Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.11, No.6, 267-278, 2024

The Japanese Society of Toxicology

Fundamental Toxicological Sciences
URL : http://www.fundtoxicolsci.org/index_e.html

Original Article

Derivation of human health hazard assessment values for tetramethylammonium hydroxide (TMAH) under the Japan Chemical Substances Control Law

Akira Kawashima, Kaoru Inoue, Kazuo Ushida, Kaoru Kai, Lucia Satiko Yoshida-Yamashita and Kenichi Masumura

Division of Risk Assessment, Center for Biological Safety and Research, National Institute of Health Sciences, 3-25-26 Tonomachi, Kawasaki-ku, Kawasaki, Kanagawa, 210-9501, Japan

(Received October 15, 2024; Accepted October 22, 2024)

ABSTRACT — Tetramethylammonium hydroxide (TMAH) and substances that release tetramethylammonium (TMA) are classified as Priority Assessment Chemical Substances (PACSs) under registration number 17 of the Japan Chemical Substances Control Law (CSCL, 1973). This classification requires a thorough human health hazard assessment and derivation of Hazard Assessment Value (HAVs) for the oral and inhalation exposure at the Assessment II stage. We analyzed their general, developmental, reproductive toxicity, genotoxicity, and carcinogenicity using hazard data from both domestic and international risk assessment agencies and subsequently proposed an HAV. For oral exposure, a no-observed-adverse-effect-level (NOAEL) of 1 mg/kg/day, based on transient or persistent salivation in parent rats from a TMAH developmental and reproductive toxicology (DART) screening study, was chosen as the point of departure (POD). The POD was then divided by uncertainty factors (UFs) totaling 1,000 (interspecies variation: 10, intraspecies variation: 10, short study duration: 10), resulting in an oral HAV of 0.001 mg/kg/day for TMAH. Due to a lack of hazard data for humans and animals via inhalation, an HAV for the inhalation route was not established.

Key words: Tetramethylammonium hydroxide (TMAH, CAS No. 75-59-2), Chemical Substances Control Law (CSCL), Assessment II for human health effects, Hazard assessment value (HAV)

INTRODUCTION

In Japan, industrial chemicals, including existing substances, are regulated under the Japan Chemical Substances Control Law (CSCL) to prevent environmental pollution. For Priority Assessment Chemical Substances (PACSs), a detailed hazard assessment involves drafting a report on general toxicity, developmental and reproductive toxicity, genotoxicity, and carcinogenicity. This draft relies on credible reports from domestic or international risk assessment organizations, and the Hazard Assessment Value (HAVs) are determined for repeated oral and inhalation exposures. The draft report and HAVs related to human health effects are then reviewed and approved by a joint council comprising the Ministry of Health, Labor and Welfare (MHLW), the Ministry of Economy, Trade and Industry (METI), and the Ministry of the Environment (MOE). A final HAV is established during Risk Assessment II stage. The risk assessment process for PACSs under the CSCL has been detailed in a previous study (Kawashima *et al*., 2022).

This paper presents the toxicological profiles of tetra-

Correspondence: Akira Kawashima (E-mail: akira_kawashima@nihs.go.jp)

methylammonium (TMA, quaternary ammonium cation $(CH_3)_4N^+$) and the draft HAV for oral exposure to tetramethylammonium hydroxide (TMAH), following the guidelines provided in the "Risk Assessment Method for Priority Assessment Chemical Substances" (MHLW, METI, and MOE, 2022a) and the "Technical Guidelines for Risk Assessment of Priority Assessment Chemical Substances" (MHLW, METI, and MOE, 2022b).

MATERIALS AND METHODS

TMAH (CAS No. 75-59-2) is highly soluble in water, forming an alkaline solution. It is commonly used in the production of electrical and electronic components, such as photoresists and liquid crystal developers. TMAH is highly toxic in acute exposures, causing severe symptoms upon skin contact (Wu *et al*., 2008; Lin *et al.,* 2010; Park *et al.,* 2013). Due to its toxicity, it was classified as a poison in 2013 under the Poisonous and Deleterious Substances Control Act (PDSC, 1950).

Under the CSCL, TMAH received a "high" priority level in the 2010 screening assessment. As a result, in 2011, TMAH and TMA-dissociating substances were classified as PACSs and assigned registration number 17 for management, as indicated in Table 1. Toxicological data were found for only four substances: TMAH, tetramethylammonium chloride (TMAC, CAS No. 75-57-0), tetramethylammonium hydrogen phthalate (TMAHP, CAS No. 79723-02-7), and tetramethylammonium bromide (TMAB, CAS No. 64-20-0; only single-dose toxicity data available). No toxicological information was available for other related chemicals, except for kinetic data. Thus, this human health assessment is based on the limited hazard data for TMA, which is released from TMAH and TMA-dissociating substances *in vivo* or in the environment. Toxicity from other constituent molecules was not considered in this evaluation.

RESULTS

Pharmacokinetics (pharmacodynamics)

The absorption, distribution, metabolism, and excretion of TMAH are summarized below. While no human data are available, some findings from animal studies exist.

TMAH, being highly alkaline, dissociates fully in animals to produce TMA. In rats, TMA administered to the intestine is rapidly absorbed, and most of the absorbed

Table 1. Chemical substances contained in the registration number 17.

CAS Number	Chemical Name	Abbreviation
64-20-0	**Tetramethylammonium bromide**	**TMAB**
75-57-0	**Tetramethylammonium chloride**	**TMAC**
75-59-2	**Tetramethylammonium hydroxide**	**TMAH**
75-58-1	*N,N,N*-Trimethylmethanaminium iodide	TMMAI
373-68-2	Tetramethylammonium fluoride	TMAFY
558-32-7	Tetramethylammonium hexafluorophosphate	TMAHFP
661-36-9	Tetramethylammonium tetrafluoroborate	TMATF
811-92-7	Tetramethylammonium methyl sulfate	TMAMS
1941-24-8	*N,N,N*-Trimethylmethanaminium nitrate	TMMAN
2537-36-2	*N,N,N*-Trimethylmethanaminium perchlorate	TMMAP
3983-91-3	Tetramethylammonium 4-methylbenzenesulfonate	TMAMBS
10424-65-4	*N,N,N*-Trimethylmethanaminium hydroxide pentahydrate	TMMAHP
10581-12-1	*N,N,N*-Trimethylmethanaminium acetate	TMMAA
14190-16-0	Bis(*N,N,N*-trimethylmethanaminium) sulfate	TMMAS
15525-13-0	*N,N,N*-Trimethylmethanaminium tetraphenylborate(1-)	TMMATPB
40768-19-2	Tetramethylammonium dihydrogen phosphate	TMADP
53803-13-7	Tetramethylammonium 2,2-dimethylpropanoate	TMADMP
58345-96-3	Tetramethylammonium carbonate	TMACB
59138-84-0	*N,N,N*-Trimethylmethanaminium formate	TMMAF
64000-88-0	*N,N,N*-Trimethylmethanaminium decanoate	TMMAD
79723-02-7	**Tetramethylammonium hydrogen phthalate**	**TMAHP**
80526-82-5	Tetramethylammonium hydrogen sulfate	TMAHS
139657-01-5	Tetramethylammonium pyridine-2-carboxylate	TMAPC
1226979-35-6	Tetramethylammonium (R)-5-((S)-1,2-dihydroxyethyl)-4-hydroxy-2-oxo-2,5-dihydrofuran-3-olate	TMADH

Toxicological information existed only for the four substances in bold.

TMA is excreted in the urine. The following pharmacokinetic, metabolic, and ion distribution data for animals are available (OECD SIDS, 2006).

Studies on TMA absorption in the rat jejunum were conducted using both the *in situ* intestinal loop method and the *in vitro* everted sac method. In one study, 0.5 mL of a 0.2 mmoL ^{14}C-labeled TMA solution was introduced into the jejunum, and the absorption was rapid, with over 80% of the solution transferred to the bloodstream within 60 min. Thin-layer chromatography of the lumen and fluid 90 min after administration showed no detectable metabolites (Tsubaki and Komai, 1986).

A kinetic analysis was conducted on 14 quaternary ammonium salts, including tetramethylammonium iodide. Anesthetized rats were intravenously injected with 0.4 μmoL of each salt, and the distribution to excretory organs, excretion rates into bile, urine, and the intestinal lumen, as well as plasma elimination half-lives were measured. All substances exhibited biphasic plasma elimination half-lives, with the first phase ranging from 0.5 to 3 min and the second phase lasting from 30 to 70 min. For TMA iodide, the percentage excreted within 2 hr post-administration was 0.6% in bile, 96.6% in urine, and 2.6% in the intestine. Thin-layer chromatography of bile, urine, and intestinal samples collected within 2 hr detected only TMA, indicating no metabolism of the absorbed ion (Neef *et al*., 1984).

These findings suggest that orally administered TMAH and TMA-dissociating substances are rapidly absorbed from the gastrointestinal tract, appear in the blood as TMA, remain largely unmetabolized, and are quickly excreted in the urine.

Non-carcinogenic effects of oral exposure

No epidemiological data were available. However, there are three repeated-dose oral toxicity studies for TMAH, TMAC, and TMAHP, as well as two developmental and reproductive toxicology (DART) screening studies for TMAH and TMAHP in rats.

Oral toxicity studies in rats

A 28-day oral toxicity study of TMAH (MHLW 2000c, OECD TG407, GLP) was conducted in Sprague–Dawley (SD) rats [Crj: CD(SD) IGS, SPF] aged 5 weeks. The rats were administered dosages of 0 (vehicle: water), 5, 10, or 20 mg/kg/day by gavage, with five animals of each sex per dose for the 28-day treatment. Additionally, five animals of each sex from the control and highest dose groups were included for a 14-day recovery study.

As a result, no deaths occurred during the treatment or recovery periods. Starting on Day 6, transient salivation that resolved within 1 hr after administration was noted in one of five males at 5 mg/kg/day, five of five males and five of five females at 10 mg/kg/day, and eight of ten males and nine of ten females at 20 mg/kg/day. From Day 13 onward, persistent salivation lasting at least 1 hr after administration was observed in one of five males at 10 mg/kg/day and in five of ten males and seven of ten females at 20 mg/kg/day (Tables 2 and 3), either sporadically or for several consecutive days. In males at doses of 10 mg/kg/day or higher and in females at 20 mg/kg/day, food consumption decreased only on Day 1, with no further changes afterward. At the end of the treatment, males at 5 mg/kg/day showed statistically significant lower absolute heart weights, while males at doses of 10 mg/kg/day or higher exhibited both significantly lower absolute and relative heart weights. However, similar changes in heart weight were not observed in females. According to another paper (Kawashima and Inoue, 2024), the statistically significant decrease in heart weight in males at the end of treatment was not due to TMAH exposure. No histopathological changes were found in any organs. There were no significant differences between the control and high-dose groups for any examination items at the end of the 14-day recovery period.

Based on these findings, the no-observed-adverse-effect-level (NOAEL) for this study was determined to be 5 mg/kg/day, considering transient salivation and sustained salivation lasting over 1 hr in males and females at doses of 10 mg/kg/day or higher.

A 90-day oral toxicity study of TMAC (ECHA registration dossier/a, OECD TG408, GLP) was conducted in Wistar Hannover rats (7 weeks old, 10 animals of each sex per dose) with dosages of 0 (vehicle: water), 3, 10, or 30 mg/kg/day administered by gavage.

Two male rats at 30 mg/kg/day died on Days 57 and 80, but no clinical symptoms, changes in body weight, or food consumption were noted before their deaths. The cause of death could not be determined during necropsy due to postmortem changes and cannibalism. Surviving males at 30 mg/kg/day exhibited clinical signs (lethargy, hunched posture, piloerection, ptosis, etc.) and reduced body weight gain, while females at the same dosage showed decreased motor activity. Serum biochemistry indicated elevated alanine aminotransferase (ALT) levels in both sexes, increased aspartate aminotransferase (AST) levels in males, and high levels of alkaline phosphatase (ALP), bilirubin, and bile acids, along with low cholesterol levels in females at 30 mg/kg/day. Additionally, females at this dosage had increased relative and absolute liver weights and decreased thymus weights. Histopathological examination revealed hepatocellular hypertrophy

Table 2. Incidence of salivation in male rats in the TMAH 28-day oral toxicity study (from MHLW, 2000c).

		Days of dosing period																											Total
Group	Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Control	1 ～ 10																													0
5 mg/kg	11																													0
	12																													0
	13																													0
	14																									● *				1
	15																													0
10 mg/kg	16								●	●			●	● *	●	● *	◎	● *	● *	● *	◎ *	● *	● *	●	●	●	●	◎	● *	19
	17																					●								1
	18																									●	●			2
	19													●							●									2
	20												●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	17
20 mg/kg	21																													0
	22																							● *						1
	23									●			●	●	●	●		●	●	◎	◎	◎	●	◎	●	◎	●	●	●	17
	24								●					●		●	●	●	●	●	◎	●	●	●	●	● *	●	●	●	16
	25						●	●	●	●	●	●	●	● *	◎	●	◎ *	◎ *	◎ *	◎	◎ *	◎ *	◎	● *	◎	◎	●	◎ *	● *	23
	26																●							●			●			3
	27						●	●	●	●	●		●	● *	◎	●	◎	●	●	●	◎ *	● *	● *	◎ *	●	◎ *	◎	◎	◎ *	22
	28												●	●		●	●	●	●	●		●	●	●	●	●	◎	●	◎	15
	29														●												●			2
	30																													0

● : transient salivation immediately after administration; ◎ : persistent salivation seen for more than 1 hr after administration
*: Salivation also seen when animals are held just before administration

Table 3. Incidence of salivation in female rats in the TMAH 28-day oral toxicity study (from MHLW, 2000c).

		Days of dosing period																											Total
Group	Animal No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Control	31 ～ 40																													0
5 mg/kg	41 ～ 45																													0
10 mg/kg	46																											●		1
	47																									●				1
	48									●		●	●	●	●	●	●	●	●		●		●	●	●	●	●	●	● *	17
	49														●	●	●								●					4
	50														●		●										●			3
20 mg/kg	51																													0
	52								●							●			●					●						4
	53							●					●	●			◎													4
	54							●	●	●			●	● *	●	●	●	●	●	●	●	◎	●	●	●	●	●	●	●	20
	55							●	●	●			●	●	●	●	●	●	●	◎	●	●	●	●	◎ *	●	●	●	●	20
	56								● *	● *	● *	●	● *	● *	● *	● *	● *	● *	● *	● *	◎ *	◎	◎ *	● *	◎ *	◎ *	◎	◎ *	◎ *	21
	57								●	●				● *		●	◎	●	●	●	●	◎	●	●	●	●	●	◎	●	17
	58						●	●	●	●	●	●	●	◎ *	◎	●	◎ *	◎	●	● *	◎ *	◎	● *	● *	◎	◎ *	●	◎	●	23
	59							●	●	●			●	◎ *	◎	●	◎	●	●	◎ *	◎	◎	●	●	● *	◎ *	◎	●	◎	20
	60																●					●								2

● : transient salivation immediately after administration; ◎ : persistent salivation seen for more than 1 hr after administration
*: Salivation also seen when animals are held just before administration

in females (4/10, 4/10, and 8/10 at 3, 10, and 30 mg/kg/day, respectively), but no changes in other liver parameters at doses of 10 mg/kg/day or lower. Therefore, the hepatocellular hypertrophy observed at ≤10 mg/kg/day was not deemed an adverse effect. Minimal thymic atrophy was noted in two females at 30 mg/kg/day.

Based on these findings, the NOAEL for this study was established at 10 mg/kg/day for both males and females due to the clinical symptoms and hepatic effects observed at 30 mg/kg/day.

A 28-day oral toxicity study of TMAHP (ECHA registration dossier/b, OECD TG407, GLP) was conducted in SD rats (5–6 weeks old, 5 animals of each sex per dose) using dosages of 0 (vehicle: water), 5, 15, or 75 mg/kg/day administered by gavage.

No deaths occurred in any of the groups. At the 75 mg/kg/day dose, both sexes exhibited clinical symptoms such as ptosis, lethargy, ataxia, and transient salivation on the dosing day. Males at this dosage also showed abdominal distension starting on Day 12, reduced body weight gain, decreased food and water intake, and prolonged blood clotting time by the end of the dosing period. Females at 75 mg/kg/day had elevated ALP, ALT, and AST levels, along with low levels of plasma protein, albumin, and urea, increased liver weight, basophilic changes, and hepatocyte hypertrophy. No toxicological effects were observed at doses lower than 15 mg/kg/day.

Based on these findings, the NOAEL for this study was established at 15 mg/kg/day due to the clinical symptoms and hepatic effects seen at 75 mg/kg/day.

Oral DART screening studies in rats

A TMAH oral DART screening study [final report by the Chemicals Evaluation and Research Institute (CERI) 2005: unpublished report, OECD TG421, GLP] was conducted in SD rats (10 weeks old, 10 animals of each sex per dose) using dosages of 0 (vehicle: water), 1, 5, or 20 mg/kg/day. Males received treatment by gavage for a total of 32 days, starting 14 days before mating and continuing until the day before necropsy. Females were treated for a total of 40–47 days, from 14 days before mating to 3 days after parturition (female parents and offspring were necropsied on Day 4).

As a general toxic effect on the parents, clinical symptoms were noted in both males and females at doses of 5 mg/kg/day or higher. In male parents (Table 4), salivation was observed sporadically or continuously in all ten males at 5 mg/kg/day from Day 6 until the end of treatment and in nine out of ten males at 20 mg/kg/day from Day 4 to the end of treatment. Most male parental animals exhibited salivation immediately before or after treatment; however, one male at each dosage (5 and 20 mg/kg/day) displayed salivation that persisted for about 1 hr after treatment (specific details regarding the occurrence and frequency of this continuous case are unknown). In female parents (Table 5), salivation immediately after treatment was seen only on Day 16 of gestation (GD16) in two out of ten at 5 mg/kg/day. At 20 mg/kg/day, salivation was observed sporadically or continuously in all ten animals from Day 5 of treatment until the end of the study. Two dams at this dosage died during the study. One died on GD22 after showing decreased motor activity, incomplete eyelid opening, and a smudge around the nose and mouth starting on GD21. The other dam exhibited decreased motor activity, reduced respiratory rate, incomplete eyelid opening, pallor, and a smudge around the nose and mouth from GD22, had closed eyes on GD23, and died during delivery on GD24. Among the eight surviving dams at 20 mg/kg/day, two displayed decreased motor activity, closed eyelids, and incomplete eyelid opening from GD20, with one also showing reduced respiratory rates. However, these two dams showed no abnormalities after delivery. In addition, another dam experienced alopecia until Day 4 of nursing and exhibited emaciation on the same day.

In addition to the clinical symptoms, decreased food consumption was noted in male parents at 20 mg/kg/day on Day 3 of treatment. In dams at the same dosage, reduced food intake was observed on GD20, along with decreased body weight on Days 0 and 4 of nursing. The weights of the reproductive organs (testis, epididymis, prostate, seminal vesicles, ovaries, and uterus) were measured during the necropsy of the parent animals. No statistically significant differences were found in the absolute or relative weights of any reproductive organs among the treatment groups.

Regarding fertility toxicity, no treatment-related effects were observed on the days to mating, mating rate, conception rate, implantation rate, gestation period, or parturition rate in either group. For offspring toxicity, there were no treatment-related effects on the number of born pups, the number of surviving pups, the sex ratio, or the birth rate. No treatment-related abnormalities were found in the external observations of the nursing pups. At 20 mg/kg/day, a statistically significant lower neonatal survival rate (1% significance level based on Fisher's exact test) was noted on postnatal day (PND) 4, which appeared to result from nursing behavior (e.g., lactation) affected by the deterioration of the dam's systemic condition. Thus, the reduced survival rate of pups at 20 mg/kg/day was attributed to the toxic effects of TMAH on the

Table 4. Incidence of salivation in F0 male rats in the TMAH oral DART screening study (from CERI, 2005).

		week 1							week 2							week 3							week 4							week 5					Total
	No./day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	
Control	1 ～ 10																																		0
1 mg/kg/day	11 ～ 20																																		0
5 mg/kg/day	21																		●											●					2
	22						●												●	●	●	●		●	●	●		●	●	●	●	●	●		14
	23						●												●	●	●		●		●		●	●	●						9
	24								●											●	●	●	●	●	●	●	●	●	●	●	●	●	●		15
	25																		●												●				2
	26																			●	●	●	●	●	●	●	●	●	●	●		●	●		13
	27																				●		●	●							●	●			5
	28																					●	●		●					●					4
	29																			●				●	●				●		●	●	●		7
	30								●										●	●	●	●	●	●	●	●	●	●	●	●	●	●			15
20 mg/kg/day	31																																		0
	32									●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		23
	33							●		●	●	●	●	●	●	●	●	●	●		●		●	●	●		●	●	●	●	●	●	●		22
	34							●				●	●		●	●		●	●		●	●	●	●	●	●	●	●	●	●	●	●	●		20
	35								●									●		●	●	●	●		●	●	●	●	●	●	●	●	●		15
	36				●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		29
	37												●		●			●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		18
	38					●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●	●	●		●		25
	39					●									●	●				●	●	●	●	●	●	●		●		●	●	●	●		15
	40				●	●		●	●		●	●				●		●		●	●	●	●	●	●	●	●	●	●	●		●			21

● : salivation immediately before or after administration

Salivation up to approximately 1 hr after administration in 1 male (no. 24) at 5 mg/kg and 1 male (no. 36) at 20 mg/kg (date and frequency not stated).

Table 5. Incidence of salivation in F0 female rats in the TMAH oral DART screening study (from CERI, 2005).

		Before mating													Mating period								Pregnant period																											Nursing period					Total frequency
	No./day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8 ～ 14	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22～	0	1	2	3	4	
Control	41 ～ 50																						**mating success up to Day 4**																																			0
1 mg/kg/day	51 ～ 60																						**mating success up to Day 4**																														**No.56: sacrifice** ＊						0
5 mg/kg/day	61																																																					0
	62																																								●															1
	63																																																				0
	64																																																					0
	65																																																				0
	66																																																				0
	67																																																				0
	68																																								●															1
	69																																																				0
	70																																																				0
20 mg/kg/day	71										●	●	●	●		●	●	●						●	●	●	●	●	●	●	●	●	●	●	●	●	●	●						●										23
	72									●		●	●	●		●	●	●						●	●	●	●	●	●	●	●	●	●	●	●	●		●		●		●		●										24
	73									●	●	●	●		●									●	●			●	●	●		●	●	●	●	●	●	●	●	●		●		●		**Death**						22
	74																						**mating on day 12**	●	●												●								●								●		●				6
	75									●	●	●	●	●	●									●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●						●	●	●		29
	76						●	●	●	●	●	●	●	●										●			●	●	●	●	●	●	●			●	●	●	●	●	●	●	●	●	●		●	**Death**					26
	77					●				●	●		●	●	●									●	●	●			●	●	●	●	●	●	●	●	●	●	●	●	●	●	●			●		●	●	●	29
	78												●											●	●	●	●	●	●	●	●	●	●			●	●	●	●			●			●							18
	79																												●	●	●	●	●	●	●	●	●	●			●	●	●			●			●	●			16
	80							●		●			●	●	●	●		●		●	●					●	●	●			●				●																		21

● : salivation immediately before or after administration, / : Skip to pregnant period due to mating success, *: sacrificed due to all the F1 being killed

mother rather than developmental or reproductive toxicity via breast milk.

Based on these findings, salivation in parent animals at doses of 5 mg/kg/day or higher was regarded as a toxic effect, leading to the determination of the NOAEL for general toxicity in parent animals at 1 mg/kg/day. In addition, the NOAEL for fertility in both parent and offspring animals was established at >20 mg/kg/day.

A TMAHP oral DART screening study (ECHA registration dossier/c, OECD TG421, GLP) was conducted with Wistar Hannover rats (10–11 weeks old, 10 animals of each sex per dose) at dosages of 0 (vehicle: water), 5, 15, or 35 mg/kg/day (initially 75 mg/kg/day until Day 6 of treatment) by gavage. Males received treatment for a total of 28 days, starting 14 days before mating and continuing until the day before necropsy, while females were treated for 41–46 days, beginning 14 days before mating and lasting at least 4 days post-parturition (dams and offspring were necropsied on Days 5–7).

As a result, two males and one female at 75 mg/kg/day died during Week 1 of treatment, and the surviving animals displayed prone positioning, piloerection, reduced body weight gain, and decreased body weight. Consequently, the dosage was lowered to 35 mg/kg/day from Day 7 onward, and no further deaths occurred. At 35 mg/kg/day, the animals exhibited sporadic piloerection, lethargy, uncoordinated movements, a supine position, and salivation. Dams at this dosage showed increased liver weight and histopathological evidence of glycogen accumulation in the liver. No treatment-related changes were observed in other clinical symptoms or necropsy findings. Regarding fertility toxicity in parent animals, there were no adverse effects on mating rate, conception rate, fertilization rate, time to complete mating, number of corpora lutea, number of implantations, parity rate, or gestational age. No premature or abnormal births (dystocia or lack of maternal behavior) were noted in any treatment groups. For offspring toxicity, no adverse effects were found regarding survival rate, clinical findings, body weight, or necropsy results.

Based on the results, the NOAEL for the toxic effects of TMAHP on parent animals in this study was established at 15 mg/kg/day, while the NOAELs for fertility in both parent and offspring animals were determined to be 35 mg/kg/day.

Non-carcinogenic effects of inhalation exposure

Regarding non-carcinogenic effects of inhalation exposure, no information is available for humans or animals.

Genotoxicity

According to the OECD SIDS (2006) and ECHA registration dossier/d, TMAH was deemed to have negative genotoxicity based on negative findings from the reverse mutation test in bacteria (MHLW, 2000a) and the chromosomal aberration test in mammalian cells (MHLW, 2000b), regardless of the presence of metabolic activation systems.

Similarly, TMAC was also deemed to have negative genotoxicity based on negative results from the reverse mutation test in bacteria, irrespective of metabolic activation systems (ECHA registration dossier/e).

TMAHP was likewise found to have negative genotoxicity since both the bacterial reverse mutation test and the chromosomal aberration test using human lymphocytes yielded negative results, regardless of metabolic activation systems (ECHA registration dossier/f).

While there were no *in vivo* test results, the *in vitro* findings mentioned above indicated that there are no concerns regarding the genotoxicity of TMAH and related chemical substances.

Carcinogenic effects of oral and inhalation exposure

No information is available regarding the carcinogenicity of TMAH or other related chemical substances in humans or laboratory animals exposed through oral and inhalation routes. However, since TMAH and its related chemical substances do not appear to pose a concern for genotoxicity, there is at least no concern for carcinogenicity resulting from genotoxicity.

Additional information

Human data (acute dermal exposure)

Wu *et al*. (2008) reported acute toxicity of TMAH in workers who handled the chemical. In a 2008 incident at a semiconductor factory in Taiwan, a 22-year-old male engineer fatally exposed his skin to a large amount of 25% TMAH solution. He was found conscious but weak with moderate salivation about 15 min after the exposure. Approximately 30 min later, he lost consciousness, exhibited a weak pulse and miosis, and ultimately died 8 days after the incident.

Lin *et al*. (2010) documented 13 incidents of TMAH exposure. TMAH is commonly used as a developer and etching agent in the semiconductor and photovoltaic industries. In one accident, three workers died from dermal exposure to a 25% TMAH aqueous solution. In another case, a worker exposed to 2.38% TMAH experienced severe effects, including muscle weakness, dyspnea, hyperglycemia, and chemical burns covering 28% of

his body. He survived after 2 days of assisted breathing with tracheal intubation. These cases indicate that acute dermal exposure to TMAH can lead to cholinergic symptoms from TMA and skin corrosion due to the substance's alkalinity.

Park *et al*. (2013) examined the causes of acute poisoning deaths resulting from dermal exposure to detergents containing 8.75% aqueous TMAH. In a fatal incident, a 39-year-old man with 7 years of experience exposed both his upper and lower limbs while manually injecting TMAH solution, the detergent's main component, from a drum into a temporary cleaning tank. After working for about 10 min, he was found in cardiopulmonary arrest in the shower room approximately 1 hr later. He was unconscious when discovered, and the autopsy revealed no injuries except for burns covering 12% of his body. The cause of death was determined to be acute poisoning from TMAH. TMAH is a neurotoxic substance that can lead to respiratory failure due to ganglion block from percutaneous absorption, and no antidote has been developed for it.

TMAH acute oral and dermal toxicity studies in rats

A TMAH acute oral toxicity study (MHLW 2000d, OECD SIDS 2006, OECD TG401, GLP) was conducted using SD rats (5 weeks old, 5 animals of each sex per dose) with single doses of 10, 15, 23, 34, or 50 mg/kg for males and 23 mg/kg for females. Results showed that one male at 34 mg/kg and four males at 50 mg/kg died on the day of administration. The deceased animals exhibited decreased locomotor activity, lowered body temperature, half-open or closed eyes, gait ataxia, clonic convulsions, salivation, and slow breathing. Four males at 23 mg/kg also had half-open or closed eyes. Survivors at 34 and 50 mg/kg showed reduced body weight 2 days post-administration. One female at 23 mg/kg displayed decreased locomotor activity, incomplete eyelid opening, and closed eyes on the day of administration, along with reduced body weight gain 2 days later, while the other four females showed no significant changes. Necropsy performed at the time of death or 15 days after administration revealed no notable changes in any of the males or females. Based on these findings, the LD_{50} value for males was determined to be between 34 mg/kg and 50 mg/kg.

A single-dose dermal acute toxicity study of TMAH (Clariant GmbH, 2001, unpublished report, GLP) was conducted using SD rats. An aqueous solution of TMAH (5 mL/kg BW) was applied to the skin at single doses of 50, 100, or 125 mg/kg in female rats (5 animals/dose) and 100 mg/kg in male rats (5 animals). Following application, females at ≥100 mg/kg and males at 100 mg/kg exhibited decreased activity, irregular breathing, incomplete eyelid opening, and tonic convulsions. Two females at 100 mg/kg and three females at 125 mg/kg died the day after administration. In the surviving animals, clinical symptoms resolved by Day 3. Erythema was noted on the skin of one deceased animal, but no changes were observed in the skin of the other animals. Necropsy conducted on Day 15 showed no macroscopic changes in any of the animals. Based on these results, the LD_{50} value was determined to be 112 mg/kg for dermal administration in females.

TMAH 28-day dermal toxicity study in rats

A 28-day dermal toxicity study of TMAH (OECD SIDS 2006, OECD TG410, GLP) was conducted using SD rats (5 weeks old, 10 animals of each sex per dose) at doses of 0, 5.5, 50, 120, or 250 mg/kg/day for males and 0, 2.5, 5.5, 10, or 50 mg/kg/day for females, with 6 hr of exposure per day, 5 days a week.

Results showed that males at 120 and 250 mg/kg/day exhibited clinical symptoms such as lethargy, convulsions, and tremors after the first application, and all died within 1.5–3 hr. Males at 50 mg/kg/day died within 1 week, while females died within 2 weeks. At doses below 50 mg/kg/day, erythema, edema, and crusting were observed on the skin of all treated rats. At 50 mg/kg/day, elevated ALP levels were noted, but there were no changes in mortality, clinical symptoms, body weight, weight gain, food intake, or clinical examination compared to controls. Necropsy revealed reddish ovaries in females at doses above 5.5 mg/kg/day and reddish lungs, bladder stones, and/or atrophy of seminal vesicles in females at 50 mg/kg/day and/or in males at doses above 50 mg/kg/day. No findings were reported from the histopathological examination.

DISCUSSION

TMA is a quaternary amine similar to acetylcholine that interacts with nicotinic and muscarinic receptors. It has been reported to impact the heart and respiration and cause fatalities in rats (Wu *et al*., 2012). TMA also exhibits muscarinic effects in the isolated perfused rat heart (Kennedy *et al*., 1995). Thus, the muscarinic effect of TMA may contribute to reducing heart weight in rats. However, aside from the statistically significant lower values observed in males during the 28-day TMAH toxicity study, no effects on heart weight were noted in other toxicity studies involving TMAH, TMAC, or TMAHP. The male controls in the TMAH 28-day oral toxicity

study had higher absolute and relative heart weights compared to background values, with some animals exceeding these ranges. Thus, it is likely that the statistically significant differences resulted from an incidental distribution of individuals with heavier heart weights among the control males and were not considered due to TMAH exposure. Further details are discussed in another paper (Kawashima and Inoue, 2024).

As indicated in the results, salivation was observed in several toxicity studies. In the TMAH 28-day oral toxicity study, transient salivation occurred in males at doses of ≥5 mg/kg/day, while prolonged salivation was seen in both males and females at doses of ≥10 mg/kg/day. In addition, the TMAH DART screening study also noted salivation at doses of ≥5 mg/kg/day. TMAH has high alkalinity in aqueous solutions (with a pH of 12.5 for a 0.2% w/v aqueous solution, MHLW, 2000c), so salivation could arise from the gavage administration of the irritating drug solution, potentially leading to salivation from the first dosing day. However, given the pharmacological effects of this substance, it likely results from repeated stimulation of the parasympathetic ganglion through the muscarinic receptor. In fact, salivation did not occur on the first treatment day but appeared several days later and persisted. This suggests that the salivation is more likely a result of the muscarinic effect of TMA (due to daily repeated stimulation of the muscarinic receptor) rather than alkaline stimulation.

A TMAH acute oral toxicity study in rats at doses of 34 or 50 mg/kg (higher than those used in the 28-day oral toxicity and DART screening studies) indicated convulsions, tremors, motor weakness, respiratory abnormalities characteristic of muscarinic effects, and salivation (due to oral administration). Both acute dermal and 28-day dermal toxicity studies in rats showed nervous system effects, such as reduced motor activity, hypothermia, half-open or closed eyes, gait ataxia, clonic convulsions, and slow breathing at doses greater than 50 mg/kg before death. Oral toxicity studies of TMAC and TMAHP also reported clinical signs of nervous system effects, including lethargy, piloerection, ptosis, and ataxia. Furthermore, acute occupational exposure to TMAH in humans resulted in skin chemical burns, hyperglycemia, bradycardia, miosis, muscle weakness, dyspnea, and death. While there is concern that the evaluated chemical substances may impact the human nervous system, assessments of human health effects in the CSCL consider exposure to the general public through the environment in everyday life, not high-concentration incidents like workplace accidents. Consequently, levels of human exposure to TMAH and TMA are expected to be low, making it unlikely that TMAH or related substances will cause severe neurological symptoms in humans through environmental exposure, which typically involves low concentrations.

Derivation of HAV for oral TMAH exposure

A review of the available toxicity data revealed salivation in both males and females at doses of ≥10 mg/kg/day in the TMAH 28-day oral toxicity study and in parents at ≥5 mg/kg/day in the TMAH DART screening study. In addition, clinical symptoms indicating nervous system effects (such as lethargy, piloerection, and ptosis) and hepatic effects (including increased liver weight and hypertrophy) were observed at 30 mg/kg/day in the TMAC 90-day toxicity study, as well as symptoms like lethargy, cramps, and tremors at 75 mg/kg/day in the TMAHP 28-day oral toxicity study. Although the two DART screening studies of TMAH and TMAHP did not allow for an evaluation of fetal teratogenicity and postnatal development, a quantitative risk assessment for fertility during pregnancy and delivery could be estimated. Carcinogenicity could not be evaluated due to a lack of information regarding carcinogenic effects in humans or animals. Regarding genotoxicity, *in vitro* tests for TMAH, TMAC, and TMAHP were all negative, indicating a low likelihood of genotoxicity and at least no concern regarding carcinogenicity stemming from genotoxic effects.

Based on the above, HAVs for TMAH were calculated for (1) general toxicity and (2) developmental and reproductive toxicity.

(1) For general toxicity, the "TMAH DART screening study" was identified as the key study providing the lowest dose without clinical signs of salivation in the parent animals. This NOAEL of 1 mg/kg/day was divided by uncertainty factors (UFs) of 1,000 (including interspecies variation: 10, intraspecies variation: 10, and short test period: 10), resulting in a derived HAV of 0.001 mg/kg/day for general toxicity.

(2) For developmental and reproductive toxicity, the "TMAH DART screening study" was also chosen as the key study. A NOAEL of >20 mg/kg/day, which showed no toxic effects on the fertility of parent animals or their offspring, was divided by UFs of 1,000 (including interspecies variation: 10, intraspecies variation: 10, and study quality due to insufficient data on effects on fetuses and offspring: 10) to derive the HAV of 0.02 mg/kg/day for developmental and reproductive toxicity.

Consequently, the lowest HAV for oral exposure derived from general toxicity effects was 0.001 mg/kg/day, which was determined to be appropriate as the representative HAV (Table 6).

Table 6. Summary of Hazard Assessment Value (HAV) for TMAH.

Route of exposure	HAV	Basis data and derivation method
Oral	0.001 mg/kg/day	A NOAEL (1 mg/kg/day) for general toxic effects (salivation of clinical symptoms) on parent rats in the TMAH oral DART screening study was selected. An oral HAV of 0.001 mg/kg/day was determined as 1 mg/kg/day divided by UFs of 1,000 (interspecies variation: 10, intraspecies variation: 10, short test period: 10).
Inhalation	—	Since there was no information on inhalation studies available in TMAH, etc., HAV for inhalation exposure was not derived.

Information for the risk assessment

The Risk Assessment (Primary) Evaluation II Conference for TMAH, a joint meeting involving the MHLW, METI, and MOE, took place in September 2023 and January 2024. The risk assessment indicated that "due to certain instances where the intake amount exceeded the HAV, actual data collection through environmental monitoring should be considered."

ACKNOWLEDGMENTS

We thank all those who supported the preparation of the manuscript. This study was financially supported by the MHLW, Japan.

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

CERI. (2005), Chemical Evaluation and Research Institute (CERI), Reproductive and Developmental Toxicity Screening Study of TMAH (OECD Test Guideline 421) Study code: E16-0033, Final Report, April 2005 (unpublished report) [in Japanese]

Clariant GmbH. (2001): Acute dermal toxicity of tetramethylammonium hydroxide, 25% aqueous solution. Report ID: PT01-0139 (unpublished report). (Quoted from OECD SIDS 2006)

CSCL. (1973): Chemical Substances Control Law: Act on the evaluation of chemical substances and regulation of their manufacture, etc. (Excerpt) (Act No. 117 of Oct. 16, 1973). https://www.nihs.go.jp/mhlw/chemical/kashin/hourei/glp_en/A-01_law_eval_reg.pdf [accessed on Oct. 10, 2024]

ECHA registration dossier/a: Tetramethylammonium hydroxide (CAS 75-59-2), Repeated dose toxicity: Oral 003 Key Read-across (Structure analog/surrogate, TMAC (CAS 75-57-0)) https://echa.europa.eu/it/registration-dossier/-/registered-dossier/14295/7/6/2/?documentUUID=bcbbb91a-757c-441c-96ee-33e1e7716609 [accessed on Oct. 10, 2024]

ECHA registration dossier/b: Tetramethylammonium hydrogen phthalate (CAS: 79723-02-7), Repeated dose toxicity: Oral https://echa.europa.eu/it/registration-dossier/-/registered-dossier/10983/7/6/2 [accessed on Oct. 10, 2024]

ECHA registration dossier/c: Tetramethylammonium hydrogen phthalate (CAS: 79723-02-7), Toxicity to reproduction: Endpoint summary https://echa.europa.eu/it/registration-dossier/-/registered-dossier/10983/7/9/1 [accessed on Oct. 10, 2024]

ECHA registration dossier/d: Tetramethylammonium hydroxide (CAS 75-59-2), Genetic toxicity: Endpoint summary https://echa.europa.eu/it/registration-dossier/-/registered-dossier/14295/7/7/1 [accessed on Oct. 10, 2024]

ECHA registration dossier/e: Tetramethylammonium chloride (CAS 75-57-0), Genetic toxicity: Endpoint summary https://echa.europa.eu/it/registration-dossier/-/registered-dossier/5540/7/7/1 [accessed on Oct. 10, 2024]

ECHA registration dossier/f: Tetramethylammonium hydrogen phthalate (CAS: 79723-02-7), Genetic toxicity: Endpoint summary https://echa.europa.eu/it/registration-dossier/-/registered-dossier/10983/7/7/1 [accessed on Oct. 10, 2024]

Kawashima, A., Inoue, K., Ushida, K., Kai, K., Suzuki, H., Matsumoto, M., Masumura, K. and Hirose, A. (2022): Derivation of human health hazard assessment values for toluene under the Japan Chemical Substances Control Law. Fundam. Toxicol. Sci., **9**, 123-133.

Kawashima, A. and Inoue, K. (2024): Re-evaluation of the reduced heart weights in male rats in a 28-day oral repeated-dose toxicity study of tetramethylammonium hydroxide. Regul. Toxicol. Pharmacol., **153**, 105712.

Kennedy, R.H., Wyeth, R.P., Gerner, P., Liu, S., Fontenot, H.J. and Seifen, E. (1995): Tetramethylammonium is a muscarinic agonist in rat heart. Am. J. Physiol., **268**, C1414-C1417.

Lin, C.C., Yang, C.C., Ger, J., Deng, J.F. and Hung, D.Z. (2010): Tetramethylammonium hydroxide poisoning. Clin. Toxicol. (Phila.), **48**, 213-217.

MHLW. (2000a): Japanese Ministry of Health, Labour and Welfare: Reverse mutation test of tetramethylammonium hydroxide on bacteria. [in Japanese]: https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF75-59-2e.pdf [accessed on Oct. 10, 2024]

MHLW. (2000b): Japanese Ministry of Health, Labour and Welfare: *In vitro* chromosomal aberration test of tetramethylammonium hydroxide on cultured Chinese hamster cells. [in Japanese]: https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF75-59-2f.pdf [accessed on Oct. 10, 2024]

MHLW. (2000c): Japanese Ministry of Health, Labour and Welfare: Twenty-eight-day repeat dose oral toxicity test of tetramethylammonium hydroxide in rats. [in Japanese]: https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF75-59-2b.pdf [accessed on Oct. 10, 2024]

MHLW. (2000d): Japanese Ministry of Health, Labour and Welfare: Single dose oral toxicity test of tetramethylammonium hydroxide in rats. [in Japanese]: https://dra4.nihs.go.jp/mhlw_data/home/pdf/PDF75-59-2a.pdf [accessed on Oct. 10, 2024]

MHLW. METI, and MOE (2022a): Risk Assessment Methodology for Priority Assessment Chemical Substances (3rd ed.), July 2022 [in Japanese]. https://www.meti.go.jp/policy/chem-

ical_management/kasinhou/files/information/ra/riskassess.pdf [accessed on Oct. 10, 2024]

MHLW. METI, and MOE (2022b): Technical Guidance for Risk Assessment of Priority Assessment Chemical Substances under the Law Concerning the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc. II. Hazard Assessment for Human Health Effects (Ver. 1.2) July 2022 [in Japanese]. https://www.meti.go.jp/policy/chemical_management/kasinhou/files/information/ra/02_tech_guidance_ii_hitoyuugaisei_v_1_2.pdf [accessed on Oct. 10, 2024]

Neef, C., Oosting, R. and Meijer, D.K. (1984): Structure-pharmacokinetics relationship of quaternary ammonium compounds. Elimination and distribution characteristics. Naunyn Schmiedebergs Arch. Pharmacol., **328**, 103-110.

OECD SIDS. (2006): Organization for Economic Cooperation and Development Tetramethylammonium hydroxide [CAS No. 75-59-2]. SIDS Initial Assessment Report for 22th SIAM. https://hpvchemicals.oecd.org/UI/handler.axd?id=25d2b783-d83e-4bbd-b778-0da407b65f92 [accessed on Oct. 10, 2024]

Park, S.H., Park, J., You, K.H., Shin, H.C. and Kim, H.O. (2013): Tetramethylammonium hydroxide poisoning during a pallet cleaning demonstration. J. Occup. Health, **55**, 120-124.

PDSC. (1950): Poisonous and Deleterious Substances Control Act (Act No. 303, Dec. 28, 1950) https://www.japaneselawtranslation.go.jp/en/laws/view/3387 [accessed on Oct. 10, 2024]

Tsubaki, H. and Komai, T. (1986): Intestinal absorption of tetramethylammonium and its derivatives in rats. J. Pharmacobiodyn., **9**, 747-754.

Wu, C.L., Su, S.B., Chen, J.L., Lin, H.J. and Guo, H.R. (2008): Mortality from dermal exposure to tetramethylammonium hydroxide. J. Occup. Health, **50**, 99-102.

Wu, C.L., Su, S.B., Chen, J.L., Chang, C.P. and Guo, H.R. (2012): Tetramethylammonium ion causes respiratory failure related mortality in a rat model. Resuscitation, **83**, 119-124.