the applied site were examined.
Result	:	OBSERVATIONS: Subcutaneous haemorrhage a ppeared after 3 minutes; brown metachromatic changes macroscopically judged to be necrosis emerged after 5 minutes. These changes showed highly necrotic appearences after 60 minutes; namely, the dark-red brown skin surfaces were slightly dried and hardened. Cut surfaces: 4 hours after application, the cut surfaces both had necrosis throughout the entire dermal layers and haemorrhage as severe as it reached the dorsal muscle.

Test substance : PURITY: 99.6%
IMPURITIES: As specified in 1.1 – 1.4
ANY OTHER INFORMATION:
Lot No.: 602 28
Water content: 0.022%
Specific gravity: 1.050

Table 1: Primary irritative symptoms in the skin

	Sex	No.	Body weight (g)	3 minutes				60 minutes				4 hours			
				Er	Ed	H	N	Er	Ed	H	N	Er	Ed	H	N
Rat	M	1	191	1	2	2	0	2	4	4	4	0	4	4	4
		2	189	1	0	2	0	1	4	4	3	0	4	4	4
		3	190	1	0	2	0	1	3	2	4	0	3	3	4
	F	4	148	2	0	1	0	1	2	2	4	0	3	3	4
		5	148	2	1	1	0	2	4	2	4	0	4	4	4
		6	149	1	1	0	0	2	4	3	4	0	3	3	4

Symptoms: Er = Erythema, Ed = Oedema, H = Haemorrhage, N = necrosis
Severity: 0 = normal, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe

Conclusion : The substance was judged to be, based on the criteria of IMO to correspond to a corrosive substance classified as Packing grade II defined in the recommendations of the United Nations.

It is considered that the substance would be corrosive to rabbit skin, the animal model normally preferred for dermal irritation and, which is considered to be more sensitive to irriation effects than rodent skin. Therefore, the classification of corrosive as defined by this study is considered to be valid.

Reliability : (2) valid with restrictions
Basic data given: comparable to guideline study with acceptable restrictions.

25.06.2001 (21)

Species : mice
Concentration : undiluted
Exposure : Open
Exposure time : 4 hour(s)
Number of animals : 12
Result : corrosive
Classification : corrosive (causes burns)
Method : other: UN Transport Regulations
Year : 1986
GLP : no data
Test substance : as prescribed by 1.1 – 1.4
Method : METHOD FOLLOWED:
Test procedure for classification and labelling of dangerous substances for transport: Corrosivity.
Method in principle equivalent to EEC Method B4 except for test species used.
Test carried out in accordance with „the test methods and evaluation criteria in the evaluation of danger of unknown substances“ established by the Maritime Techniques Safety Bureau, Ministry of Transport (December 1985).

DEVIATIONS FROM GUIDELINE:
Deviations listed in freetext: Test Conditions.
Deviations should not significally impact the interpretation of the data.

GLP: GLP not compulsory at the time the study was performed.

METHOD OF CALCULATION:
Corrosiveness of test substance evaluated based on dermal changes appearing at 3 minutes, 60 minutes and 4 hours after application in accordance with the criteria established by the International Maritime Organisation.

Test condition : TEST ORGANISMS:
Source: Male/female Cij-ICR mice, Japan Charles River Co., Ltd
Age: 5 weeks. Administration at 6 weeks.
Weight at study initiation: Initial weight not given but stated to be almost the same by sex.
Controls: No controls
ADMINISTRATION:
Area covered: 1 cm2/head
Occulsion: No covering used.
Vehicle: Substance applied as supplied.
Total volume applied: 1ml/kg
Removal of test substance: Substance not removed.
EXAMINATIONS:
Evaluation of severity of erythema, oedema, haemorrhage and necrosis were macroscopically observed whilst animals stood in fixation.
Examination after 4 -hours: Skin incised and the cut surfaces and subcutaneous region in the applied site were examined.

Result : OBSERVATIONS:
Mice exhibited almost no changes immediately after application; brown metachromatic changes judged to be necrosis appeared after 10-20 minutes. After that, subcutaneous haemorrhage appeared, and then the skin showed necrotic appearences of brown to dark red colour after 60 minutes.

Cut surfaces: 4 hours after application, the cut surfaces both had necrosis throughout the entire dermal layers and haemorrhage as severe as it reached the dorsal muscle.

: PURITY: 99.6%
IMPURITIES: As specified in 1.1 – 1.4
ANY OTHER INFORMATION:
Lot No.: 602 28
Water content: 0.022%
Specific gravity: 1.050

Table 1: Primary irritative symptoms in the skin

	Sex	No.	Body weight (g)	3 minutes				60 minutes				4 hours			
				Er	Ed	H	N	Er	Ed	H	N	Er	Ed	H	N
Mice	M	7	30	0	0	0	0	0	4	3	4	0	4	4	4
		8	30	0	0	0	0	0	1	3	3	0	1	1	4
		9	30	0	0	0	0	0	0	0	1	0	0	1	2
	F	10	24	0	0	0	0	0	2	2	3	0	3	3	4
		11	25	0	0	0	0	0	2	0	3	0	3	3	4
		12	24	0	0	0	0	0	1	2	2	0	2	3	4

Symptoms: Er = Erythema, Ed = Oedema, H = Haemorrhage, N = necrosis
Severity: 0 = normal, 1 = very mild, 2 = mild, 3 = moderate, 4 = severe

Conclusion : The substance was judged to be, based on the criteria of IMO to correspond to a corrosive substance classified as Packing grade II defined

in the recommendations of the United Nations.

It is considered that the substance would be corrosive to rabbit skin, the animal model normally preferred for dermal irritation and, which is considered to be more sensitive to irriation effects than rodent skin. Therefore, the classification of corrosive as defined by this study is considered to be valid.

Reliability : (2) valid with restrictions
Basic data given: comparable to guideline study with acceptable restrictions.

25.06.2001 (21)

Species : rabbit
Result : 750 ug per 24 hours
Classification : Corrosive
Method : Draize test
Test substance : as prescribed by 1.1 – 1. 4

25.06.2001 (39)

5.2.2 EYE IRRITATION

Remark : See section 5.2.1

The substance has been determined to be corrosive to the skin of rats and mice and has been classified as Corrosive, R34 Causes burns. It is therefore considered that the substance would also be a severe eye irritant on the basis of the result of the skin corrosion test. On the grounds of animal welfare and on the basis of the dermal result, it is considered an eye irritation study should not be performed and the substance be considered to be labelled R41: Risk ofserious damage to eyes for the purpose of risk assessment.

Reliability : (4) not assignable
Hazard end-point derived from conclusion of skin corrosion test.

25.06.2001

Species : rabbit
Result : 50 ug per 24 hours
Classification : Corrosive
Method : Draize test
Test substance : as prescribed by 1.1 – 1. 4

25.06.2001 (39)

5.3 SENSITIZATION

Type : Maximisation test
Species : Guinea pig
Concentration : 1st: Induction 10% occlusive epicutaneous
: 2nd: Challenge 2% occlusive epicutaneous
: 3rd: Challenge 1 % occlusive epicutaneous
Number of animals : 10 test+ 5 control
Vehicle : water
Result : sensitizing
Classification : sensitizing
Method : 92/69/EEC Method B6
GLP : yes
Test substance : as prescribed by 1.1 – 1.4
Conclusion : The test material gave a 7/10 sensitisation rate. The substance is therefore classified as a strong skin sensitiser.

Remark : The concentrations of test material to be used at each stage of the main study were determined by sighting tests in which groups of guinea pigs were treated with various concentrations of test material. Irritation was observed in the sighting studies at a concentration of 10 %v/v. At concentrations above 25 %v/v animals were humanely killed due to necrosis.

Very slight or well-defined erythema was noted at the intradermal induction sites of all test group animals at the 24-hour observation with well-defined erythema at the 48-hour observation. No skin reactions were noted at the intradermal induction sites of control group animals at the 24 or 48-hour observations.

After topical induction brown/yellow staining was noted at the induction sites of all test group animals at the 1 and 24-hour observations. Well defined erythema and very slight to slight oedema were noted at the induction sites of all test group animals at the 1 hour observation with very slight to well defined erythema and very slight or slight oedema in 6 test group animals at the 24-hour observation. Very slight erythema was noted at the treatment site of 2 control group animals at the 1-hour observation.

After topical challenge using 2% v/v test material in distilled water, positive skin reactions were noted at the challenge sites of 7 test group animals at the 24-hour observation and in 5 test group animals at the 48-hour observation. Other adverse dermal reactions precluded the evaluation of erythema at the challenge sites of a further 2 test group animals at the 48-hour observation. Very slight or slight oedema was noted at the challenge sites of 6 test group animals at the 24 and 48-hour observations. Other skin reactions noted at the challenge sites of test group animals were desquamation and small superficial scattered scabs. No skin reactions were noted at the challenge sites of control group animals at the 24 and 48-hour observations.

After topical challenge using 1% v/v test material in distilled water, positive skin responses (very slight or well defined erythema – grades 1 or 2) were noted at the challenge sites of 5 test group animals at he 24-hour observation and in 4 test group animals at the 48-hour observation. Very slight oedema was noted at the challenge sites of 2 test group animals at the 24 and 48-hour observations. No skin reactions were noted at the challenge sites of control group animals at the 24 and 48-hour observations.

Reliability : (1) valid without restriction
Guideline study.

25.06.2001 (38)

5.4 REPEATED DOSE TOXICITY

Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	28 days
Frequency of treatm.	:	Once daily
Post exposure period	:	1 or 15 days
Doses	:	Control (vehicle), 10, 40, 150, 600 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	ca. 150 mg/kg bw
Method	:	other: Research Institute for Animal Science in Biochemistry and Toxicology Method to Japanese Guidelines

GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Method : METHOD FOLLOWED: Guideline for 28-day Repeat Dose Toxicity Testing of Chemicals (Japan) and OECD 407
GLP: GLP compliant by order of MHW, Japan
Test condition : TEST ORGANISMS:
Age: 5 weeks at beginning of study
Weight at study initiation: Males: 173-188g, Females: 139-162g
Number of animals: 96 (6 males/6 females per group)
ADMINISTRATION/EXPOSURE:
Duration of test/exposure: 28 days
Type of exposure: oral gavage
Post exposure period: 1 or 14 days (14 day recovery groups for control and 600 mg/kg/day dose groups only)
Vehicle: JP purified water
Concentration in vehicle: Stock solution concentration not specified
SATELLITE GROUPS AND REASONS WHY THEY WERE ADDED:
Satellite groups of 6 males and 6 females dosed at 0 (vehicle) and 600 mg/kg/day were added for 14 day post-exposure observations.
CLINICAL OBSERVATIONS AND FREQUENCY:
Clinical signs: Every day
Mortality: Every day
Body weight: Every day during dosing, once weekly during recovery period.
Food consumption: 24 hour food consumption was recorded for each cage group at weekly intervals.
Water consumption: Not observed
Ophthalmoscopic examination:
Haematology: At the time of necropsy.
Biochemistry: Performed on blood samples
Urinalysis: On Day 25 of administration and Day 11 after dosing completed.
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
Macroscopic: Brain, liver, kidneys, adrenals testes/ovaries
Microscopic: Heart, liver, spleen, kidneys, adrenals, bone marrow, stomach, intestines (duodenum, jejunum, ileum, caecum, colon and rectum) and thymus.
OTHER EXAMINATIONS: None
STATISTICAL METHODS: Methods not detailed
Result : NOAEL (NOEL): The significant toxic changes detected were stomach membrane disorders. This was considered to be due to the irritant nature of the test material. Animals treated with 10, 40 and 150 mg/kg/day showed no changes considered to be attributable to the administration of the test material.
ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX:
Time of death: One male and four females in the 600 mg/kg/day dose group died on days 15 to 19.
Number of deaths at each dose: 600 mg/kg/day: 5/24, no further deaths at any dose level.
TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
Clinical signs: Among the 12 males and the 12 females of the 600 mg/kg/day dose groups, ten males and eight females showed increased salivation, three males and seven females decreased spontaneous locomotion, one male and six females pilo-erection and one male distended abdomen. No abnormalities were detected during the treatment-free period.
Bodyweight gain: Animals treated at 600 mg/kg/day weighed 285±15 g (males) and 198±17 g (females) compared to controls at 335±17 g (males)

and 204±14 g (females). Males treated with 600 mg/kg/day tended to show a reduction in bodyweight gain from Day 2. The difference from the control group tended to increase with the progress of treatment and was significant after Day 4 except Day 7. However, the bodyweight gain of the males tended to recover during the treatment free period.
Food/water consumption: Food consumption of the males of the 600 mg/kg/day dose group was smaller than that of the control group throughout the treatment period, b eing significantly smaller during week 4. In the treatment free period, however, there was no difference in food consumption between the males treated with 600 mg/kg/day and those of the control group.

Ophthalmoscopic examination:
Clinical chemistry: A s ignificant reduction in total protein and an increase in inorganic phosphorus were were detected in males from the 600 mg/kg/day dose group and an increase in triglyceride in females from the same group. The recovery group showed none of these changes, but significant increases in total cholesterol and A/G ratio and a decrease in chloride were detected in males and a decrease in albumin in females.
Haematology: Males from the 600 mg/kg/day dose group showed significant reductions in haemoglobin and haematocrit levels, an increase in prothrombin time, a reduction in activated partial thromboplastin time, an increase in segmented neutrophil ratio in differential leukocyte count, and a reduction in lymphocyte ratio. Females from the same dose group also tended to show a reduction in haematocrit level and an increase in leukocyte count primarily due to neutrophilia although these changes were not statistically significant.
The recovery group animals showed no significant changes in any investigation parameter.
Urinalysis: Urine protein of high dose males increased significantly. The urine protein value of many males from the 600 mg/kg/day dose was ++ (100 mg/dl) while the value of many control animals was + (30 mg/dl). No significant changes were observed in any investigation parameter.
Organ weights: Relative adrenal weight (23.85±4.12 mg%) of males treated at 600 mg/kg/day increased significantly compared with control animals (18.19±3.52 mg%). Absolute (81±15 mg) and relative (41.02±7.62 mg%) adrenal weights of females treated with the same dose increased significantly compared with control animals (61.3±7 mg and 30.13±3.39 mg%, respectively).
Male animals treated at 600 mg/kg/day showed a significant reduction in absolute liver weight (8.33±0.44 g) and a significant increase in relative brain weight (0.68±0.03 g%) following the reduction in body weight gain compared to control animals (10.00±0.54 g and 0.59±0.03 g%, respectively). However, there were no differences in the relative liver weight and absolute brain weight between the group and control group. No significant changes were detected in any of the organ weights of the recovery group animals.
Gross pathology: In the 600 mg/kg/day dose group, hyperplasia and ulcer of the anterior stomach and typhlectasis due to an increase in the contents of caecum were seen in all male and female rats and adrenocortical hypertrophy in half of the females. One male rat and four female rats from the same dose group which died during the treatment period showed changes in anterior stomach similar to those seen in the surviving animals, reddened mucous membrane and ulcer of the grandular stomach, and expanded stomach and intestines due to gas retention. In addition, some of the females showed red stigma on the cecal mucous mem brane, reddened adrenals, and atrophy of thymus and spleen. Thickened anterior stomach wall was also seen in almost all of the animals of the recovery group, but the changes were slighter than those seen in the animals killed after the

completion of the treatment period.
Histopathology:
Treatment-related changes were observed in stomach, adrenals, and bone marrow. In all of the 600 mg/kg/day animals that survived the 28-day exposure period, deep ulcers ranging from the muscular tunics to the serous membrane were formed in the anterior gastric mucous membrane. In the submucousa there were seen inflammatory changes. In addition, hyperplasia of the stratified squamous epithelium and thickening due to hyperkeratosthenia were observed in the peripheral mucous membrane. Erosion was detected in the mucous membrane of glandular stomach of one male and one female from the 600 mg/kg/day dose group, but the changes were smaller than those seen in the anterior stomach. Abnormalities were also detected in the adrenals, including vacuolation of the cortex, in particular, the spongiocyte in one each of the males and females treated with 600 mg/kg/day and hypertrophy in two females. In the bone marrow of four males and two females of the same dose group there was seen a slight increase in granulocytic haematopoietic cells.
The one male and four females decedants from the 600 mg/kg/day group showed congestive or atrophic changes in various organs in addition to the changes seen in the animals killed after completion of the treatment period. Furthermore, necrosis, erosion and ulcer were observed on the glandular mucous membrane of the stomach. Vacuolation in the duodenal mucosal epithelium, hyperemia of cecal mucosa, dilation of renal distal tubulus, degeneration/necrosis of adrenocortical cells were also noted in the deceased females. The recovery group animals recovered or tended to recover from the changes seen in the animals killed after completion of the treatment period. The stomach showed no mucosal coloboma, but only submucosal fibrination and slight mucosal thickening in many cases. The adrenals and bone marrow showed no abnormalities.

Table 1 Hematology in male rats after the oral administration of 1,3-bis(aminomethyl)benzene for 28 days and a recovery period for 14 days

	After administration period					After recovery period	
Dose level (mg/kg)	0	15	40	150	600	0	600
No. of animals	6	6	6	6	6	6	5
Erythrocyte (10^4/mm^3)	767 ± 38	765 ± 39	764 ± 31	748 ± 50	712 ± 36	807 ± 26	777 ± 20
Hematocrit (%)	43.1 ± 1.5	43.7 ± 1.6	42.9 ± 1.3	41.8 ± 1.5	40.1 ± 1.9*	43.6 ± 1.1	42.3 ± 1.3
Hemoglobin (g/dl)	15.1 ± 0.7	15.2 ± 0.7	15.2 ± 0.5	14.6 ± 0.5	14.1 ± 0.7*	15.4 ± 0.7	14.6 ± 0.5
MCV (fl)	56 ± 1	58 ± 2	56 ± 1	56 ± 2	57 ± 1	54 ± 1	54 ± 2
MCH (pg)	19.6 ± 0.3	19.9 ± 0.7	19.9 ± 0.4	19.6 ± 0.8	19.8 ± 0.5	19.1 ± 0.5	18.8 ± 0.8
MCHC (%)	35.0 ± 0.7	34.8 ± 0.6	35.4 ± 0.8	35.0 ± 0.3	35.1 ± 0.7	35.3 ± 0.7	34.6 ± 0.3
Reticulocyte ($^0/_{oo}$)	39 ± 8	40 ± 4	39 ± 4	53 ± 10**	42 ± 5	32 ± 8	38 ± 8
Leukocyte (10^2/mm^3)	66 ± 9	74 ± 17	67 ± 20	58 ± 23	61 ± 24	77 ± 20	70 ± 16
Differential Count (%)							
Lymphocyte	87.0 ± 3.8	86.5 ± 4.5	86.3 ± 2.4	84.8 ± 4.7	73.3 ± 9.0*	88.3 ± 4.1	89.2 ± 4.9
Neutrophil band	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
segmented	11.7 ± 3.2	11.7 ± 3.7	12.5 ± 2.9	13.7 ± 4.0	25.3 ± 8.9*	9.3 ± 2.8	10.0 ± 5.3
Eosinophil	0.0 ± 0.0	0.2 ± 0.4	0.5 ± 0.8	0.5 ± 0.5	0.2 ± 0.4	1.2 ± 1.2	0.2 ± 0.4
Basophil	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte	1.3 ± 1.2	1.7 ± 1.2	0.7 ± 0.8	1.0 ± 1.3	1.2 ± 0.8	1.0 ± 1.1	0.6 ± 0.5
Platelet (10^4/mm^3)	147 ± 9	149 ± 21	134 ± 12	144 ± 13	160 ± 18	142 ± 11	154 ± 22
PT (sec)	13.4 ± 0.2	13.7 ± 0.2	13.6 ± 0.3	13.3 ± 0.3	14.0 ± 0.3**	12.5 ± 0.2	12.7 ± 0.5
APPT (sec)	18.4 ± 1.2	18.7 ± 0.9	17.9 ± 1.0	18.6 ± 1.4	16.8 ± 0.6*	18.1 ± 1.8	18.3 ± 1.5

Values are expressed as Mean ± S.D.
Significantly different from control group (*:$p<0.05$; **:$p<0.01$)

Table 2 Hematology in female rats after the oral administration of 1,3-bis(aminomethyl)benzene for 28 days and a recovery period for 14 days

	After administration period					After recovery period	
Dose level (mg/kg)	0	15	40	150	600	0	600
No. of animals	6	6	6	6	4	6	4
Erythrocyte (10^4/mm^3)	738 ± 34	751 ± 16	756 ± 40	728 ± 33	723 ± 19	784 ± 19	801 ± 56
Hematocrit (%)	41.4 ± 1.2	41.4 ± 0.9	42.1 ± 1.1	40.6 ± 1.4	39.6 ± 1.3	42.4 ± 1.0	43.8 ± 2.4
Hemoglobin (g/dl)	14.8 ± 0.6	14.8 ± 0.6	15.2 ± 0.5	14.5 ± 0.7	14.2 ± 0.4	15.3 ± 0.5	15.7 ± 0.9
MCV (fl)	56 ± 2	55 ± 0	56 ± 2	56 ± 1	55 ± 2	54 ± 1	55 ± 1
MCH (pg)	20.0 ± 0.6	19.8 ± 0.6	20.1 ± 0.4	19.9 ± 0.3	19.6 ± 0.6	19.5 ± 0.4	19.7 ± 0.5
MCHC (%)	35.6 ± 0.5	35.9 ± 0.8	36.0 ± 0.4	35.6 ± 0.5	35.8 ± 0.3	36.0 ± 0.3	35.9 ± 0.3
Reticulocyte ($^0/_{00}$)	25 ± 8	30 ± 8	25 ± 3	25 ± 8	27 ± 6	30 ± 5	27 ± 5
Leukocyte (10^2/mm^3)	45 ± 15	41 ± 12	48 ± 15	40 ± 10	69 ± 33	52 ± 7	54 ± 19
Differential Count (%)							
Lymphocyte	86.5 ± 5.5	90.3 ± 5.4	90.8 ± 4.0	86.8 ± 5.1	67.8 ± 17.3	92.0 ± 2.2	90.0 ± 2.9
Neutrophil band	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
segmented	11.0 ± 5.0	7.7 ± 3.8	8.0 ± 4.3	11.3 ± 5.2	30.3 ± 18.0	6.8 ± 1.7	8.5 ± 4.1
Eosinophil	1.2 ± 1.0	0.8 ± 0.8	0.5 ± 0.8	0.5 ± 0.5	0.5 ± 1.0	0.8 ± 0.8	0.8 ± 0.5
Basophil	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Monocyte	1.0 ± 0.6	1.2 ± 1.3	0.7 ± 0.8	1.3 ± 2.0	1.5 ± 0.6	0.3 ± 0.5	0.8 ± 1.5
Platelet (10^4/mm^3)	144 ± 20	145 ± 10	136 ± 9	137 ± 14	163 ± 19	137 ± 12	140 ± 40
PT (sec)	13.1 ± 0.4	12.8 ± 0.3	13.2 ± 0.2	13.5 ± 0.3	13.2 ± 1.1	13.1 ± 0.4	13.3 ± 0.4
APPT (sec)	16.7 ± 1.1	15.8 ± 0.8	15.9 ± 1.1	16.0 ± 0.9	17.1 ± 1.2	16.9 ± 0.9	16.0 ± 0.6

Values are expressed as Mean ± S.D.

Table 3 Blood chemistry in male rats after the oral administration of 1,3-bis(aminomethyl)benzene for 28 days and a recovery period for 14 days

	After administration period										After recovery period			
Dose level (mg/kg)	0	15	40	150	600		0		600	
No. of animals	6	6	6	6	6		6		5	
GOT (IU/l)	62 ± 13	65 ± 6	62 ± 6	57 ± 5	67 ± 13		61 ± 5		60 ± 74	
GPT (IU/l)	29 ± 6	33 ± 8	32 ± 4	28 ± 3	29 ± 8		35 ± 3		32 ± 4	
γ-GTP (IU/l)	0.14 ± 0.14	0.35 ± 0.18	0.45 ± 0.52	0.51 ± 0.65	0.42 ± 0.53		0.46 ± 0.34		0.19 ± 0.11	
ALP (IU/l)	339 ± 75	372 ± 56	412 ± 65	402 ± 94	297 ± 83		311 ± 63		349 ± 39	
T.protein (g/dl)	6.06 ± 0.10	6.11 ± 0.18	5.98 ± 0.12	6.00 ± 0.24	5.74 ± 0.16**		6.36 ± 0.19		6.30 ± 0.05	
Albumin (g/dl)	2.95 ± 0.10	2.91 ± 0.17	2.99 ± 0.17	3.00 ± 0.20	2.90 ± 0.14		3.02 ± 0.13		3.10 ± 0.07	
A/G ratio	0.95 ± 0.07	0.91 ± 0.11	1.00 ± 0.10	1.00 ± 0.07	1.03 ± 0.12		0.91 ± 0.03		0.97 ± 0.04*	
T.cholesterol (mg/dl)	92 ± 24	75 ± 8	68 ± 10	82 ± 16	79 ± 14		80 ± 4		98 ± 7**	
Triglyceride (mg/dl)	90 ± 30	67 ± 24	103 ± 59	98 ± 41	64 ± 19		99 ± 19		75 ± 22	
Glucose (mg/dl)	143 ± 6	137 ± 20	146 ± 16	149 ± 13	132 ± 21		166 ± 14		157 ± 13	
T.bilirubin (mg/dl)	0.35 ± 0.02	0.34 ± 0.06	0.35 ± 0.06	0.36 ± 0.04	0.35 ± 0.05		0.40 ± 0.05		0.41 ± 0.03	
Urea nitrogen (mg/dl)	14.0 ± 2.0	14.6 ± 1.4	14.2 ± 1.3	17.3 ± 1.4**	13.9 ± 1.1		18.3 ± 1.8		18.3 ± 1.8	
Creatinine (mg/dl)	0.53 ± 0.05	0.55 ± 0.04	0.53 ± 0.06	0.56 ± 0.06	0.54 ± 0.06		0.59 ± 0.07		0.60 ± 0.03	
Ca (mg/dl)	10.3 ± 0.4	10.3 ± 0.5	10.2 ± 0.3	10.2 ± 0.3	10.4 ± 0.4		10.0 ± 0.2		10.0 ± 0.3	
I.phosphorus (mg/dl)	7.6 ± 0.5	7.7 ± 0.7	7.5 ± 0.5	7.9 ± 0.5	8.5 ± 0.5*		7.4 ± 0.5		7.8 ± 0.4	
Na (mEq/l)	140 ± 1	141 ± 1	140 ± 1	141 ± 1	140 ± 1		141 ± 1		140 ± 1	
K (mEq/l)	4.54 ± 0.22	4.48 ± 0.12	4.40 ± 0.24	4.31 ± 0.14	4.25 ± 0.16		4.56 ± 0.31		4.71 ± 0.25	
Cl (mEq/l)	102 ± 1	103 ± 1	102 ± 2	102 ± 1	103 ± 1		103 ± 1		101 ± 1**	

Values are expressed as Mean ± S.D.
Significantly different from control group (*:p<0.05; **:p<0.01)

Table 4 Blood chemistry in female rats after the oral administration of 1,3-bis(aminomethyl)benzene for 28 days and a recovery period for 14 days

	After administration period										After recovery period			
Dose level (mg/kg)	0	15	40	150	600		0		600	
No. of animals	6	6	6	6	4		6		4	
GOT (IU/l)	63 ± 3	61 ± 7	61 ± 7	66 ± 2	67 ± 4		59 ± 7		56 ± 4	
GPT (IU/l)	30 ± 4	26 ± 5	28 ± 5	28 ± 7	34 ± 8		29 ± 6		29 ± 2	
γ-GTP (IU/l)	0.35 ± 0.27	0.31 ± 0.29	0.43 ± 0.34	0.55 ± 0.16	0.31 ± 0.23		0.44 ± 0.37		0.44 ± 0.30	
ALP (IU/l)	223 ± 48	278 ± 74	233 ± 78	216 ± 46	231 ± 36		192 ± 29		181 ± 17	
T.protein (g/dl)	6.27 ± 0.25	6.44 ± 0.23	6.26 ± 0.26	6.25 ± 0.36	5.73 ± 0.14		6.66 ± 1.29		6.32 ± 0.08	
Albumin (g/dl)	3.34 ± 0.16	3.49 ± 0.29	3.29 ± 0.21	3.35 ± 0.26	2.90 ± 0.17		3.46 ± 0.07		3.30 ± 0.13*	
A/G ratio	1.14 ± 0.05	1.19 ± 0.15	1.11 ± 0.08	1.16 ± 0.13	1.03 ± 0.09		1.08 ± 0.06		1.10 ± 0.08	
T.cholesterol (mg/dl)	86 ± 20	87 ± 6	83 ± 7	71 ± 8	70 ± 15		84 ± 9		77 ± 12	
Triglyceride (mg/dl)	30 ± 14	45 ± 17	40 ± 21	36 ± 7	77 ± 20**		54 ± 22		65 ± 19	
Glucose (mg/dl)	126 ± 9	134 ± 10	128 ± 21	118 ± 14	103 ± 10		127 ± 11		128 ± 11	
T.bilirubin (mg/dl)	0.30 ± 0.04	0.30 ± 0.01	0.27 ± 0.03	0.27 ± 0.03	0.25 ± 0.01		0.39 ± 0.05		0.40 ± 0.05	
Urea nitrogen (mg/dl)	19.8 ± 3.2	16.9 ± 1.8	19.4 ± 3.3	19.6 ± 2.1	16.2 ± 1.8		20.0 ± 1.6		20.9 ± 1.3	
Creatinine (mg/dl)	0.59 ± 0.09	0.54 ± 0.04	0.57 ± 0.05	0.58 ± 0.03	0.52 ± 0.05		0.61 ± 0.04		0.61 ± 0.04	
Ca (mg/dl)	10.2 ± 0.3	10.2 ± 0.3	10.1 ± 0.3	10.1 ± 0.2	10.1 ± 0.1		10.2 ± 0.3		10.0 ± 0.3	
I.phosphorus (mg/dl)	7.2 ± 0.6	6.5 ± 0.7	6.7 ± 0.8	6.4 ± 0.6	7.8 ± 0.5		6.2 ± 0.6		5.9 ± 0.6	
Na (mEq/l)	140 ± 1	140 ± 1	140 ± 0	140 ± 1	139 ± 1		140 ± 1		139 ± 1	
K (mEq/l)	4.31 ± 0.14	4.34 ± 0.21	4.36 ± 0.25	4.27 ± 0.10	4.32 ± 0.15		4.57 ± 0.20		4.78 ± 0.07	
Cl (mEq/l)	104 ± 1	104 ± 1	104 ± 1	104 ± 1	104 ± 2		103 ± 1		104 ± 1	

Values are expressed as Mean ± S.D.
Significantly different from control group (*:p<0.05; **:p<0.01)

Table 5 Incidence of histopathological findings in male rats after the oral administration of 1,3-bis(aminomethyl)benzene for 28 days and a recovery period for 14 days

Organ : Findings	Dose level (mg/kg)		After administration period					After recovery period	
			0	10	40	150	600	0	600
	No. of animals		6	6	6	6	6(1)*	6	5
Heart: Myocardial fibrosis		+	2	-	-	-	1 (0)	-	-
Lung: Congestion and edema		+	-	-	-	-	- (1)	-	-
Liver: Congestion		+	0	-	-	-	0 (1)	-	-
Stomach:									
Mucosal necrosis in non-glandular portion		+	0	0	0	0	0 (1)	0	0
Ulceration in non-glandular portion		++-+++	0	0	0	0	6 (0)	0	2
Acathosis with hyperkeratosis in non-glandular portion		+	0	0	0	0	0 (0)	0	1
		++	0	0	0	0	6 (0)	0	4
Submucosal inflammation in non-glandular portion		++	0	0	0	0	6 (0)	0	0
Submucosal fibrosis in non-glandular portion		+-++	0	0	0	0	0 (0)	0	5
Erosion/ulceration in glandular portion		+-++	0	0	0	0	1 (1)	0	0
Submucosal inflammation in glandular portion		++	0	0	0	0	0 (1)	0	0
Spleen: Atrophy		+	0	-	-	-	0 (1)	-	-
Kidney:									
Congestion		+	0	-	-	-	0 (1)	-	-
Focal tubular basophilic change		+	2	-	-	-	3 (0)	-	-
Focal tubular dilatation with hyaline casts		+	2	-	-	-	0 (0)	-	-
Eosinophilic bodies in proximal tubule		+	1	-	-	-	0 (0)	-	-
Adrenal:									
Congestion		+	0	0	0	0	0 (1)	0	0
Cortical cell vacuolization		+	0	0	0	0	1 (0)	0	0
Bone marrow: Increased granulopiesis		+	0	0	0	0	4 (0)	0	0

+: Slight; ++: Moderate; +++ Marked; -: Not examined

*: animal supposed to be killed after a recovery period was found dead during the administration period.

No abnormalities detected in the thymus and intestine from animals of control and 600 mg/kg groups.

Table 6 Incidence of histopathological findings in female rats after the oral administration of 1,3-bis(aminomethyl)benzene for 28 days and a recovery period for 14 days

Organ : Findings	Dose level (mg/kg)		After administration period					After recovery period	
			0	10	40	150	600	0	600
	No. of animals		6	6	6	6	4(4)*	6	4
Thymus: Atrophy		+-++	0	0	0	0	0(4)	-	-
Liver: Congestion		+	0	-	-	-	0(4)	-	-
Hepatocellular fatty change		+	1	-	-	-	1(0)	-	-
Hepatocellular atrophy		+	0	-	-	-	0(1)	-	-
Stomach:									
Ulceration in non-glandular portion		++-+++	0	0	0	0	4(3)	0	0
Acathosis with hyperkeratosis in non-glandular portion		+	0	0	0	0	0(1)	0	2
		++	0	0	0	0	4(2)	0	1
Submucosal inflammation in non-glandular portion		+-++	0	0	0	0	4(3)	0	0
Submucosal fibrosis in non-glandular portion		+-++	0	0	0	0	0(0)	0	3
Mucosal necrosis in non-glandular and glandular portions		+++	0	0	0	0	0(1)	0	0
Erosion in glandular portion		+-+++	0	0	0	0	1(3)	0	0
Intestine:									
Epithelial vacuolization of duodenal mucosa		+	0	-	-	-	0(1)	-	-
Hypermia of cecal mucosa		+	0	-	-	-	0(1)	-	-
Spleen: Atrophy		+-++	0	-	-	-	0(4)	-	-
Kidney:									
Congestion		+	0	-	-	-	0(3)	-	-
Focal tubular basophilic change		+	3	-	-	-	2(0)	-	-
Distal tubular dilatation		+	0	-	-	-	0(2)	-	-
Adrenal:									
Congestion		+	0	0	0	0	0(2)	0	0
Cortical cell degeneration and necrosis		++	0	0	0	0	0(1)	0	0
Cortical cell vacuolization		+	0	0	0	0	1(0)	0	0
Hypertrophy of zone fasciculata		+	0	0	0	0	2(1)	0	0
Bone marrow: Increased granulopiesis		+	0	0	0	0	2(2)	0	0

+: Slight; ++: Moderate; +++ Marked; -: Not examined

*: Two animals supposed to be killed after the administration period and two after a recovery period were found dead or killed in extremis during the administration.

No abnormalities detected in the heart from animals of control and 600 mg/kg groups.

Test substance	:	SOURCE: Mistsubishi Gas Chemical Company Inc. Tokyo PURITY: 99.8% ANY OTHER INFORMATION: Batch no: 30817 Store with container closed at room temperature in the dark. MW = 136.22 Melting point: 14°C Vapour pressure: 1.6 x 10 E-03 mmHg (25°C)
Conclusion	:	There were no sex-related differences for the NOEL dose level. The NOEL = 150 mg/kg/day, therefore, the substance is not considered to be harmful by prolonged oral exposure under the conditions of the test.
Reliability	:	(1) valid without restriction The study is not to OECD or EU Annex V test guidelines, but is to a similar standard. The full study report is not available. This summary is based on the concise summary of the Government assessment work on existing chemicals.
Flag	:	Critical study for SIDS endpoint

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5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	*Salmonella typhimurium* strains: TA100, TA1535, TA98, TA1537 and *Escherichia coli* strain: WP2uvrA
Test concentration	:	312.5 to 5000 ug/plate
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Guidelines for screening mutagenicity testing of chemicals, Japan
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	METHOD FOLLOWED: Japanese study guidelines for testing chemicals. GLP: GLP compliant by order of MHW, Japan STATISTICAL METHODS: Statistical method not stated METHOD OF CALCULATION: No method given.
Test condition	:	SYSTEM OF TESTING: Deficiences/proficiences: Tested to confirm amino-acid requirement, uv sensitivity, presence of rfa wall mutation and ampicillin resistance factor pKM101. Metabolic activation system: Enzyme induction from male Sprague-Dawley rats (age 7 weeks) administered with Phenobarbital and 5,6 -benzoflavone. ADMINISTRATION: Dosing: One initial dose Dose Period: 48 hours Number of replicates: 3 plates for each dose level, 2 replicates. Application: The test substance was disolved in purified water (50mg/ml) and then further diluted with the solvent at a common ratio of 2 to approximately 3. 0.1 ml of the test material formulation, plus 0.5 ml of phosphate buffer solution or 0.5 ml of S9 mix, 0.1 ml of one of the test strain solutions and 2 ml of top agar were placed into test tubes. The contents of the test tubes were mixed , distributed onto synthetic medium plates and solidified. Positive and negative control groups and treatment: Control group: purified water

Postive controls: AF2: Furilframide
SA: Sodium azide
9AA: 9-Aminoacridine
2AA: 2-Aminoanthracene

AF2 and 2AA dissolved in DMSO and frozen until use. 9AA and SA dissolved in DMSO and distilled water respectively and used immediately.
DESCRIPTION OF FOLLOW UP REPEAT STUDY: The repeat study replicated the initial study.
CRITERIA FOR EVALUATING RESULTS: The test material was considered to be mutagenic (+ve) in the study system if the mean number of revertant colonies on the plate containing the test material should have increased to at least two times that of the respective vehicle control value and the increase should be reproducible and dose-related in at least one of the five kinds of tester strains used in the study with or without S9 mix.

Result : GENOTOXIC EFFECTS:
With metabolic activation: > 5000 ug/plate
Without metabolic activation: > 5000 ug/plate
PRECIPITATION CONCENTRATION: No precipitate observed.
MITOTIC INDEX:
CYTOTOXICITY CONCENTRATION: > 5000 ug/plate both with and without S9
TEST-SPECIFIC CONFOUNDING FACTORS: None

Table 1: Results of reverse mutation test (I) of 1,3-Bis(aminomethyl)benzene** on bacteria

With (+) or without (-) S9 Mix	Test substance dose (µg/plate)	Number of revertants (number of colonies/plate, Mean ± S.D.)					
		Base-pair substitution type			Frameshift type		
		TA100	TA1535	WP2 *uvr* A	TA98	TA1537	
S9 Mix (-)	0	123 109 118 (117 ± 7.1)	11 12 16 (13 ± 2.6)	13 18 25 (19 ± 6.0)	34 17 26 (26 ± 8.5)	8 6 6 (7 ± 1.2)	
	312.5	144 106 122 (124 ± 19.1)	11 15 14 (13 ± 2.1)	20 28 19 (22 ± 4.9)	19 24 20 (21 ± 2.6)	6 10 5 (7 ± 2.6)	
	625	92 107 105 (101 ± 8.1)	14 17 17 (16 ± 1.7)	14 23 24 (20 ± 5.5)	25 20 18 (21 ± 3.6)	2 5 6 (4 ± 2.1)	
	1250	111 102 87 (100 ± 12.1)	10 13 9 (11 ± 2.1)	19 23 25 (22 ± 3.1)	21 28 25 (25 ± 3.5)	5 9 6 (7 ± 2.1)	
	2500	104 133 139 (125 ± 18.7)	12 9 13 (11 ± 2.1)	20 24 21 (22 ± 2.1)	24 28 21 (24 ± 3.5)	14 8 3 (8 ± 5.5)	
	5000	92 127 111 (110 ± 17.5)	6 10 12 (9 ± 3.1)	23 28 24 (25 ± 2.6)	22 9 23 (18 ± 7.8)	7 8 5 (7 ± 1.5)	
S9 Mix (+)	0	130 136 123 (130 ± 6.5)	12 14 10 (12 ± 2.0)	17 16 22 (18 ± 3.2)	26 29 28 (28 ± 1.5)	7 7 8 (7 ± 0.6)	
	312.5	133 135 154 (141 ± 11.6)	17 12 13 (14 ± 2.6)	20 19 22 (20 ± 1.5)	29 51 24 (35 ± 14.4)	8 17 14 (13 ± 4.6)	
	625	110 137 126 (124 ± 13.6)	9 16 8 (11 ± 4.4)	27 16 34 (26 ± 9.1)	26 37 22 (28 ± 7.8)	11 10 8 (10 ± 1.5)	
	1250	129 130 118 (126 ± 6.7)	12 6 13 (10 ± 3.8)	20 25 27 (24 ± 3.6)	30 18 30 (26 ± 6.9)	16 13 10 (13 ± 3.0)	
	2500	136 147 125 (136 ± 11.0)	16 13 12 (14 ± 2.1)	21 36 31 (29 ± 7.6)	29 33 44 (35 ± 7.8)	11 9 14 (11 ± 2.5)	
	5000	163 148 161 (157 ± 8.1)	18 10 16 (15 ± 4.2)	26 21 19 (22 ± 3.6)	36 34 18 (29 ± 9.9)	6 12 8 (9 ± 3.1)	
Positive	Chemical	AF2	SA	AF2	AF2	9AA	

control S9 Mix (-)	Dose (µg/plate)	0.01	0.5	0.01	0.1	80	
	Number of colonies/plate	500 466 567 (511 ± 51.4)	160 163 129 (151 ± 18.8)	129 106 146 (127 ± 20.1)	874 867 804 (848 ± 38.6)	916 913 845 (891 ± 40.2)	
Positive control S9 Mix (+)	Chemical	2AA	2AA	2AA	2AA	2AA	
	Dose (µg/plate)	1	2	10	0.5	2	
	Number of colonies/plate	939 902 984 (942 ± 41.1)	217 283 244 (248 ± 33.2)	979 111 952 6 (1016 ± 87.9)	279 358 370 (336 ± 49.4)	349 367 317 (344 ± 25.3)	

AF2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, SA: Sodium azide,
9AA: 9-Aminoacridine, 2AA: 2-Aminoanthracine
**: Purity was 99.8% and H_2O (0.02%) was contained as impurity.

Table 2: Results of reverse mutation test (II) of 1,3-Bis(aminomethyl)benzene** on bacteria

With (+) or without (-) S9 Mix	Test substance dose (μg/plate)	Number of revertants (number of colonies/plate, Mean ± S.D.)					
		Base-pair substitution type			Frameshift type		
		TA100	TA1535	WP2 *uvr* A	TA98	TA1537	
S9 Mix (-)	0	136 127 127 (130 ± 5.2)	9 14 15 (13 ± 3.2)	28 18 23 (23 ± 5.0)	24 22 23 (23 ± 1.0)	15 6 12 (11 ± 4.6)	
	312.5	134 136 123 (131 ± 7.0)	10 8 9 (9 ± 1.0)	25 22 20 (22 ± 2.5)	16 16 23 (18 ± 4.0)	2 9 7 (6 ± 3.6)	
	625	146 140 145 (144 ± 3.2)	16 12 17 (15 ± 2.6)	22 35 20 (2.6 ± 8.1)	26 26 20 (24 ± 3.5)	10 5 5 (7 ± 2.9)	
	1250	126 139 119 (128 ± 10.1)	14 15 10 (13 ± 2.6)	27 21 26 (25 ± 3.2)	17 30 33 (27 ± 8.5)	5 9 11 (8 ± 3.1)	
	2500	151 157 161 (156 ± 5.0)	9 11 7 (9 ± 2.0)	27 20 34 (27 ± 7.0)	20 20 16 (19 ± 2.3)	4 8 11 (8 ± 3.5)	
	5000	155 162 154 (157 ± 4.4)	11 11 9 (10 ± 1.2)	36 30 27 (31 ± 4.6)	35 19 23 (26 ± 8.3)	5 7 8 (7 ± 1.5)	
S9 Mix (+)	0	136 149 134 (140 ± 8.1)	14 11 15 (13 ± 2.1)	31 26 37 (31 ± 5.5)	32 35 28 (32 ± 3.5)	10 11 13 (11 ± 1.5)	
	312.5	130 130 139 (133 ± 5.2)	10 7 14 (10 ± 3.5)	32 36 22 (30 ± 7.2)	33 29 28 (30 ± 2.6)	8 16 16 (13 ± 4.6)	
	625	164 148 189 (154 ± 9.0)	8 14 19 (14 ± 5.5)	32 24 30 (29 ± 4.2)	45 39 27 (37 ± 9.2)	14 9 11 (11 ± 2.5)	
	1250	149 135 140 (141 ± 7.1)	14 12 13 (13 ± 1.0)	31 28 30 (30 ± 1.5)	34 38 36 (36 ± 2.0)	18 14 17 (16 ± 2.1)	
	2500	140 148 175 (154 ± 18.3)	10 8 9 (9 ± 1.0)	31 31 29 (30 ± 1.2)	40 35 29 (35 ± 5.5)	16 11 14 (14 ± 2.5)	
	5000	173 190 185 (183 ± 8.7)	11 12 15 (13 ± 2.1)	32 31 35 (33 ± 2.1)	31 35 35 (34 ± 2.3)	18 16 10 (15 ± 4.2)	
Positive control S9 Mix (-)	Chemical	AF2	SA	AF2	AF2	9AA	
	Dose (μg/plate)	0.01	0.5	0.01	0.1	80	
	Number of colonies/plate	628 660 662 (650 ± 19.1)	185 195 234 (205 ± 25.9)	221 210 221 (217 ± 6.4)	758 843 888 (830 ± 66.0)	1723 1542 1876 (1711 ± 162.9)	
Positive control S9 Mix (+)	Chemical	2AA	2AA	2AA	2AA	2AA	
	Dose (μg/plate)	1	2	10	0.5	2	
	Number of colonies/plate	648 620 760 (676 ± 74.1)	345 296 325 (322 ± 24.6)	951 1175 1053 (1060 ± 112.1)	406 395 400 (400 ± 5.5)	279 308 356 (314 ± 38.9)	

AF2: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, SA: Sodium azide,
9AA: 9-Aminoacridine, 2AA: 2-Aminoanthracine
**: Purity was 99.8% and H_2O (0.02%) was contained as impurity.

Test substance : SOURCE: Mitsubishi Gas Chemical Company Inc. (obtained via Japan Chemical Industry Association)
PURITY: 99.8%
IMPURITY/ADDITIVE/ETC.: Water content = 0.018%

ANY OTHER INFORMATION:
Batch no: 30817
MW = 136.22

Conclusion : The substance is not mutagenic with or without metabolic activation under the conditions of the test.

Reliability : (1) valid without restriction
Summary of a Japanese Guideline study. This summary is based on the concise summary of the Government assessment work on existing chemicals.

Flag : Critical study for SIDS endpoint

27.06.2001 (24)

Type : Chromosome aberration
System of testing : Chinese hamster cells (CHL/IU)
Test concentration : -S9: 0.08 - 0.33 mg/ml, +S9: 0.12 - 0.47 mg/ml
Metabolic activation : with and without
Result : negative
Method : other: Guidelines for screening mutagenicity testing of chemicals, Japan
GLP : no data
Test substance : as prescribed by 1.1 - 1.4
Method : METHOD FOLLOWED: Japanese study guidelines for testing chemicals and OECD 473.
DEVIATIONS FROM GUIDELINE: It was impossible to analyse a sufficient number of cells due to cytotoxicity in the highest dose group (0.33 mg/ml) in which the cells were continuously exposed to the test material for 24 and 48 hours. This was not considered to have effected the validity of the study.
GLP: GLP compliant by order of MHW Japan
STATISTICAL METHODS: Fischer's exact probability test ($p<0.05$).

Test condition : SYSTEM OF TESTING:
Deficiences/proficiences: None stated.
Metabolic activation system: rat liver S9, induced with Phenobarbital and 5,6-benzoflavone.
No. of metaphases analyzed: Structural aberrations and polyploids were analysed on 200 and 800 cells in metaphase per group respectively.
ADMINISTRATION:
Dosing: One initial dose
Number of replicates: 2 plates per dose level.
Application: The test substance was dissolved in injection water and then further diluted with the solvent successively to produce the test concentrations. (No allowance was made for purity)
The test formulations were added to the culture solution so that each concentration became 10% (v/v) of the solution. The cells (2 x 10 E+04) were seeded per plate (6cm diameter) containing 5 ml of Eagles Minimal Essential Medium (with Earle's salts supplemented with 10% fetal bovine serum) and incubated (5% CO_2) at 37°C. In the continuous treatment, the test material was added on day 3 of seeding for 24 and 48-hour treatment. The short-time treatment groups were treated for 6 hours with or without S9 mix on Day 3 of seeding and then further cultivated for 18 hours in fresh media.
Positive and negative control groups and treatment:
Control group: Treatment free
Postive controls: -S9, Mitomycin C
+S9, Cyclosphosphamide
Pre-incubation time: None stated
DESCRIPTION OF FOLLOW UP REPEAT STUDY: The study was carried out with duplicate plates.

CRITERIA FOR EVALUATING RESULTS: Analysis of chromosomes made according to the classification method prepared by Mammalian Animal Test

Subcommittee, The Environmental Mutagen Society of Japan.
Chromosomes observed for the presence of structural aberrations, such as chromosome-type or chromatid-type gaps, breaks, exchanges, and polyploid cells. The number of cells observed and the type and number of the structural aberrations, and the number of polyploids totalled. Significant differences in the frequency of cells with chromosom al aberrations between the groups calculated.
Results then reviewed using the criteria of Ishidate et al.

Result : GENOTOXIC EFFECTS:
With metabolic activation: > 0.47 mg/plate
Without metabolic activation: > 0.33 mg/plate
PRECIPITATION CONCENTRATION: No precipitate observed.
MITOTIC INDEX:
CYTOTOXICITY CONCENTRATION:
With S9: > 0.47 mg/plate
Without S9: 0.33 mg/plate
TEST-SPECIFIC CONFOUNDING FACTORS: None

Table 1 Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with 1,3-bis(aminomethyl)benzene (BAB)** without S9 mix

Group	Concent-ration (mg/ml)	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations								Others[3]	No. of cells with aberrations		Polyploid[4]	Judgement[5]	
				gap	ctb	cte	csb	cse	f	mul[2]	total		TAG (%)	TA (%)	(%)	SA	NA
Control			200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.00		
Solvent[1]	0	24	200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.00		
BAB	0.08	24	200	0	1	1	0	0	1	0	3	0	3 (1.5)	3 (1.5)	0.13	-	-
BAB	0.17	24	200	0	1	0	0	0	0	0	1	0	1 (0.5)	1 (0.5)	0.13	-	-
BAB	0.33	24	33	2	10	11	0	0	1	0	24	0	9 *(27.3)	8 *(24.2)	0.00[6]	Tox	Tox
MC	0.00005	24	200	10	46	124	6	2	2	0	190	3	97*(48.5)	96*(48.0)	0.00	+	-
Solvent[1]	0	48	200	0	0	0	1	0	0	0	1	0	1 (0.5)	1 (0.5)	0.13		
BAB	0.08	48	200	0	0	0	0	1	0	0	1	0	1 (0.5)	1 (0.5)	0.13	-	-
BAB	0.17	48	200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.50	-	-
BAB	0.33	48	38	0	0	0	0	0	0	0	0	1	0 (0.0)	0 (0.0)	2.90[7]	Tox	Tox
MC	0.00005	48	200	0	34	124	14	3	8	20	203	15	101*(50.5)	101*(50.5)	0.38	+	-

Abbreviations: Gap : chromatid gap and chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange (dicentric and ring, etc), f: acentric fragment (chromatid type), mul: multiple aberrations, TAG: Total no. of cells with aberrations, TA: Total No. of cells with aberrations except gap, SA: structural aberration, NA: numerical aberration, MC: mitomycin C, Tox: toxic. 1) Water for injection was used as solvent. 2) More than 10 aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). 6) Seventy six cells were analysed. 7) Sixty nine cells were analysed. *: Significantly different from solvent control at $p<0.05$. **: Purity was 99.8%, water (0.018%) was contained as impurity.

Table 2 Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with 1,3-bis(aminomethyl)benzene (BAB)** with and without S9 mix

Group	Concent-ration (mg/ml)	S9 mix	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations								Others[3]	No. of cells with aberrations		Polyploid[4]	Judgement[5]	
					gap	ctb	cte	csb	cse	f	mul[2]	total		TAG (%)	TA (%)	(%)	SA	NA
Control				200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.13		
Solvent[1]	0	-	6 - (18)	200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.00		
BAB	0.08	-	6 - (18)	200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.00	-	-
BAB	0.17	-	6 - (18)	200	0	0	1	1	0	0	0	2	0	2 (1.0)	2 (1.0)	0.00	-	-
BAB	0.33	-	6 - (18)	200	1	3	0	0	0	0	0	4	0	3 (1.5)	3 (1.5)	0.13[6]	-	-
CPA	0.005	-	6 - (18)	200	0	1	0	1	0	0	0	2	0	2 (1.0)	2 (1.0)	0.13	-	-
Solvent[1]	0	+	6 - (18)	200	0	0	0	0	0	1	0	1	0	1 (0.5)	1 (0.5)	0.25		
BAB	0.12	+	6 - (18)	200	0	0	0	1	0	0	0	1	0	1 (0.5)	1 (0.5)	0.25	-	-
BAB	0.24	+	6 - (18)	200	0	0	0	0	0	1	0	1	0	1 (0.5)	1 (0.5)	0.50	-	-
BAB	0.47	+	6 - (18)	200	0	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.00	-	-
CPA	0.005	+	6 - (18)	200	4	58	110	3	4	1	30	210	0	94*(47.0)	93*(46.5)	0.13	+	-

Abbreviations: gap : chromatid gap and chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange (dicentric and ring, etc), f: acentric fragment (chromatid type), mul: multiple aberrations, TAG: Total no. of cells with aberrations, TA: Total No. of cells with aberrations except gap, SA: structural aberration, NA: numerical aberration, CPA: cyclophosphamide.
1) Water for injection was used as solvent. 2) More than 10 aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Judgement was done on the basis of the criteria of Ishidate et al. (1987). 6) Seven hundred and ninety four cells were analysed. *: Significantly different from solvent control at $p<0.05$. **: Purity was 99.8%, water (0.018%) was contained as impurity.

Test substance : SOURCE: Mitsubishi Gas Chemical Company Inc. (obtained via Japan Chemical Industry Association)
PURITY: 99.8%
IMPURITY/ADDITIVE/ETC.: Water content = 0.018%
ANY OTHER INFORMATION:
Batch no: 30817
MW = 136.22
Other name: BAB
Melting point: 14.1°C
Boiling point: 247°C
Vapour pressure: 1.6 x 10 E-03 mm Hg
Soluble in water, acetone and dimethylsulphoxide

Conclusion : The substance is not clastogenic to the limit of cytoxicity both with and without metabolic activation under the conditions of the test.

Reliability : (1) valid with restrictions
Summary of a Japanese Guideline study. This summary is based on the concise summary of the Government assessment work on existing chemicals.

Flag : Critical study for SIDS endpoint
27.06.2001 (23)

Type : Chromosome aberration
System of testing : Chinese Hamster Ovary (CHO) cells
Test concentration : -S9: 75 - 450 ug/ml
+S9: 200 - 800 ug/ml
Metabolic activation : with and without
Result : negative
Method : Directive 84/449/EEC, B.10

Year : 1989
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Method : METHOD FOLLOWED:
Guideline based upon:
OECD Guideline No. 473 (May 26, 1983)
EEC Method B 10 (67/548/EEC, adopted September 19, 1984)
EPA Test Guideline (TSCA, FIFRA), subchapter R, part 798, subpart F, Genetic Toxicity § 798.5375 (revised July 1, 1986)

Study conducted in accordance with generally accepted scientific principles.

DEVIATIONS FROM GUIDELINE: At the first fixation time cells were fixed 6 hours post-dosing instead of at the protocolled 4 hours post-dosing. However, this amendment was not considered to seriously affect the experimental set-up of the study.
STATISTICAL METHODS:
Chi-square test, $P < 0.05$

Test condition : SYSTEM OF TESTING:
Metabolic activation system:
Species: Rat, Adult Wistar or Sprague Dawley.
Charles River Wiga, Sulzfeld, FRG
Organ: liver
Induction substance:Aroclor 1254 in corn oil (20% w/v solution = 500 mg/kg bodyweight)
Rats injected intraperitoneally with Aroclor 1254. After five days the rats were killed (access to food was denied for at least 12 hours before sacrifice). the livers were removed and washed in 0°C sterile 0.1M sodium phosphate buffer (pH 7.4) containing 0.1mM Na2-EDTA. The livers were then minced and homogenised in 3 volumes of phosphate buffer. The homogenate was centrifuged for 15 mins and the supernatant (S9) transferred to sterile ampules. Storage in liquid nitrogen.

No. of metaphases analysed: At least 100 metaphase chromosome spreads per culture were examined.

ADMINISTRATION:
Dosing:
-S9 : 75, 200, 325 and 450 ug/ml
+S9 : 200, 400, 600 and 800 ug/ml

+S9: A concentration of 400 ug/ml was tested at the first fixation time, concentrations up to 600 ug/ml at the second fixation time and 400 and 800 ug/ml at the third fixation time.

Application: Monolayer cultures of the cells were used. The cells were exposed to test substance in F10 complete culture medium without serum, buffered with 20 mM HEPES (in the absence of CO2) (HEPES = N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.) The cells were exposed for 2 hours, washed twice with 10 ml of Hank's Balanced Salt Solution and incubated in fresh culture medium for 6, 9 and 19 hours (harvest times 8, 11 and 21 hours respectively). Colchicine was added to the highest dose levels and the positive controls in the last 2 to 2.5 to halt cell division.

Positive and negative control groups and treatment:
Negative control: The vehicle of the test article
Positive controls:
-S9: Ethylmethanesulphonate (EMS; CAS No. 62-50-0; purity 98%; Merck)

used as a direct acting mutagen at a final concentration of 8mM (solvent DMSO)
+S9: Cyclophosphamide (CP; Cas No. 50-18-0; Endoxan-Asta, Asta-Werke, F.R.G)

DESCRIPTION OF FOLLOW UP STUDY: Duplication study carried out in the same manner as the original.

Result : GENOTOXIC EFFECTS:
Only in the presence of S9 at the second fixation time a statistically significant increase was induced at the concentration of 600 ug/ml. However, as this increase was observed only when gap-containing cells were included and as there was no evidence for a dose-response relationship, a biologically significant effect was not concluded.
CYTOTOXIC CONCENTRATION:
-S9: >333 ug/ml
+S9: >333 ug/ml
STATISTICAL RESULTS:
Total number of cells with aberrations; treatment/control comparison, (inclusive/exclusive gaps)
Second fixation time

Dose (ug/ml)	S9	Gaps	P-value (two-sided)	Decision at 95% confidence level
EMS (8Mm	-	+	<0.0004	Significant
		-	<0.0004	Significant
600	+	+	=0.0108	Significant
		-	=0.1528	Not significant
CP (5)	+	+	<0.0004	Significant
		-	<0.0004	Significant

Table 1: MXDA Frequency of chromosome aberrations
KEY:
A, B: Duplicates
g' = Chromatid gap, g'' = chromosome gap, b' = chromatid break, b'' = chromosome break, cb = centromere break, d' = chromatid deletion, f' = fragment, f'' = acentric fragment, dic = dicentric chromosome, tric = tricentric chromosome, r = ring chromosome, sp = spot of chromosome material, exch. = exchange figure, intra = chromosome intrachange, p = pulverized chromosomes, ma = multiple aberrations, poly = poly ploidy, endo. = endoreduplication
* Significantly different from control group (Chi-square test): * P < 0.05, ** P < 0.01 or *** P < 0.001

a) First fixation time, -S9

Concentration ug/ml	**F10-HEPES**			**450ug/ml**		
Culture	**A**	**B**	**A+B**	**A**	**B**	**A+B**
No. of cells scored	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	7	8	15	8	6	14
No of cells with aberrations (- gaps)	1	2	3	2	1	3
g'	5	8		5	6	
g''	1			1		
b'		2		2	1	
b''						
f'						
f''	1			1		
exch.						
dic						
d'						
misc.						
Total aberrations (+ gaps)	7	10		9	7	
Total aberrations (- gaps)	1	2		3	1	

b) First fixation time, +S9

Concentration ug/ml	**F10-HEPES**			**400ug/ml**		
Culture	**A**	**B**	**A+B**	**A**	**B**	**A+B**
No. of cells scored	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	7	5	12	8	8	16
No of cells with aberrations (- gaps)	1	1	2	3	3	6
g'	7	4		4	6	
g''				2		
b'	1			3	1	
b''						
f'						
f''		1			2	
exch.						
dic						
d'						
misc.					poly	
Total aberrations (+ gaps)	8	5		9	9	
Total aberrations (- gaps)	1	1		3	3	

c) Second fixation time, -S9

Concentration ug/ml	F10-HEPES			200ug/ml			325ug/ml			450ug/ml			EMS 8mM		
Culture	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	5	4	9	5	4	9	4	9	13	6	6	12	23	20	43 ***
No of cells with aberrations (- gaps)	3	3	6	2	2	4	2	4	6	2	3	5	16	16	32 ***
g'		1		2	1		2	6		5	2		6	4	
g''	2			1	1					1	1		2		
b'	3	1		2	2		1	3		1	1		6	7	
b''															
f'															
f''		2					1	1		1	2		10	10	
exch.															
dic															
d'															
misc.										poly					
Total aberrations (+ gaps)	5	4		5	4		4	10		8	6		24	21	
Total aberrations (- gaps)	3	3		2	2		2	4		2	3		16	17	

d) Second fixation time, +S9

Concentration ug/ml	F10-HEPES			200ug/ml			400ug/ml			600ug/ml			CP 5 ug/ml		
Culture	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**
No. of cells scored	100	100	200	100	100	200	100	100	200	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	1	2	3	4	2	6	2	5	7	6	7	13*	15	21	36 ***
No of cells with aberrations (- gaps)	1	1	2	2	1	3	1	2	3	2	4	6	11	15	26 ***
g'		1		2	1		1	5		3	2		2	4	
g''				1						1	1		2	3	
b'	1	1		1	1		1	2		2	3		6	2	
b''															
f'															
f''				1							1		4	11	
exch.													3	5	
dic															
d'															
misc.							poly								
Total aberrations (+ gaps)	1	2		5	2		2	7		6	7		17	25	
Total aberrations (- gaps)	1	1		2	1		1	2		2	4		13	18	

e) Third fixation time,-S9

Concentration ug/ml	**F10-HEPES**			**450ug/ml**		
Culture	**A**	**B**	**A+B**	**A**	**B**	**A+B**
No. of cells scored	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	3	4	7	3	5	8
No of cells with aberrations (- gaps)	0	2	2	1	3	4
g'	2	2		2	2	
g''	1				1	
b'				1	1	
b''						
f'						
f''		2			2	
exch.						
dic						
d'						
misc.		poly		2 poly endo		
Total aberrations (+ gaps)	3	4		3	6	
Total aberrations (- gaps)	0	2		1	3	

f) Third fixation time, +S9

Concentration ug/ml	**F10-HEPES**			**400ug/ml**			**800ug/ml**		
Culture	**A**	**B**	**A+B**	**A**	**B**	**A+B**	**A**	**B**	**A+B**
No. of cells scored	100	100	200	100	100	200	100	100	200
No. of cells with aberrations (+ gaps)	5	5	10	10	3	13	5	9	14
No of cells with aberrations (- gaps)	3	2	5	5	1	6	2	3	5
g'	1	3		5	1		3	6	
g''	1				1				
b'		1		1				1	
b''									
f'									
f''	2	1		1	1		2	2	
exch.	1			3					
dic									
d'									
misc.	2 endo poly			3 endo 3 poly	2 endo		4 endo	endo	
Total aberrations (+ gaps)	5	5		10	3		5	9	
Total aberrations (- gaps)	3	2		5	1		2	3	

Table 2: MXDA Mitoic Index

Test substance concentration (ug/ml)	Number of metaphases per 1000 cells	
	Absolute	**% of control**
First fixation time:		
-S9		
Control	105 -79	100
75	85-60	79
200	80-57	74
325	81-55	74
450	63-53	63
+S9		
Control	42-55	100
200	45-89	138
400	91-75	171
600	8-17	26
800	2-29	32
Second fixation time:		
-S9		
Control	91-102	100
75	97-119	112
200	99-90	98
325	78-64	74
450	50-69	62
EMS (8 mM)	40-27	34
+S9		
Control	81-102	100
200	63-74	75
400	68-61	70
600	55-38	50
800	0-0	0
CP (5 ug/L)	24-25	27
***Third fixation time*:**		
-S9		
Control	110-115	100
75	116-117	104
200	125-104	102
325	107-135	108
450	107-111	97
+S9		
Control	120-108	100
200	98-96	85
400	91-91	79
600	29-28	25
800	94-98	84

Test substance : PURITY: 99.6% (+ 0.2% paraxylenediamine)
ANY OTHER INFORMATION:
Lot No.: 26589/AMZ
Keep under nitrogen in the dark

Conclusion : The number of cells with chromosome aberrations found in the solvent control cultures fell within the laboratory historical control data range. The positive control chemicals both produced statistically significant increases in the number of cells with aberrations. It was therefore concluded that the test

conditions were optimal and that the metabolic activation system functioned properly.

The test with CHO cells should therefore be considered valid and that the test substance is not clastogenic under the conditions of the test.

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

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5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	Swiss
Route of admin.	:	other: oral intubation
Exposure period	:	Single dose
Doses	:	750 mg/kg Bodyweight (Dose volume = 10 ml/kg bodyweight)
Result	:	negative
Method	:	Directive 84/449/EEC, B.12 "Other effects - Mutagenicity (micronucleus test)"
Year	:	1989
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	METHOD FOLLOWED: Study conducted in accordance with generally accepted scientific principles. Guideline based on: Method B12, 67/548/EEC (September 19, 1984) OECD Guideline No. 474 (Adopted May 26, 1983) EPA Test Guideline (TSCA, FIFRA), Subchapter R, part 798, subpart F, Genetic Toxicity § 798.5395 (revised July 1, 1986). DEVIATIONS FROM GUIDELINE: Feed was withheld overnight prior to dosing until approximately 8-9 hours after administration of the test substance instead of 3-4 hours. STATISTICAL METHOD: Wilcoxon Rank Sum test, two-sided test at $P < 0.05$
Test condition	:	TEST ORGANISMS: Age: 8 weeks at start of treatment. Weight at study initiation: Males: 26 - 34g, Females: 20 - 26g Number of animals: 5 male and 5 female animals were dosed with 750 mg/kg bodyweight ADMINISTRATION/EXPSOURE: Vehicle: Milli-RO water Duration of test/exposure: Animals were sacrificed at 24, 48 and 72 hours after dosing. (At 48 hours for the positive control). Frequency of treatment: Single dose. Control groups and treatment: Negative control: vehicle only Positive control: Cyclophosphamide (50 mg/kg bodyweight dissolved in 0.9% NaCl in Milli-RO water. Route and frequency of administration were consistent with those of the test substance. EXAMINATIONS:

Analysis of bone marrow smears of micronuclei.
General clinical observations for toxicity to the substance.
Criteria for evaluating results: The number of nuclei counted in 1000 polychromatic erythrocytes. The ratio of polychromatic to normochromatic erythrocytes was determined by counting and diferentiating the first 1000 erythrocytes at the same time.

Result : MORTALITY:
Pre-test dose selection: Three animals (two females and one male) dosed at 1000 mg/kg died with 48 hours post-dosing. At 2000 mg/kg all animals died with 24-hours of post-dosing and at 5000 mg/kg all animals died within 4 hours of treatment.
Main study: Two males died between 2 and 3 days after dosing.
CLINICAL SIGNS:
Pre-test dose selection: Animals treated at 250 mg/kg did not show any signs of reaction to the treatment. Those dosed at 500 and 750 mg/kg recovered from the letargy observed immediately after dosing.
Main study: No clinical signs noted.
BODYWEIGHT CHANGES:
Bodyweights were only measured immediately prior to dosing.
EFFECTS ON PCE/NCE RATIO:
The ratio of polychromatic/normochromatic erythrocytes does not evidence any toxic effect of the test substance on the erythropoiesis.
mPCE FREQUENCY:
No increase in the frequency of micronuclei was observed.
STATISTICAL RESULTS:
Wicoxon Rank-sum test.
Number of micronuclei per 1000 polychromatic erythrocytes; treatment/control comparison:

Group	Treatment	Dose mg/kg	sex	P-value (2-sided)	Decision at 95% C.I.
G	CP	50	M	0.01	Significant
G	CP	50	F	0.01	Significant

Table 1: MXDA Micronucleus data: Mean Number of Micronuclei per 1000 polychromatic erythrocytes and ratio polychromatic/normochromatic erythrocytes.

Males

Group	Treatment	Dose (mg/kg body weight)	Sampling time (hours)	Number of micronuclei per 1000 polychromatic erythrocytes (mean ± SD)[a]	Ratio polychromatic/ normochromatic erythrocytes (mean ± SD)
A	Vehicle[c]	--	24	0.2 ± 0.4	0.91 ± 0.06
B	Vehicle	--	48	0.2 ± 0.4	0.96 ± 0.12
C	Vehicle	--	72	0.4 ± 0.5	1.02 ± 0.09
D	METAXY[d]	750	24	0.2 ± 0.4	0.94 ± 0.09
E	METAXY	750	48	1.3 ± 1.0	0.95 ± 0.07[b]
F	METAXY	750	72	0.0 ± 0.0	0.91 ± 0.03[b]
G	CP	50	48	9.0 ± 2.2*	0.30 ± 0.05

Females

Group	Treatment	Dose (mg/kg body weight)	Sampling time (hours)	Number of micronuclei per 1000 polychromatic erythrocytes (mean ± SD)[a]	Ratio polychromatic/ normochromatic erythrocytes (mean ± SD)
A	Vehicle[c]	--	24	0.2 ± 0.4	0.96 ± 0.05
B	Vehicle	--	48	0.4 ± 0.5	0.99 ± 0.09
C	Vehicle	--	72	0.2 ± 0.4	1.04 ± 0.08
D	METAXY[d]	750	24	0.4 ± 0.5	0.86 ± 0.09
E	METAXY	750	48	0.8 ± 0.8	0.90 ± 0.07
F	METAXY	750	72	0.4 ± 0.5	1.02 ± 0.09
G	CP	50	48	10.2 ± 3.3*	0.39 ± 0.07

a Five animals per treatment group
b Four animals per treatment group, one animal died post-dosing
c Milli-RO water
d METAXYLENEDIAMINE
* Significantly different from corresponding control group (wilcoxon rank sum test $P \leq 0.05$)

Test substance	:	PURITY: 99.6% (+ 0.2% paraxylenediamine) ANY OTHER INFORMATION: Lot No.: 26589/AMZ Keep under nitrogen in the dark
Conclusion	:	The incidence of micronuclei in the control animals was found to be in the range of historical data. The groups treated with the positive control showed a decrease in the PCE/NCE ratio, which reflects a toxic effect of the compound on erythropoiesis. The positive control substance induced in both sexes a statistically significant increase in the number of nuclei. It is concluded that the test is valid and that the test substance can be considered non-mutagenic under the conditions of the test.
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

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5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type	:	other: Reproduction/developmental toxicity screening test
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	Males and females: 450 mg/kg, 150 mg/kg and 50 mg/kg. Males: 48 days Females: 41 - 45 days (until 3 days laction after parturition) or 40 days if no delivery after copulation. Offspring not dosed.
Frequency of treatm.	:	7 days/week
Premating exposure period		
Male	:	14 days before mating
Female	:	14 days before mating
Duration of test	:	7 weeks
No. of generation studies	:	1
Doses	:	450 mg/kg, 150 mg/kg and 50 mg/kg
Control group	:	yes, concurrent vehicle
NOAEL parental	:	50 mg/kg bw
NOAEL F1 offspring	:	450 mg/kg bw
Result	:	NOAEL is greater in females than males. No influences on fertility or the next generation were observed
Method	:	OECD 421
Year	:	1995
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	METHOD FOLLOWED: Study conducted in accordance with generally accepted scientific principles. Guideline based on: OECD Guideline 421 (July 1995) DEVIATIONS FROM GUIDELINE: Temperature and humidity of the animal room were deviated from the set

range on 2 days during the study period. However, each deviation was slight and for a short time, and was judged not to have affected the integrity of the study.

STATISTICAL METHOD:
Multiple comparison test applied on data of bodyweight, food intake, number of copora lutea, implantation site, number of offspring dead, sex ratio, mean length of oestrus cycle, pregant duration, implantation rate, delivery rate, birth rate, ratio of external abnormalities, ratio of offspring alive on day 4 after birth, organ weight and organ weight/bodyweight ratio (relative organ weight).
Bartlett's test: Homogenetity of variance.
Dunnett's multi-comparison test: Used when variance was homogenous, significant difference between control and test groups analysed.
Steel's test: Heterogenous variance
chi 2 test: Comaprison of birth rate, copulation index and fertility index.
Fischer's direct probit analysis: Incidence of abnormal oestrus cycle and incidence of pathology findings.
Mann-Whitney's U test: Histopathological findings which increased in severity in the treated groups.
Two tail test: Result concerning live off-spring during lactation period, mean value per one dam was used as one sample. Analysis at 5 and 1% levels of significance.

METHOD OF CALCULATION:
Body weight gains, Mean daily intake (food), Cumulative food intake, Mean duration of oestrus cycle, Incidence of abnormal oestrus cycle, Copulation index, Gestation period, Fertility index, Implantation index, Delivery index, Birth index.
Offspring: Number born, Mean body weight, viability index.

ANALYTICAL METHODS:
HPLC:
Column: NUCLEOSIL 5C18 (250 mm x 4.6 mm I.D)
Mobile phase: 0.1% sodium hexanesulfonate
Water containing 0.1% H3PO4/CH3CN (85/15 v/v)
Temperature: 50°C
Flow rate: 1.0 ml/min
Injection vol: 10ul
Dectection wavelength: 210nm

Test condition : TEST ORGANISMS:
Number: 96
Age: 8 weeks (at purchase), 10 weeks at start of study.
Sex per dose: 12 animals of each sex per dose level.
ADMINISTRATION/EXPSOURE:
Treatment:
Pre-treatment observation: 2 weeks before administration began.
Males: Duration of administration was 48 consecutive days, i.e. 14 days before mating, 14 days during mating period, and 20 days after the end of the mating period.
Females: Duration of administration was 14 days before mating, during mating period (maximum 5 days), and throughout gestation period after copulation and 3 days of lactation after parturation (41-45 days). In females which did not deliver after copulation, administration was completed for 40 days until the day before necropsy.
Confirmation of childbirth was conducted at 9.00 to 10.00 am and Day 0 of lactation applied accordingly.
Post-treatment observation: 1 day. Offspring were observed until day 4 of lactation.
Vehicle: The test substance was dissolved in official purified water (Lot

1051) to make solutions of 5, 15 and 45 mg/ml solution for e ach group.

Doses: A 2-week administration pilot study was used to determine dose levels for the reproduction study. The dose levels used in the pilot study were the same as those used in the 28-day repeat oral dose toxicity study, 600, 150, 40 and 10 mg/kg. As in the repeat dose study, there were deaths in the 600 mg/kg dose group, therefore, for the reproductive toxicity study the highest dose level was reduced to 450 mg/kg.

MATING PROCEDURES:
Females whose vaginal smear was examined for 14 days before m ating were housed together with males of the same group on a 1 to 1 basis for 5 days maximum. Copulation was judged to be successful by confirming the presence of sperms in the vaginal smear the next morning, designated day 0 of pregnacy.

STANDARDISATION OF LITTERS: No

PARAMETERS ASSESSED DURING STUDY:
Clinical observations and frequency: Observations taken daily for bodyweight, food intake, oestrus cycle (duration, incidence of abnormal cycle), gestation period, delivery, After termination; pathology, histopathology.

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
Organ weights: Male; thymus, adrenal, testes and epididymides
Female; thymus, adrenal.
Histopathology: Thymus, stomach, adrenal, ovaries, uterus, vagina, testes, epididymides, abnormal site of spleen from 2 animals, nasal wall from 1 animal, small intestine from 3 animals, large intestine from 5 animals, seminal vesicle from 1 animal, liver from 1 animal and lung from 1 animal. No histopathology carried out on offspring.

OTHER EXAMIN ATIONS:
At necropsy, examination was done on appearence, oral cavity, nostril and cranial cavity, skeleton, external appearence and dissected face of brain and spinal cord, thoracic cavity, abdominal cavity and pelvic cavity with viscera, and cervical tissue and organs. All abnormalities noted in the gross examination were recorded with the location, size, hardness and any other details.

Result : NOEL:
Male: 50 mg/kg
Female: 150 mg/kg
Offspring: 450 mg/kg

One male in the 150 mg/kg group, and one female and one male died in the 450 mg/kg group.
Male: In the groups treated with 150 mg/kg or more, salivation and nasal noise was seen in the general observations.
Female: In the 450 mg/kg group, salivation and nasal noise were seen in the general observations. In addition, suppression of bodyweight gain, reduced food intake, and ulceration of the fore-stomach in necropsy were observed.
ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX:

Male: 12/0 mg/kg, 12/50 mg/kg, 12/150 mg/kg, 12/450 mg/kg
Female: 12/0 mg/kg, 12/50 mg/kg, 12/150 mg/kg, 12/450 mg/kg

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
Death and general signs:
There was one mortality on Day 38 of one male in the 150 mg/kg group and then further deaths of 3 males in the 450 mg/kg group on days 31, 38 and 39. One female in the 450 mg/kg group died on day 2 of pregnacy. The main change in the general observations after administration of the test substance was salivation, seen in males and females of the 150 and 450 mg/kg groups, dose dependently. Salivation observed in a few animals in weeks 5 to 6 and in half the animals in week 7 of administration was a transient change which appeared immediately after dosing and disappeared at 30 minutes later. In males of the 450 mg/kg group, salivation was observed in about half of the animals and most of the animals in week 2 or later. In week 1, it appeared immediately after administration and disappeared after 30 minutes, however, salivation lasted longer accordingly as administration continued, being continuously observed at 90 to 180 minutes after administration during the latter stages of the study. In one female of the 150 mg/kg group, salivation was seen in one animal on day 2 of pregnancy, with the same transience as observed in the males. In the females of the 450 mg/kg group, salivation was observed in most animals at the pre-mating, mating periods and early stages of pregnancy, with a gradual decrease in the number of animals observed at the middle to late stages of pregnancy, and only in one animal during the lactation period after delivery. In females of the 450 mg/kg group, salivation appeared immediately after administration and lasted for 60 minutes in a few animals during the gestation period. During other periods, salivation appeared immediately after administration and disappeared 30 minutes later. In addition, the following observations were made: nasal noise (1 male, 150 mg/kg group and 3 males and females, 450 mg/kg group), irregular respiration (1 male, 150 mg/kg group), abdominal distension, emaciation, staining of fur, piloerection, and hypothermia (1 male, 150 mg/kg and 1 female, 450 mg/kg group), nasal discharge (1 male and 1 female, 450 mg/kg group), pale appearence (1 male, 150 mg/kg group and 2 females, 450 mg/kg group), ptosis (1 male, 150 mg/kg group, 2 males and 1 female of 450 mg/kg group) and prone position (1 female, 450 mg/kg group). Spontaneous changes such as eye discharge in a male, control group and alopecia in a female, 150 mg/kg group and in a male and female 450 mg/kg group were observed.

Bodyweight:
Bodyweight:
Males, 450 mg/kg group bodyweights were smaller than those of the control group on day 8 and thereafter. Bodyweight gain (62±20 g) was also significantly less than the control group (129±26 g).
Females, 450 mg/kg group had bodyweights on day 14 and 21 of pregnancy that were less than those of the control group.
Bodyweight gain during gestation (140±19 g) was also significantly less than the control group (170±31 g).

Food intake:
Food intake of the 450 mg/kg dose group was statistically significantly less than that of the control group. In males the daily intake between days 1 to 8, days 29 to 36 and days 43 and 48 and cumulatively between days 1 to 15 was smaller. In females the daily intake between days 1 to 8 and cumulatively between days 1 to 15 was smaller.

Organ weights:
Males, 450 mg/kg group; absolute weight of thymus decreased (190±60 mg compared with 277±73 mg for controls). Absolute (90±14 mg) and relative weight (20.526±3.256 %) of the adrenal increased (compared with control

values of 63±9 mg and 12.382±1.925 %, respectively), as did the relative weight of the testes (0.741±0.029 % compared with 0.667±0.047 % for the controls). Relative weight of the thymus was inclined to decrease although no statistical significance was observed (45.090±12.432 % compared with 54.228±13.221 % for the controls).

Necropsy:
Mortalities (1 male, 150 mg/kg group and 3 males and 1 female, 450 mg.kg group);
Males; Ulceration of the fore-stomach was seen in 9 animals of the 450 mg/kg group, the incidence was increased with statistical significance compared to the control group. In addition of these 9 animals, 6 had adhesipon of the stomach with surrounding tissue. Other changes were observed in both the control and test groups.
Females, natural delivery; Ulceration of the fore-stomach in 9 animals of the 450 mg/kg group and atrophy of the thymus in 6 animals of the 450 mg/kg group were statistically significant differences to the control group. 3 animals in the 350 mg/kg group had fore-stomach ulceration with stomach adhesion to surrounding tissues, 3 had mucosal-thickening of the fore-stomach and 1 animal had hypertrophy of the spleen. Other changes observed in both the control and test groups.
Female, no delivery, control group; No delivery on day 25 of pregnancy. Dead foetus found in uterus.

Histopathology:
Males; Ulceration of fore-stomach, squamous epithelium hyperplasia with hyperkeratosis in the fore-stomach and diffuse hyperplasia in the adrenal cortx occurred with higher incidence with statistical significance in comparison to the control group. For ulceration in the fore-stomach, incidence of sever cases also increased with statistical significance compared to the controls.
Females, natural delivery; Again ulceration of the fore-stomach, squamous epithelium hyperplasia with hyperkeratosis in the fore-stomach and diffuse hyperplasia in the adrenal cortex showed higher incidence with statistical significance in comparison with the control group. Again as with the male, incidence of severity of ulceration increased with statistical significance compared to the controls.

Copulation and fertility:
The female, 450 mg/kg group that died on day 2 of pregnancy was excluded from the conception observations. All females conceived.
No intergroup differences observed in the mean duration of the oestrus cycle. No intergroup differences in the incidence of abnormal oestrus cycle.

Delivery and lactation:
No abnormalities seen in delivery. The values of pregnant period, number of corpora lutea, number of implanation site, number of offspring, and number of offspring born alive were similar among each group. No differences among groups were seen in gestation index, implantation index, birth index, sex ratio or viability index on day 4.
Offspring morphology, bodyweight and necropsy findings:
External examination: Dwarfism (bodyweight less than 60% of mean bodyweight of control group) was only seen in one offspring of the 450 mg/kg group.
Bodyweight (day 0 and day 4): No intergroup differences observed.
Necropsy: Dead offspring during lactation period; only thymus remnant in the neck of one male in the control group and one female of the 450 mg/kg group was observed.
Offspring necropsied on day 4 of lactation: Thymus remnant seen in 5

males of the control group, 3 males and 1 female of the 50 mg/kg group, and 2 males of the 450 mg/kg group.
Other sporadic changes observed in the males were whitish spotting, dark spotting, adhesion with diaphram, yellowish change and anomalous nodule in the liver, pelvic dilactation of the kidney, displacement of the kidney, and reddening of the eyeball. In addition, skin bruising was seen in 3 males and females of the same litter of the 150 mg/kg group.

Table 1: Oral dose reproductive toxicity test in the rat: Toxic response and effect

KEY:
♦ = No change
↓ = Increased significantly compared to control (P=< 0.01)
↑ = Decreased significantly compared to control (P=< 0.01)
⇓ = Decrease significantly compared to control (P=< 0.05)
NE: Not examined
Day 8-49
Gestation day 14, 21
Day 1-8, 29-36, 43-48
Day 1-8
+ = Slight
++ = Moderate
+++ = High
* = p=<0.01

Duration of test substance administration	**Male: from Jan 12, 1999 to Feb 28, 1999 (total = 48 days) Female: From Jan 12, 1999 to Feb 25, 1999**							
Dose groups mg/kg	**Control Group**		**50 mg/kg**		**150 mg/kg**		**450 mg/kg**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
General appearance:								
Observations of dead animals:	♦	♦	♦	♦	1/12	♦	3/12	1/12
Salivation	♦	♦	♦	♦	♦	♦	3/3	1/1
Emaciation	♦	♦	♦	♦	1/1	♦	♦	1/1
Piloerection	♦	♦	♦	♦	1/1	♦	♦	1/1
Nasal noise	♦	♦	♦	♦	1/1	♦	2/3	1/1
Staining of fur	♦	♦	♦	♦	1/1	♦	♦	1/1
Pale	♦	♦	♦	♦	1/1	♦	♦	1/1
Hypothermy	♦	♦	♦	♦	1/1	♦	♦	1/1
Abdominal distension	♦	♦	♦	♦	1/1	♦	♦	1/1
Ptosis	♦	♦	♦	♦	1/1	♦	1/3	1/1
Nasal discharge	♦	♦	♦	♦	♦	♦	1/3	♦
Irregular respiration	♦	♦	♦	♦	1/1	♦	♦	♦
Observations of surviving animals:	12/12	12/12	12/12	12/12	11/12	12/12	9/12	11/12
Salivation	♦	♦	♦	♦	5/11	1/12	9/9	11/11
Nasal noise	♦	♦	♦	♦	♦	♦	1/9	2/11
Pale	♦	♦	♦	♦	♦	♦	♦	1/11
Nasal discharge	♦	♦	♦	♦	♦	♦	♦	1/11
Ptosis	♦	♦	♦	♦	♦	♦	♦	1/11
Prone position	♦	♦	♦	♦	♦	♦	♦	1/11
Eye discharge	1/12	♦	♦	♦	♦	♦	♦	♦
Alopecia	♦	♦	♦	♦	♦	1/11	1/9	1/11
Body weight change			♦	♦	♦	♦	↓(a)	↓(b)
Food intake			♦	♦	♦	♦	↓(c)	↓(d)

Organ weight:								
Mean bodyweight at necropsy (g)		509	316	510	313	510	306	440	296
(Absolute)	Thymus			♦	♦	♦	♦	⇓	⇓
	Adrenals			♦	♦	♦	♦	↑	♦
(Relative)	Adrenals			♦	♦	♦	♦	↑	↑
	Testes		NE	♦	NE	♦	NE	↑	NE

Dose groups mg/kg	Control Group		50 mg/kg		150 mg/kg		450 mg/kg	
	M	F	M	F	M	F	M	F
Pathological examination								
Necropsy findings *(Scheduled animals):*	12/12	11/12	12/12	12/12	11/12	12/12	9/12	11/12
Spleen: Enlargement	0/12	0/11	0/12	0/12	0/11	0/12	0/9	1/11
Thymus: Atrophy	0/12	0/11	0/12	0/12	0/11	0/12	0/9	6/11
Lung: Brownish spotting	1/12	0/11	0/12	0/12	0/11	0/12	1/9	2/11
Stomach: Mucosal thickening	0/12	0/11	0/12	0/12	0/11	0/12	0/9	3/11
Ulceration in fore-stomach	0/12	0/11	0/12	0/12	0/11	0/12	9/9	9/11
Liver: Whitish spotting	0/12	1/11	0/12	0/12	0/11	0/12	0/9	0/11
Kidneys: Cyst	2/12	0/11	0/12	0/12	0/11	0/12	0/9	0/11
Scar	0/12	0/11	0/12	0/12	0/11	1/12	0/9	0/11
Adrenals: Brownish spotting	0/12	0/11	0/12	0/12	0/11	0/12	1/9	0/11
Ovaries: Cyst		2/11		0/12		0/12		0/11
Fur: Thinning	0/12	0/11	0/12	0/12	0/11	1/12	1/9	1/11
(Mortalities):	0/12	0/12	0/12	0/12	1/12	0/12	3/12	1/12
Spleen: Atrophy	NE	NE	NE	NE	1/1	NE	0/3	1/1
Thymus: Atrophy	NE	NE	NE	NE	1/1	NE	1/3	0/1
Reddening	NE	NE	NE	NE	0/1	NE	2/3	0/1
Lung: Reddening	NE	NE	NE	NE	0/1	NE	1/3	0/1
Nasal cavity: Mucinous contents	NE	NE	NE	NE	1/1	NE	0/3	0/1
Stomach: Dilatation of lumen	NE	NE	NE	NE	1/1	NE	1/3	1/1
Reddish spotting	NE	NE	NE	NE	0/1	NE	0/3	1/1
Reddening	NE	NE	NE	NE	0/1	NE	1/3	0/1
Mucosal thickening	NE	NE	NE	NE	0/1	NE	1/3	1/1
Ulceration	NE	NE	NE	NE	0/1	NE	3/3	0/1
Small intestine: Dilatation of lumen	NE	NE	NE	NE	1/1	NE	1/3	1/1
Large intestine: Dilatation of lumen	NE	NE	NE	NE	1/1	NE	3/3	1/1
Liver: Whitish spotting	NE	NE	NE	NE	0/1	NE	0/3	1/1
Seminal vesicle: Atrophy	NE	NE	NE	NE	1/1	NE	0/3	1/1
Uterus: Small	NE	NE	NE	NE	1/1	NE		1/1
Whole body: Emaciation	NE	NE	NE	NE		NE	0/3	
Female (without natural delivery):		1/12		0/12		0/12		0/12
Uterus: Dark spotting		1/1		NE		NE		NE
Dilatation of lumen		1/1		NE		NE		NE
Histological findings *(Scheduled necropsy animals)*								
Spleen: Hyperplasia of white pulp (+)	NE	NE	NE	NE	NE	NE	NE	1/1
Thymus: Cortical atrophy (+)	0/12	2/11	0/12	1/12	0/11	0/12	0/9	6/11
Lung: Foamy cell accumulation (+)	0/1	NE	NE	NE	NE	NE	0/1	½

Bronchopneumonia (+)	1/1	NE	NE	NE	NE	NE	1/1	½
Goblet cell hyperplasia (+)	0/1	NE	NE	NE	NE	NE	1/1	2/2
Stomach: Edema (+)	0/12	0/11	0/12	0/12	0/11	0/12	0/9	1/11
Ulcration in fore-stomach (total)	0/12	0/11	0/12	0/12	0/11	0/12	9*/9	9*/11
(+)	0/12	0/11	0/12	0/12	0/11	0/12	1/9	0/11
(++)	0/12	0/11	0/12	0/12	0/11	0/12	2/9	4/11
(+++)	0/12	0/11	0/12	0/12	0/11	0/12	6/9	5/11
Dose groups mg/kg	**Control Group**		**50 mg/kg**		**150 mg/kg**		**450 mg/kg**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Fibrosis (+)	0/12	0/11	0/12	0/12	0/11	0/12	0/9	2/11
Squamous cell hyperplasia (+)	0/12	0/11	0/12	0/12	0/11	0/12	9/9	10/11
Liver: Capsular thickening (+)	NE	1/1	NE	NE	NE	NE	NE	NE
Kidneys: Cyst (+)	2/2	NE	NE	NE	NE	NE	NE	NE
Ovaries: Bursal cyst (+)		2/11		NE		NE		0/11
Adrenals: Vacuolar degeneration (+)	6/12	0/11	6/12	0/12	2/11	0/12	1/9	0/11
Hyperplasia in cortex	0/12	0/11	0/12	0/12	0/11	0/12	7/9	8/11
(Mortalities)								
Spleen: Atrophy(+)	NE	NE	NE	NE	1/1	NE	NE	1/1
Thymus: Congestion (+)	NE	NE	NE	NE	0/1	NE	2/3	0/1
Haemorrhage(+)	NE	NE	NE	NE	0/1	NE	1/3	0/1
Cortical atrophy (+)	NE	NE	NE	NE	1/1	NE	1/3	1/1
Lung: Congestion(+)	NE	NE	NE	NE	NE	NE	1/1	NE
Stomach: Congestion	NE	NE	NE	NE	0/1	NE	0/3	1/1
Edema (+)	NE	NE	NE	NE	0/1	NE	1/3	0/1
Haemorrhage (+)	NE	NE	NE	NE	0/1	NE	1/3	0/1
Ulceration in fore-stomach (total)	NE	NE	NE	NE	0/1	NE	3/3	0/1
(++)	NE	NE	NE	NE	0/1	NE	1/3	0/1
(+++)	NE	NE	NE	NE	0/1	NE	2/3	0/1
Ulceration in glandular stomach (+)	NE	NE	NE	NE	0/1	NE	0/3	1/1
Fibrosis (+)	NE	NE	NE	NE	0/1	NE	0/3	1/1
Squamous cell hyperplasia (+)	NE	NE	NE	NE	0/1	NE	3/3	0/1
Liver: Focal necrosis(+)	NE	NE	NE	NE	NE	NE	NE	1/1
Adrenals: Vacuolar degeneration (+)	NE	NE	NE	NE	0/1	NE	1/3	NE
Hyperplasia in cortex (+)	NE	NE	NE	NE	1/1	NE	3/3	NE
(Female without natural delivery)								
Uterus: Haemorrhage		1/1		NE		NE		
Pregnancy data								
Number of females with live offspring	11		12		12		11	
Birth rate (No. of females with live offspring / No. of pregnant females x 100	91.7		100.0		100.0		100.0	
Gestation rate	22.6		22.7		22.6		22.5	
Litter data								
Number of offspring	16.0		14.2		14.9		14.8	

Parturition rate (No. of offspring / No. of implantation sites x 100)	92.8	89.3	90.0	92.9
Number of offspring born alive (Day 0 of lactation)	16.0	14.2	14.9	14.8
Birth index (No. of offspring born alive / no. of offspring x 100)	100.0	100.0	100.0	100.0
Sex ratio (M:F)	1.29	1.45	1.06	1.08
Number of offspring alive on day 4 after birth	7.8:7.5	7.7:5.8	6.9:7.0	7.1:6.5

Dose groups mg/kg	Control Group		50 mg/kg		150 mg/kg		450 mg/kg	
	M	F	M	F	M	F	M	F
Viability index on day 4 (No. of offspring alive on day 4 after birth/ no. of offspring born alive x 100)	95.6:98.2		96.5:94.4		92.2:92.9		97.9:90.6	
Body weight on day of lactation	♦/♦		♦/♦		♦/♦		♦/♦	
Body weight on day 4 of lactation	♦/♦		♦/♦		♦/♦		♦/♦	
External abormalities	♦/♦		♦/♦		♦/♦		♦/♦	
Growth after birth	♦/♦		♦/♦		♦/♦		♦/♦	

Test substance : PURITY: 99.8%
IMPURITIES: As specified in 1.1 - 1.4
ANY OTHER INFORMATION:
Lot No.: 8100

Conclusion : NOEL:
Males: 50 mg/kg/day
Females: 150 mg/kg/day
Offspring: 450 mg/kg/day

Mortality:
The test substance administration influenced the death of males in the 150 mg/kg group and males and females of the 450 mg/kg group.
Clinical observations: Also considered to be caused by substance administration. It was suspected that the substance also caused stimulation of mucus secretion and caused respiratory disfuntion. Other observations such as ptosis, and fur staining were considered to be induced by exacerbation of a general condition but not induction by the substance. Salivation is considered to be an incidental occurance unrelated to the administration of the test substance.
Bodyweight: Gain was suppressed by substance administration and also food intake was influenced.
Necropsy: The action of the substance appeared to be stronger in the male than in the female. It was therefore concluded that the action of the test substance was sex related.
The substance did not cause any changes in the reproductive organs. The changes in the digestive system were considered to be induced by the corrosive nature of the substance.
Reproductive and development toxicity: The substance did not affect any part of the reproductive cycle.

Reliability : (1) valid without restriction
Guideline study: OECD Screening test for reproductive toxicity.

Flag : Critical study for SIDS endpoint
27.06.2001 (14)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9. SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Comments : In clinical observation of workers during the manufacturing process, the substance appears to act as a gastrointestinal irritant.
The substance has been shown to cause contact sensitisation reactions in workers at concentrations equal to and below the Threshold Limit Value (TLV)[41]. The TLV is listed in mg/m3 only, but the substance has a saturated vapour concentration greater than its TLV value. Therefore, the substance will exist largely as the vapour.
The substance should on the basis of human exposure data be therefore classified as a skin sensitiser at concentrations lower than the listed TLV.

27.06.2001 (31)

5.11 ADDITIONAL REMARKS

8.1 METHODS HANDLING AND STORING

Safe handling	:	Good industrial hygiene should be practiced when handling this substance. Wear full protective clothing and self-contained breathing apparatus with full faceshield
Fire/exp. protection	:	Keep away from open fire and ignition sources
Storage requirement	:	Store in sealed container in ventilated room at ambient temperature. The substance must be kept sealed as it absorbs CO2 gas. Keep cool and dry.

8.2 FIRE GUIDANCE

Hazards	:	Combustion/decomposition products may include amines and other nitrogen compounds as well as carbon monoxide and carbon dioxide.
Protective equipment	:	Full protective clothing, self contained breathing apparatus with full face shield.
Extinguishing medium	:	Dry chemical, water fog, regular foam
Unsuit. exting. medium	:	Water or foam may cause frothing which can be violent, especially if sprayed into containers of hot burning liquid.

28.02.2001

8.3 EMERGENCY MEASURES

Type	:	injury to persons (skin)
Remark	:	If substance comes into contact with the skin, wash skin thoroughly with plenty of soap and water. Remove contaminated clothing and shoes.

28.02.2001

Type	:	injury to persons (eye)
Remark	:	Immediately flush eyes with plenty of water. Seek immediate medical attention.

28.02.2001

Type	:	injury to persons (oral)
Remark	:	If swallowed, drink copious amounts of water. Seek immediate medical attention.

28.02.2001

Type	:	injury to persons (inhalation)
Remark	:	If inhaled, rem ove to fresh air immediately. Seek immediate medical attention.

28.02.2001

Type	:	accidental spillage
Remark	:	Evacuate personnel from immediate vicinity. Wear full protective clothing and self-contained breathing apparatus with a full face-shield. Small spill: Absorb spill with suitable absorbant. Dispose of by incineration. Large spill: Absorb spillage with a suitable inert material. Carefully transfer spillage to waste containers or use a sealed industrial vacuum machine. Containers filled with w aste must be labelled in same way as the original containers. Dispose of waste material in accordance with local regulations by incineration. Prevent waste from entering sewers or any other water streams.

28.02.2001

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

Domain	:	Industry/skilled trades
Process	:	Destruction
Type of destruction	:	Incineration

27.02.2001

8.5 WASTE MANAGEMENT

Remark	:	All waste from manufacture and use including packaging, which might contain the substance should be sent for disposal by incineration.

27.02.2001

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

(1) MOE, Japan (2000) Ministry of Environment : unpublished report of A growth–inhibition study to algae (*Selenastrum capricornutum*) on 1,3-benzenedimethanamine.

(2) MOE, Japan (2000) Ministry of Environment : unpublished report of A reproduction-inhibition study to *Daphnia magna* on 1,3-benzenedimethanamine.

(3) MOE, Japan (2000) Ministry of Environment : unpublished report of Acute immobilisation test to *Daphnia magna* on 1,3-benzenedimethanamine.

(4) MOE, Japan (2000) Ministry of Environment : unpublished report of Acute toxicity study to Medaka (*Oryzias latipes*) on 1,3-benzenedimethanamine

(5) Acute toxicity test of metaxylene & 1,3-bis aminomethyl cyclohexane (October 8, 1980) Mitsubishi Gas Chemical Company, (unpublished)

(6) Bioaccumulation Test (1984) MITI Evaluation of Existing Chemicals, Japan

(7) METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan).
METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan). Test No. :80245
Safety data sheet. 1997, Mitsubishi Gas Chemical Company
HEDSET submission 1995
RTECS: PF8970000

(8) METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan). Test No.: K-245.

(9) Derived using EUSES version 1.00 from AOPWIN rate. (2001)

(10) Determination of m-xylylenediamine concentration in air of a model workroom. Toray Research Center Inc. 1991 (Japan)

(11) Enninga IC, (1989) Evaluation of the ability of Metaxylenediamine to induce chromosome aberrations in cultured Chinese Hamster Ovary (CHO) cells (including multiple fixation times), RCC Notox B.V. Report No. 017324

(12) Enninga IC, (1989) Micronucleus test in bone marrow cells of the mouse with metaxylenediamine, RCC Notox B.V. Report No. 017313

(13) HEDSET submission 1995

(14) MHW, Japan(2001), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (971 – 984).

(15) MHW, Japan(1996), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (387 – 394).

(16) Jackson GC, Molloy GK, Hardy CJ, Gregson RL, Offer JM, Gopinath C (1993) MXDA: Acute inhalation toxicity in rats 4-hour exposure. Huntingdon Research Centre Ltd., Report No: MGC 2/921548 (unpublished)

(17) Manufacturer data

(18) Manufacturers safety data sheet. 1993
HEDSET submission 1995

(19) Manufacturers safety data sheet. 1997
HEDSET submission 1995

(20) RTECS: PF8970000

(21) Nagai T, Hirota M, (1986) MXDA: A skin corrosive test of metaxylene diamine founded on the Standards of the United Nations. Niigata Laboratories

(22) SafePharm Laboratories, UK 2001

(23) MHW, Japan(1996), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (399-401).

(24) MHW, Japan(1996), Ministry of Health and Welfare, Toxicity Testing Reports of Environmental Chemicals, Vol.3 (395 – 398).

(25) Sigma-Aldrich Library of Regulatory and Safety Data

(26) Specific gravity data from the test material supplier was converted from d20/20 by JS.
Report No.: K2249 (1987)
Yuhki M (2000) Bioaccumulation Test in common carp on m-Xylenediamine Test No.: 80245 K
Manufacturers safety data sheet. 1997
HEDSET submission 1995

(27) Value of 15mmHg in Sigma-Aldrich Library of Regulatory and safety Data was converted into the unit of Pa.
Yuhki M (2000) Bioaccumulation Test in common carp on m-Xylenediamine Test No.: 80245 K
Manufacturers safety data sheet. 1997
HEDSET submission 1995: Tested by Toray Research Center Inc.
1984 (Japan)

(28) METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan). Test No.: 80245

(29) METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan). Test No.: 80245
Manufacturers safety data sheet. 1997

(30) METI, Japan (2000) Ministry of Economy, Trade and Industry (former MITI), unpublished data, conducted by Chemicals Evaluation and Research Institute (CERI, Japan). Test No.: 80245

(31) Richter, G.; Kadner, H. (1990) Allergic Contact Eczema Caused by m-Xylenediamine in Polyurethane Silk Production. Derm. Beruf. Umwelt. 38 (4): 117-120

(32) Mead C (1988) Meta-Xylenediamine: Assessment of ready biodegradability; CO_2 evolution test, SafePharm Laboratories, Project No: 930/019. (unpublished). Biodegradability test on m-Xylenediamine (1983) Kurume Laboratory/ Chemicals Assessment Center, Chemicals Evaluation and Research Institute, Japan.

(33) Wetton P M (1998) Meta-Xylenediamine: Acute toxicity to Golden Orfe. Safepharm Laboratories, Project No: 930/018

(34) Mead C (1998) Meta-Xylenediamine: Acute toxicity to bacteria. Safepharm Laboratories, Project No: 930/017

(35) Whetton P M (1995): Acute toxicity to Rainbow Trout. Safepharm Laboratories, Project No: 930/003

(36) Whetton P M (1995) Acute toxicity to *Daphnia magna*. Safepharm Laboratories, Project No: 930/002

(37) Mead C (1995)Algal Inhibition Test. Safepharm Laboratories, Project No: 930/001

(38) Allen D J (1998) MXDA:Magnusson & Kligman Maximisation Study in the Guinea Pig. Safepharm Laboratories, Project No: 930/039

(39) Marhold J (1986) Prehled Prumyslove Toxikologie: Organicke Latky, Czechoslavakia, Avicenum page 447

(40) Fisk P (2001) Fugacity Model Mackay Level III

(41) Richter V G and Kadner H (1990) Allergische Kontaktekzeme durch m-Xylylen-diamin in der Polyurethanseidenproduktion, Dermatosen 38, Heft 4 117- 120