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Toxicity of tetramethylammonium hydroxide: Review of two fatal cases of dermal exposure and development of an animal model

Chung-Hsun Lee1,2, Chao-Ling Wang2,3, Hsiu-Fen Lin2,4, Chee-Yin Chai5, Ming-Yuan Hong1 and Chi-Kung Ho2,3

Abstract
To document two cases of patients who were fatally exposed to tetramethylammonium hydroxide (TMAH) on the skin and to establish a rat model to investigate the effects of dermal exposure to TMAH. The charts of two workers who died from occupational accidental exposure to TMAH were reviewed. The 4-hour lethal dose (LD50) of TMAH was determined by applying solutions mimicking the two most common industrially used concentrations (2.38% and 25%) of TMAH to the skin of Sprague-Dawley rats. Exposure of the rat’s skin to 2.38% or 25% TMAH generated LD50 values of 85.9 mg/kg and 28.7 mg/kg, respectively. Application of either concentration of TMAH to the skin produced a rapid, significant increase in the rate of respiration. The serum concentrations of tetramethylammonium (TMA) also changed significantly with time of exposure to both concentrations of TMAH. The level of blood urea nitrogen decreased significantly in rats exposed to the 2.38% TMAH, and rats exposed to the 25% solution had a significant decrease in the serum concentration of sodium. Injection of atropine after 5 minutes of exposure did not significantly overcome any of the toxic effects observed with either solution of TMAH. The preliminary results in the rat model indicated that the lethality of TMAH cannot be fully explained by the severity of the patients’ chemical burns, and the physiologic effects on respiratory and kidney functions were probably involved.

Keywords
Dermal exposure, tetramethylammonium hydroxide, tetramethylammonium, toxicity, lethal dose (LD50)

Introduction
Tetramethylammonium hydroxide (TMAH) is used widely in the manufacture of semiconductors and liquid crystal displays (LCDs; Takano et al., 1992). Its use is increasing and amounts to more than 500 tons per year in these industries. It is transported as a 25% solution in pipelines, and then diluted to a concentration of 2.38% for use in the production lines, where it often replaces sodium hydroxide and other alkaline solutions for cleaning wafers.

Despite its extensive use, little toxicologic information is available for TMAH, and it has been considered to have a relatively low level of toxicity (Sonphao and Chaisirikul, 2001). Nevertheless, a case has been reported in which a worker has died as a result of exposure to TMAH (Sonphao and Chaisirikul, 2001). The present report describes two fatal cases of dermal exposure to TMAH and provides the results of a rat model to investigate the effects of dermal exposure to TMAH.

Methods
Two workers who died from occupational accidental exposure to TMAH were reviewed. The charts of the two workers were reviewed to determine the exposure conditions, clinical manifestations, and laboratory results. The 4-hour lethal dose (LD50) of TMAH was determined by applying solutions mimicking the two most common industrially used concentrations (2.38% and 25%) of TMAH to the skin of Sprague-Dawley rats. Exposure of the rat’s skin to 2.38% or 25% TMAH generated LD50 values of 85.9 mg/kg and 28.7 mg/kg, respectively. Application of either concentration of TMAH to the skin produced a rapid, significant increase in the rate of respiration. The serum concentrations of tetramethylammonium (TMA) also changed significantly with time of exposure to both concentrations of TMAH. The level of blood urea nitrogen decreased significantly in rats exposed to the 2.38% TMAH, and rats exposed to the 25% solution had a significant decrease in the serum concentration of sodium. Injection of atropine after 5 minutes of exposure did not significantly overcome any of the toxic effects observed with either solution of TMAH.

The preliminary results in the rat model indicated that the lethality of TMAH cannot be fully explained by the severity of the patients’ chemical burns, and the physiologic effects on respiratory and kidney functions were probably involved.

Keywords
Dermal exposure, tetramethylammonium hydroxide, tetramethylammonium, toxicity, lethal dose (LD50)
result of dermal exposure to a solution of TMAH (Wu et al., 2008), prompting further investigations into the effects of this type of exposure.

Aside from the caustic effects of 25% TMAH, which is a strong base (pH ≥ 13), the breakdown of TMAH produces tetramethylammonium (TMA) or tetraamine, a toxic quaternary ammonium compound. The effects of TMA in humans have long been known to be the cause of many cases of intoxication resulting from eating foods such as marine gastropods (Henry, 1948). The extensive studies of the physiologic effects of TMA taken orally or by injection have been reviewed by Anthoni (Anthoni et al., 1989a, b). However, little is known about the effects of dermal exposure.

In this report, we review the cases of two patients who were fatally injured in an occupational accident involving dermal exposure to TMAH that was similar to the one reported by Wu et al. (Wu et al., 2008). Given the widespread use of TMAH and the potential for accidental exposure, further study of its toxicity and possible antidotes is crucial. Therefore, we developed a rat model to investigate the physiologic effects of the two most common industrially used concentrations of TMAH when absorbed through the skin. We also evaluated the utility of atropine as an antidote in the rat model.

Materials and methods

Experiment animals and drugs

We used male Sprague-Dawley rats (BioLASCO Taiwan Co., Ltd, Taipei, Taiwan), 8 weeks of age, with a mean weight of 334.8 ± 9.3 (range 300–350) g. The rats were housed in the Laboratory Animal Center at Kaohsiung Medical University under conditions of 22 ± 3°C, relative humidity of 40% to 60%, and a 12-hour light/dark cycle.

LD50 assays

The rats (for both the control and experimental groups) were anesthetized by intraperitoneal injection with thiamylal sodium (50 mg/kg; Shinlin Sinseng Pharmaceutical Co., Ltd, authorized by Kyorin Pharmaceutical Co. Ltd, Japan). The hair on the backs of the rats was shaved with an electric razor, and glass rings of 3.1-cm internal diameter, 3.5-cm external diameter, and 2.5-cm height were affixed to the shaved skin with quick-drying glue. To determine the 4-hour lethal dose (LD50) of TMAH applied to the skin, we used two stock solutions of TMAH (2.38% and 25% solutions in pure water, supplied by Kemitek Industrial Corp, Hsin Chu, Taiwan). We varied the dose by applying different amounts of the stock solutions onto the area of the back within the glass rings to achieve a total of 5 different doses of exposure. Six rats were assigned to each dose, along with six rats in a control group. The mortality rate was calculated after a 4-hour exposure. The LD50 analysis for chemical toxicology was based on the Organization for Economic Cooperation and Development (OECD) 2001 guideline. To establish an LD50 for the stock solutions of TMAH administered by subcutaneous injection, three doses of TMAH were tested, six rats for each dose. Control rats for each dose were injected with equal volume of PBS.

Physiologic tests and biochemical analysis

The 2.38% and 25% stock solutions of TMAH were applied to the rats’ skin in amounts corresponding to the LD50; and the rats’ blood pressure, pulse rates, and respiration frequency were recorded at 15, 30, 60, 90, and 120 minutes post exposure (n = 10 at each time point). Blood pressure and pulse rates were measured with a non-invasive rat sphygmomanometer that did not require preheating (MK-2000, Muromachi Kikai CO., Ltd, Tokyo, Japan). The respiration frequency was measured with a four-channel physiologic recording system, IWX214 Data Recorder (iWorx/CB Sciences, Inc., Dover, New Hampshire, USA). After measurement of the physiologic parameters at each time point, 6 to 8 mL of whole blood was taken from the heart without anti-coagulants. After total coagulation, the serum was obtained by centrifugation for 30 minutes at 3000 RPM in a CI8000 (Toshiba Co., Ltd, Tokyo, Japan) and analyzed for biochemistry profiles, the serum level of acetylcholinesterase (AChE), and TMA. AChE concentration was measured by the immunoturbidimetric method with the DXC 800 (Beckman Coulter, Inc., Fullerton, California, USA). The concentration of TMA in the serum was estimated by LC/MS/MS as described by Ariffin and Anderson (Ariffin and Anderson, 2006).

Atropine administration

The 2.38% and 25% stock solutions of TMAH were applied to the skin of six rats at the LD50 concentration, and atropine (Atropine Sulfate Injection, 1 mg/mL/amp, Taiwan Biotech, Taiwan) was injected subcutaneously at a dose of 0.1 mg/kg after 5 minutes. The effect of atropine on the rate of mortality was evaluated 4 hours later.
Statistical analysis

The LD$_{50}$ was calculated with Probit analysis. The physiologic and biochemical variables are presented as mean and standard deviation, and their changes over time were analyzed with a general linear model. Fisher’s exact test was applied to test the effects of atropine as an antidote. Data were analyzed using SAS 9.0 (SAS Institute Inc., Cary, North Carolina, USA), and a p value less than 0.05 was considered statistically significant.

Results

Case reviews

A 35-year-old man arrived at the Emergency Department of Kaohsiung Municipal Hsiao-Kang Hospital on 16 February 2007, after an occupational injury involving a spill of TMAH onto his skin. Upon arrival, the patient had no spontaneous breathing or heartbeat but a pulse was detected after intubation and cardiac resuscitation were performed. The patient suffered second- to third-degree burns over 7% of his body, primarily in the face and neck area. Evaluation of blood chemistry showed respiratory acidosis, leukocytosis, hyperglycemia, and abnormal liver enzymes (Table 1). Chest X-ray examination revealed no abnormalities. Although the patient was in deep coma, computed tomography (CT) showed only slight brain edema. The patient was transferred to the Burn Injury Unit for treatment, but he died that afternoon.

The second patient was a 28-year-old man who was injured in the same accident and also arrived in the Emergency Department with no pulse or breathing. A pulse was obtained after intubation and resuscitation, and bronchoscopic examination revealed no obvious inhalation injury. This patient also had second- and third-degree burns over less than 8% of his body; and blood tests revealed leukocytosis, hyperglycemia, and abnormal liver enzymes (Table 1). This patient died the next day.

Experimental rat model

In the experimental animal model, the LD$_{50}$ values after exposures of the rats’ skin to 2.38% and 25% TMAH were 85.9 mg/kg (95%CI: 6.9-110.7) and 28.7 mg/kg (15.8-55.6), respectively. For comparison, when the TMAH stock solutions were diluted and administered by subcutaneous injection, the values of LD$_{50}$ were 12.9 mg/kg and 11.9 mg/kg, respectively.

| Table 1. Initial blood chemistry analyses for two patients exposed to TMAH on the skin |
|-----------------------------------------------|----------------|----------------|
| Analytes (normal range) | Patient 1, 35-year-old man | Patient 2, 28-year-old man |
| WBC × 10$^3$/μL (4.8-10.8) | 16.99 | 6.60 |
| RBC × 10$^6$/μL (4.7-6.1) | 4.85 | 5.48 |
| Hgb g/dL (14-18) | 14.5 | 15.5 |
| PLT × 10$^3$/μL (140-500) | 209 | 331 |
| Glucose mg/dL (75-112) | 395 | 233 |
| GOT IU/L (10-33) | 82 | 66 |
| GPT IU/L (3-34) | 125 | 80 |
| BUN mg/dL (8-21.1) | 12.8 | 10 |
| CRTN mg/dL (0.6-1.5) | 1.65 | 1.4 |
| Na mmol/L (133-145) | 141 | 141 |
| K mmol/L (3.5-5.3) | 3.7 | 6.5 |
| Acetylcholinesterase U/L (4499-13320) | 11833 | 11998 |

Arterial blood gas (after administration of O$_2$)

<table>
<thead>
<tr>
<th>Analytes (normal range)</th>
<th>Patient 1, 35-year-old man</th>
<th>Patient 2, 28-year-old man</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (7.35-7.45)</td>
<td>6.848</td>
<td>7.384</td>
</tr>
<tr>
<td>pCO$_2$ mmHg (35-45)</td>
<td>107.9</td>
<td>74.5</td>
</tr>
<tr>
<td>pO$_2$ mmHg (75-100)</td>
<td>195.8</td>
<td>37.8</td>
</tr>
<tr>
<td>HCO$_3^-$ mmol/L (22-26)</td>
<td>18.3</td>
<td>19.7</td>
</tr>
<tr>
<td>Base excess mEq/L (±3)</td>
<td>-17.8</td>
<td>-8.2</td>
</tr>
<tr>
<td>O$_2$ SAT % (95-98)</td>
<td>97.8</td>
<td>99.0</td>
</tr>
</tbody>
</table>


When the rats’ skin was exposed to amounts of the 2.38% and 25% solutions of TMAH corresponding to the LD$_{50}$ levels, there was a significant increase in the rate of respiration ($p < 0.007$ and $p = 0.0153$ for 2.38% and 25% TMAH, respectively) as shown in Figure 1. Rats exposed to the 25% solution also had a significant increase in systolic blood pressure ($p = 0.0328$; Figure 2).

As shown in Table 2, the concentrations of AChE in serum were not significantly changed after dermal exposure to TMAH.

The concentrations of TMA in the serum changed significantly with time of exposure when the LD$_{50}$ levels, both stock solutions of TMAH were applied to the skin ($p < 0.0001$ and $p = 0.0355$ for 2.38% and 25% TMAH, respectively), as shown in Figure 3. The maximum concentrations of TMA were attained at 60 minutes. The concentration of blood urea nitrogen (BUN) decreased significantly in rats exposed to the LD$_{50}$ of TMAH administered as the 2.38% stock (Figure 4). When rats were exposed to the 25%
solution at the LD₅₀, there was a significant decrease in the serum concentration of sodium from 141.25 mEq/L at 0 minutes to 136.6 mEq/L at 120 minutes ($p = 0.037$).

Injection of atropine after 5 minutes of exposure to TMAH did not significantly overcome the toxic effects observed with either stock solution of TMAH (data not shown).

**Discussion**

We have described the cases of two patients who died as a result of dermal exposure to TMAH in an occupational accident. The deaths of these patients could not be satisfactorily explained by the severity of the burns they suffered, and direct damage to the respiratory tract may have been involved. These cases are similar to the one reported by Wu et al., in which the patient also had neither pulse nor breathing upon arrival at the hospital (Wu et al., 2008). In that case, the laboratory results also revealed leukocytosis, hyperglycemia, and metabolic and respiratory acidosis. Although that patient’s burns were more severe than those of the patients we have described (second-degree burns over 24% of the total body surface area and third-degree burns over 5%), those authors also concluded that burn syndrome could not fully explain the patient’s death and that TMA intoxication should be considered as the most probable cause of death.

Most cases of TMA intoxication in humans have occurred as a result of ingestion of food containing TMA, for example marine gastropods (Anthoni et al., 1989a; Henry, 1948; Kawashima et al., 2004; Zhao et al., 1997); and TMA has been shown to be rapidly absorbed by the intestines in rats (Tsubaki and Komai, 1986). The symptoms of such TMA intoxication include headache, dizziness, and nausea, which generally are mild and transient because of rapid elimination of TMA from the body (Neef et al., 1984).

When TMA is injected as a bolus into animals, many of the symptoms, such as excessive salivation, suggest that TMA exerts an effect on the autonomic nervous system (Anthoni et al., 1989b). In the autonomic ganglion, TMA acts directly on nicotinic and muscarinic receptors that are responsible for depolarization and, at larger doses, blockage of depolarization (Gebber and Volle, 1966). Also, sodium accumulates intracellularly, which causes a depression of ganglionic excitability. However, the responses of individual ganglia are complex (Volle, 1980); and the effects of more prolonged exposure, such as would occur after a spill on the skin, may be somewhat different from those observed after a bolus injection (Anthoni et al., 1989b). When TMA was delivered by intravenous infusion, which would produce a more prolonged exposure, only a depolarization was observed (Gebber and Snyder, 1968). TMA also interferes with neuromuscular transmission, which first influences the fast-moving muscles, such as the orbital muscles and

![Figure 1](image1.png) Effect of tetramethylammonium hydroxide (TMAH) on respiration. Rats were exposed to final doses of 85.9 mg/kg and 28.7 mg/kg by applying 2.38% and 25% solutions of TMAH, respectively, to the skin of the back for 2 hours ($n = 10$ at each time point).

![Figure 2](image2.png) Effect of 25% tetramethylammonium hydroxide (TMAH) on systolic blood pressure. Rats were exposed to a final dose of 28.7 mg/kg by applying the 25% solution of TMAH to the skin of the back for 2 hours ($n = 10$ at each time point).
muscles of the fingers, then the muscles of the four limbs, trunk, and neck, and finally paralyzes the intercostal muscles and diaphragm. Paralysis of the respiratory muscles has been proposed as the cause of death in animals (Anthoni et al., 1989b).

We have established an animal model to further investigate how the toxicity of TMAH after dermal exposure is related to its activity on the nervous system. Because TMAH is transported in pipelines for the semiconductor industry as a 25% solution, which is then diluted to 2.38% in the production line, we applied these concentrations to the skin of Sprague-Dawley rats to mimic an accidental cutaneous exposure.

We first compared the LD$_{50}$ values of TMAH applied to the skin to those for subcutaneous injection. The LD$_{50}$ values when 2.38% and 25% solutions were diluted and injected subcutaneously in the rats were 12.9 mg/kg and 11.9 mg/kg, respectively. These data are in good agreement with those of Anthoni et al. (1989a). In contrast, we observed that the LD$_{50}$ when 2.38% TMAH was applied to the rats’ skin was 85.9 mg/kg whereas that of the 25% solution was 28.7 mg/kg. These results suggest that the skin served as a somewhat effective barrier against uptake of TMAH, but that the corrosive effects of the 25% solution destroyed the skin structure and increased the uptake of the more concentrated TMA ion across the skin.

The most striking physiologic effect we observed in the rat model was the increase in the rate of respiration, which was evident within 15 minutes and continued throughout the 120 minutes of exposure to both the 2.8% and 25% solutions on the skin. The initial increase in the rates of respiration may have been the result of the pain sensation caused by the corrosive solution (TMAH). The short duration of the anesthesia (thiamylal sodium) could induce a hypnotic state; but the rats could still feel the severe pain induced by corrosive agents (due to skin injury), and no analgesics were administered. Paralysis of the diaphragm is the proposed cause of death, but it is also possible that the function of the brain or central nervous system was inhibited. The inhibition of AChE, for example through organophosphate and carbamate intoxication, will cause similar toxic effects. In cases of poisoning with organophosphate, most victims died because of bronchorrhea, bronchospasm, and bradycardia (muscarinic effect). However, the concentrations of AChE would decrease obviously in cases of severe poisoning (Tafuri and Roberts, 1987). In our study, the effects on the levels of AChE did not correspond to the severity of the poisoning caused by TMAH. In other words, TMA was just a weak AChE inhibitor, and this effect could only partially explain of the mechanism of toxicity. We observed that decompensation of respiratory function followed by apnea and death occurred in most of the rats after exposure to TMAH for 2 to 3 hours, which supports the hypothesis that death was due to paralysis of the diaphragm.

### Table 2. The acetylcholinesterase activity in the serum of rats after dermal exposure to TMAH

<table>
<thead>
<tr>
<th>TMAH concentration</th>
<th>Acetylcholinesterase activity (U/L, mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of exposure to TMAH (n = 6 for each time period)</td>
</tr>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>2.38%</td>
<td>249 ± 83</td>
</tr>
<tr>
<td>25%</td>
<td>249 ± 83</td>
</tr>
</tbody>
</table>

**Figure 3.** Change in serum levels of tetramine (TMA) at different time points after dermal exposure to 2.38% or 25% solutions of tetramethylammonium hydroxide (TMAH; n = 3 at each time point).
The laboratory analyses of blood chemistry revealed a significant decrease in BUN after the rats were exposed to 2.38% TMAH, suggesting that kidney function may have been damaged. Previous investigations of the tissue distribution of 15C-TMA injected intraperitoneally into mice revealed that the highest concentrations of TMA were found in the liver, urinary bladder, kidney, intestine, and salivary gland from 5 minutes to 24 hours after injection, with small but uniform distributions in skeletal and cardiac muscles (Tsubaki et al., 1986). Neef et al. (1984) have shown that TMA is rapidly cleared from the serum, with more than 95% excreted by the kidneys; thus, it is not surprising that its toxic effects would be manifested in the kidneys. However, the lack of a significant effect on BUN induced by exposure to the 25% solution was unexpected and remains unexplained. In addition, the rats exposed to the 25% solution showed a significant decrease in the plasma concentration of sodium ion, the cause of which is still unknown. Although we did not observe significant hyperglycemia in the rats after exposure to 2.38% TMAH, there was a trend toward hyperglycemia ($p = 0.0501$) in the rats exposed to the 25% solution. Hyperglycemia was also noted in the two cases of human exposure we reviewed and in that reported by Wu et al. (2008). These authors noted that the hyperglycemia might result from nicotinic effects.

TMA and other mono- or bis-quaternary ammonium compounds are reversible inhibitors of AChE (Adamic, 1972; Bakry et al., 1982). Furthermore, Takeshige et al. showed that the prolonged ganglion depolarization caused by TMA was unaffected and the block of transmission was only partially prevented by atropine (Takeshige and Volle, 1964). However, Luo et al. (2010) have recently reported a novel blocker against both nicotinic and muscarinic receptors that showed better protection than atropine against AChE inhibitors. It will be interesting to test this compound in the animal model we have described.

In conclusion, we have documented two cases of fatal exposure to TMAH in humans and describe an animal model to investigate the toxic effects of dermal exposure to TMAH. The deaths of these two patients call further attention to the hazardous nature of this compound. The results of the preliminary investigation in the rat model showed that the primary physiologic effect was on the respiratory system and could not be fully explained by chemical burns. This finding is consistent with previous proposals that TMA can cause death by paralysis of the respiratory system, and suggests that intoxication with TMA was responsible for the severity of the injuries and death. However, many questions remain to be addressed in future research, especially the identification of antidotes.

Conflict of interest
None

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References


