FOREWORD INTRODUCTION

o-ACETOACETOTOLUIDIDE

CAS N°: 93-68-5

SIDS Initial Assessment Report

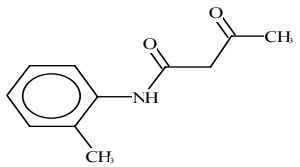
For

SIAM 16

Paris, France, 27-30 May 2003

1. Chemical Name:	o-Acetoacetotoluidide
2. CAS Number:	93-68-5
3. Sponsor Country:	Japan Mr. Yasuhisa Kawamura Director, Second International Organizations Div. Ministry of Foreign Affairs 2-2-1 Kasumigaseki, Chiyoda-ku Tokyo 100-8919
4. Shared Partnership with:	The industry consortium collected new data and prepared the updated IUCLID, and drafted versions of SIAR and SIAP.
5. Roles/Responsibilities of the Partners:	Mr. Kiminori Nagayama, Mitsuboshi Chemical Co., Ltd. e-mail: nagayama@mitsuboshi-chem.co.jp
• Name of industry sponsor /consortium	The industry contact point is Mr. K. Nagayama, Mitsuboshi Chemical Co., Ltd. acting on behalf of the AAOT consortium (other consortium members: Clariant GmbH (Germany), Eastman Chemical Company (USA), Lonza Ltd. (Switzerland)).
• Process used	
6. Sponsorship History	
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	This substance is sponsored by Japan under the ICCA Initiative and is submitted for first discussion at SIAM 16.
7. Review Process Prior to the SIAM:	Japanese government peer-reviewed the documents and audited selected studies.
8. Quality check process:	Japanese government peer-review committee performed spot checks on randomly selected endpoints and compared original studies with data in the SIDS Dossier.
9. Date of Submission:	February 21, 2003
10. Date of last Update:	July 16, 2003
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	93-68-5
Chemical Name	o-Acetoacetotoluidide
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

The oral LD50 of o-Acetoacetotoluidide (AAOT) in rats was 1854 mg/kg in males and 1945 mg/kg in females [OECD TG401]. Toxicological effects such as decreased locomotor activity, adoption of a prone position, hypotonia, ptosis, deep respiration, piloerection, hypothermia, lacrimation and pale skin were observed at 819 mg/kg and higher in both sexes in a dose dependent manner.

In addition, the following data was available, although they were insufficient for adequate assessment. AAOT caused slight irritation to the rabbit eyes, and caused slight to moderate irritation to the guinea pig skin. There was a potential for it to induce contact sensitization to guinea pig. Erythema was found in one of ten guinea pigs.

In a Combined Repeat Dose and Reproduction/Developmental Toxicity Screening Test in rats [OECD TG422], AAOT was administered by gavage at the dose levels of 0, 8, 25, 80 and 250 mg/kg/day.
The blood findings in males in the 250 mg/kg/day group were: decreases of erythrocyte count, hemoglobin concentration and hematocrit value, also increases of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocyte count, methemoglobin concentration, bilirubin and potassium. Other findings in the 250 mg/kg/day group were: increase of pituitary weight in males; increases of weight of spleen, weight of liver, extramedullary hematopoiesis and congestion in spleen, also blackening of spleen and hemosiderin deposit in liver and spleen in both sexes.
The blood findings in males in the 80 mg/kg/day group were: decrease of erythrocyte count and increase of MCV and bilirubin. Other findings in the 80 mg/kg/day group were: increase of congestion in spleen in females, blackening of spleen and hemosiderin deposit in liver and spleen in both sexes.
In all dose groups up to 250 mg/kg/day, no changes in mortality, behavior or toxic effects on the body weight and food consumption were observed in any sexes. No toxic effects were observed in any dose groups up to 25 mg/kg/day.
Based on these results, the NOAEL for repeat dose toxicity is considered to be 25 mg/kg/day in both sexes.

AAOT was not mutagenic in bacteria up to 5,000 ug/plate [OECD TG471, 472]. Although AAOT showed marginal response in induction of chromosomal aberrations in CHL/IU cells at 2.5 or 5.0 mg/mL, the response was observed only at concentration levels higher than 10 mM (1.91 mg/mL) [OECD TG473]. Therefore, the response was regarded as a biologically irrelevant phenomenon under unphysiological (high osmolality) culture condition. Both the unscheduled DNA synthesis test in rat CD-1 cells and HGPRT assay in CHO cells were negative. Considering all of the *in vitro* studies available, AAOT is not genotoxic.

For reproduction/developmental toxicity, AAOT was administered in the above described screening test [OECD TG422] for 44 days in males and 41 – 45 days (from 14 days before mating to 3 days after parturition) in females. No toxic effects were observed in the following test parameters in parental animals; copulation index, fertility index, gestation index, number of corpora lutea or implantations, implantation index, gestation index and maternal behavior, at up to 250 mg/kg/day.

As for pups; no compound-related effects on the number of pups, delivery index, sex ratio, body weight and viability index were observed in any dose groups. No pups with malformations were found in any groups. No changes in histopathological findings were observed in offspring.
Based on these results, the NOAEL for reproduction/developmental toxicity is considered to be 250 mg/kg/day.

Environment

AAOT is soluble in water (3.0 g/L at 25°C) and the vapour pressure is low (0.00066 Pa at 20°C by calculation) [MPBPWIN v1.40]. AAOT is inherently biodegradable with pre-adapted inoculum (78.5% on DOC after 7 days incubation) [OECD TG302B]. AAOT is stable to hydrolysis in water at pH 4, 7 and 9 [OECD TG111]. The bioaccumulation potential is estimated to be low (BCF = 3.2: calculated from log Pow = 0.85 [OECD TG107]). If AAOT is released into the atmosphere, it will react with photo-chemically produced hydroxyl radicals and will be decreased with a half-life of 8.0 hours. The Fugacity Model [Mackey level III] suggests that if released to water, the majority of the substance would remain in the water compartment, if released into air, 41 % would distribute to water and 58 % distribute to soil compartment, and if released to soil, 36 % would distribute to water and 64 % remain in soil compartment.

In acute toxicity tests with algae, daphnids and fish [OECD TG201, 202, 203 and other methods], the EC50 for algae (*Selenastrum capricornutum*) was 383 mg/L (0 - 72hr biomass) and 654 mg/L (24 - 72hr growth rate), the EC50 for daphnids was 931 mg/L (*Daphnia magna*, 48hr) and the LC50s for fish were > 100 mg/L (*Oryzias latipes*, 96hr limit test), 316.2 mg/L (*Pimephales promelas*, 96hr) and > 500 mg/L (*Brachydanio rerio*, 96hr).

In chronic toxicity tests with daphnids and algae [OECD TG211, 201], the NOEC for daphnids was 10 mg/L (*Daphnia magna*, 21 days reproduction), and the NOEC for algae (*Selenastrum capricornutum*) was 95.3 mg/L (0 - 72hr biomass) and 171 mg/L (24 - 72hr growth rate).

Exposure

The production volume of AAOT in 2001 is estimated to be 1,000 - 1,500 tons/year in Japan and ca. 4,000 tonnes/year in the world. The production countries are Germany, India, Japan, P.R. China, Switzerland, U.S.A and maybe in Eastern Europe. In total there are about 15 manufacturing sites and about 55 use sites in the world.

AAOT is produced in closed systems, and the packing process is performed in semi-closed or open systems. The user may use it in semi-closed systems. The only recognized use is an industrial intermediate in the synthesis of organic pigments. These pigments are utilized in ink, paint and coloring of various materials. There are no known direct uses of AAOT in any consumer product.

The concentration of non-reacted AAOT in the pigments is unknown. However, migration of the pigments is expected to be very limited and there are no adverse health reports from such exposure. Therefore, significant consumer exposure is not expected.

Because of its use limited to the pigment industry, the releases to the environment are estimated to be low.

A survey of users and producers show that the chemical is usually used in well controlled processes and therefore worker exposure is likely to be low.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

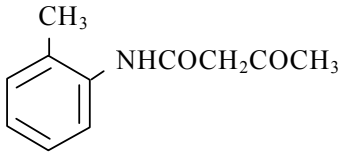
The chemical possesses properties indicating a potential hazard for human health. Based on data presented by the Sponsor country, exposure to humans and the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 93-68-5
IUPAC Name: Butanamide, N-(2-methylphenyl)-3-oxo-
Molecular Formula: C11H113NO2
Structural Formula:



Molecular Weight: 191.23
Synonyms: AAOT
Acetoacet-o-toluidide
o-Acetoacetotoluidide
Acetoacetyl-2-methylanilide
N-(2-Methylphenyl)-3-oxobutanamide
2'-Methylacetoacetanilide
Acetoacetic acid 2-methylanilide
o-Methylacetoacetanilide

1.2 Purity/Impurities/Additives

Purity: ca. 99.9% by HPLC

Impurity: o-Toluidine trace

Additives: none

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Protocol
Physical state	solid/powder	visual inspection
Melting point	106 °C	JIS K4101-1993 5.1
Boiling point	> 170 °C (scorched)	OECD TG103
Relative density	1.307 g/cm3	JIS K7112-1980
Vapour pressure	< 130 Pa at 40 °C 0.00066 Pa at 25 °C	OECD TG104 calculation (MPBPWIN v 1.40)
Water solubility	3.0 g/L at 25 °C	OECD TG105
Partition coefficient n-octanol/water (log value)	0.85 at 25 °C	OECD TG107 (flask shaking)
pH	5.8 at 20 °C, 3.0g/L	Unknown (pH meter)
pKa	no dissociation	OECD TG112

Reference: CITI Japan, 1999, etc.

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

1) Manufacture

The production volume of this substance (o-Acetoaceto-toluidide; AAOT) in 2001 is estimated to be 1000-1500 tons/year in Japan and ca. 4000 tons/year in the world. The producing countries are Germany, India, Japan, P.R.China, Switzerland, U.S.A and maybe in Eastern Europe. A total of about 15 manufacturing sites are existing in the world. Though it is produced in a closed system by a chemical reaction process, possibility of limited leakage to the air (as dust) and the waste water at workplace (for example, at packing process) can be expected.

The product is marketed as a powder in 20 - 25 kg net paper or plastic bags, in 20 120 kg net drums or in 200 1000 kg net big bags.

2) Uses

The only recognized use is an industrial intermediate in the synthesis of Pigment Yellow 9, 14, 16, 174 and Orange 1. These pigments are utilized in ink, paint, stationery goods, and coloring of resin, fiber, leather, paper, rubber, etc. There are no known direct uses of AAOT in any consumer product. A total of about 55 use site exist in the world.

The concentration of non-reacted AAOT in those pigments is unknown. However; (1) about 0.09% excess volume is used at chemical synthesis of some of those pigments (according to the pigment producer in Japan), (2) in some cases human exposure of the pigments and the non-reacted AAOT by stationery goods are possible, however the quantity is very limited and there are no adverse health reports from such exposures, and (3) exposure volume of ink, paint, etc. to workers in industry in its synthesis or use is limited due to good hygiene practices.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Sources of potential release to the environment are, (1) emission to the air (as dust) and waste water at the producer’s chemical factories and (2) emission to the air (as dust) and waste water at the user’s chemical factories.

Release to the out side of each factory through; (1) the air is low due to the low vapour pressure (< 130 Pa at 40 degrees C [OECD TG104] and 0.00066 Pa at 20 degrees C [calculated: MPBPWIN v1.40]), (2) the soil is very low as floors are covered with concrete, etc., (3) the waste water is considerable. However the concentration in the effluent from the waste water treatment plant of the production site in Japan was about 0.024 mg/L [Mitsuboshi Chemical; unpublished report, 2002]. The environmental release volume through waste water at the production site in Japan is estimated to be 86 kg/year.

2.2.2 Photodegradation

AAOT, if released to the air compartment, will react with photochemically-produced hydroxyl radical with a half life of 8.0 hours [calculated: SRC AOP Win v.1.90].

2.2.3 Stability in Water

AAOT was stable to hydrolysis in water at pH 4, 7 and 9 [OECD TG111] (METI 1999).

2.2.4 Transport between Environmental Compartments

A generic Fugacity Model (Mackay level III) suggests that if released to water, the majority would remain in the water compartment, if released into air, 41 % would distribute to water and 58 % to soil, and if released to soil, 36 % would distribute to water and 64 % remain in soil. Those data are shown in Table 2 below.

Table 2: Environmental distribution of AAOT using the Fugacity Model (Mackey level III)

compartment	release: 100% to air	release: 100% to water	release: 100% to soil
air	0.0 %	0.0 %	0.0 %
water	41.4 %	99.6 %	36.2 %
soil	58.4 %	0.0 %	63.7 %
sediment	0.2 %	0.4 %	0.2 %

2.2.5 Biodegradation

The result from an inherent biodegrability test [OECD TG302B] (Hoechst report 1989) indicated that AAOT was inherently biodegradable with pre-adapted inoculum (78.5% biodegradation based on BOD during a 7 day incubation period).

2.2.6 Bioaccumulation

The log Pow value is 0.85 [OECD TG107] (CITI 1999). The calculated value of BCF is 3.2 [EPI Suite v3.10 (U.S. EPA 2002)].

2.2.7 Other Information on Environmental Fate

As a conclusion, the preferred environmental compartment of AAOT is water, and the total volume released is considered to be very low.

2.3 Human Exposure

2.3.1 Occupational Exposure

No official workplace exposure limit value is assigned for AAOT.

Occupational exposure by the dust of AAOT at the producer's workplace (for example, packing process) and user's workplace (for example, dumping process to reactor or storage) may occur through the inhalation and dermal route.

At a producer's workplace in Japan, AAOT is produced in a closed system by a chemical reaction process, and the drying, sampling and packing process is semi-closed or open. Basically all of the semi-closed or open systems are designed with local ventilators.

The atmospheric concentration was measured at the production site in Japan in 2002. The monitoring data and the Estimated Human Exposures (EHEs) are shown in Table 3.

The monitoring data at a workplace in Japan suggests that if all processes are operated by the same worker, the Estimated Human Exposure by inhalation (EHE inh) would be 0.28 mg/kg/day (worst case). And the EASE model suggests that if all processes are operated by same worker and if absorption occurred through hands, the calculated EHE der would be 13.05 mg/kg/day.

Table 3: Workplace monitoring data and EHEs of AAOT

operation	**monitoring data (mg/m^3)**		**working time (hours/day)**	**maximum EHE (mg/kg/day)**
	maximum	**average**		
sampling for process evaluation	0.28	0.19	0.2	EHE inh = 0.28 x 1.25 x 0.2 /70 = 0.00 EHE der = 840 x 1 x 0.2/8 /70 = 0.30
analysis	0.13	0.1	1.0	EHE inh = 0.13 x 1.25 x 1.0 /70 = 0.00 EHE der = 840 x 1 x 1.0/8 /70 = 1.50
monitoring of transferring process 1	0.07	0.07	1.0	EHE inh = 0.07 x 1.25 x 1.0 /70 = 0.00 EHE der = 840 x 1 x 1.0/8 /70 = 1.50
monitoring of transferring process 2	3.53	2.94	0.5	EHE inh = 3.53 x 1.25 x 0.5 /70 = 0.03 EHE der = 840 x 1 x 0.5/8 /70 = 0.75
monitoring of transferring process 3	7.27	2.76	1.0	EHE inh = 7.27 x 1.25 x 1.0 /70 = 0.13 EHE der = 840 x 1 x 1.0/8 /70 = 1.50
monitoring of rinse process	0.02	0.02	1.0	EHE inh = 0.02 x 1.25 x 1.0 /70 = 0.00 EHE der = 840 x 1 x 1.0/8 /70 = 1.50
monitoring of packing process and sampling	1.56	1.00	4.0	EHE inh = 1.56 x 1.25 x 4.0 /70 = 0.11 EHE der = 840 x 1 x 4.0/8 /70 = 6.00
total				EHE inh = 0.28 mg/kg/day EHE der = 13.05 mg/kg/day grand total = 13.33 mg/kg/day

Source: Japan Industrial Safety and Health Association report 2003

Monitoring method: Air sample was suctioned at the breathing zone (1.5 m in height) of the worker at the suction rate of 2 L/min for 4 - 34 minutes and was passed through a filter after an impactor. AAOT collected on the filter was dissolved in acetonitrile, and analyzed by HPLC.

EHEs were calculated with the following parameters.

body weight = 70kg, respiratory volume = $1.25 m^3$/hr, open hands area = $840 cm^2$,

dermal absorption rate = $1 mg/cm^2$/day (EASE model)

Normally, workers wear protective clothing, gloves and breathing protection during the work. And, in fact each process is operated by another worker. Therefore, the actual exposure is considered to be substantially lower than the calculated value.

The occupational monitoring and working time data at user's workplace were not available. However normally workers wear protective clothing, gloves and breathing protection during the work, and local ventilators are equipped appropriately.

2.3.2 Consumer Exposure

As mentioned in section 2.1 2) Consumer Exposure by stationery goods is very limited and there are no adverse health reports from such exposures.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

There is no available information on toxicokinetics and metabolism of AAOT.

3.1.2 Acute Toxicity

Studies in Animals

Oral

An oral rat study (MHW Japan, 1999a) is identified as the best quality and the key study, because it was well conducted according to OECD TG401, following GLP and described in detail. In the Single Dose Oral Toxicity test in rats; AAOT was administered at the doses of 0, 819, 1024, 1280, 1600, 2000, 2500 mg/kg to both sexes.

In males, one of five rats died at 1280 mg/kg and three of five died at 2000mg/kg. In females, one of five rats died at 1600 mg/kg and two of five died at 2000 mg/kg. Then, all rats died at 2500 mg/kg. Those results were consistent with another study, LD_{50} = ca.1600 mg/kg [Eastman 1975]. However the former study was more robust and is therefore retained for the present assessment.

Toxicological effects such as decreased locomotor activity, adoption of a prone position, hypotonia, ptosis, deep respiration, piloerection, hypothermia, lacrimation and pale skin were found at 819 mg/kg and higher groups in both sexes in a dose-dependent manner. In surviving rats they returned to normal after 1 - 12 days recovery. At necropsy, bloody material in the stomach and intestine, petechiae in the glandular stomach and distension of the urinary bladder were observed in the dead animals.

Considering the results of Repeat Dose Toxicity (see section 3.1.5), those toxic effects are assumed to be caused by hemolytic anemia.

Studies in Humans

There is no adequate information on humans.

Conclusion

The oral LD_{50} is 1,854mg/kg in male rats and 1,945mg/kg in females.

3.1.3 Irritation

Studies in Animals

Though the quality of data is not sufficiently robust, following information is available.

Skin Irritation

AAOT was a slightly irritating to guinea pig skin at 250, 500, 1000 mg/kg after a 24 hours exposure (Eastman report 1975). It produced moderate edema and slight erythema. Seven days after 24 hours exposure, the skin appeared normal.

In a repeat dose dermal irritation study in guinea pigs: AAOT (0.165 mg added to a lotion) was applied 5 days/week for 2 weeks. One of the ten animals exhibited a severe erythema, eight exhibited mild erythema, and one was non-reactive. Those results also are suggestive of a possible contact sensitization reaction.

Eye Irritation

AAOT was a slightly irritating to rabbit eyes (Eastman report 1975). One hour after a 100 mg exposure, the conjunctivae and nictitating membranes were slightly erythematous, however they returned to normal after 24 hours and remained so over the next 13 days of the test. In another report [OECD TG405] (Lonza MSDS), similar results were described.

Conclusion

AAOT may cause slight irritation to rabbit eyes, also may cause slight to moderate irritation to guinea pig skin.

3.1.4 Sensitisation

Though the quality of the data is not sufficiently robust, the following information is available.

Studies in Animals

AAOT has a slight potential to induce contact sensitization in guinea pigs. A compound-heparinized-whole rabbit-blood reaction product was injected in the footpads of ten animals. One week later they were challenged by a dermal application. One of ten reacted with a strong erythema, while nine of ten were normal.

Conclusion

There may be a potential for it to induce contact sensitization to guinea pig. Erythema ability was found in one of ten guinea pigs.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Oral

One adequate oral rat study and one supporting study are available.

A Combined Repeat Dose and Reproduction/Developmental Toxicity Screening Test (MHW Japan 1999b) was well conducted according to OECD TG422, following GLP. The test results are described as follows.

AAOT was administered to Sprague-Dawley rats (10/sex/dose) at doses of 0, 8, 25, 80, 250 mg/kg/day by oral gavage. The dosing period was 44 days for males and 41 - 45 days (including 14 days before mating and 3 days after pregnancy) for females.

In the 250 mg/kg/day group the following effects were observed: decreases of erythrocyte count, hemoglobin concentration and hematocrit value in males; increases of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocyte count, methemoglobin concentration, bilirubin and potassium in males; increase of pituitary weight in males; increases of weight of spleen, weight of liver, extramedullary hematopoiesis and congestion in spleen in both sexes; and blackening of spleen and hemosiderin deposit in liver and spleen in both sexes.

In the 80 mg/kg/day group the following effects were observed: decrease of erythrocyte count and increases of MCV and bilirubin in male; increase of congestion in spleen in female; and blackening of spleen and hemosiderin deposit in liver and spleen in both sexes.

Those changes are known as typical toxic symptoms of hemolytic anemia caused by aromatic amine compounds.

No changes in mortality, behavior or toxic effect on the body weight and food consumption were observed in any groups. Increase of specific gravity of urine was observed in males of the 250 mg/kg group. However no related changes were observed in other findings.

In the other test (Eastman report 1975), although the quality of testing rats was a little questionable, similar results were reported. That was, hemolytic anemia related toxic symptoms including liver and spleen at 88 mg/kg and in higher dose groups.

Studies in Humans

There is no available information on humans.

Conclusion

Toxicological effects and the target organs are hemolytic anemia and the related changes on the blood, spleen, liver and kidney, including male kidney (increasing of eosinophilic bodies) and female liver (increasing of the weight). The NOAEL for repeat dose toxicity to rats is 25 mg/kg/day in both sexes.

3.1.6 Mutagenicity

Studies in Animals

In vitro Studies

There are four results from bacterial tests (including three adequate studies) and three results from non-bacterial *in vitro* tests (including three adequate studies) reported on AAOT. The summary of adequate studies is shown in Table 4.

Table 4: Summary of adequate genetic toxicity studies of AAOT

Type	species	protocol	dose	S9	result	reference
Bacterial test						
Ames test	*S.typh.* (TA100, TA1535, TA98, TA1537), *E.coli* (WP2*uvrA*)	OECD TG471 & TG472	up to 5,000 ug/plate	-	negative	MHW Japan 1999c
				+	negative	
Ames test	*S.typh.* (TA102, TA2638), *E.coli* (WP/pKM101, WP2*uvrA*/pKM101)	Maron and Ames	up to 5,000 ug/plate	-	negative	Mutat.Res, 1996
Ames test	*S.typh.* (TA100, TA1535, TA98, TA1537, TA1538)	other	up to 10,000 ug/plate	-	negative	Eastman report 1985a
				+	negative	
Non-bacterial *in vitro* test						
Chromosomal aberration test	CHL/IU cell	OECD TG473	up to 3,600 or 5,000 ug/mL	-	positive	MHW Japan 1999d
				+	ambiguous	
HGPRT assay	CHO-K1-BH4 cell	other	up to 1.5 mg/mL	-	negative	Eastman report 1985b
				+	negative	
Unscheduled DNA synthesis	hepatocytes from CD-1 rat	other	up to 3,300 ug/mL	+	negative	Eastman report 1985c

There are four key studies on AAOT, because they are well conducted and giving detailed information. They are described below.

Bacterial test:

The Ames test study (MHW Japan 1999c) was well conducted and reported according to OECD TG 471 & 472 following GLP. All results were negative in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvrA* with and without a metabolic activation system.

Non-bacterial test:

The chromosomal aberration study with CHL cell (MHW Japan 1999d) was well conducted and reported according to OECD TG 473 following GLP. At short-term treatment, slight structural aberration was observed in the 5,000 ug/mL dose with S9 mix (5%) and without (9%). At continuous treatment without S9 mix, slight structural aberration was observed in the 2,500 ug/mL dose of 24hr (10%) and in the 1,800 ug/mL dose of 48hr (5%). At confirmative 24hr continuous treatment without S9 mix, structural aberration was induced (8.5%) at 2,000ug/mL. However those responses above 5% were observed only at concentration levels higher than 10 mM (1,910 ug/mL). While, 50 % cell viability concentrations calculated by Probit method (ug/mL) were as follows. With S9 short-term = 3,699: without S9 short-term = 3,392: 24hr continuous = 1,565: 48hr continuous = 940. Therefore, the response was regarded as a biologically irrelevant phenomenon under unphysiological (high osmolality) culture condition.

The forward mutation (HGPRT) study with CHO cell (Eastman report 1985b) was well conducted and reported following GLP. The mutation frequency without S9 was less than in the negative control. And with S9, they were within the spontaneous level (less than 20 mutants per million clonable cells), also there was no dose-response relationship for the mutation frequency. Therefore, AAOT is considered to be negative in this study.

The unscheduled DNA synthesis (UDS) study with hepatocytes isolated from CD-1 rat (Eastman report 1985c) was well conducted and reported following GLP. From both results of the "number of net UDS grains/nucleus" and the "% of cells with more than 5 UDS grains/nucleus" compared with each negative control, AAOT is considered to be negative in this study.

In vivo Studies

There is no available in vivo information.

Studies in Humans

There is no available information on humans.

Conclusion

Considering all of the in vitro studies available, AAOT is not genotoxic.

3.1.7 Carcinogenicity

There is no available information on carcinogenicity.

3.1.8 Toxicity for Reproduction

Studies in Animals

Effects on Fertility

A Combined Repeat Dose and Reproduction/Developmental Toxicity Screening Test (MHW Japan, 1999b), was well conducted according to OECD TG 422, following GLP, and reported detailed information. Regarding test condition, histopathological finding, etc., please refer to section 3.1.5 above.

In this study, at all dose levels up to 250 mg/kg/day, no toxic effects were observed on the copulation index, fertility index, gestation length, number of corpora lutea or implanations, implanation index, gestation index and maternal behavior.

Developmental Toxicity

In the above Combined Test, no compound-related effects on pups, delivery index, sex ratio, body weight and viability index were observed in any dose groups. No pups with malformation were found in any groups. No changes in histopathological findings were observed in offspring.

Studies in Humans

There is no available information on humans.

Conclusion

No toxicological effect on reproduction/developmental parameter was found at any doses up to 250 mg/kg/day. The NOAEL for reproduction/developmental toxicity is considered to be 250 mg/kg/day.

3.2 Initial Assessment for Human Health

The oral LD_{50} value in rats is 1,854 mg/kg in males, and 1,945 mg/kg in females [OECD TG401]. Toxicological effects such as decreased locomotor activity, adoption of a prone position, hypotonia, ptosis, deep respiration, piloerection, hypothermia, lacrimation and pale skin were found at 819 mg/kg and higher in both sexes in a dose-dependent manner. At necropsy, bloody material in the stomach and intestine, petechiae in the glandular stomach and distension of the urinary bladder were observed in the dead animals.

In a Combined Repeat Dose and Reproduction/Developmental Toxicity Screening Test in rats [OECD TG422], AAOT was administered by gavage at dose levels of 0, 8, 25, 80 and 250 mg/kg/day.

At 250 mg/kg/day, the following blood findings were observed in males: Decreases of erythrocyte count, hemoglobin concentration and hematocrit value, also increases of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocyte count, methemoglobin concentration, bilirubin and potassium. Other findings in the 250 mg/kg/day group were: Increases of weight of spleen, weight of liver, extramedullary hematopoiesis and congestion in spleen, also blackening of spleen and hemosiderin deposit in liver and spleen in any sexes.

At 80 mg/kg/day, the following blood findings were observed in males: Decrease of erythrocyte count and increases of MCV and bilirubin. Other findings in the 80 mg/kg/day group were: Increase of congestion in spleen in females, also blackening of spleen and hemosiderin deposit in liver and spleen in any sex.

Through all dose groups up to 250 mg/kg/day, no changes in behavior, death or toxic effects on the body weight and food consumption were observed in any sexes.

Based on these results, the NOAEL for repeat dose toxicity is considered to be 25 mg/kg/day for both sexes.

Those changes are known as typical toxic symptoms of hemolytic anemia caused by aromatic amine compounds.

From the aspect of reproduction/developmental toxicity of this test, no toxic effects were observed in the following test parameters in dams; copulation index, fertility index, gestation index, number of corpora lutea or implanations, implanation index, gestation index and maternal behavior up to 250 mg/kg/day. No changes in the number of pups, delivery index, sex ratio, body weight and viability index were observed in any dose groups. No pups with malformation were found in any groups. No changes in histopathological findings were observed in offspring. Based on those results, the NOAEL for reproduction/developmental toxicity is considered to be 250 mg/kg/day.

AAOT was not mutagenic in bacteria up to 5,000 ug/plate [OECD TG 471, 472]. Although AAOT showed marginal response in induction of chromosomal aberration in CHL/IU cells at 2.5 or 5.0 mg/mL, the response was observed only at concentrations higher than 10 mM (1.91 mg/mL) [OECD TG473]. Therefore, the response was regarded as a biologically irrelevant phenomenon under unphysiological (high osmolality) culture condition. Both the unscheduled DNA synthesis in rat CD-1 cells and the HGPRT assay in CHO cells were negative. Considering all of the *in vitro* studies available, AAOT is not genotoxic.

In addition, the following data was available, although they were insufficient for adequate assessment. AAOT causes slight irritation to rabbit eyes, also causes slight to moderate irritation to guinea pig skin. There was a potential for it to induce contact sensitization to guinea pig. Erythema was found in one of ten guinea pigs.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute toxicity studies on three species of fish [OECD TG203 and other] (EA 1999a, Eastman 1975, Hoechst 1989) were well conducted and documented. The 96hr LC_{50} was 100 - 500 mg/L or more depending on the species. An acute toxicity study to daphnids [OECD TG202] (EA 1999b) was also well conducted and documented.

Chronic toxicity studies with daphnids [OECD TG211] (EA 1999c) and algae [OECD TG201] (EA 1999d) were also well conducted and documented.

The summary of reliable studies is shown in Table 5.

Table 5: Aquatic toxicity of AAOT

organism	test method	result (mg/L)	reference
Fish			
Medaka (*Oryzias latipes*)	OECD TG203 96hr (ss)	LC_{50} (96hr) > 100 (mc) LC_0 (96hr) > 100 (mc)	EA Japan 1999a
Pimephales promelas	other 96hr (s)	LC_{50} (96hr) = 316 (nc) LC_0 (96hr) = 100 (nc) LC_{100} (96hr) = 1000 (nc)	Eastman report 1975
Brachydanio rerio	OECD TG203 96hr (s)	LC_{50} (96hr) > 500 (nc*) LC_0 (96hr) > 500 (nc*)	Hoechst report 1989
Daphnia			
Water flea (*Daphnia magna*)	OECD TG202 48hr (s)	EC_{50} (imm. 48hr) = 931 (nc*) NOEC (imm. 48hr) = 667 (nc*)	EA Japan 1999b
Water flea (*Daphnia magna*)	OECD TG211 21days (ss)	EC_{50} (rep. 21day) = 16.5 (nc*) NOEC (rep. 21day) = 10 (nc*) LOEC (rep. 21day) = 20 (nc*)	EA Japan 1999c
Algae			
Green algae (*Selenastrum capricornutum*)	OECD TG201 72hr (s)	EC_{50} (bms. 0-72hr) = 383 (nc*) NOEC(bms. 0-72hr) = 95.3 (nc*) EC_{50} (gr. 24-48hr) = 607 (nc*) NOEC(gr. 24-48hr) = 171(nc*) EC_{50} (gr. 24-72hr) = 654 (nc*) NOEC(gr. 24-72hr) = 171(nc*)	EA Japan 1999d

s: static, ss: semi-static, mc: measured concentration, nc: nominal concentration,
nc*: nominal concentration (actual concentration measured and greater than 80% of nominal),
bms: biomass, gr: growth rate, imm: immobility, rep: reproduction

Acute Toxicity Test Results

The EC_{50} for algae (*Selenastrum capricornutum*) was 383 mg/L (0 - 72hr biomass) and 654 mg/L (24 - 72hr growth rate), the EC_{50} for daphnids was 931 mg/L (*Daphnia magna*, 48hr) and the LC_{50}s for fish were > 100 mg/L (*Oryzias latipes*, 96hr limit test), 316.2 mg/L (*Pimephales promelas*, 96hr) and > 500 mg/L (*Brachydanio rerio*, 96hr).

Chronic Toxicity Test Results

The NOEC for daphnids was 10 mg/L (*Daphnia magna*, 21 days reproduction), and the NOEC for algae (*Selenastrum capricornutum*) was 95.3 mg/L (0 - 72hr biomass) and 171 mg/L (24 - 72hr growth rate).

4.2 Terrestrial Effects

There is no available information.

4.3 Other Environmental Effects

There is no available information.

4.4 Initial Assessment for the Environment

AAOT is soluble in water (3.0g/L at 20°C) [OECD TG105] and vapor pressure is low (< 130 Pa at 25°C [OECD TG104] and 0.00066 Pa at 20°C [calculation: MPBPWIN v1.40]). AAOT is inherently biodegradable with pre-adapted inoculum (78.5% during 7 days) [OECD TG302B] and is stable to hydrolysis in water at pH 4, 7 and 9 [OECD TG111]. The bioaccumulation potential is estimated to be low (BCF = 3.2: calculated from log Pow = 0.85). AAOT, if released into the atmosphere, will react with photochemically- produced hydroxyl radical and decrease with a half-life of 8.0 hours.

AAOT could be released into the aquatic environment from waste water at manufacturer's or user's chemical factory site, and it is expected to remain almost entirely in the water compartment based on calculations using the Fugacity Model [Mackey level III].

The concentration in effluent water from manufacturer's waste water treatment plant in Japan was about 0.024mg/L.

In acute toxicity tests with algae, daphnids and fish [OECD TG201, 202, 203 and other methods], the EC50 for algae (*Selenastrum capricornutum*) was 383 mg/L (0 - 72hr biomass) and 654 mg/L (24 - 72hr growth rate), the EC50 for daphnids was 931 mg/L (*Daphnia magna*, 48hr) and the LC50s for fish were > 100 mg/L (*Oryzias latipes*, 96hr limit test), 316.2 mg/L (*Pimephales promelas*, 96hr) and > 500 mg/L (*Brachydanio rerio*, 96hr).

In chronic toxicity tests with daphnids and algae [OECD TG211, 201], the NOEC for daphnids was 10 mg/L (*Daphnia magna*, 21 days reproduction), and the NOEC for algae (*Selenastrum capricornutum*) was 95.3 mg/L (0 - 72hr biomass) and 171 mg/L (24 - 72hr growth rate).

The predicted no effect concentration (PNEC) of 0.10 mg/L for aquatic organisms was calculated from the lowest NOEC (*Daphnia magna*, 21 days reproduction, 10 mg/L), using an assessment factor of 100 (as recommended by the OECD), because two chronic test results (daphnids and algae) are available.

5 RECOMMENDATIONS

The chemical is currently of low priority for further work.

AAOT possesses properties indicating a potential hazard for human health. Based on data presented, exposure to humans and the environment is anticipated to be low, and therefore this chemical is currently of low priority for further work. Countries may desire to investigate any exposure scenarios that were not presented by the Sponsor country.

6 REFERENCES

CITI Japan, 1999: Report No. 80240K, Chemical Inspection and Testing Institute Japan, unpublished report on physical properties of N-Acetoacetyl-2-methyl aniline

Eastman report, 1975: Report No. TOX-75-16, Basic Toxicity of Acetoacet-o-toluidide, Eastman Kodak Company; unpublished report

Eastman report, 1985a: Report No. 188466L TOX-85-13, EVALUATION OF ACETOACET-O-TOLUIDIDE BLEND IN THE SALMONELLA/MICROSOME MUTAGENICITY ASSAY, Eastman Kodak Company; unpublished report

Eastman report, 1985b: Report No. 188473L TOX-85-20, Evaluation of Acetoacet-o-toluidide in the CHO/HGPRT Forward Mutation Assay, Eastman Kodak Company; unpublished report

Eastman report, 1985c: Report No. 188468N TOX-85-15, EVALUATION OF ACETOACET-O-TOLUIDIDE BLEND IN THE UNSCHEDULED DNA SYNTHESIS TEST, Eastman Kodak Company; unpublished report

EA Japan, 1999a: Report No. 92052, Environment Agency, Japan; unpublished report on acute toxicity to Oryzias latipes

EA Japan, 1999b: Report No. 92050, Environment Agency, Japan; unpublished report on acute toxicity to daphnia

EA Japan, 1999c: Report No. 92051, Environment Agency, Japan; unpublished report on chronic toxicity to daphnia

EA Japan, 1999d: Report No. 92049, Environment Agency, Japan; unpublished report on toxicity to algae

Hoechst report, 1989: Report No. 89.1780, Hoechst AG, unpublished report

Lonza MSDS: Lonza Ltd.; MSDS 25.03.99

MHW Japan, 1999a: Toxicity Testing Reports of Environmental Chemicals, vol.7, 1999, p273-274, "Single Dose Oral Toxicity Test of o-Acetoacetotoluidide in Rats", Ministry of Health and Welfare, Japan

MHW Japan, 1999b: Toxicity Testing Reports of Environmental Chemicals, vol.7, 1999, p275-287, "Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test of o-Acetoacetotoluidide by Oral Administration in Rats", Ministry of Health and Welfare, Japan

MHW Japan, 1999c: Toxicity Testing Reports of Environmental Chemicals, vol.7, 1999, p288-291, "Reverse Mutation Test of o-Acetoacetotoluidide on Bacteria", Ministry of Health and Welfare, Japan

MHW Japan, 1999d: Toxicity Testing Reports of Environmental Chemicals, vol.7, 1999, p292-296, "In Vitro Chromosomal Aberration Test of o-Acetoacetotoluidide on Cultured Chinese Hamster Cells", Ministry of Health and Welfare, Japan

Mutat.Res, 1996: K.Watanabe et al. Comparisons of chemically-induced mutagenicity among four bacterial strains, Mutation Research 361 (1996), p143-155

SIDS Dossier

Existing Chemical	:	ID: 93-68-5
Memo	:	AAOT
CAS No.	:	93-68-5
EINECS Name	:	2'-methylacetoacetanilide
EC No.	:	202-267-0
Molecular Formula	:	$C_{11}H_{13}NO_2$

Producer related part

Company	:	Mitsuboshi Chemical Co., Ltd.
Creation date	:	18.04.2002

Substance related part

Company	:	Mitsuboshi Chemical Co., Ltd.
Creation date	:	18.04.2002
Status	:	
Memo	:	
Printing date	:	14.07.2003
Revision date	:	
Date of last update	:	14.07.2003
Number of pages	:	62
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 organization
Name	:	Mitsuboshi Chemical Co., Ltd.
Contact person	:	Kiminori Nagayama
Date	:	08.07.2003
Street	:	1-49-4 Takashimadaira, Itabashi-ku
Town	:	175-0082 Tokyo
Country	:	Japan
Phone	:	+81-3-3932-5231
Telefax	:	+81-3-3932-5230
Telex	:	
Cedex	:	
Email	:	nagayama@mitsuboshi-chem.co.jp
Homepage	:	http://www.mitsuboshi-chem.co.jp

Remark : AAOT consortium
08.07.2003

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

Type	:	manufacturer
Name of plant	:	Clariant GmbH
Street	:	
Town	:	
Country	:	Germany

Flag : non confidential
08.07.2003

Type	:	manufacturer
Name of plant	:	Eastman Chemical Company
Street	:	
Town	:	
Country	:	United States

Flag : non confidential
08.07.2003

Type	:	manufacturer
Name of plant	:	Lonza Ltd.
Street	:	
Town	:	
Country	:	Switzerland

Flag : non confidential
08.07.2003

Type	:	manufacturer
Name of plant	:	Mitsuboshi Chemical Co., Ltd. Fukui Plant
Street	:	3-3-7 Technoport Shirakata-cho
Town	:	910-3138 Fukui-shi Fukui
Country	:	Japan
Phone	:	+81-776-85-1816
Telefax	:	+81-776-85-1820

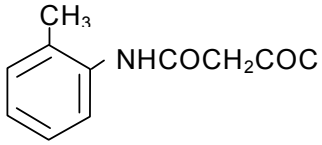
Flag : non confidential
08.07.2003

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : Butanamide, N-(2-methylphenyl)-3-oxo-
Smiles Code :
Molecular formula : $C_{11}H_{13}NO_2$
Molecular weight : 191.2
Petrol class :
Structural formula :



Remark : OECD name: o-Acetoacetotoluidide
Flag : non confidential
08.07.2003

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : typical for marketed substance
Substance type : organic
Physical status : solid
Purity : ca. 99.9 % w/w
Colour : white
Odour : no distinct odour

Remark : Mitsuboshi internal data
Flag : non confidential
08.07.2003

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

AAOT

Flag : non confidential
08.07.2003

ACETOACET-O-TOLUIDIDE

Flag : non confidential
08.07.2003

O-ACETOACETOTOLUIDIDE

Flag **:** non confidential
08.07.2003

Acetoacetyl-2-methylanilide

Flag **:** non confidential
08.07.2003

Butanamide, N-(2-methylphenyl)-3-oxo

Flag **:** non confidential
08.07.2003

N-(2-Methylphenyl)-3-oxobutanamide

Flag **:** non confidential
08.07.2003

2'-methylacetoacetanilida

Flag **:** non confidential
08.07.2003

2-Methylacetoacetanilide

Flag **:** non confidential
08.07.2003

2-(Acetoacetylamino)toluene

Flag **:** non confidential
08.07.2003

Acetoacetic acid 2-methylanilide

Flag **:** non confidential
08.07.2003

o-Methylacetoacetanilide

Flag **:** non confidential
08.07.2003

ACETESSIGSAEURE-O-TOLUIDID

Flag **:** non confidential
08.07.2003

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : ca. 4000 tonnes produced in 2001

Source : AAOT consortium
Flag : non confidential
08.07.2003

Quantity : 1000 - 1500 tonnes produced in 2001

Remark : annual production in Japan
Source : Mitsuboshi Chemical Co., Ltd.: unpublished report
Flag : non confidential
08.07.2003

1.6.1 LABELLING

Labelling : as in Directive 67/548/EEC
Specific limits : no
Symbols : Xn, , ,
Nota : , ,
R-Phrases : (20/21/22) Harmful by inhalation, in contact with skin and if swallowed
S-Phrases : (24/25) Avoid contact with skin and eyes
(28) After contact with skin, wash immediately with plenty of ...
(36/37/39) Wear suitable protective clothing, gloves and eye/face protection
(45) In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Flag : non confidential
08.07.2003 (2) (4) (10) (32)

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category : Chemical industry: used in synthesis

Flag : non confidential
08.07.2003

1.7.1 DETAILED USE PATTERN

Industry category : 3 Chemical industry: chemicals used in synthesis
Use category : 33 Intermediates
Extra details on use category :
No extra details necessary
Emission scenario document : available

Product type/subgroup :
Tonnage for Application :
Year :
Fraction of tonnage for application :
Fraction of chemical in formulation :
Production : :
Formulation : :
Processing : :
Private use :
Recovery :

Source : AAOT consortium
Flag : non confidential
08.07.2003

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Remark : This substance can be produced by reaction of o-toluidine ($C_6H_4CH_3NH_2$: CAS No. 95-53-4) and diketene ($CH_2=CCH_2OCO$: CAS No. 674-82-8). In Japan, the chemical reaction is operated in closed system, and the drying and packing are operated in semi-closed or open system.
Source : AAOT consortium
Flag : non confidential
08.07.2003

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : other: Time Weighted Average (TWA)
Limit value : 1 mg/m^3

Remark : No official limit has been established as of August 2002.
This figure is Eastman Chemical Company's private reference or recommendation.
Flag : non confidential
08.07.2003 (4)

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information : EU: 202-267-0

08.07.2003

Type : TSCA
Additional information : U.S.A.

08.07.2003

Type : ENCS
Additional information : Japan: 3-204

08.07.2003

Type : DSL
Additional information : Canada

08.07.2003

Type : other: PICCS
Additional information : Philippines

08.07.2003

Type : ECL
Additional information : Korea: KE-24832

08.07.2003

Type : CHINA
Additional information : China

08.07.2003

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : Human: exposure by production
Exposure to the : Substance

Source : AAOT consortium
Flag : non confidential
08.07.2003

Source of exposure : Human: exposure of the operator by intended use
Exposure to the : Substance

Source : AAOT consortium

Flag : non confidential
08.07.2003

1.11 ADDITIONAL REMARKS

Memo : HMIS Hazard Ratings (USA): Health-2, Flammability-1, Chemical Reactivity-0

Reliability : (2) valid with restrictions
Flag : non confidential
12.12.2002 (4)

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered :
Date of search :

Remark : Japanese governments and the agencies provided available published and unpublished reports through JCIA. And members of AAOT consortium, which were established top four manufacturer of this substance in the world (having total about 80-90 % of the market share), provided available in-house reports.
Supplementary literature search were conducted in on-line and CD-ROM database - RTECS, TOXNET, IRIS, ECOTOX, etc. - in the interest of comprehensive cover page.
Flag : non confidential
08.07.2003

1.13 REVIEWS

2.1 MELTING POINT

Value : = 106 °C
Sublimation : no
Method : other: JIS K4101-1993 5.1
Year : 2002
GLP : no
Test substance : other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%

Test condition : By using Melting Point measurement apparatus.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
10.10.2002 (11)

Value : = 104 - 106 °C
Sublimation :
Method :
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (28)

Value : = 106 °C
Sublimation :
Method :
Year : 2002
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Flag : non confidential
14.07.2003 (30)

Value : > 105 °C
Sublimation :
Method :
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (2)

Value : = 106 °C
Sublimation :
Method :
Year :
GLP : no data
Test substance : other TS: Eastman Chemical Company

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (4)

Value : = 103.5 - 105 °C
Sublimation :
Method :
Year :
GLP : no data
Test substance : other TS: Lonza Ltd.

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (10)

2.2 BOILING POINT

Value : > 170 °C at
Decomposition : yes
Method : OECD Guide-line 103 "Boiling Point/boiling Range"
Year : 1999
GLP : no
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Remark : The color became yellow at 170°C
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
09.07.2003 (16)

2.3 DENSITY

Type : density
Value : = 1.307 g/cm³ at 25 °C
Method : other: JIS K 7112-1980
Year : 1999
GLP : no
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Result : 1st. 1.310; 2nd 1.307; 3rd 1.305: average 1.307
Test condition : pycnometer method
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
09.07.2003 (16)

Type : density
Value : = 1.3 g/cm³ at 20 °C
Method :
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (2)

Type : density
Value : = 1.062 g/cm³ at 106 °C
Method :
Year :
GLP : no data
Test substance : other TS: Eastman Chemical Company

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (4)

Type : density
Value : = 1.062 g/cm³ at 20 °C
Method :
Year :
GLP : no data
Test substance : other TS: Lonza Ltd.

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (10)

Type : bulk density
Value : ca. 0.6 g/cm³ at 20 °C
Method :
Year : 2002
GLP : no
Test substance : other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%

Reliability : (2) valid with restrictions
Flag : non confidential
21.11.2002 (11)

Type : bulk density
Value : = 0.45 - 0.5 g/cm³ at °C
Method :
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (2)

Type : bulk density
Value : ca. 0.7 g/cm³ at °C
Method :
Year :
GLP : no data
Test substance : other TS: Lonza Ltd.

Reliability : (4) not assignable
Flag : non confidential
08.07.2003 (10)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : < 130 Pa at 40 °C
Decomposition : no
Method : OECD Guide-line 104 "Vapour Pressure Curve"
Year : 1999

GLP : no
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Remark : As the value was less than detection limit by Static method (130 Pa), this study should continue by another method (for example, Gas saturation method) that can detect very low vapour pressure.
Result : All of the results were less than quantitative limit, 130 Pa .
Test condition : Static method
replication: 3
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
09.07.2003 (16)

Value : = 0.00066 Pa at 20 °C
Decomposition :
Method : other (calculated): MPBPWIN v 1.40
Year : 2003
GLP : no
Test substance : other TS: based on 100% pure

Source : Mr. Naitou of Mitsubishi Chemical Safety Institute Ltd.
Test condition : Modified Grain Method
PARAMETERS
boiling point: 364.4°C (estimated)
melting point: 106.0°C (measured)
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
09.07.2003

Value : = 1.3 Pa at °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (28)

Value : = 1.3 Pa at 20 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (2)

Value : = 1.33 Pa at 20 °C
Decomposition :
Method :
Year :
GLP : no data
Test substance : other TS: Eastman Chemical Company

Reliability : (4) not assignable
Flag : non confidential

09.07.2003 (4)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = 0.85 at 25 °C
pH value : = 6.1 6.3
Method : OECD Guide-line 107 "Partition Coefficient (n-octanol/water), Flask-shaking Method"
Year : 1999
GLP : yes
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Result :

	A		B	
condition	pH	log Pow	pH	log Pow
1	6.1	0.85	6.2	0.85
2	6.3	0.85	6.3	0.84
3	6.3	0.85	6.3	0.84

rem. average log Pow = 0.85
pH value is at water layer.

Test condition : sample weight: 7.41mg (= 5mL x 1.480g/L)
component of test solution:

case	condition -1 mL	condition -2 mL	condition -3 mL
1-octanol saturated by water	-	5	15
water saturated by 1-octanol	30	25	15

temperature: 25(24-26) °C
revolution: 20/min x 5min
number of replicate: 2
analysis: HPLC

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
14.09.2002 (15)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : = 3 g/L at 25 °C
pH value : = 5.8
concentration : 3 g/L at 20 °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : soluble (1000-10000 mg/L)
Stable : yes
Deg. product : no
Method : OECD Guide-line 105
Year : 1999
GLP : no
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Remark : The pH value was measured by Mitsuboshi Chemical, and was non OECD, non GLP study.

Result : shaking time concentration
hr g/L
---
24 1st 3.0
2nd 3.0
48 1st 3.0
2nd 3.0
72 1st 3.0
2nd 3.0
---
average 3.0
Test condition : pre-shaking: 24hr, 48hr, 72hr at 30°C
shaking: 24hr at 25°C
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
09.07.2003 (11) (16)

Solubility in : Water
Value : = 3 g/L at 25 °C
pH value : ca. 7
concentration : 3 g/L at 25 °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : soluble (1000-10000 mg/L)
Stable : yes
Deg. product :
Method :
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (2)

Solubility in : Water
Value : = 2 g/L at 20 °C
pH value : = 7
concentration : 2 g/L at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method :
Year :
GLP : no data
Test substance : other TS: Lonza Ltd.

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (10)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : = 143 °C
Type : closed cup
Method : other: Pensky-Martens closed cup
Year :
GLP : no data
Test substance : other TS: Eastman Chemical Company

Reliability : (2) valid with restrictions
Flag : non confidential
21.11.2002 (4) (10)

Value : = 143 °C
Type :
Method :
Year :
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (28)

2.8 AUTO FLAMMABILITY

Value : = 516 °C at
Method : other: ASTM D2155
Year :
GLP : no data
Test substance : other TS: Eastman Chemical Company

Reliability : (2) valid with restrictions
Flag : non confidential
21.11.2002 (4) (10)

Value : >= 220 °C at
Method :
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (2)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

Acid-base constant : No dissociation was observed.
Method : OECD Guide-line 112
Year : 1999
GLP : no
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Result : All of the result was less than 2.00uS/cm, which is within a conductivity of pure water. Therefore no dissociation was observed.
Source : METI Japan
Test condition : concentration: 1.00, 10.0 and 100 mg/L
temperature: 25(24-26) °C
detection: electric conductivity meter
replication: 5
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
09.07.2003 (16)

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

Memo : combustion number: BZ2 Short flaming up without spreading, rapid extinction (20.0°C)

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (2) (10)

Memo : thermal decomposition: >400°C (Hazardous decomposition product: Nitrous gases)

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (2)

Memo : no exothermic to 450°C

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (4)

Memo : Material reacts with strong oxidizing agents.

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (4)

Memo : deposited dust; (BZ1) no ignition > 365.0°C

Reliability : (4) not assignable
Flag : non confidential
09.07.2003 (10)

Memo	:	dust explosion class: St(H)2 strong dust explosion, indicator 2 30.0g/m^3 (Modified Hartmann tube)
Reliability	:	(4) not assignable
Flag	:	non confidential
09.07.2003		(10)
Memo	:	thermal decomposition: exothermic at 290.0°C
Reliability	:	(4) not assignable
Flag	:	non confidential
09.07.2003		(10)

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
DIRECT PHOTOLYSIS		
Halflife t1/2	:	= 0.7 day(s)
Degradation	:	% after
Quantum yield	:	
Deg. product	:	
Method	:	other (calculated): AOP Win v.1.90(Syracuse Research Corporation)
Year	:	2002
GLP	:	
Test substance	:	other TS: based on 100% pure
Result	:	Hydrogen Abstraction = 0.7634 x10^{-12} cm^3/molecule-sec Addition to Aromatic Ring* = 15.2209 x10^{-12} cm^3/molecule-sec --- total OH Rate Constant = 15.9843 x10^{-12} cm^3/molecule-sec *Designates estimation using assumed value HALF-LIFE = 8.030hr = 0.669day (12hr/day; concentration of sensitizer: 1.5x10^6 OH/cm^3)
Source	:	calculated by Mr.Shinoda of CERI Japan (Sep.2002)
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

09.07.2003

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	> 5 day(s) at 50 °C
t1/2 pH7	:	> 5 day(s) at 50 °C
t1/2 pH9	:	> 5 day(s) at 50 °C
Deg. product	:	
Method	:	OECD Guide-line 111 "Hydrolysis as a Function of pH"
Year	:	1999
GLP	:	no
Test substance	:	other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%
Result	:	At pre-test this substance had no activity of hydrolysis and was stable at pH4, pH7 and pH9.
Test condition	:	PRE-TEST CONDITION concentration: about 300mg/L temperature: 50(49-51) °C pH 4, 7 and 9 replication: 2 term: 5 days
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

14.07.2003 (16)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level III
Year : 2001

Result :

compartment	amount % release 100% to air	release 100% to water	release 100% to soil
air	0.0	0.0	0.0
water	41.4	99.6	36.2
soil	58.4	0.0	63.7
sediment	0.2	0.4	0.2

Cited from Attached document (Table 3).

Source : CERI Japan
Attached document : The Fugacity Model (Mackay Level III)
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
14.07.2003 (25)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, industrial, adapted
Concentration : 191 mg/L related to DOC (Dissolved Organic Carbon)
related to
Contact time : 7 day(s)
Degradation : = 78.5 (±) % after 7 day(s)
Result : inherently biodegradable
Kinetic of testsubst. : 1 day(s) > 35.5 %
3 day(s) > 65.7 %
%
%
%
Deg. product :
Method : OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year : 1989
GLP : no
Test substance : other TS: Clariant GmbH: purity >99%

Reliability : (2) valid with restrictions

Flag : Critical study for SIDS endpoint
14.07.2003 (7)

Type : aerobic
Inoculum : activated sludge
Contact time :
Degradation : > 97 (±) % after 5 day(s)
Result : inherently biodegradable
Deg. product :
Method : OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year :
GLP : no data
Test substance : other TS: Clariant GmbH

Reliability : (2) valid with restrictions
Flag : non confidential
09.07.2003 (2) (10)

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to Test substance
related to
Contact time : 14 day(s)
Degradation : = 17.6 (±) % after 14 day(s)
Result : inherently biodegradable
Control substance : Aniline
Kinetic : 7 day(s) > 40 %
14 day(s) > 60 %
Deg. product : yes
Method : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year : 1977
GLP : no
Test substance : other TS: Dainippon Ink & Chemicals, Incorporated: purity >99.8%
Deg. products : 95-53-4 202-429-0 o-Toluidine
1. o-Toluidine was not detected in the effluent water from waste water treatment plant in Japan.
2. According to this study, it existed only in the sludge compartment.
3. Usually the sludge in waste water treatment plant is taken out and is incinerated periodically.
Therefore, the release of o-Toluidine to an environmental water is low.

Result : 14 days biodegradation detected by consumed oxygen: 17.6 %
14 days biodegradation detected by Total Organic Carbon: 35.7 %
The reason why this substance is assumed to be changed to o-Toluidine in sludge:
1. UV chart pattern in sludge became same as o-Toluidine.
(The pattern in water has not changed.)
2. If all of this substance became o-Toluidine, the decrease rate of organic carbon is 36% (= 4/11 x 100), that is very close to the above TOC result (35.7 %).
3. Chloroform extracted test solution was clearly separated into this substance and o-Toluidine by Gel Permeation Chromatograph.
Source : METI Japan
Test condition : test substance conc.: 100mg/L, sludge conc.: 30mg/L
remark: Actual kinetic % of control substance (aniline) was not described. Those are guaranteed criterion of this study.
Conclusion : This substance has almost changed to o-Toluidine (CAS 95-53-4) by biodegradation in sludge within 14 days. The biodegradation of o-Toluidine is 65.4% (see reference (1)) or 90-97% (see reference (6)) after 28 days. So, this substance can be regarded as inherently biodegradable.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
14.07.2003 (1) (6) (24)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method :
Year : 1975
Concentration : related to
BOD5 : = 0 mg/L
GLP : no data
COD
Method :
Year : 1975
COD : = 2000 mg/g substance
GLP : no data
RATIO BOD5 / COD
BOD5/COD : = 0

Remark : BOD-20 = 1680mg/g
ThOD = 2280mg/g
Test condition, etc. have not described.
Reliability : (4) not assignable
Flag : non confidential
14.07.2003 (4) (23)

3.7 BIOACCUMULATION

Species : other: calculated
Exposure period : at °C
Concentration :
BCF : = 3.2
Elimination :
Method : other: calculated
Year : 2002
GLP :
Test substance :

Method : calculated by using Down load EPI Suite v3.10 (U.S. EPA)
As log Pow = 0.85, estimated log BCF = 0.500 (BCF = 3.162).
Reliability : (2) valid with restrictions
Flag : non confidential
25.11.2002

3.8 ADDITIONAL REMARKS

Memo : Powdered material may form explosive dust-air mixtures.

Reliability : (2) valid with restrictions
Flag : non confidential
12.12.2002 (4)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : semistatic
Species : *Oryzias latipes* (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/L
LC0 : > 100 measured
LC50 : > 100 measured
Limit test : yes
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1999
GLP : yes
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Result : CONCENTRATIONS

nominal concentration (mg/L)	measured concentration (mg/L) 0hr fresh	16hr expired	mean
control	nd	nd	nd
solvent control	nd	nd	nd
100	104	96.6	100

nd : < 0.500 mg/L
The values are expressed as time-weighted means calculated by the following equation: (C0-C16)/(lnC0-lnC16) where,
C0: the measured concentration at 0hr
C16: the measured concentration at 16hr
lnC0: the natural logarithm of C0
lnC16: the natural logarithm of C16
As the result, measured concentration was equivalent to nominal one.

EFFECTS
No abnormal behavior, abnormal respiration nor dead one were observed in any of those dose levels.
MONITORING DATA
water temperature: 23.7-24.1°C
dissolved oxygen: 7.8-8.4 mg/L
(Saturated concentration at 24°C is 8.25 mg/L.)
pH: 7.3-7.6

Source : EA Japan
Test condition : TEST ORGANISMS
strain: not described
supplier: Nakajima fish firm (Kumamoto, Japan)
size/weight: 18mm (17-20mm), n=10; 0.098g (0.082-0.13g), n=10
feeding: "TETRAMIN", till 24hr before test
pretreatment: acclimated for more than 12days
feeding during test: none
reference substance: Copper(II)Sulfate Pentahydrate (96hr LC_{50} = 1.22mg/L)
PREPARATION OF TEST SOLUTION
Reagent (Hardened Castor Oil; HCO-40): test substance = 1:10 acetone solution was prepared. Then, after evaporation of acetic acid, it was diluted by dilution water so that the concentration became 1000mg/L. Then, the 100mg/L test solution was prepared by 10 times dilution.
While, "control" was dilution water only, and "solvent control" was HCO-40 100mg/L solution.
DILUTION WATER

source: tap water, treated and dechlorinated (Cl < 0.02mg/L) by activated carbon
aeration: yes
hardness: 52.0mg/L as $CaCO_3$
pH: 7.5
TEST SYSTEM
concentration: 0(control), 0(solvent control) and 100mg/L
renewal of test solution: 2 times/day
exposure vessel: 2.5L solution in a 3.0L glass vessel (16cm diameter x 17cm depth)
aeration: none
number of replication: 2
number of fish per dose: 5
water temperature: 24.0(23.0-25.0) °C
photoperiod: 16hr-8hr light-dark cycle by room light
test parameter: mortality, abnormal behavior, abnormal respiration

Conclusion : 96hr LC_{50} (and LC_0) for *Oryzias latipes* is > 100mg/L.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
14.07.2003 (20)

Type : static
Species : *Brachydanio rerio* (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/L
LC0 : = 500 measured
LC50 : > 500 measured
Limit test :
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1989
GLP : yes
Test substance : other TS: Clariant GmgH: purity >99%

Result : CONCENTRATIONS
Measured concentration of nominal 500mg/L:
510mg/L (0h), 506mg/L (48h), 496mg/L (96h)
EFFECTS
No dead one was observed in 0 and 500 mg/L dose levels.
Following abnormal behaviors were observed at 96hr in 500mg/L dose level.

No. of fishes	behavior
several	decrease of respiration frequency
several	irregular respiration
several	staying in the bottom of vessel
several	swimming in the bottom of vessel
all (10)	tail heavy swimming
all (10)	dark body color
all (10)	no reaction when tapping the vessel

MONITORING DATA
water temperature: 21.8-22.2 °C
dissolved oxygen: 6.3-9.0 mg/L
pH: 7.6-8.1
REMARK
This study was a limit test at 500mg/L only.

Test condition : TEST ORGANISMS
strain: Hamilton-Buchanan
supplier: West Aquarium, Germany
size/weight: 28mm(26-31mm), n=10; 0.18g

feeding during test: no
TEST SYSTEM
concentration: 0, 500 mg/L
renewal of test solution: none
number of replication: 1
number of fish per dose: 10
photo period: 16hr-8hr light-dark cycle
TEST PARAMETER
mortality

Reliability : (1) valid without restriction
Flag : non confidential
14.07.2003 (7) (10)

Type : static
Species : *Pimephales promelas* (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/L
LC0 : = 100 nominal
LC50 : = 316.2 nominal
LC100 : = 1000 nominal
Limit test :
Analytical monitoring : yes
Method :
Year : 1975
GLP : no data
Test substance : other TS: Eastman Chemical Company

Result : EFFECTS

nominal concentration (mg/L)	number of survival fish at 24hr	48hr	72hr	96hr
control	20	20	20	20
10	20	20	20	20
100	20	20	20	20
1000	0	0	0	0

LC_{50} value at 24hr, 48hr, 72hr and 96hr was 316.2mg/L each.
At 96hr of 100mg/kg dose level, behavior of one or more fishes were "resting"/positioned at the bottom of the vessel. Behavior of others of all control, 10 and 100 mg/L dose levels were "normal".

remark: Actual concentrations of this test substance have not confirmed during this study.

MONITORING DATA
water temperature: 15-20°C
dissolved oxygen: 1.3-9.7 mg/mL
pH: 7.3-8.0

Test condition : TEST ORGANISMS
juvenile fathead minnow, with average wet weight 0.55g per fish.
No other data was available.
PREPARATION OF TEST SOLUTION
no data available
DILUTION WATER
source: polypropylene filtrated, activated carbon treated and dechlorinated lake water of Lake Ontario (USA)
aeration: yes (by open aeration basin)
TEST SYSTEM
concentration: 0, 10, 100, 1000 mg/L of dilution water
renewal of test solution: none
exposure vessel: 20L solution in 30.5cm cuboidal chromatography jar

number of replication: 1
number of fish per dose: 20
photo period: 16hr-8hr light-dark cycle
TEST PARAMETER
mortality
CALCULATION
used nominal concentration
by SAS statistical software program, EC_LC50.SAS(Ver.1)

Reliability : (2) valid with restrictions
Flag : non confidential
14.07.2003 (4) (21) (23)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : *Daphnia magna* (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/L
NOEC : = 667 nominal
EC50 : = 931 nominal
24hr EC50 : > 1000 nominal
Limit Test : yes
Analytical monitoring : yes
Method : OECD Guide-line 202
Year : 1999
GLP : yes
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Result : CONCENTRATIONS

nominal concentration (mg/L)	measured concentration (mg/L) (percentage of nominal) 0hr	48hr	mean
control	nd	nd	nd
solvent control	nd	nd	nd
198	199 (101)	191 (96.4)	195 (98.6)
296	306 (103)	292 (98.5)	299 (101)
444	452 (102)	453 (102)	452 (102)
667	644 (96.5)	683 (102)	663 (99.4)
1000	1000 (100)	977 (97.7)	988 (98.8)

nd : < 0.500 mg/L
The values are expressed as time-weighted means calculated by the following equation: (C0-C48)/(lnC0-lnC48) where,
C0: the measured concentration at 0hr
C48: the measured concentration at 48hr
lnC0: the natural logarithm of C0
lnC48: the natural logarithm of C48
As the result measured concentration was 96.4-103% of nominal one.

EFFECTS (immobilization)
24hr EC_{50} > 1000 mg/L
48hr EC_{50} = 931 mg/L
48hr NOEC = 667 mg/L

nominal concentration (mg/L)	cumulative number of immobilized daphnid (% of immobility) 24hr	48hr

control	0 (0)	0 (0)
solvent control	0 (0)	0 (0)
198	0 (0)	0 (0)
296	0 (0)	0 (0)
444	0 (0)	0 (0)
667	0 (0)	0 (0)
1000	6 (30)	13 (65)

The values include dead daphnia.

MONITORING DATA
water temperature: 20.1-20.3°C
dissolved oxygen: 8.1-8.9mg/L (Saturated concentration at 20°C is 8.84mg/L.)
pH: 7.7-7.9

Source : EA Japan

Test condition : TEST ORGANISMS
supplier: Sheffield Univ. (Sheffield, United Kingdom)
age: less than 24hr old
feeding in acclimation: Chlorella vulgaris, 0.1-0.2mgC/day/one daphnia
pretreatment: 2-4 weeks
feeding during test: none
reference substance: Potassium Dichromate (48hr EC_{50} = 0.135mg/L)
PREPARATION OF TEST SOLUTION
Following solutions were prepared for test.
A. dilution water ("control")
B. 100mg/L HCO-40(Hardened Castor Oil) + dilution water ("solvent control")
C. 198, 296, 444, 667, 1000 mg/L each test substance + 100mg/L HCO-40 + dilution water
DILUTION WATER
source: active carbon treated and dechlorinated(Cl < 0.02mg/L) tap water
aeration: yes
hardness: 52.0mg/L as $CaCO_3$
pH: 7.5
TEST SYSTEM
renewal of test solution: none
exposure vessel: 200mL solution in a deep petri dish (8.5cm diameter x 5.7cm depth)
number of replication: 4
number of daphnia per replicate: 5
water temperature: 20(19-21) °C
photoperiod: 16hr-8hr light-dark cycle by room light
test parameter: immobility

Conclusion : 48hr EC_{50} for *Daphnia magna* is 931mg/L.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (18)

Type : static
Species : *Daphnia magna* (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/L
EC50 : = 41.1 nominal
EC100 : = 1000 nominal
Analytical monitoring : yes
Method :
Year : 1975
GLP : no data
Test substance : other TS: Eastman Chemical Company

Remark : Immobility of the control was 20% (4 in 20) at 96hr, and the partially low dissolved oxygen concentration may have contributed to the toxicity. So, the quality of this study is a little questionable.
According to MSDS of Eastman Chemical Company as of August 2002, the 96hr LC_{50} value is 37mg/L.

Result : EFFECTS

nominal concentration (mg/L)	number of mobile daphnia at 24hr	48hr	72hr	96hr
control	17N	17N	17N	16N
10	16N	16N	16R	16R
100	17R	17R	16R	2R
1000	4R	0	0	0
LC_{50} value (mg/L)	412.5	278.3	244.8	41.1

N = "normal" swimming behavior
R = "resting"/positioned at the bottom of the mesh basket
remark: Actual concentrations of this test substance have not confirmed during this study.

MONITORING DATA
water temperature: 15-20°C
dissolved oxygen: 1.3-9.7 mg/mL
pH: 7.3-8.0

Test condition : TEST ORGANISMS
age: juvenile *Daphnia magna* less than 24hr old
acclimation of adult daphnid: in 100L culturing tank for at least two weeks; then gravid daphnides were transferred into glass bowls
PREPARATION OF TEST SOLUTION
no data available
DILUTION WATER
source: polypropylene filtrated, activated carbon treated and dechlorinated lake water of Lake Ontario (USA)
aeration: yes (by open aeration basin)
TEST SYSTEM
concentration: 0, 10, 100, 1000 mg/L of dilution water
renewal of test solution: none
exposure vessel: 20L solution in 30.5cm cuboidal chromatography jar; Each daphnid was put in stainless steel mesh basket suspended in the jar.
number of replication: 1
number of daphnid per dose: 20
photo period: 16hr-8hr light-dark cycle
TEST PARAMETER
mobility
CALCULATION
used nominal concentration
by SAS statistical software program, EC_LC50.SAS(Ver.1)

Reliability : (3) invalid
Flag : non confidential
14.07.2003 (4) (22) (23)

Type :
Species : *Daphnia magna* (Crustacea)
Exposure period : 96 hour(s)
Unit : mg/L
EC50 : = 10 - 100
Method :
Year :
GLP : no data
Test substance : other TS: Lonza Ltd.

Reliability : (4) not assignable
Flag : non confidential
10.07.2003 (10)

Type :
Species : other aquatic mollusk: Ramshorn snail
Exposure period : 96 hour(s)
Unit : mg/L
EC50 : > 1000
Method :
Year :
GLP : no data
Test substance : other TS: Eastman Chemical Company

Reliability : (4) not assignable
Flag : non confidential
14.07.2003 (4) (23)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : *Selenastrum capricornutum* (Algae)
Endpoint : biomass
Exposure period : 72 hour(s)
Unit : mg/L
NOEC : = 95.3 nominal
EC50 : = 383 nominal
Limit test : yes
Analytical monitoring : yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1999
GLP : yes
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.

Result : CONCENTRATIONS

nominal concentration (mg/L)	measured concentration (mg/L) (percentage of nominal) 0hr	72hr	mean
control	nd	nd	nd
solvent control	nd	nd	nd
95.3	95.6(100)	89.1(93.5)	92.3(96.9)
171	165 (96.3)	155 (90.6)	160 (93.4)
309	311 (101)	289 (93.7)	300 (97.1)
556	548 (98.6)	529 (95.2)	539 (96.9)
1000	994 (99.4)	978 (97.8)	986 (98.6)

nd : < 0.500 mg/L
The values are expressed as time-weighted means calculated by the following equation: (C0-C72)/(lnC0-lnC72) where,
C0: the measured concentration at 0hr
C72: the measured concentration at 72hr
lnC0: the natural logarithm of C0
lnC72: the natural logarithm of C72
As the result measured concentration was 90.6-101% of nominal one.

EFFECTS
biomass;
EbC_{50} (0-72hr) = 383 mg/L (95% c.l.: 257-572 mg/L)

NOECb (0-72hr) = 95.3 mg/L
growth rate;
ErC_{50} (24-48hr) = 607 mg/L (95% c.l.: 391-942 mg/L)
NOECr (24-48hr) = 171 mg/L
ErC_{50} (24-72hr) = 654 mg/L (95% c.l.: none)
NOECr (24-72hr) = 171 mg/L

AVERAGE CELL DENSITY DURING 72HR EXPOSURE

nominal concentration (mg/L)	cell density (x 10^4 cells/mL) 0hr	24hr	48hr	72hr
control	1.0	7.3	37.8	112.1
solvent control	1.0	7.4	35.1	104.9
95.3	1.0	7.4	37.2	113.0
171	1.0	6.5	32.6	102.8
309	1.0	6.0	23.4	79.7
556	1.0	4.3	9.3	19.7
1000	1.0	2.4	4.1	5.0

AVERAGE GROWTH INHIBITION DURING 72HR EXPOSURE

nominal concentration (mg/L)	biomass (0-72hr) %	growth rate (24-48hr) %	growth rate (24-72hr) %
control	-	-	-
solvent control	6.28	5.00	2.75
95.3	0.0246	1.93	0.293
171	10.8	1.66	-1.26
309	32.4	16.9	5.03
556	78.7	53.4	44.3
1000	93.4	67.2	72.6

CELL OBSERVATION AFTER 72HR EXPOSURE
Swelling was observed in 1000mg/L level. No other abnormal was observed in any of another levels.
MONITORING DATA
water temperature: 21.8-23.0°C

pH: Nominal conc.(mg/L)	at 0hr	at 72hr
control	8.0	10.1
algal medium	7.8	10.1
95.3	7.9	10.0
171	7.8	9.9
309	7.8	9.2
556	7.8	8.6
1000	7.8	8.3

There is no explanation why the pH increased in the original report. However, by consumption of CO_2, pH deviation is frequently notices in test system and environment.

intensity of irradiation: 4200-4800 lux

Source : EA Japan

Test condition : TEST ORGANISMS
strain: ATCC22662
supplier: American Type Culture Collection
pretreatment: 72hr
initial cell concentration: $1x10^4$ cells/mL
growth/test medium: OECD medium
reference substance: Potassium Dichromate (72hr EbC_{50} = 0.295mg/L)
PREPARATION OF TEST SOLUTION
Following solutions were prepared for test.

A. OECD medium ("control")
B. 100mg/L HCO-40 (Hardened Castor Oil) + OECD medium ("solvent control")
C. 95.3, 171, 309, 556, 1000 mg/L each test substance + 100mg/L of HCO-40 + OECD medium
TEST SYSTEM
exposure vessel: 100mL medium in a 500mL conical flask with a cap, which allows ventilation.
number of replication: 3
water temperature: 23(21-25) °C
pH: no treatment
intensity of irradiation: 4000-5000 lux
photo period: continuous
shaking: 100 rpm
test parameter: cells/mL

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
14.07.2003 (17)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : *Pseudomonas putida* (Bacteria)
Exposure period : 16 hour(s)
Unit : mg/L
EC10 : ca. 800
Method : DIN 38412, part8
Year : 1989
GLP : no
Test substance : other TS: Clariant GmbH: purity >99%

Reliability : (4) not assignable
Flag : non confidential
11.07.2003 (2) (10)

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 (reproduction) : = 10 nominal
LOEC : = 20 nominal
EC50 : = 16.5 nominal
21day LC50 (parent) : > 80 nominal
Analytical monitoring : yes
Method : OECD Guide-line 211
Year : 1999
GLP : yes
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: purity 99.9%

Result : CONCENTRATIONS
nominal measured concentration (mg/L) (% of nominal)
concentration 0day 2day 9day 12day 14day 16day 21day

(mg/L)	new	old	new	old	new	old	mean
control	nd	nd	nd	nd	nd	nd	-
solvent control	nd	nd	nd	nd	nd	nd	-
5.00	4.88	4.60	4.95	4.62	4.66	4.66	4.74
	(97.6)	(92.0)	(99.0)	(92.4)	(93.1)	(93.2)	(94.7)
10.0	9.96	9.54	10.1	9.22	9.76	9.18	9.63
	(99.6)	(95.4)	(101)	(92.2)	(97.6)	(91.8)	(96.3)
20.0	20.0	18.9	20.4	18.8	19.7	16.3	19.1
	(100)	(94.4)	(102)	(94.1)	(98.7)	(81.5)	(95.4)
40.0	40.3	37.6	37.8	37.9	37.7	34.2	37.6
	(101)	(93.9)	(94.6)	(94.7)	(94.2)	(85.4)	(94.0)
80.0	77.5	75.7	77.4	74.4	77.8	75.9	76.4
	(96.8)	(94.6)	(96.8)	(93.1)	(97.2)	(94.9)	(95.5)

rem. nd : < 0.500 mg/L
new = fresh solution
old = expired solution
mean = time-weighted mean during 21days
The values are expressed as time-weighted means calculated by the following equation:
{2(C0-C2)/(lnC0-lnC2)+3(C9-C12)/(lnC9-lnC12)+2(C14-C16)/(lnC14-lnC16)}/7
where, CX: the measured concentration at X-day
lnCX: the natural logarithm of CX
As the result measured concentration was 81.5-102% of nominal one.

EFFECTS
21day LC_{50} > 80.0 mg/L
21day EC_{50} = 16.5 mg/L (95% c.l.: 15.0-18.0 mg/L)
21day NOEC = 10.0 mg/L
21day LOEC = 20.0 mg/L

CUMULATIVE NUMBER OF DEAD PARENTAL DAPHNIA AND THE MORTALITY AFTER 21 DAY

nominal concentration (mg/L)	number of dead parent	mortality %
control	2	20.0
solvent control	1	10.0
5.00	1	10.0
10.0	0	0.0
20.0	1	10.0
40.0	2	20.0
80.0	1	10.0

MEAN DAYS REQUIRED TO FIRST BROOD PRODUCTION DURING EXPOSURE

nominal concentration (mg/L)	mean (day)
control	8
solvent control	8
5.00	8
10.0	8
20.0	8
40.0	8
80.0	14.7

MEAN CUMULATIVE NUMBER OF JUVENILES PRODUCED PER ADULT DURING 21DAYS EXPOSURE

nominal concentration (mg/L)	mean (number of juveniles)
control	130
solvent control	149
5.00	137
10.0	137
20.0	55.9
40.0	3.9
80.0	0.3

ANOTHER OBSERVATIONS
Some growth inhibition were observed to the adult in 20, 40, 80 mg/L level. Also, change of body color and attachment of Chlorella to feelers were observed in those levels.
Non hatching egg was not observed in all levels.
Dead juveniles and dropped egg were observed in all levels, however the number was increased in higher concentration.
MONITORING DATA
water temperature: 20.0-20.2°C
dissolved oxygen: 8.4-8.7 mg/L
(Saturated concentration at 20°C is 8.84mg/L.)
pH: 7.5-7.8
hardness: 41.8-45.4mg/L as $CaCO_3$

Source : EA Japan

Test condition : TEST ORGANISMS
supplier: Sheffield Univ. (Sheffield, United Kingdom)
age: juveniles less than 24hr old
feeding in acclimation: *Chlorella vulgaris*, 0.1-0.2mgC/day/one daphnia
pretreatment: 2-4 weeks
feeding during test: same condition as acclimation
reference substance: Potassium Dichromate (48hr EC_{50} = 0.135mg/L)
PREPARATION OF TEST SOLUTION
Following solutions were prepared for test.
A. dilution water ("control")
B. 100mg/L HCO-40(Hardened Castor Oil) + dilution water ("solvent control")
C. 5.00, 10.0, 20.0, 40.0, 80.0 mg/L each test substance + 100mg/L HCO-40 + dilution water
DILUTION WATER
source: tap water , treated and dechlorinated (Cl < 0.02mg/L) by active carbon
TEST SYSTEM
renewal of test solution: 3 times a week
exposure vessel: 80mL solution in a glass beaker for 100mL
number of replication: 10
number of daphnia per replicate: 1
water temperature: 20(19-21) °C
photoperiod: 16hr-8hr light-dark cycle by room light
test parameter: number of dead daphnia per day, and number of juveniles produced per adult

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (19)

4.6.1 TOXICITY 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 REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type : LD_{50}
Value : = 1854 mg/kg bw
Species : rat
Strain : Crj: CD(SD)
Sex : male/female
Number of animals : 5
Vehicle : other: 1% methylcellulose solution
Doses : 0, 819, 1024, 1280, 1600, 2000, 2500 mg/kg for both sexes
Method : OECD Guide-line 401 "Acute Oral Toxicity"
Year : 1999
GLP : yes
Test substance : other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%

Result : MORTALITY

dose mg/kg	number of animals per sex	number of deaths male	female
0	5	0	0
819	5	0	0
1024	5	0	0
1280	5	1(Hr.3)	0
1600	5	0	1(Day3)
2000	5	3(Hr.3, Day3)	2(Hr.3,6)
2500	5	5(Hr.3, Day3)	5(Hr.3,6,Day2,3)

Hr.: hours after dose, Day: days from dose

LETHAL DOSE

	male (95% confidential)	female (95% confidential)
LD_0 :	= 1024 mg/kg	= 1280 mg/kg
LD_{50} :	= 1854 (1549-2298) mg/kg	= 1945 (1654-2318) mg/kg
LD_{100}:	> 2000 mg/kg	> 2000mg/kg

OBSERVATIONS
From 10 minutes after dose, decreased locomotor activity and adoption of prone position were observed in all treated groups, and hypomyotonia, ptosis and deep respiration were observed in many of treated groups.
From 1 to 3 hours later, piloerection, hypothermia and lacrimation were observed in all treated groups dose-dependently.
From the Day 2, pale skin was observed in all treated groups dose-dependently.
Dead animals showed serious those clinical signs and weak respiration before die.
Body weights in treated groups were dose-dependently lower than those of the control group on the day after treatment. At necropsy, bloody material in the stomach and intestine, petechiae in the glandular stomach and distension of the urinary bladder were observed in the animals that died.
Except pale skin all of those symptoms on live animals were recovered by Day 5, and pale skin was recovered by day 12.
Body weight showed recovery trend on day 3, then normally increased after day 7.

Source : MHW Japan

Test condition : TEST ORGANISMS
source: Japan Charles River Co. Ltd.
age: 5 weeks old
weight at initiation: 120-137g for males, 106-118g for females
pellet food: free take till 17:00 on the day before test and from 3 hours after dose onward
water: free take
ADMINISTRATION
vehicle: 1% Methylcellose water solution
route: 1.0mL/100g body weight by gavage
post dose observation: till 14 days after administration

Conclusion : The LD50 value by oral for rat is 1854 mg/kg for male and 1945 mg/kg for female. The major toxicity is hemolytic anemia (please refer section 5.4).

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (29)

Type : LD_{50}

Value : ca. 1600 mg/kg bw

Species : rat

Strain : other: Caesarean-derived, barrier-reared

Sex : male

Number of animals : 2

Vehicle : other: 10% suspension in a 0.5% aqueous jaguar medium

Doses : 200, 400, 800, 1600, 3200 mg/kg

Method :

Year : 1975

GLP : no data

Test substance : other TS: Eastman Chemical Company

Result : MORTALITY

dose mg/kg	No. of animals	No. of death	(time of death)
200	2	0	
400	2	0	
800	2	0	
1600	2	1	(Day 5)
3200	2	2	(Hour 5)

OBSERVATIONS
Clinical signs such as prostration, labored breathing and jerking motions were observed in the 1600 and 3200 mg/kg groups. Hypersensitivity to touch and sound was also observed in the 1600 mg/kg group. Severe weakness was noted in the 200 and 400 mg/kg groups and slight to moderate weakness was observed in 200 mg/kg group on the day of dosing.
On the next day, all animals in 200 and 400mg/kg groups appeared normal. All surviving animals gained weight over the study 15 days later.

OTHER DATA
LD_{50} = 1600mg/kg bw (mouse) (Test method, etc. were not described.)

Test condition : source: Charles River Co.
route of administration: oral, gavage
post dose observation: till 15 days after administration

Reliability : (2) valid with restrictions

Flag : non confidential

14.07.2003 (4) (23) (27)

Type : LD_{50}

Value : = 2500 - 5000 mg/kg bw

Species	:	rat
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: Clariant GmbH
Reliability	:	(4) not assignable
Flag	:	non confidential

11.07.2003 (2)

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: Lonza Ltd.
Reliability	:	(4) not assignable
Flag	:	non confidential

11.07.2003 (10)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD_{50}
Value	:	> 1000 mg/kg bw
Species	:	guinea pig
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	no data
Reliability	:	(4) not assignable
Flag	:	non confidential

14.07.2003 (23) (27)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD_{50}

Value	:	= 800 - 1600 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Reliability	:	(4) not assignable
Flag	:	non confidential

14.07.2003 (5) (23)

Type	:	LD_{50}
Value	:	= 400 - 800 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Reliability	:	(4) not assignable
Flag	:	non confidential

14.07.2003 (5) (23)

5.2.1 SKIN IRRITATION

Species	:	guinea pig
Concentration	:	250, 500, 1000 mg/kg
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: Eastman Chemical Company
Result	:	24 hrs later, moderate edema and slight erythema were produced. One week later, desquamation was noted. One week after the test, the skin appeared normal.
Reliability	:	(4) not assignable
Flag	:	non confidential

14.07.2003 (4) (23)

Species	:	guinea pig
Concentration	:	0.2 mg
Exposure	:	Occlusive
Exposure time	:	14 day(s)
Number of animals	:	10
Vehicle	:	other: see Test condition
PDII	:	

Result	:	moderately irritating
Classification	:	
Method	:	other: see Test condition
Year	:	1975
GLP	:	no
Test substance	:	other TS: Eastman Kodak Company
Result	:	after the first application: 6 Pigs were no reaction, and 4 were minimal erythema. after two weeks' test: 1 Pig was no reaction, 8 were minimal erythema and 1 was severe erythema.
Test condition	:	This substance was added to a lotion (33% w/v) consisting 3A alcohol : glycerin (1:9, v/v). 1/2mL of this mixture (= 0.165mg of substance) was rubbed on the clipped back of 10 guinea pigs five days a week for two weeks.
Conclusion	:	As the author said, "Repeated exposure probably does not exacerbate its irritation potential. However the possibility of an occasional incident of contact dermatitis should be anticipated."
Reliability	:	(4) not assignable
Flag	:	non confidential
14.07.2003		(23)
Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: Clariant GmbH
Reliability	:	(4) not assignable
Flag	:	non confidential
11.07.2003		(2) (10)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	100 %
Dose	:	100 other: mg
Exposure time	:	24 hour(s)
Comment	:	
Number of animals	:	6
Vehicle	:	none
Result	:	slightly irritating
Classification	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS
Result	:	immediately after treatment: The eyelids were held shut for about 30 seconds. one hour later: The conjunctivae and nictitating membranes were slightly

erythematous.
24 hours later: All eyes appeared normal and no tissues stained with fluorescein.
post exposure: The eyes remained normal during the subsequent 13 days. While, three of the eyes were washed one minute after the application, with distilled water. The only reaction was a slightly increased blinking rate.

Test condition : Approximately 100mg of the substance was placed in the lower eye sack of six albino rabbit eyes.
Three of the eyes were washed one minute later with distilled water.
post dose observation for 13 days

Reliability : (4) not assignable
Flag : non confidential
14.07.2003 (2) (4) (23)

Species : rabbit
Concentration :
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result : moderately irritating
Classification :
Method : OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year :
GLP : no data
Test substance : other TS

Reliability : (4) not assignable
Flag : non confidential
11.07.2003 (10)

5.3 SENSITIZATION

Type : other: see Test condition
Species : guinea pig
Number of animals : 10
Vehicle :
Result : ambiguous
Classification :
Method :
Year : 1975
GLP : no data
Test substance : other TS: Eastman Chemical Company

Result : Nine of the ten reacted similarly to their control.
One of the ten reacted with a strong erythema both at 24 and 48 hours after application.

Test condition : A compound-heparinized-whole-rabbit-blood reaction product was injected into the footpads of ten guinea pigs.
One week later they were challenged with topical application.

Conclusion : "The pig reacted with a strong erythema was sensitized and that an occasional human may, after repeated exposures, also become sensitized." - the author said.

Reliability : (4) not assignable
Flag : non confidential
14.07.2003 (4) (23)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	males: 44days, females: from 14days before mating to Day 3 of lactation (41 - 45days)
Frequency of treatm.	:	one administration/day
Post exposure period	:	none
Doses	:	0, 8, 25, 80, 250 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 25 mg/kg bw
Method	:	OECD combined study TG422
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%
Remark	:	This data is a part of OECD TG422 (combined study). Please refer to section 5.8.1 and 5.8.2.
Result	:	PRELIMINARY EXAMINATION 4 males and 4 females were used for 14days Preliminary Repeat Dose Test. Several symptoms to blood, liver and kidney were observed at >250mg/kg/day for male and >100mg/kg/day for female. So, highest dose was set up to 250mg/kg/day. CLINICAL OBSERVATIONS General: No change in mortality and behavior were observed in any groups. Body weight and food consumption: No toxicological effect was observed in any groups. Urinary findings in male: Increases of specific gravity was observed in 250mg/kg group. However as the author said, it's likely within normal range, and no related change was observed in another check items. HEMATOLOGICAL AND BLOOD CHEMICAL FINDINGS IN MALE

dose (mg/kg/day)	0	8	25	80	250
erythrocyte count:	-	-	-	D	D
mean corpuscular volume (MCV):	-	-	-	I	I
hemoglobin concentration:	-	-	-	-	D
hematocrit value:	-	-	-	-	D
mean corpuscular hemoglobin (MCH):	-	-	-	-	I
reticulocyte count:	-	-	-	-	I
methemoglobin concentration:	-	-	-	-	I
Heinz-body in erythrocytes:	-	-	-	-	O
bilirubin:	-	-	-	I	I
potassium:	-	-	-	-	I

-: normal or nothing, I: increased, D: decreased, O: observed

HISTOPATHOLOGICAL FINDINGS, ETC. IN MALE

dose (mg/kg/day)	0	8	25	80	250
blackening of spleen:	-	-	-	O	O
enlargement of spleen:	-	-	-	-	-
weight of spleen:	-	-	-	-	I
weight of pituitary:	-	-	-	-	I

		weight of liver: - - - - - hemosiderin deposit in liver and spleen: - - - O O extramedullary hematopoiesis: - - - - I congestion in spleen: - - - - I eosinophilic body in tuble of kidneys: - - - - I --- -: normal or nothing, I: increased, D: decreased, O: observed HISTOPATHOLOGICAL FINDINGS, ETC. IN FEMALE dose (mg/kg/day) 0 8 25 80 250 --- blackening of spleen: - - - O O enlargement of spleen: - - - - O weight of spleen: - - - - I weight of pituitary: - - - - - weight of liver: - - - - I hemosiderin deposit in liver and spleen: - - - O O extramedullary hematopoiesis: - - - - I congestion in spleen: - - - I I eosinophilic body in tuble of kidneys: - - - - - --- -: normal or nothing, I: increased, D: decreased, O: observed NOEL for repeat dose toxicity is 25mg/kg/day for both sexes.
Source	:	MHW Japan
Test condition	:	TEST ORGANISMS Age: 9 weeks for male, 8 weeks for female Weight at initiation: 343-391 g for male, 211-241 g for female Number of animals: 10 per sex per dose Pellet food and water: free take ADMINISTRATION Vehicle: 1% methylcellulose water solution, 0.5mL/100g body weight Type of administration: gavage, once a day Duration of administration: male; 44 days (including 14 days before mating) female: 41-45 days (from 14 days before mating to 3 days after parturition) MATING PROCEDURE one by one in each cage (All of those 10 pairs had finished mating by Day 4.) CLINICAL OBSERVATIONS AND FREQUENCY Clinical signs and mortality: every day Body weight: once a week, and the time of termination Food consumption: at every body weight check (24hr consumption) Water consumption: not checked HISTOPATHOLOGICAL OBSERVATIONS Urinalysis: by male at Day 39 - 43; pH, blood, protein, ketones, bilirubin, urobilinogen, specific gravity, deposit and appearance Hematology: by male at day 45 (stopped feeding at 17:00 on the day before terminal kill); erythrocyte count, hemoglobin, hematocrit, MCV, MCH, mean corpuscular hemoglobin(MCHC), leukocyte count, platelet count, reticulocyte count, Heinz-body and methemoglobin Blood biochemical: Same sample as hematology was used.; total protein, albumin, albumin/globulin(A/G) ratio, glucose, triglyceride, total cholesterol, total bilirubin, nitrogen of urea, creatinine, GOT, GPT, gamma-GTP, lactate dehydrogenase(LDH), alkaline phosphatase, cholin esterase, calcium, phosphate, sodium and potassium

Organs: by male after extraction of blood, and by female at day 4 after (estimated) pregnant;
for weight check; brain, liver, kidney, spleen, heart, thymus, thyroid, pituitary, adrenals, testes and epididymides
for observation; above mentioned ones plus, lung, stomach, bladder, medulla, spinal cord, sciatic nerve, etc.

Attached document : Findings of rats and the organ weights treated orally with AAOT in the combined repeat dose and reproductive/developmental toxicity screening test (Table 6, 7, 8)

Conclusion : Main toxicity by the repeat dose was hemolytic anemia and the related changes on the blood, spleen, liver and kidney. Also, slight changes were observed in the kidneys' eosinophilic bodies (increased) of male and on the liver weight (increased) of female.
NOAEL for repeat dose toxicity to rats is considered to be 25mg/kg/day in both sexes.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (3)

Type : Sub-acute
Species : rat
Sex : male
Strain :
Route of admin. : oral feed
Exposure period : 11 days
Frequency of treatm. :
Post exposure period :
Doses : 0, 88, 96, 760, 816 mg/kg/day
Control group : other: yes, concurrent chow and historical data
NOAEL : < 88 mg/kg bw
Method :
Year : 1975
GLP :
Test substance : other TS: Eastman Kodak Company

Remark : "It appears that red cell lifespan may be decreased in these animals." - this author said.
Under mentioned Results and Test condition are all data available from the original report.

Result : Remark: According to this author, the control animals used for Experiment I (namely, dose rate 88 and 760 mg/kg/day) were anomalous, because they did not gain weight normally. So, replicated test (Experiment II; 96 and 816 mg/kg/day) was made.

HISTOLOGICAL AND STATISTICAL RESULTS
general: No gross changes in appearance, coat, behavior or stools were observed in any of them.
body weight and food consumption: Decreased food intake with an associated decreased weight gain was observed in 96 and 816 mg/kg groups.
hematological finding: Slight dose-related decrease in hemoglobin concentration and hematocrit in 88 and 760 mg/kg groups, and increase in circulating white cell number were observed. And two animals in 760mg/kg group exhibited moderate polychromatophilia.
blood chemical finding: The values of lactic acid dehydrogenase and alkaline phosphatase in 88 and 760 mg/kg groups were increased. While, glutamic oxalacetic transaminase and urea nitrogen were equivalent to historical control.
necropsy finding: One control animal had focal interstitial nephritis. Bone marrow hematopoiesis was more intense in one treated animal than control.

weight of organs: Decreased liver weight was observed in 816mg/kg group.
histological finding: Increased amounts of splenic hemosiderin and congestion of the spleen were observed in four of five treated animals.

Test condition : TEST ORGANISMS
male rat; More than 5, but the detail was not described.
ADMINISTRATION
blended into PURINA Laboratory Chow
duration: 11 days
CLINICAL OBSERVATION
appearance, coat, behavior, stool, body weight and food consumption
HISTOLOGICAL OBSERVATIONS by light microscopy
trachea, lung, esophagus, stomach, small intestine, cecum, colon, liver, kidney, urinary bladder, heart, adrenal gland, pancreas, thyroid, testis, spleen, bone marrow, mesenteric lymph node, cerebrum, cerebellum, medulla and eye

Conclusion : As still some change were observed on weight gain and food consumption in 88 and 96 mg/kg levels, NOAEL is < 88 mg/kg/day range.

Reliability : (2) valid with restrictions

Flag : non confidential

14.07.2003 (23)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test

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

Test concentration : -S9mix and +S9mix: 0, 156, 313, 625, 1250, 2500, 5000 ug/plate

Cycotoxic concentr. : Toxicity was not observed up to 5000ug/plate in five strains with or without S9mix.

Metabolic activation : with and without

Result : negative

Method : other: OECD Test Guidelines 471 and 472 "Genetic Toxicology (*Salmonella typhimurium* and *Escherichia coli*)

Year : 1999

GLP : yes

Test substance : other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%

Result :

	Salmonella typhimurium TA100, TA1535, TA100, TA1537			*Escherichia coli* WP2*uvrA*		
	+	?	-	+	?	-
-S9mix:	[ ]	[ ]	[*]	[ ]	[ ]	[*]
+S9mix:	[ ]	[ ]	[*]	[ ]	[ ]	[*]

OBSERVATION
Number of revertant colonies per plate in all doses with/without S9mix were equivalent to control. On the other hand, more than 2 times revertant colonies were observed in all positive controls. Visible precipitation was not observed in any plates.

Source : MHW Japan

Test condition : TEST SYSTEM
metabolic activation system: S9 from male rat liver, induced with phenobarbital and 5,6-benzoflavon
ADMINISTRATION
number of replicate: 2
plates per dose: 3
application: pre-incubation
solvent: DMSO (Concentration was not described.)
positive control groups:
without S9mix; 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA98, TA100,

WP2), sodium azide (TA1535), 9-aminoacridine hydrochloride (TA1537) with S9mix; 2-aminoanthracene (all five strains)
test parameter: revertant colonies per plate

Conclusion : This substance is not mutagenic to *Salmonella typhimurium* and *Escherichia coli*.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
14.07.2003 (26)

Type : Ames test
System of testing : *Salmonella typhimurium* (TA102, TA2638); *Escherichia coli* (WP2/pKM101, WP2*uvrA*/pKM101)
Test concentration : 0, 20, 78, 313, 625, 1250, 2500, 5000 ug/plate
Cycotoxic concentr. : Toxicity was not observed up to 5000ug/plate in all strains.
Metabolic activation : without
Result : negative
Method : other: plate incorporation method essentially as described by Maron and Ames
Year : 1996
GLP : no data
Test substance : other TS: Tokyo Kasei Kogyo Co., Ltd.: (most probably) purity 99.9%

Result : MUTAGENIC ACTIVITY

dose	number of revertant/plate							
ug/plate	TA102		TA2638		WP2/pKM101		WP2uvrA/pKM101	
	lab1	lab2	lab1	lab2	lab1	lab2	lab1	lab2
0	441	342	36	43	50	89	97	103
20	-	352	-	43	-	76	-	114
78	-	344	-	40	-	81	-	128
313	447	339	31	35	45	78	105	125
625	456	-	35	-	49	-	102	-
1250	395	317	29	36	48	81	99	134
2500	416	-	24	-	38	-	69	-
5000	307	223	16	29	35	58	56	98

rem. This study was operated by two different laboratories. "lab1" is the one and "lab2" is the other.
All values are the average of three plates at each laboratory.
There was no description about the result of those positive controls. However the results of simultaneous 28 chemicals were reported. On them was Formaldehyde, of which positive results were observed at following doses (ug/plate). TA102: 50-400, TA2638: 50-500, WP2/pKM101: 25-700, WP2*urvA*/pKM101: 25-800

GENOTOXIC EFFECT
without metabolic activation;
Salmonella typhimurium TA102, TA2638; negative
Escherichia coli WP2/pKM101, WP2*uvrA*/pKM101; negative
with metabolic activation; (This is not a part of this report.)

Test condition : BACTERIAL STRAINS
source: TA102, TA2638; Professor B.N.Ames (Univ. California, USA)
WP2, WP2uvrA/; National Institute of Genetic (Japan)
introduction of R-factor resistance plasmid pKM101;
at Institute of Environmental Toxicology (Japan) by Ishizawa's method
ADMINISTRATION
number of replicate: 2 (different laboratories)
plates per test: 3
application: pre-incubation
positive control groups:

Mitomycin C ; TA102, TA2638
2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; WP2/pKM101, WP2*uvrA*/pKM101
solvent: DMSO

Reliability : (2) valid with restrictions
Flag : non confidential
14.07.2003 (9) (31)

Type : Ames test
System of testing : *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)
Test concentration : -S9mix and +S9mix: 0, 25, 250, 2500, 5000, 10000 ug/plate
Cycotoxic concentr. : Toxicity was not observed in TA100 up to 10000ug/plate with or without S9mix.
Metabolic activation : with and without
Result : negative
Method : other: no description for standard protocol number
Year : 1985
GLP : yes
Test substance : other TS: mixture of Hoechst, Kodak and Lonza

Result : Number of revertant colonies per plate in all doses with/without S9mix were equivalent to negative control.
On the other hand, more than 2 times revertant colonies were observed in all positive controls. While, there was no description about visible precipitation.
Test condition : ADMINISTRATION
number of replicate: 1 (2 for TA98 only)
plates per dose: 3
solvent: DMSO 100mg/mL solution
positive control groups: not described
test parameter: revertant colonies per plate
Reliability : (2) valid with restrictions
Flag : non confidential
14.07.2003 (12)

Type : Ames test
System of testing :
Test concentration :
Cycotoxic concentr. :
Metabolic activation :
Result : negative
Method :
Year :
GLP : no data
Test substance : other TS: Lonza Ltd.

Remark : OTHER RESULTS
Gene mutation in mammalian cells: negative
DNA repair assay in vitro: negative
Reliability : (4) not assignable
Flag : non confidential
11.07.2003 (10)

Type : Chromosomal aberration test
System of testing : CHL/IU cell
Test concentration : See under mentioned Test condition.
Cycotoxic concentr. : See under mentioned Result.
Metabolic activation : with and without
Result : positive
Method : OECD Guide-line 473
Year : 1999
GLP : yes

Test substance : other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%

Result :

	clastogenicity			polyploid		
	+	?	-	+	?	-
-S9mix 24hr continuous	[*]	[ ]	[ ]	[ ]	[ ]	[*]
-S9mix 48hr continuous	[ ]	[*]	[ ]	[ ]	[ ]	[*]
-S9mix 6hr short term	[ ]	[*]	[ ]	[ ]	[ ]	[*]
+S9mix 6hr short term	[ ]	[*]	[ ]	[ ]	[ ]	[*]
-S9mix 24hr continuous (confirmative test)	[ ]	[*]	[ ]	[ ]	[ ]	[*]

Please refer to the attached documents, too.

CYTOTOXIC CONCENTRATION (50% growth inhibition calculated by Probit method)
-S9mix 24hr continuous : 1565 ug/mL
-S9mix 48hr continuous : 940 ug/mL
-S9mix 6hr short term : 3392 ug/mL
+S9mix 6hr short term : 3699 ug/mL
OBSERVATION
Some cytotoxicity were observed as per attached documents (Fig. 1).
Visible precipitation was shown as per attached documents (Table 3, 4, 5).
At continuous treatment, slight structural aberration was observed in 24hr (10%) and in 48hr (5%) at highest dose. On the other hand, remarkable aberration was observed in positive control.
At short-term treatment, slight structural aberration was observed in with S9 mix (5%) and in without (9%) at highest dose. On the other hand, remarkable aberration was observed in positive control of the case with S9 mix.
CONFIRMATIVE 24HR CONTINUOUS TREATMENT (EXTRACTED)

dose ug/mL	0	1500	2000	2500	3000	3500
s.aberration %	0.5	4.0	8.5	2.5	3.9	toxic

---

rem. Due to cytotoxicity of AAOT, possible number of analyze cell was 180 at 3000ug/mL (others were 200), and it was almost nothing at 3500ug/mL.

CONSIDERATION
Those more than 5% responses were observed only at concentration levels higher than 10 mM (1,910 ug/mL). Therefore the response was regarded as a biologically irrelevant phenomenon under unphysiological (high osmolality) culture condition.

Source : MHW Japan

Test condition :
CONCENTRATIONS (doses)
-S9mix 24hr continuous : 0, 625, 1250, 2500, 5000 ug/mL
-S9mix 48hr continuous : 0, 450, 900, 1800, 3600 ug/mL
-S9mix 6hr short term : 0, 1250, 2500, 5000 ug/mL
+S9mix 6hr short term : 0, 1250, 2500, 5000 ug/mL
-S9mix 24hr continuous (confirmative test) :
0, 1500, 2000, 2500, 3000, 3500 ug/mL
ADMINISTRATION
metabolic activation: S9 from male rat liver, induced with phenobarbital and 5,6-benzoflavone
number of replicates: 2 (plates/test)
positive control:
-S9mix 24 and 48hr continuous; *Mitomycin C*
-S9mix and +S9mix 6hr short term; cyclophosphamide
number of cells analyzed: 200/dose (= 100/plate x 2plates)
test parameter:
Less than 5% aberration is to be "negative".
Between 5% and 10% is to be "ambiguous".
More than 10% is to be "positive".

Final judge of "positive" is done, if dose-dependency or repeatability have confirmed.

Attached document : Chromosome aberration test on CHL cells;
continuous and short-term treatment, also the confirmative examination (Table 3, 4, 5)
Dose-survival curves (Fig.1)

Conclusion : AAOT induces weak clastogenicity at only concentration levels higher than 10 mM. AAOT dose not induce polyploid. Therefore, AAOT is considered to be not induces Chromosomal aberration.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (8)

Type : HGPRT assay

System of testing : forward mutation in the CHO-K1-BH4, Chinese hamster ovary cell line

Test concentration : -S9mix and +S9mix: 0, 0.3, 0.6, 0.9, 1.2, 1.5 mg/mL

Cycotoxic concentr. : % cell survival at 1.5mg/mL dose: -S9mix= 91%, +S9mix= 85%, that was acceptable range.

Metabolic activation : with and without

Result : negative

Method : other: see Test condition

Year : 1985

GLP : yes

Test substance : other TS: mixture of Hoechst, Kodak and Lonza

Result : MUTATION FREQUENCY

-S9mix:

dose (mg/mL)	% absolute cloning efficiency	total mutant colonies	mutation frequency (mutans/mil.cells)
control	73.0	13	8.9
solvent control	83.8	19	11.3
0.3	63.4	1	0.8
0.6	80.6	12	7.4
0.9	81.4	5	3.1
1.2	75.2	2	1.3
1.5	77.6	1	0.6
positive control	71.8	343	238.9

$$\text{mutation frequency} = \frac{\text{total mutant colonies}}{(\text{cells seeded per test}) \times (\text{absolute cloning efficiency})}$$

cells seeded per test = 2×10^5/dish x 10dishes = 2×10^6

positive control: ethyl methanesulfonate, 0.25mg/mL

+S9mix:

dose (mg/mL)	% absolute cloning efficiency	total mutant colonies	mutation frequency (mutans/mil.cells)
control	72.0	1	0.7
solvent control	90.0	2	1.1
0.3	78.8	16*	10.2*
0.6	57.6	13*	11.3*
0.9	78.6	0	0.0
1.2	64.2	6	4.7
1.5	65.6	8	6.1
positive control	51.6	51	49.4

* Significant different from controls.

$$\text{mutation frequency} = \frac{\text{total mutant colonies}}{}$$

(cells seeded per test) x (absolute cloning efficiency)
cells seeded per test = $2x10^5$/dish x 10dishes = $2x10^6$
positive control: dimethylnitrosamine, 0.25mg/mL
Remark: "None of these induced mutation frequency values (*) is in excess of the spontaneous background range normally observed for this assay. A report from --- EPA --- gives the approximate range of spontaneous mutation frequency as 0-20 mutants per million clonable cells." - the author said.

Dose response relationship was not observed for either with and without S9mix.

Test condition : CELL STRAINS
type: Chinese hamster ovary cell, CHO-K1-BH4
source: from Dr.Hsie (Oak Ridge National Labo., USA)
selection of HGPRT+ cells: prior to assay
MEDIA
culture medium: Nutrient Mixture F12 supplemented with L-glutamine and heat-inactivated dialyzed fetal bovine serum (5% by volume)
selection medium: hypoxanthine-free F12 containing 10 u mol of 6-thioguanine
CONTROLS
control: mentioned above culture medium
solvent control: culture medium + 1% DMSO
S9
induced from rat liver, 1mg protein per mL
TEST SYSTEM
number of replicates for cloning: 5 (flasks/treatment)
number of replicates for mutant selection: 1 (10 dishes total/treatment)
positive control: see above "Result"
PROTOCOL
Cells were seeded into 25 cm^2 flasks at 5 $x10^5$ cells per flask.
After 24hr incubation, test substances were added in each 2 flasks.
After 4hr exposure, those were washed and incubated in F2 overnight.
The cell monolayers were trypsinized 16-24hr and suspended, then were seeded at about 100 per flask and incubated for 7 days. (The rest of colonies were used for counting cytotoxicity.)
The cell suspension were used to replant at 10^6 cells per 75 cm^2 flask, then were incubated to permit growth and expression and subcultured every 2 or 3 days. At each subculture, two cultures each were combined and reseeded at 10^6 cells into each of 2 flasks.
At the end of expression period, each culture was reseeded at 2 $x10^5$ cells per dish x 10 dishes in selection medium. (The rest of colonies were used for counting absolute cloning efficiency.)
After 7 days incubation, colonies in both dishes and flasks were checked.
TEST PARAMETER
survival to treatment, absolute cloning efficiency and mutant frequency

Conclusion : This substance is considered negative in the CHO/HG Forward Mutation assay at dose levels up to 1.5mg/mL. Because, induced mutation frequencies in the without S9 were rather smaller than negative control. And, though some of the ones in the with S9 were higher than the negative control, it was within the spontaneous normal value (less than 20 mutants per million clonable cells), and also, dose-response relationship was not observed.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
14.07.2003 (14)

Type : Unscheduled DNA synthesis
System of testing : non bacteria
Test concentration : 0, 165, 330, 825, 1650, 3300 ug/mL
Cycotoxic concentr. : 3300ug/mL (12.9% survival) (at 1650ug/mL - 105.4% survival)

Metabolic activation : with
Result : negative
Method : other: see Test condition
Year : 1985
GLP : yes
Test substance : other TS: mixture of Hoechst, Kodak and Lonza

Result : CONCENTRATIONS
The measured concentration of the nominal 330mg/mL DMSO solution (for making 3300ug/mL medium by 100x dilution) was 288.6mg/mL. The 12% difference was similar enough.
UDS FREQUENCY

dose (ug/mL)	survival %	UDS grains/nucleus mean ± sd	% of cells with >5 UDS grains
control	100.0	-1.3 ± 0.6	2.0 ± 2.0
solvent control	124.9	-1.0 ± 0.4	5.3 ± 1.2
165	115.5	-0.3 ± 0.6	4.7 ± 1.2
330	114.4	-1.0 ± 0.6	6.7 ± 2.3
825	118.7	-0.6 ± 0.5	3.3 ± 2.3
1650	105.4	-0.6 ± 0.2	6.0 ± 2.0
3300	12.9	-0.4 ± 0.8	1.3 ± 2.3
positive control	90.1	40.0 ± 1.8	100

positive control: 2-Aminoanthracene, 0.4ug/mL
harvest viability: 92.1%
attachment efficiency: 77%
The majority of the cells at the 3300ug/mL dose were necrotic with very few S-phase cells observed.

Test condition : TEST ORGANISMS
cell type: hepatocytes isolated from male Charles River CD-1 rat weighing 200-330g
pre-incubation: 2-3hr on cover slip mounted plastic tissue culture dish by William's Medium E at 37°C in 95% air and 5% CO_2
CONTROLS
control: William's Medium E
solvent control: 1% DMSO in William's Medium E
positive control: 2-Aminoanhracene 0.4ug/mL in William's Medium E
TEST SYSTEM
Each 5 culture dishes were prepared for pre-incubation and test.
exposure: 18hr by William's Medium E with 1.0-2.0uCi/mL of tritiated thymidine at 37°C in 95% air and 5% CO_2
detection: After treatment and dried, the cells were mounted coverslip on slides, then were stained. The number of grains on video screen were detected and counted by electronic counter. While, "UDS grains/nucleous" and "% of cells with > 5 UDS grains" were based on net nuclear grain (NNG) calculated by following formula.
NNG = (grains appearing over the nucleus) - (average number of grains appearing in three nuclear sized area of the cytoplasm adjacent to the nucleus)
Nuclei in undergoing replicative DNA synthesis were excluded.
number of replicate: 3 sets for UDS, 2 sets for cytotoxicity; Each 50 cells per plate was used for score.
test parameter: cytotoxicity (survival), number of net UDS grains/nucleus and % of cells with more than 5 UDS grains/nucleus

Conclusion : This substance failed to produce a significant amount of UDS compared with negative control, and can be judged to negative.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
14.07.2003 (13)

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Type : One generation study
Species : rat
Sex : male/female
Strain : Crj: CD(SD)
Route of admin. : gavage
Exposure period : male: 44days, female: from 14days before mating to 3 days after parturition (41-45days)
Frequency of treatm. : once a day, every day
Premating exposure period
Male : 14days
Female : 14days
Duration of test : male: 44days, female: 41-45days
No. of generation studies : 1
Doses : 0, 8, 25, 80, 250 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL parental : = 250 mg/kg bw
NOAEL F1 offspring : = 250 mg/kg bw
Method : OECD Guide-line 422
Year : 1999
GLP : yes
Test substance : other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%

Remark : This data is a part of OECD TG422 (combined study).
Please refer to section 5.4 and 5.8.2

Result : PRELIMINARY EXAMINATION
4 males and 4 females were used for 14days Preliminary Repeat Dose Test.
Several symptoms to blood, liver and kidney were observed at >250mg/kg/day for male and >100mg/kg/day for female. So, highest dose was set up to be 250mg/kg/day.
STATISTICAL RESULTS
(As you can see on under mentioned tables;)
No effects were observed in the couplation index, fertility index, gestation length, number of corpora lutea or implanations, implanation index, gestation index, nurturition or maternal behavior.
No compound-related effects on the number of pups, delivery index, sex ratio, body weight and viability index were observed in any dose groups.
No pups with malformation were found in any groups. No changes in histopathological findings were observed in offspring.

REPRODUCTIVE PERFORMANCE

dose (mg/kg)	0	8	25	80	250
No. of pairs mated	10	10	10	10	10
No. of pairs coupled	10	10	10	10	10
pairing days till couplation	2.3±1.16	2.4±1.26	2.9±0.88	2.3±0.82	2.3±1.06
No. of pregnant females	9	9	10	9	9
fertility index (%)	90	90	100	90	90

No. of corpora lutea	21.8±2.0	21.7±2.1 20.9±2.2 20.7±1.2 21.2±2.5
No. of implanation sites	17.2±1.9	15.9±2.1 16.0±3.2 16.1±0.9 18.0±0.9
implanation index (%)	79.5±9.5	73.7±9.2 77.1±16.2 78.4±8.3 85.7±8.8
No. of pregnant females with parturition	9	9 10 9 9
gestation length	22.7±0.5	23.0±0 22.7±0.5 22.7±0.5 22.4±0.5
No. of pregnant females with live pups	9	9 10 9 9
No. of pregnant females with live pups on day 4	9	9 10 9 9
weight of Testes (g)	3.49±0.23	3.10±0.64 3.55±0.25 3.44±0.27 3.42±0.18
weight of Epididymides (g)	1.53±0.14	1.41±0.21 1.46±0.09 1.49±0.18 1.43±0.14

All couplation index (= (No. of pairs with successful couplation/No. of pairs mated)x100) were 100%.
fertility index = (No. of pregnant females/No. of pairs with successful couplation) x 100
All gestation index (= (No. of females with live pups/No. of pregnant females)x100) were 100%
Some values are expressed as mean±sd.

Source : MHW Japan

Test condition : TEST ORGANISMS
age: 9 weeks old for male, 8 weeks old for female
weight at initiation: 343-391 g for male, 211-241 g for female
number of animals: 10 per sex per dose
pellet food and water: free take
ADMINISTRATION
vehicle: 1% methylcellulose water solution, 0.5mL/100g body weight
type of administration: oral feed by tube to stomach, once a day
duration of administration:
male; 44 days (including 14 days before mating)
female: before mating 14 days, during mating and gestation, after pregnant 3 days; total 41-45 days
MATING PROCEDURE
one by one in each cage (All of those 10 pairs had finished mating by Day 4.)
CLINICAL OBSERVATIONS AND FREQUENCY FOR PARENTAL ANIMALS
clinical signs and mortality: every day
body weight: once a week, and the time of termination
food consumption: at every body weight check (24hr consumption)
water consumption: not checked
mating, parturition and the related count: everyday
HISTOPATHOLOGICAL OBSERVATIONS FOR PARENTAL ANIMALS
necropsy: to all animals of 0mg/kg and 250mg/kg doses, and to the couples failed pregnant; general organs plus prostate gland, testis, epididyms for males, and ovary, uterus, number of corpora lutea, number of implants for females
While, regarding to those of urinalysis, hematology, blood biochemical and organs, please refer to section 5.4.

Attached document : Finding of rats and the organ weight treated orally with AAOT in the combined repeat dose and reprocuct/developmental toxicity screening test (Table 6, 7, 8)

Conclusion : The NOAEL for reproductive/developmental toxicity are considered to be 250mg/kg/day.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (3)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	male: 44 days, female: from 14 days before mating to 3 days after parturition
Frequency of treatm.	:	once a day, every day
Duration of test	:	male: 44 days, female: 41-45 days
Doses	:	0, 8, 25, 80, 250 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 250 mg/kg bw
NOAEL teratogen.	:	= 250 mg/kg bw
Result	:	of low toxicity to offspring
Method	:	other: OECD TG421
Year	:	1999
GLP	:	yes
Test substance	:	other TS: Mitsuboshi Chemical Co., Ltd.: purity 99.9%
Remark	:	This data is a part of OECD TG422 (combined study). Please refer to section 5.4 and 5.8.1.
Result	:	STATISTICAL RESULTS No compound-related effects on the number of pups, delivery index, sex ratio, body weight and viability index were observed in any dose groups. No pups with malformation were found in any groups. No changes in histopathological findings were observed in offspring.

OBSERVATIONS ON PUPS (F1)

dose (mg/kg)	0	8	25	80	250
No. of pups born	21.8±2.0	21.7±2.1	20.9±2.2	20.7±1.2	21.2±2.5
delivery index (%)	94.5±7.4	96.2±5.0	95.6±6.6	94.2±8.5	92.5±7.2
No. of pups alive on day 0 of lactation					
male	7.7±2.1	8.6±1.7	7.6±3.3	6.8±1.6	9.0±1.7
female	8.1±2.1	6.3±1.5	7.4±2.2	7.9±2.7	9.0±1.4
live birth index (%)	97.5±5.5	97.6±3.2	98.2±4.2	96.2±4.8	97.4±4.4
sex ratio (male/female)	0.92	1.36	0.99	0.88	1.24
No. of pups alive on Day 4 of lactation					
male	7.4±1.8	8.6±1.7	7.6±3.3	6.8±1.6	8.9±1.5
female	8.1±2.1	6.3±1.5	7.3±2.3	7.9±2.7	6.9±1.9
viability index (%)	98.6±2.8	100±0.0	99.5±1.6	99.3±2.1	97.4±3.1
body weight of live pups on day 0 (g)					
male	7.4±0.7	7.8±0.4	7.7±0.9	7.5±0.5	7.0±0.4
female	6.9±0.7	7.4±0.3	7.1±0.9	7.2±0.6	6.6±0.4
body weight of live pups on Day 4 (g)					
male	12.0±1.1	13.0±0.9	12.6±2.3	12.4±1.3	11.1±0.5
female	11.3±1.2	12.5±0.7	11.7±2.0	12.1±1.5	10.6±0.5

delivery index = (No. of pups born/No. of implanation sites)x100
live birth index = (No. of live pups on day 0/No. of pups born)x100
viability index = (No. of live pups on day 4/No. of live pups on day 0)x100
Each value is expressed as mean±sd, except sex ratio.

Source	:	MHW Japan
Test condition	:	TEST ORGANISMS age: 9 weeks old for male, 8 weeks old for female weight at initiation: 343-391 g for male, 211-241 g for female number of animals: 10 per sex per dose pellet food and water: free take ADMINISTRATION

vehicle: 1% methylcellulose water solution, 0.5mL/100g body weight
type of administration: oral feed by tube to stomach, once a day
duration of administration:
male; 44 days (including 14 days before mating)
female: before mating 14 days, during mating and gestation, after pregnant 3 days; total 41-45 days
MATING PROCEDURE
one by one in each cage (All of those 10 pairs had finished mating by Day 4.)
CLINICAL OBSERVATIONS AND FREQUENCY FOR PARENTAL ANIMALS
clinical signs and mortality: every day
body weight: once a week, and the time of termination
food consumption: at every body weight check (24hr consumption)
water consumption: not checked
mating, parturition and the related count: everyday
HISTOPATHOLOGICAL OBSERVATIONS FOR PARENTAL ANIMALS
necropsy: to all animals of 0mg/kg and 250mg/kg doses, and to the couples failed pregnant; general organs plus prostate gland, testis, epididyms for males, and ovary, uterus, number of corpora lutea, number of implants for females
While, regarding to those of urinalysis, hematology, blood biochemical and organs, please refer to section 5.4.
CLINICAL AND PATHOLOGICAL OBSERVATIONS FOR PUPS
general: appearance (including oral cavity), mortality and body weight by litter on Day 0 and Day 4
necropsy: on Day 4 or when died; major organs by eye observation

Attached document : Findings of rats and the organ weights treated orally with AAOT in the combined repeat dose and reproduct/developmental toxicity screening test (Table 6, 7, 8)

Conclusion : NOAEL for Developmental Toxicity and Teratogenicity is considered to be 250 mg/kg/day.

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

14.07.2003 (3)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

(1) BIODEGRADATION AND BIOACCUMULATION DATA OF EXISTING CHEMICALS BASED ON THE CSCL JAPAN, (1992). Ministry of International Trade & Industry Japan

(2) Clariant GmbH: MSDS 29.06.2001

(3) Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test of o-Acetoacetotoluidide by Oral Administration in Rats, (1999). Toxicity Testing Reports of Environmental Chemicals, vol.7, p275-287, Ministry of Health & Welfare Japan

(4) Eastman Chemical Company: MSDS 09/06/2001

(5) ECDIN on line data; generated on Mar. 2001

(6) EUROPEAN COMMISSION, IUCLID CD-ROM ver.4.0.1

(7) Hoechst AG (1989). unpublished internal report

(8) In Vitro Chromosomal Aberration Test of o-Acetoacetotoluidie on Cultured Chinese Hamster Cells, (1999). Toxicity Testing Reports of Environmental Chemicals, vol.7, p292-296, Ministry of Health & Welfare Japan

(9) K. Watanabe et al. (1996). Comparisons of chemically-induced mutagenicity ....., Mutation Research 361, p143-155

(10) Lonza Ltd.: MSDS 25.03.99

(11) Mitsuboshi Chemical Co., Ltd.: unpublished report

(12) Report No. 188466L TOX-85-13, (1985). EVALUATION OF ACETOACET-O-TOLUIDIDE BLEND IN THE SALMONELLA/MICROSOME MUTAGENICITY ASSAY, Eastman Kodak Company, unpublished report

(13) Report No. 188468N TOX-85-15, (1985). EVALUATION OF ACETOACET-O-TOLUIDIDE BLEND IN THE UNSCHEDULED DNA SYNTHESIS TEST, Eastman Kodak Company, unpublished report

(14) Report No. 188473L TOX-85-20, (1985). Evaluation of Acetoacet-o-toluidide in the CHO/HGPRT Forward Mutation Assay, Eastman Kodak Company, unpublished report

(15) Report No. 80240K, (1999). Chemical Inspection and Testing Institute, Japan, unpublished report on partition coefficient of 1-Octanol/Water of N-Acetoacetyl-2-methyl aniline

(16) Report No. 80240K, (1999). Chemical Inspection and Testing Institute, Japan, unpublished report on physical properties of N-Acetoacetyl-2-methyl aniline

(17) Report No. 92049, (1999). Environment Agency Japan, unpublished report on toxicity to algae

(18) Report No. 92050, (1999). Environment Agency Japan, unpublished report on acute toxicity to daphnia

(19) Report No. 92051, (1999). Environment Agency Japan, unpublished report on chronic toxicity to daphnia

(20) Report No. 92052, (1999). Environment Agency Japan, unpublished report on acute toxicity to Oryzias latipes

(21) Report No. ES-2000-044, (2000). AN ACUTE AQUATIC EFFECTS TEST WITH THE FATHEAD MINNOW, Eastman Kodak Company, unpublished report

(22) Report No. ES-2000-045, (2000). AN ACUTE AQUATIC EFFECTS TEST WITH THE DAPHNID, Eastman Kodak Company, unpublished report

(23) Report No. TOX-75-16, (1975). Basic Toxicity of Acetoacet-o-toluidide, Eastman Kodak Company, unpublished report

(24) report on Biodegradation of N-Acetoacetyl-2-methyl aniline, (1977). Chemical Inspection and Testing Institute, Japan

(25) Report on generic Fugacity Model (Mackay Level III), (2001). Chemicals Evaluation and Research Institution, Japan

(26) Reverse Mutation Test of o-Acetoacetotoluidide on Bacteria, (1999). Toxicity Testing Reports of Environmental Chemicals, vol.7, p288-291, Ministry of Health & Welfare Japan

(27) RTECS, 2001 version

(28) Sigma Aldrich on line Catalog, accessed Apr. 24, 2002

(29) Single Dose Oral Toxicity Test of o-Acetoacetotoluidie in Rats, (1999). Toxicity Testing Reports of Environmental Chemicals, vol.7, p273-274, Ministry of Health & Welfare Japan

(30) Tokyo Kasei Organic Chemicals, Catalog 35

(31) TOXNET, National Library of Medicine (USA): on line data generated on Jul. 2002

(32) web Acros Organics N.V.: MSDS Rev.#1 8/02/2000

Remark: This substance, o-Acetoacetotoluidide, is referred to AAOT, hereafter.

3.3.2 Distribution

Table 1. The Fugacity Model (Mackay level III) treated with AAOT.

scenario 1

	emission rate [kg/h]	conc. [g/m³]	amount [kg]	percent [%]	transformation rate [kg/h]	
					reaction	advection
air	1,000	2.1.E-08	2.1.E+02	0.0	1.9E+01	2.1.E+00
water	0	4.9.E-02	9.7.E+05	41.4	2.8E+00	9.7.E+02
soil	0	8.6.E-01	1.4.E+06	58.4	4.0E+00	
sediment		4.3.E-02	4.3.E+03	0.2	4.1E-03	8.5.E-02
		total amount	2.3.E+06			

scenario 2

	emission rate [kg/h]	conc. [g/m³]	amount [kg]	percent [%]	transformation rate [kg/h]	
					reaction	advection
air	0	1.8.E-13	1.8.E-03	0.0	1.6.E-04	1.8.E-05
water	1000	5.0.E-02	1.0.E+06	99.6	2.9.E+00	1.0.E+03
soil	0	7.2.E-06	1.2.E+01	0.0	3.3.E-05	
sediment		4.4.E-02	4.4.E+03	0.4	4.2.E-03	8.7.E-02
		total amount	1.0.E+06			

scenario 3

	emission rate [kg/h]	conc. [g/m³]	amount [kg]	percent [%]	transformation rate [kg/h]	
					reaction	advection
air	0	3.6.E-11	3.6.E-01	0.0	3.1.E-02	3.6.E-03
water	0	5.0.E-02	9.9.E+05	36.2	2.9.E+00	9.9.E+02
soil	1000	1.1.E+00	1.7.E+06	63.7	5.0.E+00	
sediment		4.3.E-02	4.3.E+03	0.2	4.2.E-03	8.7.E-02
		total amount	2.7.E+06			

scenario 4

	emission rate [kg/h]	conc. [g/m³]	amount [kg]	percent [%]	transformation rate [kg/h]	
					reaction	advection
air	600	1.3.E-08	1.3.E+02	0.0	1.1.E+01	1.3.E+00
water	300	4.9.E-02	9.8.E+05	49.5	2.8.E+00	9.8.E+02
soil	100	6.2.E-01	1.0.E+06	50.3	2.9.E+00	
sediment		4.3.E-02	4.3.E+03	0.2	4.1.E-03	8.6.E-02
		total amount	2.0.E+06			

3.3.2 Distribution (continued)

Table 2. The Fugacity Model (Mackay level III) treated with AAOT (continued).

molecular weight		191.23	Calculated	Temp. [℃]	20
melting point [℃]		106	Measured		
vapor pressure [Pa]		6.60E-04	Calculated		
water solubility [g/m^3]		3000	Measured		
log Kow		0.85	Measured		
half life [h]	in air	8	Calculated		
	in water	240000	Estimated		
	in soil	240000	Estimated		
	in sediment	720000	Estimated		

Environmental parameter

		volume [m^3]	depth [m]	area [m^2]	organic carbon [-]	lipid content [-]	density [kg/m^3]	residence time [h]
bulk air	air	1.0E+13					1.2	100
	particles	2.0E+03						
	total	1.0E+13	1000	1E+10				
bulk water	water	2.0E+10					1000	1000
	particles	1.0E+06			0.04		1500	
	fish	2.0E+05				0.05	1000	
	total	2.0E+10	10	2E+09				
bulk soil	air	3.2E+08					1.2	
	water	4.8E+08					1000	

	solid	8.0E+08			0.04		2400	
	total	1.6E+09	0.2	8E+09				
bulk sediment	water	8.0E+07					1000	
	solid	2.0E+07			0.06		2400	50000
	total	1.0E+08	0.05	2E+09				

Intermedia Transport Parameters [m/h]

air side air-water MTC	5	soil air boundary layer MTC	5
water side air water MTC	0.05	sediment-water MTC	1E-04
rain rate	1E-04	sediment deposition	5E-07
aerosol deposition	6E-10	sediment resuspension	2E-07
soil air phase diffusion MTC	0.02	soil water runoff	5E-05
soil water phase diffusion MTC	1E-05	soil solid runoff	1E-08

5.5 Genetic Toxicity in 'Vitro'; Chromosomal aberration test

Table 3 Chromosome aberration test on CHL cells treated with AAOT [continuous treatment]

compound	Dose (ug/ml)	Time of exposure (hr)	Number of cells analyzed	Number of cells with structural aberrations gap	ctb	cte	csb	cse	oth	Total (+gap) (%)	Total (-gap) (%)	Polyp. cells (%)	Final judgment SA	Pol
AAOT	0	24	200	0	0	0	0	0	0	0.0	0.0	0.5	-	-
	625	24	200	0	0	0	0	0	0	0.0	0.0	0.0	-	-
	1250	24	200	0	3	1	0	0	0	2.0	2.0	0.0	-	-
	2500	24	200	2	6	13	0	0	0	10.0	9.0	0.0	+	-
	5000#	24	Toxic											
MMC*	0.05	24	200	4	44	81	0	0	0	51.5	51.5	0.5	+	-
AAOT	0	48	200	0	1	0	0	1	0	1.0	1.0	0.5	-	-
	450	48	200	0	3	0	1	0	0	1.5	1.5	0.0	-	-
	900	48	200	0	2	4	1	0	0	3.5	3.5	0.0	-	-
	1800	48	200	0	4	7	0	0	0	5.0	5.0	0.5	+/-	-
	3600#	48	Toxic											
MMC*	0.25	48	200	5	44	78	1	1	0	50.0	50.0	1.0	+	-

*: Positive control (Mitomycin C)

ctb: Chromatid break cte: Chromatid exchange csb: Chromosome exchange oth: others

SA: structural aberration Pol: polyploid cell

#: Visible precipitation was shown at the end of exposure period.

Table 4 Chromosome aberration test on CHL cells treated with AAOT [short-term treatment]

compound	Dose (ug/ml)	S9 mix	Time of exposure (hr) **	Number of cells analyzed	Number of cells with structural aberrations gap	ctb	cte	csb	cse	oth	Total (+gap) (%)	Total (-gap) (%)	Polyp. cells (%)	Final judgment SA	Pol
AAOT	0	-	6-(18)	200	0	0	1	0	0	0	0.5	0.5	0.5	-	-
	1250	-	6-(18)	200	0	2	1	0	0	0	1.5	1.5	0.5	-	-
	2500#	-	6-(18)	200	0	4	4	0	0	0	3.5	3.5	0.5	-	-
	5000#	-	6-(18)	200	1	10	11	0	0	0	9.0	8.5	0.0	+/-	-
CP*	12.5	-	6-(18)	200	0	4	1	0	0	0	2.5	2.5	0.5	-	-

AAOT	0	+	6-(18)	200	0	0	0	0	1	0	0.5	0.5	0.0	-	-
	1250	+	6-(18)	200	0	0	0	0	0	0	0.0	0.0	0.5	-	-
	2500	+	6-(18)	200	0	0	1	0	0	0	0.5	0.5	0.5	-	-
	5000#	+	6-(18)	200	1	6	8	0	0	0	5.0	5.0	0.5	+/-	-
CP*	12.5	+	6-(18)	200	11	58	177	0	1	0	89.5	89.5	0.0	+	-

*: Positive control (Cyclophosphamide)

** 6-(18): means 18hr treatment in fresh control culture after 6hr treatment in each test substance.

ctb: Chromatid break cte: Chromatid exchange csb: Chromosome exchange oth: others

SA: structural aberration Pol: polyploid cell

#: Visible precipitation was shown at the end of exposure period.

5.5 Genetic Toxicity in 'Vitro'; Chromosomal aberration test (continued)

Table 5 Results of the confirmative examination of AAOT [continuous treatment]

compound	Dose	Time of exposure	Number of cells	Number of cells with structural aberrations						Total (+gap)	Total (-gap)	Polyp. cells	Final judgment	
	(ug/ml)	(hr)	analyzed	gap	ctb	cte	csb	cse	oth	(%)	(%)	(%)	SA	Pol
AAOT	0	24	200	1	0	0	0	0	0	0.5	0.0	0.5	-	-
	1500	24	200	1	5	3	0	0	0	4.0	3.5	0.0	-	-
	2000	24	200	1	11	6	0	0	0	8.5	8.0	0.0	+/-	-
	2500	24	200	0	2	3	0	0	0	2.5	2.5	0.0	-	-
	3000	24	180	0	3	4	0	0	0	3.9	3.9	0.0	-	-
	3500#	24	Toxic											
MMC*	0.05	24	200	12	56	85	0	0	0	57.0	56.0	0.5	+	-

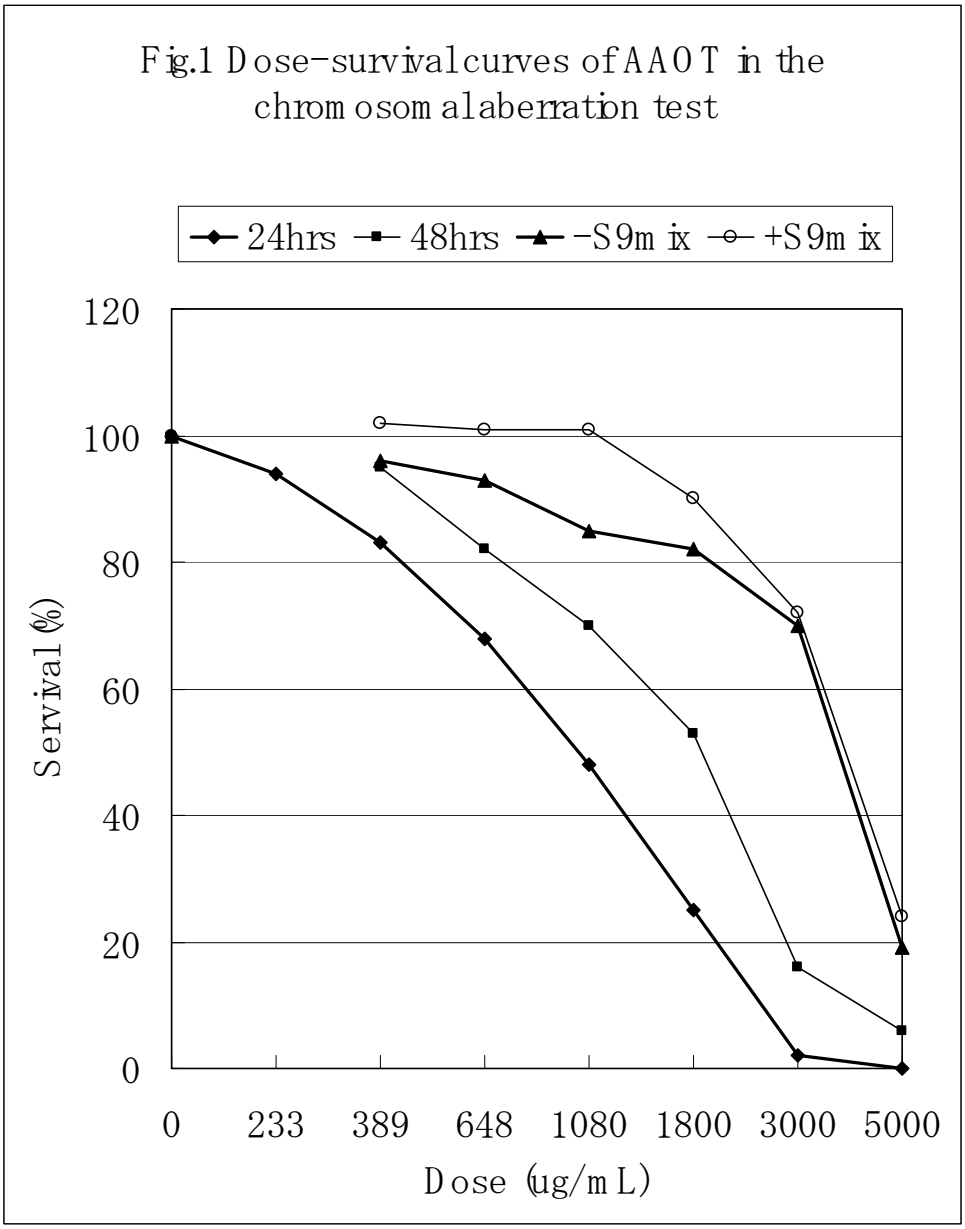
*: Positive control (Mitomycin C)

ctb: Chromatid break cte: Chromatid exchange csb: Chromosome exchange oth: others

SA: structural aberration Pol: polyploid cell

#: Visible precipitation was shown at the end of exposure period.

5.5 Genetic Toxicity in 'Vitro'; Chromosomal aberration test (continued)



5.4, 5.8.1, 5.8.2 Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test

Table 6 Hematological findings of male rats treated orally with AAOT in the combined repeat dose and reproductive/developmental toxicity test

Item Dose level (mg/kg/day)	0	8	25	80	250
No. of animals	10	10	10	10	10
RBC (10^4/uL)	810 ± 36	804 ± 32	779 ± 22	756 ± 42**	681 ± 28**
Hb (g/dL)	14.8 ± 0.5	14.8 ± 0.6	14.5 ± 0.5	14.3 ± 0.7	13.3 ± 0.4**
Ht (%)	44.5 ±1 .2	44.3 ± 1.8	43.3 ± 1.3	43.0 ± 2.0	40.1 ± 1.0**
MCV (fL)	55 ± 2	55 ± 1	56 ± 1	57 ± 1*	59 ± 2**
MCH (pg)	18.3 ± 0.7	18.4 ± 0.4	18.6 ± 0.5	18.9 ± 0.3	19.5 ± 0.7**
MCHC (%)	33.3 ± 0.5	33.4 ± 0.5	33.5 ± 0.5	33.2 ± 0.3	33.1 ± 0.5
Ret. ($^0/_{00}$)	35 ± 8	41 ± 7	46 ± 12	50 ± 13	94 ± 20**
Met-Hb (%)	0.8 ± 0.5	0.6 ± 0.5	0.5 ± 0.7	0.8 ± 0.6	1.3 ± 0.7
Hein-B ($^0/_{00}$)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	27 ± 25**
Plat. (10^4/uL)	136 ± 16	140 ± 15	137 ± 14	132 ± 15	148 ± 13
PT (sec)	12.9 ± 0.5	13.5 ± 1.1	13.0 ± 0.4	13.2 ± 0.3	13.2 ± 0.7
APTT (sec)	17.6 ± 1.1	18.4 ± 1.6	18.1 ± 1.3	17.7 ± 1.3	18.5 ± 1.5
WBC (10^2/uL)	79 ± 14	72 ± 14	81 ± 26	74 ± 21	77 ± 20

Each value is expressed as Mean ± S.D.
Significantly different from control (*: $p<0.05$, **: $p<0.01$)

Table 7 Blood biochemical findings of male rats treated orally with AAOT in the combined repeat dose and reproductive/developmental toxicity screening test

Item Dose level (mg/kg/day)	0	8	25	80	250
No. of animals	10	10	10	10	10
LDH (IU/L)	280 ± 114	262 ± 85	304 ± 97	302 ± 141	302 ± 78
GOT (IU/L)	61 ± 6	66 ± 7	66 ± 9	65 ± 7	62 ± 5
GPT (IU/L)	34 ± 6	36 ± 6	38 ± 6	37 ± 8	38 ± 7
ALP (IU/L)	245 ± 61	226 ± 42	228 ± 44	243 ± 68	225 ± 55
gamma-GTP	0.71 ± 0.54	1.14 ± 0.50	0.67 ± 0.56	0.48 ± 0.33	0.91 ± 0.65
(IU/L)	53 ± 15	41 ± 12	49 ± 12	44 ± 19	54 ± 27
ChE (IU/L)	6.21 ± 0.25	6.26 ± 0.18	6.42 ± 0.12	6.26 ± 0.15	6.12 ± 0.27
T.protein (g/dL)	2.81 ± 0.21	2.88 ± 0.31	2.98 ± 0.18	2.74 ± 0.28	2.60 ± 0.37
Albumin (g/dL)	0.83 ± 0.11	0.87 ± 0.14	0.87 ± 0.09	0.78 ± 0.13	0.74 ± 0.15
A/G ratio	68 ± 14	67 ± 15	84 ± 13	76 ± 17	74 ± 17
T.cholesterol	83 ± 27	81 ± 15	83 ± 30	91 ± 34	94 ± 36
(mg/dL)	137 ± 19	136 ± 27	135 ± 23	133 ± 27	124 ± 17
Triglyceride	0.27 ± 0.03	0.28 ± 0.02	0.29 ± 0.02	0.31 ± 0.02*	0.33 ± 0.03**
(mg/dL)	15.4 ± 1.4	15.7 ± 1.6	15.6 ± 1.9	16.2 ± 2.2	17.3 ± 1.1
Glucose (mg/dL)	0.51 ± 0.05	0.54 ± 0.07	0.51 ± 0.03	0.51 ± 0.07	0.54 ± 0.04
T.bilirubin	10.3 ± 0.3	10.5 ± 0.3	10.5 ± 0.3	10.5 ± 0.2	10.5 ± 0.3
(mg/dL)	7.1 ± 0.8	7.2 ± 0.4	7.0 ± 0.6	7.2 ± 0.5	7.1 ± 0.6
BUN (mg/dL)	142 ± 1	142 ± 1	143 ± 1	143 ± 1	142 ± 1
Creatinie (mg/dL)	4.69 ± 0.28	4.76 ± 0.18	4.92 ± 0.31	4.97 ± 0.29	5.47 ± 0.40**
Ca (mg/dL)	103 ± 1	103 ± 1	104 ± 1	103 ± 2	104 ± 1
P (mg/dL)					
Na (mEq/L)					
K (mEq/L)					
Cl (Eq/L)					

Each value is expressed as Mean ± S.D.
Significantly different from control (*: $p<0.05$, **: $p<0.01$)

5.4, 5.8.1, 5.8.2 Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test (continued)

Table 8 Absolute and relative organ weights of rats treated orally with AAOT in the combined repeat dose and reproductive/developmental toxicity screening test

Sex	Item	Dose level (mg/kg/day) 0	8	25	80	250
Male	No. of animals	10	10	10	10	10
	Body weight	486 ± 30	472 ± 29	475 ± 32	482 ± 30	475 ± 37
	Absolute weight					
	Brain (g)	2.07 ± 0.10	2.10 ± 0.05	2.10 ± 0.09	2.13 ± 0.11	2.11 ± 0.05
	Liver (g)	13.29 ± 1.58	12.63 ± 1.46	13.28 ± 1.14	13.93 ± 1.59	13.8 ± 1.58
	Kidneys (g)	3.14 ± 0.21	3.03 ± 0.11	3.11 ± 0.22	3.21 ± 0.35	2.99 ± 0.17
	Spleen (g)	0.83 ± 0.11	0.84 ± 0.09	0.87 ± 0.09	0.94 ± 0.18	1.11 ± 0.14**
	Heart (g)	1.38 ± 0.13	1.34 ± 0.07	1.37 ± 0.07	1.42 ± 0.11	1.33 ± 0.10
	Thymus (g)	0.33 ± 0.06	0.32 ± 0.08	0.32 ± 0.08	0.36 ± 0.07	0.29 ± 0.05
	Thyroid (mg)	33.7 ± 2.9	32.7 ± 3.8	34.9 ± 2.8	36.0 ± 5.2	35.2 ± 3.9
	Pituitary (mg)	13.6 ± 1.4	13.4 ± 0.8	14.6 ± 1.6	14.2 ± 1.0	15.4 ± 1.6*
	Adrenals (mg)	66.8 ± 12.2	63.5 ± 10.2	61.6 ± 4.0	61.8 ± 16.1	55.7 ± 7.9
	Testes (g)	3.49 ± 0.23	3.10 ± 0.64	3.55 ± 0.25	3.44 ± 0.27	3.42 ± 0.18
	Epididymides (g)	1.53 ± 0.14	1.41 ± 0.21	1.46 ± 0.09	1.49 ± 0.18	1.43 ± 0.14
	Relative weight	0.43 ± 0.03	0.45 ± 0.02	0.44 ± 0.04	0.44 ± 0.03	0.45 ± 0.03
	Brain (g%)	2.73 ± 0.22	2.67 ± 0.17	2.79 ± 0.13	2.89 ± 0.23	2.90 ± 0.18
	Liver (g%)	0.65 ± 0.03	0.64 ± 0.04	0.66 ± 0.03	0.67 ± 0.05	0.63 ± 0.04
	Kidneys (g%)	0.17 ± 0.02	0.18 ± 0.02	0.18 ± 0.02	0.19 ± 0.03	0.23 ± 0.02**
	Spleen (g%)	0.28 ± 0.02	0.29 ± 0.02	0.29 ± 0.02	0.29 ± 0.01	0.28 ± 0.02
	Heart (g%)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
	Thymus (g%)	7.0 ± 0.6	6.9 ± 0.6	7.4 ± 0.9	7.5 ± 1.1	7.5 ± 1.1
	Thyroid (mg%)	2.8 ± 0.3	2.8 ± 0.3	3.1 ± 0.4	3.0 ± 0.3	3.3 ± 0.4*
	Pituitary (mg%)	13.7 ± 2.1	13.5 ± 2.6	13.0 ± 0.8	12.8 ± 3.2	11.8 ± 2.0
	Adrenals (mg%)	0.72 ± 0.05	0.66 ± 0.14	0.75 ± 0.07	0.71 ± 0.04	0.72 ± 0.06
	Testes (g%)	0.32 ± 0.03	0.30 ± 0.04	0.31 ± 0.02	0.31 ± 0.04	0.30 ± 0.03
	Epididymides (g%)					
Female	No. of animals	9	9	10	9	9
	Body weight	359 ± 17	359 ± 13	347 ± 20	353 ± 14	358 ± 13
	Absolute weight					
	Brain (g)	1.98 ± 0.06	1.99 ± 0.11	1.95 ± 0.09	1.99 ± 0.07	1.98 ± 0.08
	Liver (g)	14.8 ± 0.74	14.35 ± 0.89	14.61 ± 0.91	14.81 ± 1.14	16.10 ± 1.16
	Kidneys (g)	2.10 ± 0.15	2.18 ± 0.19	2.04 ± 0.14	2.11 ± 0.17	2.16 ± 0.14
	Spleen (g)	0.71 ± 0.16	0.67 ± 0.10	0.74 ± 0.11	0.77 ± 0.10	1.12 ± 0.19**
	Heart (g)	1.07 ± 0.09	1.02 ± 0.07	1.04 ± 0.08	1.05 ± 0.04	1.10 ± 0.08
	Thymus (g)	0.27 ± 0.03	0.27 ± 0.10	0.25 ± 0.09	0.23 ± 0.03	0.25 ± 0.05
	Thyroid (mg)	23.4 ± 3.9	22.0 ± 3.2	22.4 ± 3.1	24.2 ± 3.0	26.8 ± 2.8
	Pituitary (mg)	18.1 ± 2.5	16.0 ± 1.6	14.4 ± 1.1**	17.1 ± 1.5	17.5 ± 2.1
	Adrenals (mg)	81.7 ± 14.0	72.2 ± 8.5	75.0 ± 12.0	76.1 ± 8.9	72.6 ± 4.1
	Relative weight					
	Brain (g%)	0.55 ± 0.03	0.55 ± 0.02	0.56 ± 0.04	0.56 ± 0.02	0.55 ± 0.03
	Liver (g%)	4.12 ± 0.26	4.00 ± 0.18	4.21 ± 0.21	4.19 ± 0.31	4.50 ± 0.23**
	Kidneys (g%)	0.58 ± 0.03	0.61 ± 0.06	0.59 ± 0.04	0.60 ± 0.05	0.61 ± 0.04
	Spleen (g%)	0.20 ± 0.05	0.18 ± 0.03	0.21 ± 0.03	0.22 ± 0.03	0.31 ± 0.05**
	Heart (g%)	0.30 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.30 ± 0.01	0.31 ± 0.02
	Thymus (g%)	0.07 ± 0.01	0.07 ± 0.03	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
	Thyroid (mg%)	6.5 ± 1.1	6.1 ± 0.9	6.5 ± 0.8	6.9 ± 0.9	7.5 ± 0.6
	Pituitary (mg%)	5.0 ± 0.7	4.5 ± 0.4	4.1 ± 0.3*	4.8 ± 0.4	4.9 ± 0.6
	Adrenals (mg%)	22.7 ± 3.9	20.1 ± 2.4	21.7 ± 3.8	21.5 ± 2.7	20.3 ± 1.3

Each value is expressed as Mean ± S.D. Significantly different from control (*: $p<0.05$, **: $p<0.01$)