FOREWORD INTRODUCTION

1,1',2-TRICHLOROETHANE

CAS N°: 79-00-5

Assessment Report for Post-SIDS Testing Results

SIAM 16
(Paris, 27-30 May 2003)

Chemical Name: **1,1,2-Trichloroethane,**

CAS No: **79-00-5**

Sponsor Country: **Japan**

National SIDS Contact Point in Sponsor Country: Mr. Yasuhisa Kawamura
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HISTORY:

The SIDS Initial Assessment Report of this chemical had been discussed at SIAM 8 and 9, and the conclusion and recommendation were agreed at SIAM 10 as a candidate for further work. The recommendation was "An *in vivo* genotoxicity study such as an *in vivo* micronucleus test is recommended because some non-core genotoxicity studies indicate positive results".

The original assessment documents are published by UNEP Chemicals (2002, Volume 8, part 2). The current documents include an update of the SIDS Profile as well as addenda to the SIDS Initial Assessment Report and the SIDS Dossier, based on the results of post-SIDS testing.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	79-00-5
Chemical Name	1,1,2-Trichloroethane
Structural Formula	$Cl_2CH\text{-}CH_2Cl$

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

The acute toxicity (LD50) of 1,1,2-trichloroethane is 837 mg/kg by oral administration in rats, 9 g/m^3 /6 hr by inhalation in rats and 5.38 g/kg by dermal administration in rabbits. This chemical is considered to be irritating to skin, eyes, the upper respiratory tract and the stomach. There is no available information on skin sensitisation.

In a 90 days drinking water study of mice at the concentration of 0, 20, 200, or 2,000 mg/l, a reduction of P-450 contents in liver were observed and the NOEL was considered to be 3.9 mg/kg/day. Repeated inhalation exposure (7 hours/day, 5 days/week) to 83 mg/m^3 air for 6 months did not lead to any chemical-related changes in the rat, guinea pig and rabbit. The daily intake is equivalent to roughly 11 mg/kg/day in rats, 7.4 mg/kg/day in guinea pigs, and 25 mg/kg/day in rabbits. In humans, this chemical was reported to act as a narcotic at low concentrations, and to irritate the conjunctiva, the mucosa of the respiratory tract and the external skin. Moreover, gastrointestinal tract complaints, fatty degeneration of the kidneys and lung damage by prolonged exposure were reported.

A carcinogenicity study with this chemical by gavage showed hepatocellular carcinomas and pheochromocytomas in mice but the chemical was not carcinogenic in rats. Initiation/promotion screening studies on male rat liver demonstrated that this chemical has neither initiation nor promotion activity. A carcinogenicity study in skin of rats given 0, 2.05 or 6.24 mg by subcutaneous injection once a week for two years indicated no chemical related changes.

A bacterial mutagenicity study showed negative results in all strains of *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 with and without metabolic activation. Unscheduled DNA synthesis was not observed in livers of treated mice. On the other hand, a mutation study in *Saccharomyces cerevisiae* and an *in vitro* micronucleus test with human lymphocytes was positive. However, the latest micronucleus test in mice *in vivo* showed negative results. Therefore the weight of evidence suggests that this chemical is not genotoxic *in vivo*.

In a developmental toxicity study, the chemical was administered by gavage to mice on days 8 through 12 of gestation at dose of only 350 mg/kg/day. Any changes including teratogenicity and embryo/fetal viability, and/or postnatal growth and viability were not observed. Therefore, the NOEL for developmental toxicity was considered to be 350 mg/kg/day.

Environment

1,1,2-Trichloroethane is a stable liquid and is not readily biodegradable (OECD TG 301C). Its measured bioconcentration factor is 0.7 – 4.0 (OECD TG 305C).

As the lowest acute toxicity test results for algae, zooplankton and fish, a 96 h-EC50 for *Phaeodactylum tricornutum* (60 mg/l), a 48 h EC50 for *Daphnia magna* (18 mg/l) and a 7 d LC50 for *Poecilia reticulata* (40 mg/l) were selected. As the lowest chronic toxicity test results for algae, zooplankton and fish, a 72 h NOEC (growth) for *Selenastrum capricornutum* (51.4 mg/l), a 21d NOEC (reproduction) for *Daphnia magna* (32 mg/l) and a 56d NOEC (mortality during early life stage) for *Pleuronectes platessa* (3.0 mg/l) were selected. An assessment factor of 10 was used on the chronic toxicity data to determine a PNEC, which is 0.3 mg/l in the present report.

Exposure

The production volume of this chemical was ca. 153,000 tonnes/year in 1996 in Japan. This chemical is used as an

intermediate for the production of vinylidene chloride and is not included in consumer products in the Sponsor country. The potential environmental distribution of 1,1,2-trichloroethane obtained from a generic fugacity model (Mackey level III) showed that this chemical would be distributed mainly to air and water. The main route of human exposure is inhalation with a limited number of workers potentially exposed during sampling, subsequent analysis, tank filling and maintenance operations.

RECOMMENDATION

The chemical is currently of low priority for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

As the original recommendation was "An *in vivo* genotoxicity study such as an *in vivo* micronucleus test is recommended because an *in vitro* chromosomal aberration test indicates clear positive results", an *in vivo* micronucleus test was conducted as post-SIDS work. Based on the negative result, this chemical is currently of low priority for further work.

Addendum
to
SIDS Initial Assessment Report

Note: Only the sections of the SIDS Initial Assessment Report which are affected by the results of the post-SIDS testing are presented below. For the original SIDS initial assessment documents, see Volume 8, part 2.

Genetic toxicity

Previous Information and Evaluation

An *in vitro* reverse mutation study in *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 demonstrated negative results with or without metabolic activation (Barber *et al.*: 1981). Unscheduled DNA synthesis was not observed in livers of treated mice (Mirsalis *et al.*: 1989).

On the other hand, positive results were reported in *Saccharomyces cerevisiae* (Bronzetti *et al.*: 1987). There is an indication of an aneuploidy-inducing effect from studies with *Aspergillus nidulans* (Crebelli *et al.*: 1988). This chemical showed weakly positive results in a micronucleus test *in vitro* and clear positive results in an alkaline single cell gel electrophoresis test (comet assay) of human lymphocytes both with and without metabolic activation (Tafazoli and Krisch-Volders: 1996).

In summary, core genotoxicity studies such as reverse mutation in *Salmonella typhimurium* and unscheduled DNA synthesis *in vivo* demonstrate negative results. However, some uncertainties of genotoxic potential remain because non-core *in vitro* studies indicate positive results.

New Testing Results

Genetic toxicity *in vivo*

To elucidate the genotoxic potential of 1,1,2-trichloroethane *in vivo*, a mammalian erythrocyte micronucleus test according to OECD Test Guideline 474 was conducted (MHLW, Japan: 2003). 1,1,2-Trichloroethane was administered by gavage in olive oil to male CD-1 mice (5 animals in the control group, 5 treated animals per dose) at 100, 200 and 400 mg/kg bw, based on severe toxicity at 800 mg/kg and no sex differences on the toxicity in a previous range-finding study. The number of micronucleated PCEs in 2000 PCEs of bone marrow cells was counted at 24 and 48 h after the administration. Cyclophosphamide, a positive control, induced significant increase of micronucleated PCEs. No increases in the number of micronucleated PCEs in bone marrow cells were observed both 24 and 48 h after administration in any treated groups.
Based on this result, it was considered that 1,1,2-trichloroethane is not genotoxic *in vivo*.

Carcinogenicity

In a bioassay conducted by NCI (1978), 1,1,2-trichloroethane was administered by gavage in corn oil to Osborne-Mendel rats and $B6C3F_1$ mice.
Low-dose and high-dose rats received respectively, 35 and 70 mg/kg b.w./day for 20 weeks, then 50 and 100 mg/kg b.w./day for 58 weeks (Time-weighted average doses: 33 and 66 mg/kg b.w./day). No statistically significant increase in tumour incidence was found, either in males or in female rats.

Low-dose and high-dose mice received 150 and 300 mg/kg b.w./day, respectively for eight weeks and then 200 and 400mg/kg b.w./day for 70 weeks (Time-weighted average doses: 139 and 278 mg/kg b.w./day). The incidence of hepatocellular neoplasms [reported as carcinomas] was increased significantly ($p < 0.01$) in all treated groups: males - 2/17 (untreated controls), 2/20 (vehicle controls), 18/49 (low-dose animals) and 37/49 (high-dose animals); in females- 2/20 (untreated controls), 0/21 (vehicle controls), 16/48 (low -dose animals) and 40/45 (high-dose animals). Adrenal pheochromocytomas were present in 8/48 high-dose males and in 12/43 high-dose females, but not in the other groups.

Additionally, several mechanistic studies of carcinogenesis were conducted. 1,1,2-Trichloroethane bound to calf thymus DNA *in vitro* (DiRenzo *et al*.: 1982) and to DNA, RNA and proteins of the liver, kidney, lung and stomach after intraperitoneal injection into rats and mice (Mazzullo *et al*., 1986). The extent of interaction of 1,1,2-trichloroethane with mouse liver DNA was about 2.5 times higher than that with rat liver DNA. A cell transformation assay performed without metabolic activation on mouse BALB/c-3T3 cells resulted in weakly positive (Tu *et al*: 1985). Strong S-phase induction was observed in livers of treated mice (Mirsalis *et al*.: 1989).

Initial Assessment of Genotoxicity and Carcinogenicity

In oral carcinogenicity studies, hepatocellular carcinomas and pheochromocytomas in one strain of mice were observed but carcinogenicity was not shown in rats. The initiation/promotion screening studies on male rat liver demonstrated that 1,1,2-trichloroethane has neither initiation nor promotion activity. In an assessment by USEPA, this chemical is classified to group C, a possible human carcinogen and is indicated to structurally related to 1,2-dichloroethane, a probable human carcinogen. On the other hand, International Agency for Research on Cancer (IARC) had evaluated in 1991 that 1,1,2-trichloroethane was not classifiable as its carcinogenicity to humans (Group 3) because of limited evidence for the carcinogenicity in experimental animals and no available data in humans.

Core genotoxicity studies such as bacterial mutagenicity in *Salmonella typhimurium* and unscheduled DNA synthesis *in vivo* demonstrate negative results, although some positive results are given in non-core *in vitro* genotoxicity study. However, a micronucleus test in mice *in vivo* showed clearly negative results. The weight of evidence suggests that this chemical is not genotoxic *in vivo*. Furthermore, the results in a cell transformation assay (Tu *et al*.: 1985) and S-phase synthesis assay (Mirsalis *et al*: 1989) might support that this chemical has promotion activity in mice rather than initiation activity. Therefore the formation of tumors in mice may take place exclusively due to non-genotoxic mechanism rather than genotoxic mechanism.

Conclusions and Recommendations

Conclusions

Bacterial mutagenicity study showed negative results in all strains of *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, TA100 with and without metabolic activation. Unscheduled DNA synthesis was not observed in livers of treated mice. On the other hand, a mutation study in *Saccharomyces cerevisiae* and an *in vitro* micronucleus test of human lymphocytes showed positive. However, the latest micronucleus test in mice *in vivo* showed negative results. Therefore the weight of evidence suggests that this chemical is not genotoxic *in vivo*. Carcinogenicity studies of this chemical by gavage showed hepatocellular carcinomas and pheochromocytomas in mice but no carcinogenicity in rats. Base on these results, the formation of tumors in mice may take place

exclusively due to non-genotoxic mechanism rather than genotoxic mechanism.

Recommendations

Human Health

As negative results were found in an *in vivo* micronucleus test, this chemical is currently of low priority for further work.

References

Barber,E.D. *et al., Mutat.Res.,* 90, 31-48 (1981)

Bronzetti,G. *et al., Eur.J.Cancer Clin.Oncol*., 23, 1737-1738 (1987)

Crebelli,R. *et al., Mutat.Res.,* 201, 401-411 (1988)

DiRenzo,A.B. *et al., Toxicol.Lett*., 11, 243-253 (1982)

Ministry of Health, Labour and Welfare: Japan, *Toxicity Testing Reports of Environmental Chemicals* 10 (2003) Publication in progress.

Mazzullo,M. *et al., Jpn.J.Cancer.Res*., 77, 532-539 (1986)

Mirsalis,J.C. *et al., Environ.mol.Mutagenesis*, 14, 155-164 (1989)

National Cancer Institute: Technical Report No. 74 (1978)

Tafazoli,M. and Krisch-Volders,M., *Mutat.Res.*, 371, 185-202 (1996)

Tu,A.S. *et al.,Cancer Lett*., 28, 85-92 (1985)

Addendum to SIDS Dossier

ROBUST STUDY SUMMARY for Post SIDS Testing of 1,1,2 Trichloroethane CAS No. 79-00-5

Sponsor country: Japan
DATE: August 8, 2002

GENETIC TOXICITY *IN VIVO* (Micronucleus Test)

TEST SUBSTANCE

- **Identity** 1,1,2-Trichloroethane (CAS No 79-00-5)
  Source: Sigma-Aldrich Japan Co., Lot No. 01404MQ, Purity: 97.5 % (impurity: unknown, stabilizer: 2-propanol (0.5 %)), Kept at 4C until use

METHOD

- **Method/guideline** OECD Test Guideline 474
- **Test type** Mammalian Erythrocyte Micronucleus Test/ Bone Marrow Cells
- **GLP** Yes
- **Year** 2001
- **Species** Mouse
- **Strain** Crj: CD-1(ICR)
- **Sex** Male
- **Route of administration** By gavage
- **Doses levels** 0, 100, 200 and 400 mg/kg
- **Exposure period** 24 and 48 hours
- **Statistical methods** The incidences of micronucleated polychromatic erythrocytes in negative control group and positive control group were analyzed whether they are within the range of the background variance. The significances between the treatment groups and negative control were analyzed by Fisher's exact test, following to Bonderroni's correction. Dose dependency was analyzed by Cochran-Armitage trend test.

Test conditions

- Age of study initiation: 9 weeks old
- Number of animals: 5 males per dose
- Vehicle: Olive oil
- April 25, 2001 to June 12, 2001 (48 days) including preliminary study
- Frequency of treatment: Single administration
- Sampling times and number of samples: 24 and 48 hours after administration and 5 samples
- Negative control: Methyl cellulose, Positive control: Cyclophosphamide
- Clinical observation: Every 1 hour at the treatment day and every 6 hours after that
- Organ examination: None
- Staining of bone marrow cells: Acridine Orange fluorescence dye on slide glass
- Evaluation: Number of micronucleated polychromatic erythrocytes in 2000 polychromatic erythrocytes in bone marrow cells
- At preliminary experiment (5 males and 5 females per dose: 100, 200, 400 and 600 mg/kg), 3 males died and all males and females showed abdominal position at 600 mg/kg. As 600 mg/kg was considered to be M.T.D. and there were no clear sex differences, it was concluded that only males were used and the highest dose was 400 mg/kg.

RESULTS

- **MNPCE/PCE (No. of animals)**

24 hours		
	Negative Control	2, 2, 5, 1, 3 / 2000
	100 mg/kg	3, 4, 5, 5, 2 / 2000
	200 mg/kg	5, 0, 2, 1, 0 / 2000
	400 mg/kg	0, 1, 10, 3, 4 / 2000
	Positive Control	30, 49, 42, 13, 23 / 2000*
48 hours		
	Negative Control	2, 0, 2, 2, 5 / 2000

	100 mg/kg	0, 2, 4, 3, 5 / 2000
	200 mg/kg	4, 2, 3, 3, 3 / 2000
	400 mg/kg	5, 2, 3, 2, 0 / 2000
		*: Significant against negative control ($p < 0.1$ %)

- **Genotoxic effects** Negative
- **Statistical results** No significant changes between negative control and any chemical treatment

CONCLUSIONS

(Study author) The 1,1,2-trichloroethane-exposed male mice showed low locomotor activity at 400 mg/kg. No increase of micronucleated polychromatic erythrocyte counts in 2000 polychromatic erythrocytes in bone marrow cells were observed in any dose groups at 24 and 48 hours after administration. Based on these results, 1,1,2-trichloroethane was considered not genotoxic *in vivo*.

DATA QUALITY

- **Reliabilities** 1. Reliable without restrictions because this study was conducted under OECD test guideline and GLP.

REFERENCES

MHLW, Japan (2003) Ministry of Health, Labour and Welfare, Toxicity Testing Reports of Environmental Chemicals, 10 (Publication is expected early 2003).

OTHER Prepared by Japanese Government

- **Last changed** 8 August 2002