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RESEARCH ARTICLE

Decontamination of tetramethylammonium hydroxide (TMAH) splashes: promising results with Diphoterine® *in vitro*

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Abstract

Tetramethylammonium hydroxide (TMAH), used in microelectronic industries and research and development, has both corrosive properties and systemic toxicity. Two fatal TMAH occupational exposure cases have been published. Studies comparing initial TMAH decontamination with Diphoterine® versus tap water were performed: an *in vitro* pH titration study and an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) *in vitro* cytotoxicity cell viability assay. For pH normalization, 17 times more tap water than Diphoterine® was required. In the cytotoxicity test, two-thirds of the cells remained viable after Diphoterine® washing, compared with only one-third after tap water washing ($p < .001$). Diphoterine® washing is a promising TMAH decontamination method.

Keywords: *TMAH; tetramethylammonium hydroxide; chemical injury; chemical toxicity; MTT test; Diphoterine®*

Introduction

Tetramethylammonium hydroxide (TMAH; CAS No. 75-59-2, molecular formula C4H12N.HO; Figure 1) is a quaternary ammonium compound, one of a group of ammonium salts in which organic radicals have been substituted for all 4 hydrogens of the original ammonium cation. They have 1 central nitrogen atom, which is bound to 4 organic radicals and 1 alcohol radical (OH^-). Usually, quaternary ammonium compounds are used as surface-active agents, solvents, chemical intermediates, active ingredients for conditioners, antistatic agents, detergent sanitizers, softeners for textiles and paper products, phase-transfer catalysts, antimicrobial agents, disinfection agents and sanitizers, slimicides, algaecides, emulsifying agents, and pigment dispersers.

TMAH can mainly be found in the research and development field, or as a methylating and/or hydrolyzing agent. It is used in the microelectronics industry as an anisotropic etchant of silicon for the manufacture of semiconductors (1). A 25% TMAH aqueous solution is widely used for this purpose, although more dilute solutions may also be used. It is usually transported through piping and valves as a 25% solution, but it is often diluted to a 2.38% solution for use (2).

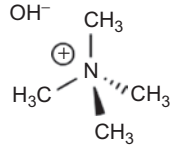

Figure 1. Structural formula of tetramethylammonium hydroxide (TMAH).

In Europe, TMAH is classified as *corrosive* (R34–Causes burns) because of its strong alkaline properties

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and as *toxic* (R24/25–Toxic in contact with skin and if swallowed) (3). There are few data on the mechanisms of TMAH toxicity, although rare cases of fatal human occupational exposure have been reported (2,4). TMAH has been reported to cause symptoms of intense burning of the eyes, nose, throat, lungs, and skin (3,5).

Previous experimental studies have indicated that the tetramethylammonium ion ($(CH_3)_3NH^+$; TMA^+) is a weak inhibitor of acetylcholinesterase and acts as a cholinergic (muscarinic and nicotinic) agonist (6). Because of its severe eye and skin corrosive potential, it is necessary to perform effective decontamination as soon after exposure as possible. Two *in vitro* studies of 25% TMAH decontamination using either an amphoteric, hypertonic washing solution, Diphoterine® (Laboratoire Prevor, Valmondois, France) (7), or tap water are described here.

Materials and methods

An initial *in vitro* experiment was performed at the Prevor Laboratory (Laboratoire Prevor) to evaluate the efficacy of different washing solutions (Diphoterine®, tap water). The experiment was a titration of 1 mL of 25% TMAH aqueous solution obtained from Sigma-Aldrich (St. Louis, MO, USA; 331635, batch 015482/1) by an increasing volume of Diphoterine® (batch D550505B) versus tap water. This method has been described previously (8).

The human body tolerates a pH range between 5.5 and 9.0, which was evaluated in this *in vitro* experiment. Variation in pH was measured with a pH meter (pH meter radiometer PHM240, pH electrode, Schott Instuments, Mainz, Germany, N6280).

A second study was performed under contract with Laboratoire Prevor by ABICH Laboratories (ABICH Srl, Verbano, Italy) consisting of *in vitro* evaluation of the protective efficacy of Diphoterine® washing solution versus tap water on TMAH-induced damage in a reconstructed 3-dimensional human skin model (EpiSkin, SkinEthic Laboratories, Nice, France) (9). An MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assay was performed as the endpoint measurement. This colorimetric and quantitative assay allows the percentage of living cells within a cell culture to be determined. The MTT cell viability assay, first developed by Mossman (10), is based on the determination of mitochondrial dehydrogenase activity using an enzymatic reaction with generation of a chromophore from MTT. Only viable cells exhibit this enzyme activity, and hence an increase or decrease in viable cell numbers results in a concomitant change in the color reaction, indicating the degree of cytotoxicity caused by the test compound (TMAH in the study described here).

The results of the cell viability assay are expressed in terms of cell viability, as compared with untreated control cultures using the following formula:

$$\text{Viability}\,(\%) = \left[\frac{\text{OD}\,(550-690\,\text{nm})\ \text{test product}}{\text{OD}\,(550-690\,\text{nm})\ \text{negative control}}\right]\times 100$$

where OD represents optical density. All test points were performed in triplicate.

The optical densities of the negative and positive control preparations met the acceptance criteria.

EpiSkin (ref. RHE/HTS/17, batch 07022A1202) is a reconstituted human epidermis, supplied as specimens with a surface area of 0.33 cm^2 on 24-well High-Throughput Screening (HTS) plates, age day 17.

MTT from Sigma-Aldrich (M2128, batch 056K5323) is the key reagent in the MTT test. A 25% TMAH aqueous solution (Sigma-Aldrich 331635, batch 015482/1) was used to induce chemical injury on the reconstituted human epidermis model. This concentration was chosen because the authors were aware of 2 published fatal cases related to occupational exposure to 25% TMAH (2,4) and to evaluate the differences between decontaminations with Diphoterine® washing solution and tap water in maximally injurious concentrations that might reasonably be encountered in an industrial facility during failure of piping or valves.

Preliminary tests were performed to determine the time of contact and the concentration of TMAH necessary to induce moderate damage (about 50% of viable cells) to the reconstituted skin.

An EpiSkin 0.33-cm^2 specimen was exposed to 60 µL of a 25% TMAH aqueous solution for either 30 or 60 seconds. The sample was then flushed for 7 minutes 30 seconds with tap water (20 times with 150 µL of tap water each time) immediately after removing TMAH. This rinsing was designed to ensure that no residual TMAH remained on the reconstituted skin sample. After the last washing, the tested solution was removed and the skin cultures were placed for 30 minutes in an incubator at 37°C with a 5% carbon dioxide (CO_2) atmosphere. At the end of this incubation period, an MTT test was performed to evaluate cell survival, compared with that for untreated control cultures.

A 30-second exposure with 25% TMAH aqueous solution induced a decrease in cell viability by 65%, and this appeared to be the maximum exposure time to produce moderate damage to the skin.

The skin protection assay was performed with 0.33-cm^2 EpiSkin specimens, which met validity and acceptance criteria treated with 60 µL of 25% TMAH

in aqueous solution, with exposure times of 10 and 30 seconds. Immediately after these exposures, the TMAH solution was removed and the specimens were washed with either 150 µL of Diphoterine® washing solution or an equal volume of tap water. This washing step was repeated 20 times for a total washing time of 7 minutes 30 seconds for each tested decontamination solution. After the last wash, the skin cultures were placed in an incubator at 37°C with a 5% CO_2 atmosphere for 30 minutes. At the end of this incubation period, an MTT assay was performed to evaluate cell viability.

Statistical analysis

Statistical significance was assessed with a 2-tailed Student's t-test on the raw triplicate data sets of optical densities obtained by the MTT cytotoxicity assay. Results were considered significant at probability values for the null hypothesis of $p \leq .05$.

Results

Figure 2 shows a more rapid decrease of the pH with Diphoterine® washing compared with tap water. This *in vitro* washing simulation shows that the physiological pH was reached when only 60 mL of Diphoterine® washing solution was added, whereas the volume of tap water required to reach physiologically acceptable pH was 17 times greater.

Table 1 shows the results obtained for the preliminary EpiSkin MTT cell viability test for 30 and 60 seconds of exposure to a 25% TMAH aqueous solution. Table 2 shows the improved results with Diphoterine® washing solution versus tap water washing as shown by the MTT cell viability test. Diphoterine® washing solution was more efficacious than tap water in preserving cell viability following 25% TMAH exposure in this *in vitro* model, with 10.9% better cell viability preservation after 10 seconds of TMAH exposure and 32.7% better cell viability preservation after 30 seconds of TMAH exposure.

Discussion

Chemical skin or eye injuries result from chemical reactions between corrosive or irritating molecules and biochemical components of exposed tissues. The severity of the chemical injury depends mainly on the chemical–physiological nature and concentration of

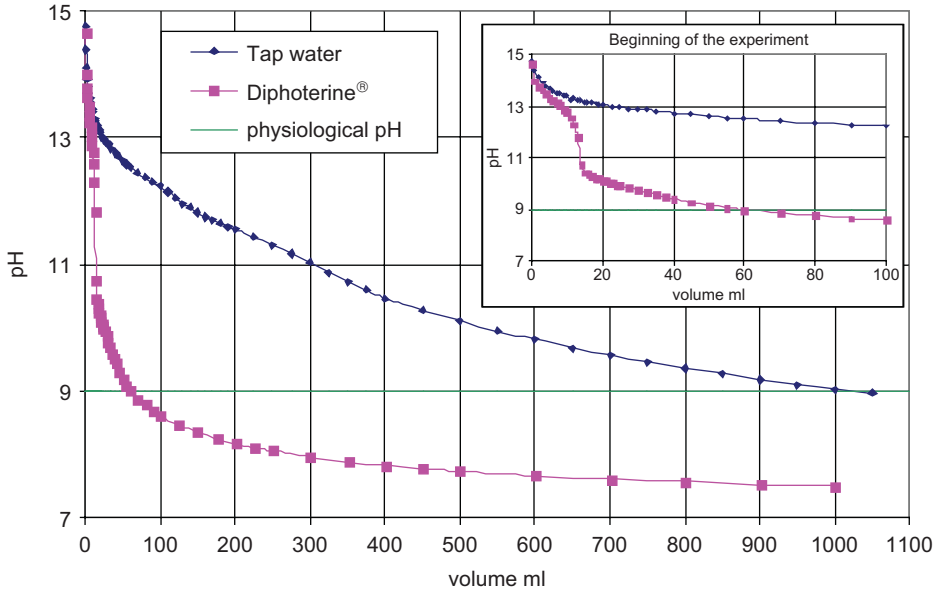

Figure 2. Evolution of pH during the measurement of 1 mL of 25% tetramethylammonium hydroxide solution according to the amount of emergency washing solution added. (See colour version of this figure online at www.informahealthcare.com/cot)

Table 1. Results obtained for the preliminary test of the MTT cell viability test.

Rinsing procedure	Cell viability (%) after 30 seconds of TMAH exposure (SD %)	Cell viability (%) after 60 seconds of TMAH exposure (SD %)
Tap water 150 µL, 20 times	34.4 (18.2)	10.7 (17.6)

MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SD % = standard deviation expressed as percentage; TMAH = tetramethylammonium hydroxide.

Table 2. Results obtained in the skin protection assay.

Rinsing procedure	Cell viability (%) after 10 seconds of TMAH exposure (SD %)	% Protection vs. tap water	Cell viability (%) after 30 seconds of TMAH exposure (SD %)	% Protection vs. tap water
Diphoterine® 150 µL, 20 times	98.7 (1.6)	10.9	66.5 (8.5)	32.7
Tap water 150 µL, 20 times	87.8 (2.3)	—	33.8 (6.5)	—

Student's t-test performed on the raw triplicate data sets of optical densities generated by the MTT cytotoxicity assay.
10-second TMAH exposure, Diphoterine® vs. tap water treatment group: $p < .005$.
30-second TMAH exposure, Diphoterine® vs. tap water treatment group: $p < .001$.
MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SD % = standard deviation expressed as percentage; TMAH = tetramethylammonium hydroxide.

the involved chemical agent, the energy of the reaction, and the duration of the eye/skin contact (11). It also depends on physical factors such as pressure or temperature, the area of the affected tissue (total body surface area [TBSA]), and whether the exposed tissue was previously healthy.

The efficacy of emergent decontamination influences the development and extent of a chemical injury and, consequently, the significance of possible sequelae (8). Early washing of a chemical splash can potentially prevent or decrease the severity of the injury. Rinsing and dilutional effects were the initial interests of washing with generally available tap water. However, water sometimes has a limited action on major corrosive product splashes (12), and the often-recommended time to intervention of 10 seconds (13) may be difficult to achieve in practice.

Diphoterine® washing solution is an amphoteric, *mildly* hypertonic molecule with multiple binding sites, capable of reacting with corrosive and irritating chemical agents without a significant release of heat, and preventing or decreasing their damaging action on exposed tissues (7). This decontamination solution is used at work sites as an emergent decontamination measure against corrosive or irritant chemicals (acids, bases, oxidizers, reducing agents, etc.), providing an *active* amphoteric hypertonic washing, compared with hypotonic tap water washing, which acts only by *passive* rinsing and dilution (12). Because of its physical nature (aqueous solution), Diphoterine® washing solution retains the same rinsing effect and dilutional capacity as an equal volume of tap water. Diphoterine® washing solution has not shown any irritating, sensitizing, or toxic effects on either normal or damaged skin (7,14,15) or eyes (7,14).

In vitro methods represent interesting alternatives to traditional *in vivo* testing for evaluation of chemical and biological properties of chemical products for cosmetic or topical use, as required or recommended by current European Commission (EC) regulations such as Directive 2003/15/EC (Cosmetics) and Regulation 1907/2006/EC (REACH [Registration, Evaluation, and Authorisation of Chemicals]), and are strongly recommended by UNI (ente Nazionale Itialono di Unificazione), 10993 rules that govern safety assessment for the testing of medical devices. Diphoterine® washing solution is currently registered as a medical device in the European Union, Australia, Brazil, and Canada.

EpiSkin is a model validated by the European Centre for the Validation of Alternative Methods (ECVAM) that very likely reacts quite similarly to living human skin exposed to chemicals (16).

The Organisation for Economic Co-operation and Development (OECD) TG 431 *In Vitro* Skin Corrosion Guideline describes the MTT test as an alternative, quantitative method to determine the corrosivity of chemical substances. The MTT test used in the cytoxicity study presented here was performed according to the method first described by Mossman (10), and was used to evaluate the impact of washing solutions, Diphoterine® versus tap water, on cells exposed to 25% TMAH. The MTT test was chosen in preference to other tests, such as the tryptan blue test, because it detects living, but not dead, cells and the generated signal is dependent on the degree of cellular activation. This method can therefore be used to measure cellular cytotoxicity, proliferation, or activation. In the study described here, it was used to test the efficacy of washing solutions. The MTT test has not previously been used for this purpose.

TMAH has the molecular formula of $((CH_3)_4N.OH)$. It is a white crystalline powder, odorless, and freely soluble in water with a specific gravity of 1.014 at 20°C (3). As it is a strong alkaline corrosive ($pH \geq 12$), cutaneous or ocular exposure can generate chemical tissue injuries, and cutaneous splashes can even result in death (2,4).

The systemic toxicity of TMAH is probably due to the toxicity of the corresponding tetramethylammonium ion ($(CH3)_3NH^+$; TMA^+). TMA^+ seems to act as a cholinergic agonist that can bind to nicotinic and muscarinic receptors in ganglion cells (6), diaphragmatic muscle (17), smooth muscle (18), and cardiac muscle (19).

The initial *in vitro* experiment showed that a much lesser volume of Diphoterine® permitted a physiological pH to be reached than was the case with tap water.

This increased efficacy may be linked to the properties of Diphoterine® to prevent or decrease TMAH skin penetration and to bind the hydroxyl moiety of TMAH (OH^-). The mechanism of TMAH skin penetration might be explained by its alkaline properties, causing superficial skin damage that then might facilitate absorption of the systemically toxic TMA^+ ion. Prevention or mitigation of the alkaline corrosive injury might theoretically decrease percutaneous absorption of the TMA^+ ion and prevent or mitigate the severity of systemic toxicity.

In the fatal industrial exposure case reported by Wu et al. (2), chemical burns occurred but were judged by the authors to be of insufficient extent and severity to have caused death. The authors also noted by physical examination and chest x-ray that there was no inhalational or ingestion-related exposure. Based on this and the occurrence of cholinergic signs, they postulated a role for the systemic toxicity of the TMA^+ ion following dermal exposure. In this case, nearly immediate tap water washing in an industrial safety shower did not prevent chemical burns and fatal systemic toxicity.

The results of the *in vitro* cell viability test after TMAH exposure and subsequent washing with Diphoterine® washing solution compared with tap water showed that a much larger portion of cells remain viable after Diphoterine® washing. After 30 seconds of exposure to a 25% TMAH aqueous solution, cell viability is significantly higher (32.7%) in the reconstituted human epidermis model (EpiSkin) washed with Diphoterine® ($p < .001$).

Conclusion

TMAH is a strong corrosive chemical. Its capacity to generate the TMA^+ ion is most likely responsible for the systemic effects observed following TMAH exposures. In the event of a TMAH exposure, it is important to wash the exposed eye/skin tissue as soon as possible to prevent or decrease the extent of chemical injury and to decrease skin absorption.

In the initial *in vitro* study, titration with Diphoterine® washing solution was shown to be more beneficial than tap water, as the pH decreased more rapidly to a value where burns are usually not observed. In MTT *in vitro* experiments on reconstituted human skin (EpiSkin), washing with Diphoterine® after 30 seconds of exposure to a 25% TMAH solution was more efficacious than tap water for washing cells exposed to TMAH. This appeared to be due to its amphoteric and hypertonic properties.

Other than its corrosivity, the toxicologic mechanism of TMAH is not completely known, and more toxicologic data on TMAH should be obtained because of the suggested systemic toxicity of the TMA^+ ion. The finding that more cells remained viable in the MTT EpiSkin study following TMAH exposure and Diphoterine® washing compared with tap water is promising. The studies described here suggest that the corrosivity and systemic toxicity of TMAH, and the potentially better impact of washing with Diphoterine® washing solution than tap water on preventing or mitigating them, deserve further study.

Declaration of interest

Mlle. Fosse and Drs. Mathieu and Burgher are employees of Laboratoire Prevor, Valmondois, France, manufacturer of Diphoterine®. Drs. Bocchietto and Fischbach are employees of ABICH Srl, Verbano, Italy, a contract laboratory that performs studies for Laboratoire Prevor. Drs. Hall and Maibach are consultants to Laboratoire Prevor.

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