METABOLITES OF HEXAMETHYLDISILOXANE AND DECAMETHYL CYCLOPENTASILOXANE IN FISCHER 344 RAT URINE—A COMPARISON OF A LINEAR AND A CYCLIC SILOXANE

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ABSTRACT:

Hexamethyldisiloxane (MM or HMDS) and decamethylcyclopentasiloxane (D5) are examples of a linear and a cyclic siloxane, respectively. These volatile low molecular weight siloxanes are of significant commercial importance. To aid in the pharmacokinetic and metabolite elution was carried out on a C18 column using an acetonitrile/water mobile phase. The structural assignments were based on GC-MS analysis of the tetrahydrofuran extract of urine containing the metabolites. Some of the metabolites in the extracts were first protected with trimethylsilyl groups prior to GC-MS analysis using bis(trimethylsiloxy)trifluoroacetamide or highly purified hexamethyldisiloxane. The structures were also confirmed by comparisons with synthetic 14C-labeled metabolite standards. The following are among the major metabolites identified in the case of MM: Me2Si-(OH)2, HOME-SiCH2OH, HOCH2Me2SiOSiMe2-CH2OH, HOME-SiOSiMe2-CH2-OH, HOCH2Me2SiOSiMe2, and Me2SiOH. The metabolites of D5 are as follows: Me2Si(OH)2, MeSi(OH)3, MeSi(OH)2OSi(OH)3, MeSi(OH)3OSi(OH)2Me, MeSi(OH)OSi(OH)Me2, MeSi(OH)2OSi(OH)Me2, MeSi(OH)OSi(OH)Me2, MeSi(OH)OSiMe2OSi(OH)Me2, nonamethylcyclopentasiloxanol, and hydroxymethylnonamethylcyclopentasiloxane. No parent MM or D5 was present in urine. The presence of certain metabolites such as HOME-SiCH2OH and Me2Si(OH)2 in MM and D5, respectively, clearly established the occurrence of demethylation at the silicon-methyl bonds. Metabolites of the linear siloxane are structurally different from that obtained for cyclic siloxane except for the commonly present Me2Si(OH)2. Mechanistic pathways for the formation of the metabolites were proposed.

Hexamethyldisiloxane (HMDS), the smallest member of the polydimethylsiloxane polymers, and decamethylcyclopentasiloxane (D5), a cyclic siloxane are colorless volatile fluids. MM is quite volatile with a vapor pressure of 42.2 mm Hg at 25°C and a boiling point of 100°C (Flanningham, 1986). D5 is relatively less volatile with a vapor pressure of 2 mm Hg at 50°C and a boiling point of 210°C. The aqueous solubilities of MM and D5 are 930 and 17 ppb, respectively (Varaprath et al., 1996). The primary use of MM and D5 is as intermediates in the manufacturing of high molecular weight siloxane polymers. MM and D5 also find use as vehicles or ingredients in a wide range of consumer product formulations (Cameron et al., 1986) since they have several favorable properties such as low surface tension, adequate evaporation rate, lack of odor, high degree of compatibility with many consumer product ingredients, and low toxicity. Typical examples of applications include moisturizing creams, lotions, bath oils, colognes, shaving products, and perfumes. Besides these product applications, they are also used as cleaners, lubricants, and penetrating oils.

The rigorously purified MM (Dow Corning OS-10, purity >99.9%) is one of the many ozone-safe volatile methylsiloxanes that is exempt from federal volatile organic compound regulations and hence is accepted as an alternative for other organic solvents. Another important industrial use of MM is as a chain-terminating agent in siloxane polymerizations. The use of MM and D5 in various product formulations necessitated conducting chemical and environmental fate/effects tests of them.

Potential human exposure to MM and D5 can result at the workplace during the manufacturing process, as well as through the normal use of consumer products that contain them. Only sparse toxicological information is available on these siloxanes since they are believed to be relatively inert and of low toxicity. However, octamethylcyclotetrasiloxane (D4), a homolog of D5 had been extensively studied. In rodents, inhalation exposure to D4 results in dose-related hepatomegaly, transient hepatic hyperplasia, hypertrophy, and induction of hepatic cytochrome P450 enzymes in a fashion similar to phenobarbital (McKim et al., 1998, 2001). Very limited toxicity data are available on HMDS and D5 in biological systems. In a 13-week subchronic MM whole-body inhalation, renal histopathology consis-
tent with male rat-specific α-2u-globulin nephropathy accompanied by slight increases in plasma urea and creatinine concentrations were seen in male Fischer F344 rats at vapor concentrations of 600 to 5000 ppm. No other treatment-related pathological changes were seen in MM-exposed rats (Cassidy et al., 2001).

In a 13-week subchronic D₅ inhalation study (Burns-Naas et al., 1998) with male and female Fischer F344 rats, exposure-related increases in absolute and/or relative liver weights were observed in both sexes, although histopathology of the liver was uneventful. The histopathology evaluation following D₅ inhalation exposure indicated lung as the primary target organ. An increase in focal macrophage accumulation and interstitial inflammation were observed in the lungs of male and female rats at high concentrations (224 ppm) of D₅.

A comprehensive program has been initiated to assess the kinetics, metabolism and toxicity of MM and D₅ in rats after relevant routes of exposure. To aid in the pharmacokinetic investigations, identification of MM and D₅ metabolites in urine collected from rats following exposure to these materials were undertaken, and the results are presented in this paper.

Materials and Methods

Instrumentation/Reagents. Radioactivity measurements were made using a liquid scintillation counter (Packard, model 2500 TR; PerkinElmer Life Sciences, Boston, MA). HPLC analyses were performed with a Hewlett Packard 1050 liquid chromatograph (Hewlett Packard, Palo Alto, CA) equipped with an HP autosampler (model 79855A), and a Radiomatic (model 500 TR series) flow scintillation analyzer from PerkinElmer Life Sciences. The detector was equipped with an HP flow cell 500 μl in size. HPLC conditions in the water/acetonitrile mobile phase were as follows: 100% water, 0 to 20 min; 100% water to 100% acetonitrile, 20 to 40 min; 100% acetonitrile, 40 to 50 min; 100% acetonitrile to 100% water, 50 to 60 min. A C₁₈ Altima column (4.6 × 250 cm and 5 μm from Alttech Associates, Deerfield, IL) was used as the stationary phase; Ultima-Flo M liquid scintillation cocktail was used in the flow cell. The ratio of column effluent to scintillation cocktail was 3:1.

HPLC fractions were collected using an automated fraction collector from Isco (model Foxy 200; Isco Inc., Lincoln, NE) coupled to the HPLC systems. GC-MS was performed using a Hewlett Packard 6890A series II gas chromatographs coupled with either an HP 5970 mass-selective detector or HP 5973 Turbo mass-selective detector. The GC-MS systems were also equipped with HP 7673 GC/SFC injectors as well as electronic pressure control units. Data analyses were performed using a Microsoft Windows-based ChemStation software (Agilent Technologies Inc., Wilmington, DE). GC-MS conditions are as follows: GC oven, 70°C/3-min ramp, 20°C/min to 220°C/3-min hold; ramp at a rate of 20°C/min to 210°C and then at a rate of 20°C/min to 250°C; GC column, HP-5 (5% phenylmethylsilicone; 30-m length, 0.25-mm i.d.; 0.25-μl thick; MS detector, 280°C, scan m/z 50 to 550).

Samples or reagents were mixed with either a VWR vortex mixer (Scientific Industries Inc., Bohemia, NY), a horizontal platform shaker (Eberbach Corporation, Ann Arbor, MI) or a wrist action shaker (model 75; Burrell Scientific, Bohemia, NY), a horizontal platform shaker (Eberbach Corporation, Ann Arbor, MI) or a wrist action shaker (model 75; Burrell Scientific, Bohemia, NY) was used. A Pasteur pipette and collected in a clean 20-ml vial. Fresh CH₂Cl₂ was added to the sample residue, and the extraction procedure was repeated as before.

Animals administered [¹⁴C]MM orally received a nominal dose of 300 mg/kg. In one study, two female rats (169 and 150 g) received doses of 49.5 and 38.4 mg of [¹⁴C]MM (original-specific activity, 8.13 mCi/mg) diluted with unlabeled hexamethyldisiloxane to a specific activity of 2.2 μCi/mg. In another study, two female rats (150 g each) received 38.8 and 82 mg, respectively, of [¹⁴C]MM (original specific activity 24.4 mCi/mmol) diluted to a specific activity of 7.02 μCi/mg with unlabeled hexamethyldisiloxane.

For i.v administration, camouflaged (jugular vein) female rats (141 and 138 g) were used. The rats received a nominal dose of 80 mg/kg [¹⁴C]MM as an emulsion. [¹⁴C]MM was emulsified (v/v) as follows: 7 parts saline, 1 part Emulphor EL-620/liter, 1 part ethanol, and 1 part [¹⁴C]MM. The radioactivity of the emulsion was determined to be 0.25 μCi/mg. The rats received 196.1 and 153.6 μg of the emulsion, respectively.

In the case of D₅, two female Fischer 344 rats were each administered [¹³C]D₅ orally. Original [¹³C]D₅ (specific activity 25.08 mCi/mmol) was diluted with unlabeled D₅ to a specific activity of 17.377 mCi/mmol. The rats received 7.6 and 8.7 mg, respectively, of the diluted [¹³C]D₅.

After dosing, all rats were housed in glass metabolism cages to facilitate the collection of urine. Urine samples were collected over a 24-h period following dosing and stored at −20°C until analysis. Following the collection, the urine samples were centrifuged, and the clear supernatant fluids that separated were collected and kept frozen at −80°C until use.

Solvent Extraction of MM Metabolites. A 500-μg aliquot was measured into a 7-ml glass vial with an aluminum-lined screw cap. Dichloromethane (2 ml) was added. The vial was then tightly capped, and the contents were first vortex mixed at high-speed settings for 3 min and then centrifuged (3000 RPM) for 4 min. The clear bottom layer of CH₂Cl₂ was carefully removed with a Pasteur pipette and collected in a clean 20-ml vial. Fresh CH₂Cl₂ was added to the sample residue, and the extraction procedure was repeated as before. Extractions were repeated until no significant amount of radioactivity was left in the urine residue. The extracts were combined, treated with anhydrous MgSO₄ to remove water, vortex mixed (4–5 min), and finally centrifuged (5 min) to obtain a clear, dry fr. The extract of the metabolites, where dry extract was then concentrated to about 250 to 300 μl by gently blowing a nitrogen stream via Pasteur pipette along the sides of the container vial. Extraction was also performed on aliquots of samples in a similar fashion using THF.

Solid Phase Extraction of D₅ Metabolites. Since most of the D₅ metabolites are not soluble in CH₂Cl₂, the extractions were performed with THF only. Also, in an effort to increase the efficiency, solid phase extractions (Varapragh and Cao, 2000) were performed as follows.

Sodium chloride (3 g) was placed on top of the sorbent (500 mg, ENV+) contained in a SPE cartridge purchased from Jones Chromatography Inc. (Lakewood, CO). The sorbent was conditioned using 6 ml of methanol.
Methanol was drawn through the cartridge using a vacuum manifold at a flow rate of 3 ml/min. This was followed by washing with 6 ml of milli-Q water (3 ml/min). The cartridge was then loaded with 8 g of urine and the latter drawn at a flow rate of 1 ml/min into a 20-ml scintillation vial. The eluate (herein referred to as sample eluate) was retained for repeat loading with fresh SPE cartridges and further solvent extractions. The sorbent was dried by suction of air for a minute. The sorbed D₃ metabolites were then eluted three times, each with 6 ml of dry THF and the THF eluates collected into separate vials. The process was repeated a total of 6 times with the sample elute using fresh SPE cartridges each time. The THF eluates from all SPE runs were combined, dried with 3 g of anhydrous MgSO₄ by shaking for 2 h, and then centrifuged. The clear THF solution was concentrated using Savant Concentrator (Speed Vac, SC 100) to approximately 300 µl in a 12-ml capacity glass centrifuge tube.

**Derivatization.** The dried and concentrated CH₃Cl₄ or THF extract of the metabolites was treated with an equivalent amount (v/v) of BSTFA, vortex mixed for 2 to 5 min, and then shaken using a horizontal shaker for 2 h at ambient temperatures. BSTFA treatment was repeated as needed, if partial derivatization became apparent from GC-MS analyses. Derivatization with specially purified hexamethyldisiloxane (99.9%) was carried out in a similar way, except that a catalytic amount of 10% (w/w) hydrochloric acid or CF₃SO₂H was also added. The reagent hexamethyldisiloxane was used only to derivatize a metabolite such as dimethylsilanediol containing one Si per molecule, since other metabolites containing Si-O-Si bonds will undergo chemical transformation (hydrolysis) under acidic conditions. The trimethylsilyl derivatives of the metabolite sample were then subjected to analysis by GC-MS for structural identification.

**Synthesis of Metabolite Standards and Precursors.** Synthesis of the standards for identification of metabolites of MM are described below with the exception of [¹⁴C]Me₃SiOH, which was synthesized following the literature procedure (Varaprath, 1999). It should be pointed out unless otherwise indicated, no special attempts were made to isolate or purify metabolite standards and precursors from the by-products. Their presence was verified from GC-MS and in some cases by HPLC radiochromatographic analyses. All the metabolites of D₃ with the exception of nonamethylcyclopentasiloxanol and hydroxymethylnonamethycyclopentasiloxane were synthesized following the literature procedure (Varaprath et al., 1999). Nonamethylnonamethycyclopentasiloxanol was available internally at Dow Corning Corp. (Midland, MI). Hydroxymethylnonamethycyclopentasiloxane was inferred by GC-MS analysis.

**Unlabeled HOCH₂SiMe₂OSiMe₂CH₂OH (1,3-bis(Hydroxymethyl)tetramethyldisiloxane).** The chloro precursor CICH₂SiMe₂OSiMe₂CH₂Cl (23.1 g) was placed in a one-necked 250-ml round bottom flask equipped with a water condenser, a magnetic stir bar, and a Drierite moisture trap. Potassium acetate (19.6 g), glacial acetic acid (50 ml), and a catalytic amount (0.5 g) of potassium fluoride were added. The reaction mixture was heated at reflux temperature for 2 h. The flask was then allowed to cool to room temperature. The solution was transferred to a 500-ml separatory funnel. The contents were washed with water (3 x 50 ml). Pentane (50 ml) was added, and the solution was washed with a 5% sodium bicarbonate solution. The organic layer was then washed several times with water until the wash water remained neutral to a pH paper. Pentane was removed using a rotary evaporator at 40°C. Pure product (23 g, 80%) AcOCH₂SiMe₂OSiMe₂CH₂OAc was obtained by distillation at 85°C/1.6 mm Hg.

The acetate, AcOCH₂SiMe₂OSiMe₂CH₂OAc (13.9 g, 0.05 mol) was added dropwise via an addition funnel to a suspension of sodium borohydride (2.2 g) in 10 ml of dry THF in a 100-ml flask equipped with a magnetic stir bar and an argon inlet. This was followed by the addition of BF₃·THF complex (10.3 g). The addition funnel was then replaced with a water condenser, and the contents were allowed to reflux for 2 h. The flask was then cooled to room temperature, and the contents were filtered through a sintered funnel to remove the white precipitate. The filtrate was collected, the THF removed using a rotary evaporator, and ether (10 ml) was added. The ether solution was placed in a beaker, and while stirring, 5% HCl (25 ml) was added slowly. A 10% solution of NaOH was slowly added in sufficient quantities to make the reaction mixture slightly alkaline. The ether layer was collected and the aqueous layer extracted (3 x 50 ml) with ether. The ether solutions were combined and dried over anhydrous MgSO₄. A vacuum distillation (65°C/0.05 mm) yielded the desired product.

**[¹⁴C]HOCH₂SiMe₂OH (Hydroxymethyltrimethylsilanol).** Urine containing MM metabolites was subjected to fractionation by HPLC. The fraction eluting during the time interval of 25.5 to 28.0 min that contained [¹⁴C]HOCH₂SiMe₂OH was collected from repeat injections. The individual fractions were combined. A 500-µl aliquot of the combined fraction was placed in a 7-ml vial with Teflon-lined screw cap. A 10% HCl (20 µl) was added, and the contents were shaken for 2 h in a water bath maintained at 50°C to generate the [¹⁴C]HOCH₂SiMe₂OH in aqueous solution. Unlabeled HOCH₂SiMe₂OH was synthesized by hydrolysis at 37°C for an hour of HOCH₂SiMe₂OSiMe₂CH₂OH (50 µl) with rat liver microsomes (50 µl) and NADPH (2 mM) in a pH 7.7 phosphate buffer (800 µl) containing MgCl₂. Product was extracted with 1 ml of THF in presence of NaCl (2 g). GC-MS analysis showed the formation of desired product.

**[¹⁴C]Me₃SiOSiMe₂CH₂OH (Hydroxymethylpentamethyldisiloxane).** MM (300 µmol) was added to the aqueous solution of [¹⁴C]HOCH₂SiMe₂OH synthesized above, and the contents were shaken at ambient temperature for 18 h using the Eberbach horizontal shaker. The sample was centrifuged for 4 min, and the MM layer was collected and treated with anhydrous Na₂CO₃. HPLC analysis of the MM solution showed that the product [¹⁴C]Me₃SiOSiMe₂CH₂OH eluted at 39.1 min. GC-MS analysis confirmed its presence. The same product was also obtained shaking (3.5 h) HOCH₂SiMe₂OSiMe₂CH₂OH (50 µl) and [¹⁴C]Me₃SiOSiMe₂ (200 µl) in the presence of CF₃SO₂H. The product was extracted with ether and neutralized with anhydrous Na₂CO₃.

**Unlabeled Me₃SiOSiMe₂CH₂OH.** Diethyl ether (250 ml) and potassium trimethylsilanolate (32 g) were placed in a 500-ml round bottom flask equipped with an addition funnel and a magnetic stir bar. Chloromethyltrimethylchlorosilane (35.5 g) was added dropwise. The reaction mixture was stirred overnight at room temperature. It was filtered to remove the solid by-product. The filtrate was collected and washed with water until the wash water was neutral. The filtrate was distilled to obtain chloromethylpentamethyldisiloxane (20 g). The latter (18.07 g) was added to Me₃SiOSiMe₂CH₂OAc (followed by the procedure described above for HOCH₂SiMe₂OSiMe₂CH₂OH) using potassium acetate (9.22 g), potassium iodide (200 mg), and glacial acetic acid (20 ml). The ether extract dried over anhydrous MgSO₄ was distilled (42°C/4 mm) to obtain the pure acetate. Using 5.6 g of the acetoxy derivative thus prepared, 1.1 g of NaBH₄ in 5 ml THF and 5.15 g of BF₃·THF (a 3 M solution in THF from Aldrich Chemical Co.), the procedure described above for HOCH₂SiMe₂OSiMe₂CH₂OH was followed to obtain the desired Me₃SiOSiMe₂CH₂OH.

**[¹⁴C]Me₃SiOH (Trimethylsilanol).** A 250-µl solution of [¹⁴C]Me₃SiOSiMe₂OH, in acetonitrile was placed in a 2-ml Nalgene vial with a screw cap. A 10% (w/w) HCl solution (500 µl) was added. The contents were shaken overnight for 14 h. A 200-µl aliquot of the acidic solution of [¹⁴C]Me₃SiOH was then neutralized with anhydrous Na₂CO₃. The product eluted at 31.90 min by HPLC.

**[¹⁴C]Me₃SiOSiMe₂OH (Pentamethyldisiloxane).** HOCH₂SiMe₂OH (1,1,3,3-Tetramethyldisiloxane) (45 mg solution in unlabeled MM), and 10% HCl (10 ml) were placed in a 20-ml scintillation vial and shaken at room temperature for 24 h using a horizontal shaker. The aqueous phase was discarded. The organic phase was washed with water until wash water was neutral. The reaction mixture was placed in a 10 ml beaker and while stirring, a slurry of 92 mg of 10% Pd on charcoal in 1 ml of water was added. It was filtered, and the filtrate was subjected to fractionation by HPLC. The fraction eluting at retention time 38.7 min was collected from repeat injections. The individual fractions were combined.

**Unlabeled Me₃SiOSiMe₂OH.** Water (803 ml) was placed in a 3-liter round-bottomed flask equipped with a magnetic stir bar and an addition funnel. The flask was cooled to 0°C. Water was vigorously stirred, and a mixture of Me₂SiCl (120 g, 0.904 mol) and Me₃SiCH₂Cl (209 g) was added slowly via the addition funnel. The temperature of the reaction mixture was maintained below 10°C. Following the addition, the stirring was continued overnight. The contents were allowed to warm to ambient temperature. The organic layer was separated, washed with water until the wash water was neutral to a pH paper. The solution was dried with anhydrous Na₂SO₄ overnight and distilled using a spinning band column to obtain the intermediate Me₃SiOSiMe₂H. A slurry of 10% palladium on carbon (0.35 g) in 3 ml of dioxane was placed in a 500-ml beaker and stirred using a magnetic stir bar. The slurry Me₃SiOSiMe₂H (50 g) was added slowly, and the temperature of the reaction mixture was maintained at room temperature by cooling the beaker in a water bath. After the brisk effervescence ceased, the beaker was covered with aluminum foil and the stirring continued overnight. The mixture was stirred with anhydrous Na₂SO₄.
extraction efficiency for MM metabolites from urine was determined also quite efficient in extracting metabolites of MM from urine. The prath et al., 1998), we have experimentally determined that THF was urinary metabolites of silicones has already been established (Vara-GC-MS, the metabolites were first extracted into an organic solvent. Unlike the Si-OH functional endogenous materials in the extracts. Unlike the Si-OH functional CH2 OH), which are readily soluble in dichloromethane, the latter was also used on fresh aliquots of samples for extraction. Use of methylene chloride significantly reduced the presence of endogenous materials in the extracts. Unlike the Si-OH functional functions (\(\text{CH}_2 \text{OH}\)), which are readily soluble in dichloromethane, the latter was also used on fresh aliquots of samples for extraction. Use of methylene chloride significantly reduced the presence of endogenous materials in the extracts. Unlike the Si-OH functional

Results

Metabolites of MM. \textit{HPLC profile of MM metabolite}. Using a radioisotope detector, HPLC analyses of the urine samples (from oral as well as i.v.-dosed rats) containing metabolites of MM revealed the presence of several metabolites. For the purpose of illustration, the profile obtained from one of the rats administered orally with \(^{14}\text{C}\text{MM}\) is shown in Fig. 1. There were some minor variations with respect to the presence of trace levels of metabolites in the HPLC profiles among rats administered MM by the same route as well as between those administered MM via different routes. In spite of the variations, there were 5 metabolites that were commonly present in all samples. These were the metabolites eluting at retention times centered around 4.0, 13.10, 24.2, 31.9, and 39.0 min. These metabolites constituted 72 to 74% and 80 to 85% of the total radioactivity eluted in HPLC for the oral and intravenous routes, respectively. The major focus of this work was to establish the structure of these common metabolites. Other metabolites revealed from GC-MS analyses, the retention times of which were not assigned in HPLC, are also presented. The HPLC and GC-MS retention times of the metabolites are compiled in Table 1. The total radioactivity of the metabolites eluted in HPLC from a known volume of urine sample accounted for essentially 100% of the activity measured for the same volume of urine by liquid scintillation.

Identification of MM metabolites\(^2\). For structure elucidation by GC-MS, the metabolites were first extracted into an organic solvent. Extraction was performed using THF as well as methylene chloride. Although the utility of THF as an efficient solvent for extraction of urinary metabolites of silicones has already been established (Varaprath et al., 1998), we have experimentally determined that THF was also quite efficient in extracting metabolites of MM from urine. The extraction efficiency for MM metabolites from urine was determined to be 97.7 ± 0.3%. However, since a preliminary investigation revealed the presence of several metabolites containing hydroxymethyl functions (\(-\text{CH}_2\text{OH}\)), which are readily soluble in dichloromethane, the latter was also used on fresh aliquots of samples for extraction. Use of methylene chloride significantly reduced the presence of endogenous materials in the extracts. Unlike the Si-OH functional

metabolites, the \(-\text{CH}_2\text{OH}\) functional metabolites readily elute by GC. Therefore the extracts were analyzed as such by GC-MS, in addition to analyzing them following trimethylsilyl derivatization. The details on the structural elucidation aspects for the individual metabolite identified are given below.

\textit{Metabolite HOCH}_2\text{SiMe}_2\text{OSiMe}_2\text{CH}_2\text{OH} \ (1,3\text{-bis(\text{hydroxymethyl}) tetramethyldisiloxane}). GC-MS profile of a methylene chloride extract of the urine sample revealed a component of \(m/z\) 163 (M\(-\text{CH}_2\text{OH}\)) at a retention time of 7.6 min. It was established from HPLC fractionation of the urine sample, followed by methylene chloride extraction and GC-MS analysis, that this component in urine eluted at 24.6 min. The GC-MS retention time and spectral characteristic of this component matched with the synthetic material (Fig. 2). The trimethylsilyl derivatives of the synthetic standard and of the 24.6-min metabolite fraction also matched with respect to retention time and mass fragmentation patterns. The trimethylsilyl derivatization gave rise to mass \(m/z\) 323 (M\(-\text{CH}_3\)) expected for the fully derivatized molecule of the structure \(\text{Me}_3\text{SiOH} \text{OSiMe}_2\text{CH}_2\text{OH}\) (mol. wt. = 338).

It should be pointed out that another metabolite \(\text{Me}_3\text{SiOSiMe}_2\text{CH}_2\text{OH}\) (to be discussed later) also showed a mass \(m/z\) 163. However, its fragmentation pattern and retention time were quite different.

\textit{Metabolite HOSiMe}_2\text{CH}_2\text{OH} \ (\text{hydroxymethyldimethylsilanol}). The component eluting in HPLC at retention time 4.0 min in Fig. 1 was assigned the structure HOSiMe$_2$CH$_2$OH. Oxidation of one of the methyl groups in MM followed by hydrolysis of Si-O-Si linkage can give rise to HOSiMe$_2$CH$_2$OH. Its presence was confirmed by its synthesis by two different routes.

In one, the metabolite \(^{14}\text{C}\text{HOCH}_2\text{SiMe}_2\text{OSiMe}_2\text{CH}_2\text{OH}\), the presence of which was confirmed (see discussion above), was isolated from urine and hydrolyzed with hydrochloric acid. The HPLC retention time (3.9 min) of the product \(^{14}\text{C}\text{HOSiMe}_2\text{CH}_2\text{OH}\) matched with one of the metabolite components in the urine sample. The HPLC fraction at 3.9 min was then subjected to derivatization with MM. GC-MS analysis showed the presence of Me$_3$SiOSiMe$_2$CH$_2$OH (\(m/z\) 163 from M-CH$_3$ and \(m/z\) 147 from M-CH$_2$OH) expected from HOSiMe$_2$CH$_2$OH. The mass spectral characteristics of this derivative (Fig. 3) matched with that of the authentic material.

During an attempted enzyme-mediated oxidation reaction of HOCH$_2$SiMe$_2$OSiMe$_2$CH$_2$OH using rat liver microsomes in the presence of NADPH, it was observed that the hydrolysis product HOCH$_2$SiMe$_2$OH was being formed. The material extracted into THF and analyzed by GC-MS eluted at 3.76 min and gave rise to the expected fragments \(m/z\) 91 and 75 for M-CH$_3$ and M-CH$_2$OH, respectively. Partial protection with trimethylsilyl group gave rise to

\(^2\) HPLC and GC-MS of standards were not included in this manuscript. They will be available upon request.
Me$_3$SiOSiMe$_2$CH$_2$OH, which matched in GC-MS characteristics to the component in urine.

**Metabolite HOSiMe$_2$OH (dimethylsilanediol).** An authentic sample of $[^{14}$C]HOSiMe$_2$OH was available and its HPLC retention time (13.1 min) matched with one of the metabolite components in urine sample. When the urine sample was fortified with this standard and analyzed by HPLC, coelution at 13.1 min was observed. For structural analysis, the component in urine eluting at 13.1 min was collected by HPLC fractionation and subjected to trimethylsilyl derivatization with MM in presence of a catalytic amount of CF$_3$SO$_3$H. The organic layer containing the derivative was analyzed by GC-MS following neutralization with anhydrous Na$_2$CO$_3$ and it showed the expected formation of Me$_3$SiOSiMe$_3$OSiMe$_3$ (m/z 221 from M-CH$_3$). The retention time and mass spectral characteristics of the latter matched to a commercially available standard thus confirming the presence of the metabolite HOSiMe$_2$OH in urine.

**Metabolite Me$_3$SiOH (trimethylsilanol).** The component in urine eluting at 31.9 min in HPLC was unambiguously assigned the structure as $[^{14}$C]Me$_3$SiOH by comparison with a synthetic standard. An acetonitrile solution of $[^{14}$C]Me$_3$SiOSiMe$_2$OH was hydrolyzed using a 10% HCl solution and then neutralized with solid sodium carbonate to obtain $[^{14}$C]Me$_3$SiOH as an aqueous solution. This synthetic material eluted at 31.9 min and matched to the component in urine. The metabolite Me$_3$SiOH is highly volatile, and due to its loss during fractionation and subjected to trimethylsilyl derivatization with MM in presence of a catalytic amount of CF$_3$SO$_3$H. The organic layer containing the derivative was analyzed by GC-MS following neutralization with anhydrous Na$_2$CO$_3$ and it showed the expected formation of Me$_3$SiOSiMe$_2$OSiMe$_2$CH$_2$OH based on the mass fragments 149 (M-CH$_3$) and 133 (M-OCH$_3$). Comparison of its GC-MS data (Fig. 4) with an authentic sample synthesized confirmed the structure of this metabolite in urine.

To determine where this component eluted in HPLC, $[^{14}$C]-labeled Me$_3$SiOSiMe$_2$OH was synthesized. The synthesis yielded several by-products. However, by HPLC fractionation and GC-MS analysis, it was established that the desired $[^{14}$C]Me$_3$SiOSiMe$_2$OH eluted near 38.7 min. This is in close proximity (39 min) to the component present in urine.

**Metabolite Me$_3$SiOSiMe$_2$CH$_2$OH (hydroxymethylpentamethyldisiloxane).** The component eluting at 4.84 min in GC-MS analysis of the methylene chloride extract of urine was assigned the structure Me$_3$SiOSiMe$_2$CH$_2$OH based on the mass fragments 163 (M-CH$_3$) and 147 (M-CH$_2$OH) expected for the siloxane moiety of mass 178. The GC-MS data were identical to that in Fig. 3 and also matched with that of an authentic sample synthesized thus confirming the structure of this metabolite present in urine.

To determine the retention time of the component in HPLC, the $[^{14}$C]-labeled Me$_3$SiOSiMe$_2$CH$_2$OH was synthesized. The structure of the synthetic material was verified by GC-MS and then subjected to HPLC analysis. The HPLC retention time was determined to be 39.3 min, which matched to the component present in urine. Incidentally, the $[^{14}$C]Me$_3$SiOSiMe$_2$CH$_2$OH synthesized from another route in connection with confirming the structure of the metabolite HOSiMe$_2$OH (see discussion above) also further substantiated the presence of this metabolite.

**Other Metabolites of MM Revealed by GC-MS Analysis.** There were a few minor metabolites for which HPLC data comparisons could not be made due to lack of availability of $[^{14}$C]-labeled standards. However, their presence was apparent from the GC-MS analysis. The
presence of these metabolites was confirmed by comparison of the GC-MS data with the synthetic materials that were not labeled.

Metabolite HOSiMe₂OSiMe₂CH₂OH (3-hydroxymethyl-1,1,3,3-tetramethyldisiloxanol). From the list of the confirmed metabolites that indicated the presence of −CH₂OH and −OH functions, it was logical to expect a metabolite of the structure HOSiMe₂OSiMe₂CH₂OH to be formed from MM with Si-O-Si linkage intact. THF extract of urine did show by GC-MS a component eluting at 6.41 min with mass fragments 165 (M-CH₃) and 149 (M-CH₂OH) expected for this siloxane moiety of mol. wt. 180. The synthetic material eluted at 6.37 min, and its fragmentation pattern was identical to the 6.41-min component in urine (Fig. 5).

Metabolite 2,2,5,5-tetramethyl-2,5-disila-1,3-dioxalane. GC-MS data on the methylene chloride extract of urine showed a component eluting at 3.52 min of m/z 162. This component was assigned the structure shown below.

![Scheme 1. 2,2,5,5-Tetramethyl-2,5-disila-1,3-dioxalane.](image)

GC-MS analysis of the product mixture from the synthetic procedure (Simmler et al., 1969) showed a product eluting at 3.45 min with m/z 162. The fragmentation characteristic of this material was identical to the component in the methylene chloride extract of urine that eluted at 3.52 min (Fig. 6).

Metabolite 2,2,5,5-tetramethyl-1,4-dioxa-2,5-disilacyclohexane. GC-MS data on the THF extract of urine showed a component eluting at 5.78 min with a mass of 176. This component was assigned the structure shown below.

![Scheme 2. 2,2,5,5-Tetramethyl-1,4-dioxa-2,5-disilacyclohexane.](image)

The material synthesized following a published procedure (Tacke et al., 1983) yielded a trace amount of the product, which eluted at 5.6 min. The fragmentation pattern of the latter was quite similar to the component found in the urine sample (Fig. 7).

Control urine samples collected from rats that were not exposed to [¹⁴C]MM were also subjected to extraction, derivatization, and GC-MS analysis. The data from these experiments verified the absence of the metabolites in solvents and derivatization agents.

**Metabolites of D₅**

**HPLC profile of D₅ metabolites.** The HPLC radiochromatogram for D₅ metabolites is shown in Fig. 8. It revealed two major metabolites (A and C) and at least three minor metabolites (B, D, and E). Combined, the two major components A and C constituted ~75% of the total radioactivity and B, D, and E accounted for the rest. The total radioactivity of the metabolites eluted in HPLC from a known volume of urine sample accounted for essentially 100% of the activity measured for the same volume of urine by liquid scintillation. It should be pointed out that although HPLC showed essentially five metabolites, there were other metabolites that were revealed by GC-MS but present at levels below detection by HPLC.

**Identification of D₅ metabolites.** As far as the major metabolites were concerned, the HPLC profile of D₅ metabolites was essentially identical to that reported by the author for D₄ in rat urine (Varaprath et al., 1999). The HPLC components of identical retention times were therefore expected to have the corresponding structures. The methodology used in D₄ metabolites identification was applied to the case of D₅. The hydroxy groups of the metabolites were protected with trimethylsilyl groups, and structures of the resultant derivatives were assigned by comparison to that of the standards. The GC-MS retention times of the TMS derivatives of all the metabolites and their respective major mass (m/z) fragments are compiled in Table 2.

There were two additional metabolites in the case of D₅ that were not detected for D₄. These were m/z 429 (Fig. 9) and m/z 443 (Fig. 10). Presence of m/z 429 was indicative of the structure D₅D'OSiMe₃ (Fig. 9).
An authentic sample of D₄D/OSiMe₃ was prepared by trimethylsilylation of an unlabeled standard of D₄D/H₁₁₀₃₂OH with BSTFA. Its GC-MS characteristics in terms of retention time and mass spectra were in agreement with that observed for the component extracted from the urine. The data thus confirmed the presence of the metabolite D₄D/H₁₁₀₃₂OH in urine.

With respect to m/z 443 seen in GC-MS (Figure not included), there were four isomers. The components of retention times 9.25, 9.37, and 9.47 were assigned Me₂Si(OSiMe₃)O-Si(OSiMe₃)₃, MeSi(OSiMe₃)₂OSi(OSiMe₃)₂Me, and MD₄M based on the rationale described in case of D₄ metabolism (Varaprath et al., 1999). The fourth isomer of the same mass of 443 eluting at 9.58 min was therefore assigned the structure D₄D/CH₂OSiMe₃. The latter then suggested the presence of D₄D/CH₂OH in urine. The fragmentation pattern for this component is shown in Fig. 10.

Control urine samples collected from rats that were not exposed to [¹⁴C]D₅ were also subjected to extraction, derivatization, and GC-MS analysis. The control experiments verified the absence of the potential metabolites in solvents and derivatization agents.

**Discussion**

Since potential human exposure to MM and D₅ can result at the workplace during the manufacturing process, as well as through the normal use of consumer products that contain them, comprehensive pharmacokinetic studies combined with toxicity studies will be helpful in characterizing the risk, if any, to human populations exposed to MM and D₅. Preliminary pharmacokinetic data following a single inhalation exposure of 5000 ppm [¹⁴C]MM to male and female Fischer F344 rats (unpublished data) has indicated that most of the recovered radioactivity was in urine (~40%) and expired volatiles (~50%), with minor amounts, either excreted via CO₂ (1–2%) and feces (1–2%) or remaining in the carcass (~5%) 168-h post exposure.

In the case of D₅, following a single inhalation exposure of 160 ppm [¹⁴C]D₅ to male and female Fischer F344 rats, most of the recovered radioactivity (unpublished data) was found in urine (~30%) or excreted in feces (~50%). The remaining radioactivity was either excreted via expired volatiles (4–10%), CO₂ (5%), or remained in the carcass (10%) after 168-h post exposure. The disposition of radioactivity for both MM and D₅ following inhalation exposure is similar to that observed with other siloxanes (Bennett and Aberg, 1975; Plotzke et al., 2000; Andersen et al., 2001).

More comprehensive mass balance studies and pharmacokinetics analysis on MM and D₅ from various routes of administration are currently underway and will be published later. The objective of the work presented in this paper is to identify the major metabolites of MM and D₅ in urine collected from rats following exposure, since metabolism plays a significant role in elimination of these materials from the body.
As demonstrated by the HPLC profile, with both MM and D5 the radioactivity that was excreted in the urine contained only polar metabolites and no parent material. It was apparent from the results that except for the commonly present dimethylsilanediol, the urinary metabolites of the linear siloxane MM were different from those obtained for cyclic siloxane D4. Presence of a hydroxymethyl group, the primary oxidation product of the methyl group, was found in most of the metabolites of MM. The bis(hydroxymethyl) metabolite with Si-O-Si bond in tact was the major metabolite. Some metabolites, in addition, have hydroxy groups. With D5 on the other hand, presence of multiples of hydroxy groups was a common feature. Metabolites containing –CH2OH groups were absent with the exception of the presence of D4D3CH2OH at trace level. The reasons for the meager presence of –CH2OH functional metabolites in D4 are not clear, but may be related to factors such as relative stability when the –CH2OH is on a ring system and solubility of the metabolites. In a smaller cyclic siloxane D5 (Varaprauth et al., 1999), the corresponding metabolite D5D4CH2OH was not detected.

The possible pathways for the formation of the metabolites of decamethylcyclopentasiloxane in Fischer 344 rat urine.

Fig. 12. Possible pathways for the formation of the metabolites of decamethylcyclopentasiloxane in Fischer 344 rat urine.

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The possible pathways for the formation of the metabolites of MM and D5 are shown in Figs. 11 and 12. Metabolites such as 2,2,5,5-tetramethyl-2,5-disila-1,3-dioxalane and 2,2,5,5-tetramethyl-1,4-dioxo-2,5-disilacyclohexane were unexpected. Due to lack of availability of 14C-labeled standards, it was not established that these metabolites were actually present in urine. It is quite possible that the mixed ether linkage (CH2-O-Si) make these metabolites quite water soluble and thus contribute to their formation and subsequent elimination in urine. As shown in the mechanistic scheme, these metabolites could well be artifacts arising from inadvertent cyclization, at the injection port of the GC-MS, of the corresponding linear metabolites containing –OH and CH2OH present in urine. The presence of metabolites such as dimethylsilanediol in MM and methylsilanetriol in D5 clearly established that some demethylation occurs at the silicon-methyl bond.

References


