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

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ABSTRACT:

Hexamethyldisiloxane (MM or HMDS) and decamethylcylclopenta-siloxane (D_5) 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 investigations, major metabolites of MM and D_5 were identified in urine collected from Fischer (F-344) rats administered [^{14}C]MM and [^{14}C]D_5 orally and via intravenous injection. The metabolite profiles were obtained using a high-pressure liquid chromatography (HPLC) system equipped with a radioisotope detector. The metabolite elution was carried out on a C_{18} 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 ^{14}C-labeled metabolite standards. The following are among the major metabolites identified in the case of MM: $Me_2Si(OH)_2$, $HOMe_2SiCH_2OH$, $HOCH_2Me_2SiOSiMe_2CH_2OH$, $HOMe_2SiOSiMe_2CH_2$-OH, $HOCH_2Me_2SiOSiMe_3$, and Me_3SiOH. The metabolites of D_5 are as follows: $Me_2Si(OH)_2$, $MeSi(OH)_3$, $MeSi(OH)_2OSi(OH)_3$, $MeSi(OH)_2OSi(OH)_2Me$, $MeSi(OH)_2OSi(OH)Me_2$, $Me_2Si(OH)OSi(OH)Me_2$, $Me_2Si(OH)OSiMe_2OSi(OH)Me_2$, nonamethylcyclopentasi-loxanol, and hydroxymethylnonamethylcyclopentasiloxane. No parent MM or D_5 was present in urine The presence of certain metabolites such as $HOMe_2SiCH_2OH$ and $Me_2Si(OH)_2$ in MM and D_5, 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 $Me_2Si(OH)_2$. Mechanistic pathways for the formation of the metabolites were proposed.

Hexamethyldisiloxane (MM, HMDS[1]), the smallest member of the polydimethylsiloxane polymers, and decamethylcyclopentasiloxane (D_5), 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 (Flanningam, 1986). D_5 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 D_5 are 930 and 17 ppb, respectively (Varaprath et al., 1996). The primary use of MM and D_5 is as intermediates in the manufacturing of high molecular weight siloxane polymers. MM and D_5 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 D_5 in various product formulations necessitated conducting chemical and environmental fate/effects tests of them.

Potential human exposure to MM and D_5 can result at the work place 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 (D_4), a homolog of D_5 had been extensively studied. In rodents, inhalation exposure to D_4 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 D_5 in biological systems. In a 13-week subchronic MM whole-body inhalation, renal histopathology consis-

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[1] Abbreviations used are: MM, (HMDS) hexamethyldisiloxane; D_5, decamethylcyclopentasiloxane; D_4, octamethylcyclotetrasiloxane; HPLC, high performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; THF, tetrahydrofuran; BSTFA, bis(trimethylsilyl)trifluoroacetamide; BF_3-THF, borontrifluoride-THF; SPE, saline/phosphate/EDTA; and the following abbreviations are based on General Electric's siloxane notation [Hurd (1946)]: M, $Me_3SiO_{1/2}$; D, $-Me_2SiO_{2/2}$; and D′, $MeRSiO_{2/2}$, where R is any group as indicated.

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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 D$_5$ 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 D$_5$ 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 rates at high concentrations (224 ppm) of D$_5$.

A comprehensive program has been initiated to assess the kinetics, metabolism and toxicity of MM and D$_5$ in rats after relevant routes of exposure. To aid in the pharmacokinetic investigations, identification of MM and D$_5$ 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 installed with a flow liquid 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 C$_{18}$ Alltima column (4.6 × 250 cm and 5 μm from Alltech 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 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-μ 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, Pittsburgh, PA). [^{14}C]MM and [^{14}C]D$_5$ used in animal exposures were custom synthesized at Wizard Laboratories (Sacramento, CA), and labeling was done at random. Purities of [^{14}C]MM and [^{14}C]D$_5$, as determined by GC and HPLC were ~99%.

Animals administered [^{14}C]MM and [^{14}C]D$_5$ were female Fischer-344 rats, CDF(F-344)/CrlBr, approximately 7 to 10 weeks of age at the time of administration. The animals were purchased from Charles River Laboratories (Raleigh, NC).

Concentration of samples (urine or HPLC fractions of the metabolites) was carried out using the Speed Vac system consisting of the following components: refrigerated condensation trap, Model RT 100; chemical trap, Model SC 120; rotary vacuum pump, Model VP 100; Speed Vac concentrator, Model SC 100. The system was purchased from Savant Instrument, Inc. (Holbrook, NY).

Extraction of urine metabolites was conducted with either reagent grade tetrahydrofuran (THF) and/or dichloromethane (CH$_2$Cl$_2$) purchased from Aldrich Chemical Co. (Milwaukee, WI). For HPLC analysis, HPLC grade acetonitrile purchased from Fisher Scientific Co. (Fair Lawn, NJ) and Milli-Q

water generated using Ultrapure Water Systems (Millipore Corporation, Bedford, MA) were used. For liquid scintillation counting, the cocktails Hionic-Fluor and Ultima-Gold obtained from PerkinElmer Life Sciences were used.

End capping (protection) of the hydroxy functions of the metabolites was performed using bis(trimethylsilyl)trifluoroacetamide (BSTFA) purchased from Aldrich Chemical Co. or specially purified hexamethyldisiloxane (99.9%, obtained by spinning band distillation of Dow Corning OS-10). Trimethylchlorosilane (Me$_3$SiCl) and dimethylchlorosilane (Me$_2$SiClH) and chloromethyldimethylchlorosilane (ClCH$_2$SiMe$_2$Cl), 1,3-bis(chloromethyl)-1,1,3,3-tetramethydisiloxane (ClCH$_2$SiMe$_2$OSiMe$_2$CH$_2$Cl) were purchased from Gelest Inc. (Morrisville, PA). Anhydrous sodium sulfate, sodium carbonate, sodium bicarbonate, sodium borohydride, potassium iodide, potassium acetate, potassium trimethylsilanolate, potassium phosphate-monobasic, potassium phosphate-dibasic, magnesium chloride, lithium aluminum hydride (LiAlH$_4$), n-butyllithium (n-BuLi, 2.5 M solution in hexane), borontrifluoride-THF (BF$_3$-THF, a 3 M solution in THF), trifluoromethanesulfonic acid (CF$_3$SO$_3$H), glacial acetic acid, toluene, triethylamine, diethyl ether, and 10% palladium on charcoal were purchased from Aldrich Chemical Co.. Liver microsomes from phenobarbital-treated male Sprague-Dawley rats were purchased from Xeno-Tech (Kansas City, KS). NADPH was purchased from Roche Diagnostics (Indianapolis, IN).

Dose Administration and Sample Collection. Urine samples used in the metabolite investigation were from female rats administered [^{14}C]MM by two routes, oral gavage and i.v.

Animals administered [^{14}C]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 [^{14}C]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 (150g each) received 38.8 and 82 mg, respectively, of [^{14}C]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, canulated (jugular vein) female rats (141 and 138 g) were used. The rats received a nominal dose of 80 mg/kg [^{14}C]MM as an emulsion. [^{14}C]MM was emulsified (v/v) as follows: 7 parts saline, 1 part Emulphor EL-620/liter, 1 part ethanol, and 1 part [^{14}C]MM. The radioactivity of the emulsion was determined to be 0.25 μCi/mg. The rats received 196.1 and 153.6 mg of the emulsion, respectively.

In the case of D$_5$, two female Fischer 344 rats were each administered [^{14}C]D$_5$ orally. Original [^{14}C]D$_5$ (specific activity 25.08 mCi/mmol) was diluted with unlabeled D$_5$ to a specific activity of 17.377 mCi/mmol. The rats received 7.6 and 8.7 mg, respectively, of the diluted [^{14}C]D$_5$.

After dosing, all rats were housed in glass metabolism cage to facilitate the collection of urine. Urine samples were collected over a 24-h period following the exposure into 20 ml of scintillation vials that were kept frozen with dry ice. 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-mg 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 CH$_2$Cl$_2$ was carefully removed with a Pasteur pipette and collected in a clean 20-ml vial. Fresh CH$_2$Cl$_2$ 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 residues. The extracts were combined, treated with anhydrous MgSO$_4$ to remove water, vortex mixed (4–5 min), and finally centrifuged (5 min) to obtain a clear, dry CH$_2$Cl$_2$ extract of the metabolites. The 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 D$_5$ Metabolites. Since most of the D$_5$ metabolites are not soluble in CH$_2$Cl$_2$, the extractions were performed with THF only. Also, in an effort to increase the efficiency, solid phase extractions (Varaprath 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 after 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 D_5 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 eluate using fresh SPE cartridges each time. The THF eluates from all SPE runs were combined, dried with 3 g of anhydrous $MgSO_4$ 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 CH_2Cl_2 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 CF_3SO_3H 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 trimethyl-silyl 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 $[^{14}C]Me_2Si(OH)_2$ that 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 D_5 with the exception of nonamethylcyclopentasiloxanol and hydroxymethylnonamethylcyclopentasiloxane were synthesized following the literature procedure (Varaprath et al., 1999). Nonamethylcyclopentasiloxanol was available internally at Dow Corning Corp. (Midland, MI). Hydroxymethylnonamethylcyclopentasiloxane was inferred by GC-MS analysis.

Unlabeled $HOCH_2SiMe_2OSiMe_2CH_2OH$ (1,3-bis(Hydroxymethyl)tetramethyldisiloxane). The chloro precursor $ClCH_2SiMe_2OSiMe_2CH_2Cl$ (23.1 g) was placed in a one-necked 250-ml round bottomed 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 iodide were added. The contents were heated to a gentle reflux for 48 h using an oil bath, and then the flask was cooled to room temperature. The solution was transferred to a 500-ml separatory funnel. The contents were washed with water (3 × 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%) $AcOCH_2SiMe_2OSiMe_2CH_2OAc$ was obtained by distillation at 85°C/1.6 mm Hg.

The acetate, $AcOCH_2SiMe_2OSiMe_2CH_2OAc$ (13.9g, 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 BF_3-THF complex (10.3 g). The addition funnel was then replaced with a water condenser, and the contents were then heated at reflux temperature 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 × 50 ml) with ether. The ether solutions were combined and dried over anhydrous $MgSO_4$. A vacuum distillation (65°C/0.05 mm) yielded the desired product.

$[^{14}C]HOCH_2SiMe_2OH$ (Hydroxymethyldimethylsilanol). Urine contain-

ing MM metabolites was subjected to fractionation by HPLC. The fraction eluting during the time interval of 25.5 to 28.0 min that contained $[^{14}C]HOCH_2SiMe_2$-O-$SiMe_2CH_2OH$ 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 $[^{14}C]$ $HOCH_2SiMe_2OH$ in aqueous solution. Unlabeled $HOCH_2SiMe_2OH$ was synthesized by hydrolysis at 37°C for an hour of $HOCH_2SiMe_2OSiMe_2CH_2OH$ (50 μl) with rat liver microsomes (50 μl) and NADPH (2 mM) in a pH 7.7 phosphate buffer (800 μl) containing $MgCl_2$. Product was extracted with 1 ml of THF in presence of NaCl (2 g). GC-MS analysis showed the formation of desired product.

$[^{14}C]Me_3SiOSiMe_2CH_2OH$ (Hydroxymethylpentamethyldisiloxane). MM (300 μl) was added to the aqueous solution of $[^{14}C]$-$HOCH_2SiMe_2OH$ 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 Na_2CO_3. HPLC analysis of the MM solution showed that the product $[^{14}C]Me_3SiOSiMe_2CH_2OH$ eluted at 39.1 min. GC-MS analysis confirmed its presence. The same product was also obtained shaking (3.5 h) $HOCH_2SiMe_2OSiMe_2CH_2OH$ (50 μl) and $[^{14}C]Me_3SiOSiMe_3$ (200 μl) in the presence of CF_3SO_3H. The product was extracted with ether and neutralized with anhydrous Na_2CO_3.

Unlabeled $Me_3SiOSiMe_2CH_2OH$. Diethyl ether (250 ml) and potassium trimethylsilanolate (32 g) were placed in a 500-ml round bottomed flask equipped with an addition funnel and a magnetic stir bar. Chloromethyldimethylchlorosilane (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 chloromethylpenatmethyldisiloxane (20 g). The latter (18.07 g) was converted to $Me_3SiOSiMe_2CH_2OCOCH_3$ (following the procedure described above for $HOCH_2SiMe_2OSiMe_2CH_2OH$) using potassium acetate (9.22 g), potassium iodide (200 mg), and glacial acetic acid (20 ml). The ether extract dried over anhydrous $MgSO_4$ 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 $NaBH_4$ in 5 ml THF and 5.15 g of BF_3-THF (a 3M solution in THF from Aldrich Chemical Co.), the procedure described above for $HOCH_2SiMe_2OSiMe_2CH_2OH$ was followed to obtain the desired $Me_3SiOSiMe_2CH_2OH$.

$[^{14}C]Me_3SiOH$ (Trimethylsilanol). A 250-μl solution of $[^{14}C]Me_3SiOSiMe_3$ 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 $[^{14}C]Me_3SiOH$ was then neutralized with anhydrous Na_2CO_3. The product eluted at 31.90 min by HPLC.

$[^{14}C]Me_3SiOSiMe_2OH$ (Pentamethyldisiloxanol). 1,1,3,3-Tetramethyldisiloxane (166 mg), $[^{14}C]Me_3SiOSiMe_3$ (405 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 $Me_3SiOSiMe_2OH$. 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 Me_3SiCl (120 g, 0.904 mol) and Me_2SiClH (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 for 4 h, and 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 Na_2SO_4 overnight and distilled using a spinning band column to obtain the intermediate $Me_3SiOSiMe_2H$. 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 silane $Me_3SiOSiMe_2H$ (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 Na_2SO_4
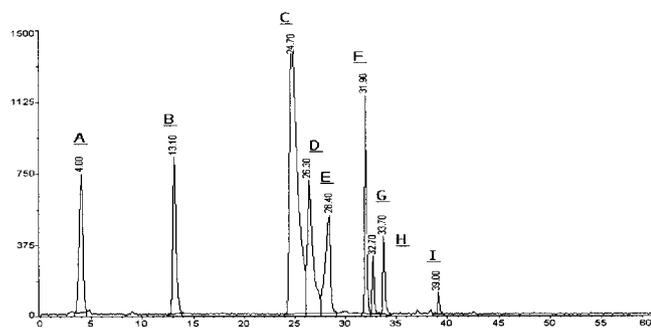
FIG. 1. *HPLC radiochromatogram of the metabolites of hexamethyldisiloxane in Fischer 344 female rat urine.*

A, HOCH$_2$SiMe$_2$OH; B, HOSiMe$_2$OH; C, HOCH$_2$SiMe$_2$OSiMe$_2$CH$_2$OH; F, Me$_3$SiOH; I, Me$_3$SiOSiMe$_2$CH$_2$OH; peaks D, E, G, and H were not assigned due to unavailability of radiolabeled standards.

(2 h) and filtered through a bed of anhydrous MgSO$_4$. The filtrate was subjected to distillation. Pentane and dioxane were removed at 25°C/60 mm Hg and 30°C/32 mm Hg, respectively. The product Me$_3$SiOSiMe$_2$OH was distilled at 54°C/16 mm Hg.

Results

Metabolites of MM. *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}C]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 (−CH$_2$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
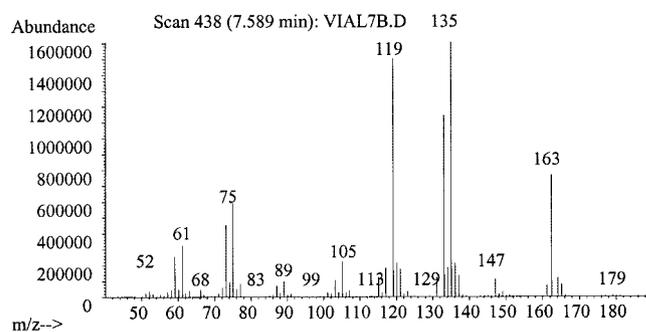
[2] HPLC and GC-MS of standards were not included in this manuscript. They will be available upon request.

FIG. 2. *Mass spectrum of the urinary component eluting at 26.5 min in HPLC identified as HOCH$_2$SiMe$_2$OSiMe$_2$CH$_2$OH.*

m/z fragments are as follows: 179, M-CH$_3$; 163, M-CH$_2$OH; 89, M-OSiMe$_2$CH$_2$OH.

metabolites, the −CH$_2$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.

Metabolite HOCH$_2$SiMe$_2$OSiMe$_2$CH$_2$OH (1,3-bis(hydroxymethyl) tetramethyldisiloxane). GC-MS profile of a methylene chloride extract of the urine sample revealed a component of m/z 163 (M-CH$_2$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-CH$_3$) expected for the fully derivatized molecule of the structure Me$_3$SiOCH$_2$SiMe$_2$OSiMe$_2$CH$_2$OSiMe$_3$ (mol. wt. = 338).

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

Metabolite HOSiMe$_2$CH$_2$OH (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}C]HOCH$_2$SiMe$_2$OSiMe$_2$CH$_2$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}C]HOSiMe$_2$CH$_2$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

TABLE 1

Structures of the metabolites of MM and the comparison of GC-MS and HPLC retention times-synthetic vs. component present in urine

Structure of the Metabolite	HPLC Retention Time: Extracts of Urine	HPLC Retention Time: Synthetic Material	GC-MS Retention Time: Extracts of Urine	GC-MS Retention Time: Synthetic Material
		min		
$HOSiMe_2CH_2OH$	4.0	3.9	N/A	3.76
$HOSiMe_2OH$	13.1	13.1	4.32 (TMS derivative)	4.18 (TMS derivative)
$HOCH_2SiMe_2OSiMe_2CH_2OH$	24.7	N/A	7.59	7.64
Me_3SiOH	31.9	31.8	N/A	2.1
$Me_3SiOSiMe_2OH$	38.3	38.7	3.18	3.17
$Me_3SiOSiMe_2CH_2OH$	39.3	39.1	4.84	4.75
$HOSiMe_2OSiMe_2CH_2OH$	N/A	N/A	6.41	6.38
2,2,5,5-Tetramethyl-2,5-disila-1,3-dioxalane	N/A	N/A	3.52	3.4
2,2,5,5-Tetramethyl-1,4-dioxa-2,5-disilacyclohexane	N/A	N/A	5.78	5.48

N/A, not available; TMS, trimethylsilyl.

$Me_3SiOSiMe_2CH_2OH$, which matched in GC-MS characteristics to the component in urine.

Metabolite $HOSiMe_2OH$ (dimethylsilanediol). An authentic sample of [^{14}C]$HOSiMe_2OH$ 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_3SO_3H. The organic layer containing the derivative was analyzed by GC-MS following neutralization with anhydrous Na_2CO_3 and it showed the expected formation of $Me_3SiOSiMe_2OSiMe_3$ (*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 $HOSiMe_2OH$ in urine.

Metabolite Me_3SiOH (trimethylsilanol). The component in urine eluting at 31.9 min in HPLC was unambiguously assigned the structure as [^{14}C]Me_3SiOH by comparison with a synthetic standard. An acetonitrile solution of [^{14}C]$Me_3SiOSiMe_3$ was hydrolyzed using a 10% HCl solution and then neutralized with solid sodium carbonate to obtain [^{14}C]Me_3SiOH as an aqueous solution. This synthetic material eluted at 31.9 min and matched to the component in urine. The metabolite Me_3SiOH is highly volatile, and due to its loss during concentration, the extracts of urine containing MM metabolites did not reveal its presence in GC-MS analysis.

Metabolite $Me_3SiOSiMe_2OH$ (pentamethyldisiloxanol). Analysis of the methylene chloride extract of urine by GC-MS showed a component eluting at 3.17 min. This was assigned the structure

$Me_3SiOSiMe_2OH$ based on the mass fragments 149 (M-CH$_3$) and 133 (M-CH$_3$O). 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 $Me_3SiOSiMe_2OH$ was synthesized. The synthesis yielded several by-products. However, by HPLC fractionation and GC-MS analysis, it was established that the desired [^{14}C]$Me_3SiOSiMe_2OH$ eluted near 38.7 min. This is in close proximity (39 min) to the component present in urine.

Metabolite $Me_3SiOSiMe_2CH_2OH$ (hydroxymethylpentamethyldisiloxane). The component eluting at 4.84 min in GC-MS analysis of the methylene chloride extract of urine was assigned the structure $Me_3SiOSiMe_2CH_2OH$ 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 $Me_3SiOSiMe_2CH_2OH$ 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]$Me_3SiOSiMe_2CH_2OH$ synthesized from another route in connection with confirming the structure of the metabolite $HOSiMe_2CH_2OH$ (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
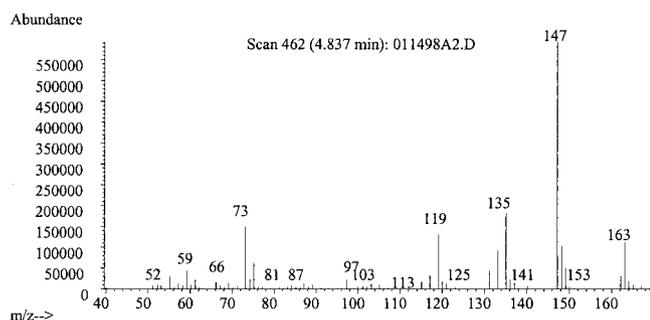

FIG. 3. *Mass spectrum of the urinary component eluting at 4 min in HPLC (identified as HOMe$_2$SiCH$_2$OH) that was protected with a trimethylsilyl group to obtain Me$_3$SiOSiMe$_2$CH$_2$OH.*

m/z fragments are as follows: 163, M-CH$_3$; 147, M-CH$_2$OH; 73, Me$_3$Si.
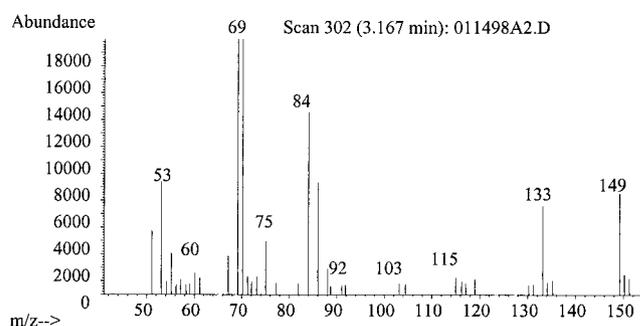

FIG. 4. *Mass spectrum of the component identified as Me$_3$SiOSiMe$_2$OH in CH$_2$Cl$_2$ extract of urine.*

m/z fragments are as follows: 149, M-CH$_3$; 133, M-OCH$_3$; 75, M-OSiMe$_3$.
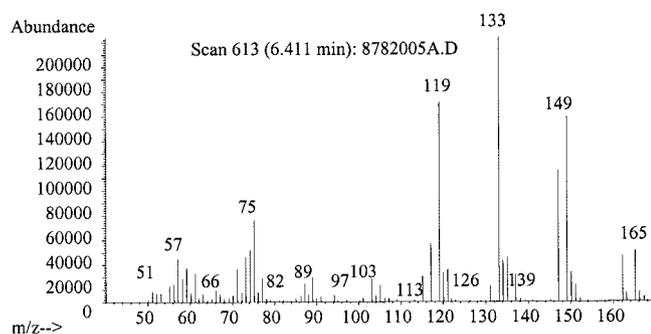
FIG. 5. *Mass spectrum of the component identified as HOMe$_2$SiOSiMe$_2$CH$_2$OH in CH$_2$Cl$_2$ extract of urine.*

m/z fragments are as follows: 165, M-CH$_3$; 149, M-CH$_2$OH; 75, M-OSiMe$_2$CH$_2$OH.

presence of these metabolites was confirmed by comparison of the GC-MS data with the synthetic materials that were not labeled.

Metabolite HOSiMe$_2$OSiMe$_2$CH$_2$OH (3-hydroxymethyl-1,1,3,3 tetramethyldisiloxanol). From the list of the confirmed metabolites that indicated the presence of −CH$_2$OH and −OH functions, it was logical to expect a metabolite of the structure HOSiMe$_2$OSiMe$_2$CH$_2$OH 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-CH$_3$) and 149 (M-CH$_2$OH) 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.
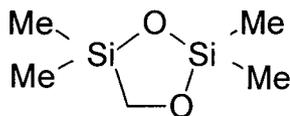
SCHEME 1. *2,2,5,5-Tetramethyl-2,5-disila-1,3-dioxalane.*

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:
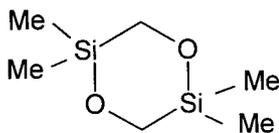
SCHEME 2. *2,2,5,5-Tetramethyl-1,4-dioxa-2,5-disilacyclohexane.*

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).

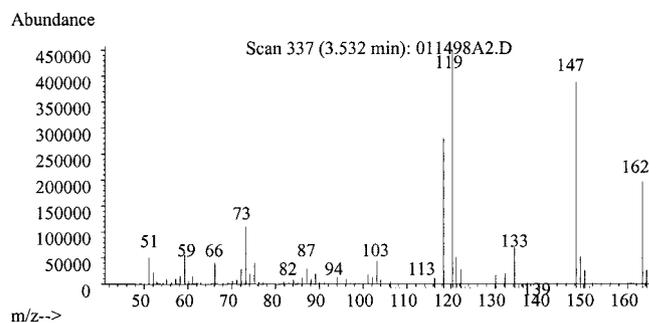Control urine samples collected from rats that were not exposed to

FIG. 6. *Mass spectrum of the component identified as 2,2,5,5-tetramethyl-2,5-disila1,3-dioxalane in CH$_2$Cl$_2$ extract of urine.*

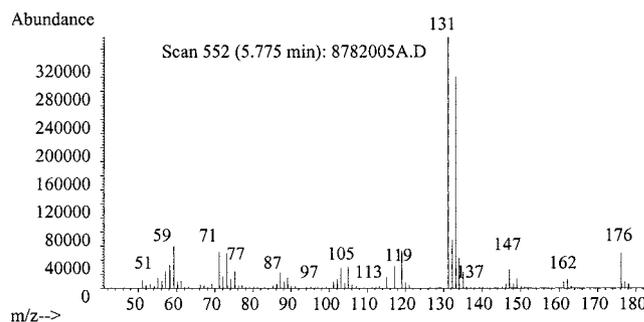m/z fragments are as follows: 162, M-CH$_3$; 73, Me$_3$Si.

FIG. 7. *Mass spectrum of the component identified as 2,2,5,5-tetramethyl-1,4-dioxa-2,5-disilacyclohexane.*

m/z fragments are as follows: 176, M; 161, M-CH$_3$.

[^{14}C]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 D$_5$. *HPLC profile of D$_5$ metabolites.* The HPLC radiochromatogram for D$_5$ 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 D$_5$ metabolites[3]. As far as the major metabolites were concerned, the HPLC profile of D$_5$ metabolites were essentially identical to that reported by the author for D$_4$ 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 D$_4$ metabolites identification was applied to the case of D$_5$. 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 D$_5$ that were not detected for D$_4$. These were m/z 429 (Fig. 9) and m/z 443 (Fig. 10). Presence of m/z 429 was indicative of the structure D$_4$D'OSiMe$_3$ (Fig. 9).

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[3] HPLC and GC-MS of standards were not included in this manuscript. They will be available upon request. Also, only the GC-MS data of those metabolites not available in the literature are given here.
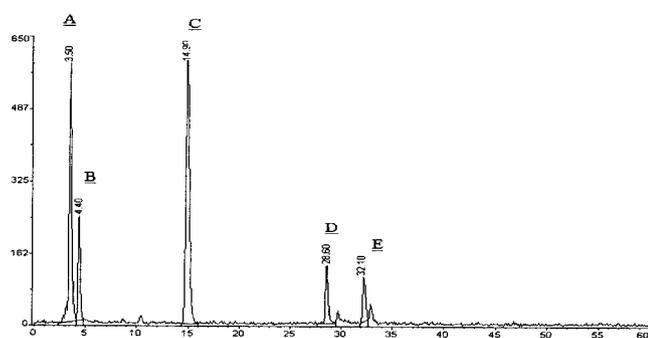
FIG. 8. *HPLC radiochromatogram of the metabolites of decamethylcyclopentasiloxane in Fischer 344 female rat urine.*

A, $MeSi(OH)_3$; B, $Me(OH)_2SiOSi(OH)_3$; C, $Me_2Si(OH)_2$; $Me_2(OH)Si\text{-}SiMe(OH)_2$; $HO(SiMe_2O)_3\text{-}H$.

FIG. 10. *Mass spectrum of the trimethylsilyl-protected hydroxymethylnonamethyl-cyclopentasiloxane.*

m/z fragments are as follows: 443, $M\text{-}CH_3$; 355, $M\text{-}CH_2OSiMe_3$.
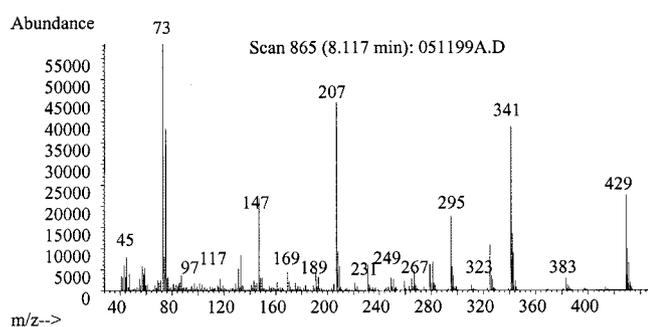
FIG. 9. *Mass spectrum of the trimethylsilyl-protected nonamethylcyclopentasiloxanol.*

m/z fragments are as follows: 429, $M\text{-}CH_3$; 73, Me_3Si.

An authentic sample of $D_4D'OSiMe_3$ was prepared by trimethylsilylation of an unlabeled standard of $D_4D'OH$ 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 $D_4D'OH$ 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 $Me_2Si(OSiMe_3)O\text{-}Si(OSiMe_3)_3$, $MeSi(OSiMe_3)_2OSi(OSiMe_3)_2Me$, and MD_4M based on the rationale described in case of D_4 metabolism (Varaprath et al., 1999). The fourth isomer of the same mass of 443 eluting at 9.58 min was therefore assigned the structure $D_4D'CH_2OSiMe_3$. The latter then suggested the presence of $D_4D'CH_2OH$ in urine. The fragmentation pattern for this component is shown in Fig. 10.

Control urine samples collected from rats that were not exposed to $[^{14}C]D_5$ 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 D_5 can result at the work place 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 D_5. Preliminary pharmacokinetic data following a single inhalation exposure of 5000 ppm $[^{14}C]MM$ to male and female Fischer F344 rats (unpublished data) has indicated that most of the recovered radioactivity was in urine (~40%) and expired volatiles
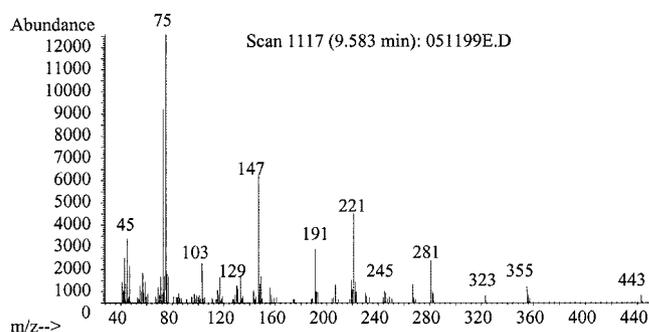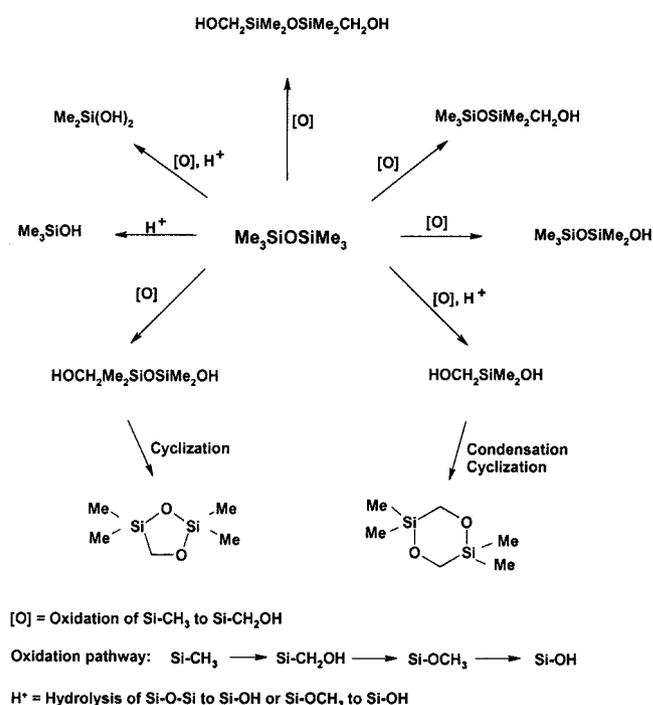

FIG. 11. *Possible pathways for the formation of the metabolites of hexamethyldisiloxane in Fischer 344 rat urine.*

(~50%), with minor amounts, either excreted via CO_2 (1–2%) and feces (1–2%) or remaining in the carcass (~5%) 168-h post exposure.

In the case of D_5, following a single inhalation exposure of 160 ppm $[^{14}C]D_5$ 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%), CO_2 (5%), or remained in the carcass (10%) after 168-h post exposure. The disposition of radioactivity for both MM and D_5 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 D_5 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 D_5 in urine collected from rats following exposure, since metabolism plays a significant role in elimination of these materials from the body.

TABLE 2

Structures of the TMS derivatives confirmed from GC-MS data, retention times, major mass (m/z) fragments and the corresponding silanol metabolites of D$_5$ present in urine

TMS Derivatives of The Metabolites & (GC-MS Retention Time, min)	Major GC-MS Mass Fragments (*m/z*)	Metabolites Present in Urine; HPLC Retention Time
MeSi(OSiMe$_3$)$_3$ (5.66)	73, 207, 295	MeSi(OH)$_3$, 3.5 min
Me$_3$SiO-(SiMe$_2$O)$_2$-SiMe$_3$ (5.86)	73, 207, 295	HO-(SiMe$_2$O)$_2$-H, 32.1 min
Me$_2$Si(OSiMe$_3$)$_2$ (4.59)	73, 133, 147, 205, 221	Me$_2$Si(OH)$_2$, 14.9 min
MeSi(OSiMe$_3$)$_2$-O-Si(OSiMe$_3$)Me$_2$ (8.22)	73, 147, 281, 369	MeSi(OH)$_2$-O-Si(OH)Me$_2$, 28.6 min
Me$_3$SiO-(SiMe$_2$O)$_3$-SiMe$_3$ (8.36)	73, 147, 281, 369	HO-(SiMe$_2$O)$_3$-H*
MeSi(OSiMe$_3$)$_2$-O-Si(OSiMe$_3$)$_2$ Me (9.37)	75, 147, 221, 281, 355, 443	MeSi(OH)$_2$-O-Si(OH)$_2$Me*
Me$_2$Si(OSiMe$_3$)-O-Si(OSiMe$_3$)$_3$ (10.31)	75, 147, 221, 281, 355, 443	Me$_2$Si(OH)-O-Si(OH)$_3$, 4.40 min
MeSi(OSiMe$_3$)$_2$-O-Si(OSiMe$_3$)$_3$ (9.37)	73, 147, 221, 341, 355, 427, 519	MeSi(OH)$_2$-O-Si(OH)$_3$
D$_4$D'OSiMe$_3$ (8.12)	73, 147, 207, 295, 341, 429	D$_4$D'OH*
D$_4$D'CH$_2$OSiMe$_3$ (9.58)	75, 147, 221, 281, 355, 443	D$_4$D'CH$_2$OH*

* Metabolite that was revealed by GC-MS but the presence not established in HPLC.
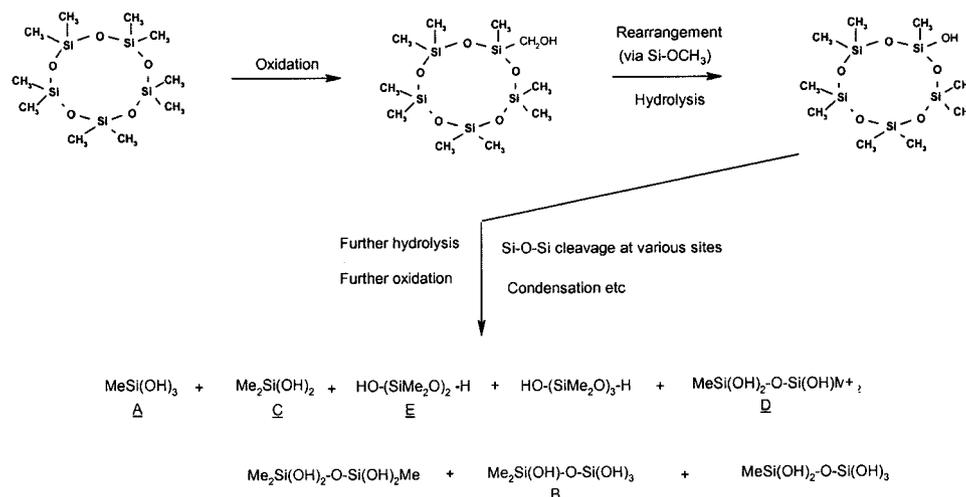

FIG. 12. *Possible pathways for the formation of the metabolites of decamethylcyclopentasiloxane in Fischer 344 rat urine.*

As demonstrated by the HPLC profile, with both MM and D$_5$ 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 D$_5$. 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 D$_5$ on the other hand, presence of multiples of hydroxy groups was a common feature. Metabolites containing $-$CH$_2$OH groups were absent with the exception of the presence of D$_4$D'CH$_2$OH at trace level. The reasons for the meager presence of $-$CH$_2$OH functional metabolites in D$_5$ are not clear, but it may be related to factors such as relative stability when the $-$CH$_2$OH is on a ring system and solubility of the metabolites. In a smaller cyclic siloxane D$_4$ (Varaprath et al., 1999), the corresponding metabolite D$_3$D'CH$_2$OH was not detected.

The possible pathways for the formation of the metabolites of MM and D$_5$ 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-dioxa-2,5-disilacyclohexane were unexpected. Due to lack of availability of ^{14}C-labled standards, it was not established that these metabolites were actually present in urine. It is quite possible that the mixed ether linkage (CH$_2$-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 CH$_2$OH present in urine. The presence of metabolites such as dimethylsilanediol in MM and methylsilanetriol in D$_5$ clearly established that some demethylation occurs at the silicon-methyl bond.

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