

DOW CORNING

July 24, 2007

Document Control Office (7407)  
TSCA Data Processing Center - CBIC  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
Room 6428  
1200 Pennsylvania Avenue, NW  
Washington, DC 20460

RECEIVED  
TSCA

07 JUL 27 AM 10:44

*mpt*  
306120

**Contains No CBI**

Attn: TSCA Docket Clerk

Re: For Your Information Submission:

The enclosed information is submitted on behalf of Dow Corning Corporation, Midland, Michigan, 48686-0994, on a For-Your-Information (FYI) basis as a follow-up to submissions made concerning decamethylcyclopentasiloxane (DMCPS), which chemical substance was the subject of a health and safety data rule issued under Section 8(d) of the Toxic Substances Control Act (TSCA) and with an effective date of June 14, 1993 (sunset date June 30, 1998), as codified at 40 CFR 716 (Health and Safety Data Reporting). The information presented in this submission was generated as part of our Siloxane Research Program. This program was the subject of a memorandum of understanding, dated April 9, 1996, between Dow Corning and EPA.

**Listed Chemical Substance:**

541-02-6 Decamethylcyclopentasiloxane (DMCPS, D<sub>5</sub>)

**Final Study Report:**

*In Vivo* Percutaneous Absorption of <sup>14</sup>C-Decamethylcyclopentasiloxane in the Rat

Dow Corning Corporation  
2003-I0000-52915  
November 4, 2003



**Manufacturer:**

Dow Corning Corporation  
PO Box 994  
2200 West Salzburg Road  
Midland, Michigan 48686-0994

For purposes of this TSCA For-Your-Information (FYI) submission, the general INTERNAL designation on the attached health and safety report is waived by Dow Corning.

If you require further information regarding this submission, please contact Michael Thelen, Manager of U.S. EPA Regulatory Affairs, at 989-496-4168 or at the address provided herein.

Sincerely,

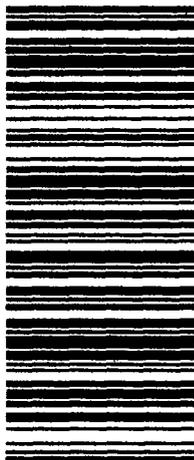
A handwritten signature in cursive script that reads "Kathleen P. Plotzke". The signature is written in black ink and is positioned above the printed name and title.

Kathleen P. Plotzke  
Director, Health and Environmental Sciences  
(989) 496-8046

**UPS Internet Shipping: View/Print Label**

1. **Print the label(s):** Select the Print button on the print dialog box that appears. Note: If your browser does not support this function select Print from the File menu to print the label.
2. **Fold the printed label at the dotted line.** Place the label in a UPS Shipping Pouch. If you do not have a pouch, affix the folded label using clear plastic shipping tape over the entire label.
3. **GETTING YOUR SHIPMENT TO UPS**  
**Customers without a Daily Pickup**
  - Schedule a same day or future day Pickup to have a UPS driver pickup all of your Internet Shipping packages.
  - Hand the package to any UPS driver in your area.
  - Take your package to any location of The UPS Store<sup>®</sup>, UPS Drop Box, UPS Customer Center, UPS Alliances (Office Depot<sup>®</sup> or Staples<sup>®</sup>) or Authorized Shipping Outlet near you. Items sent via UPS Return Services<sup>SM</sup> (including via Ground) are accepted at Drop Boxes.
  - To find the location nearest you, please visit the 'Find Locations' Quick link at ups.com.**Customers with a Daily Pickup**
  - Your driver will pickup your shipment(s) as usual.

FOLD HERE

<p>KATHLEEN PLOTZKE 989-496-5393 DOW CORNING CORPORATION 2200 W. SALZBURG RD. AUBURN MI 48611</p> <p><b>SHIP TO:</b> OPPT PUBLIC DOCKET #42071-A US ENVIRONMENTAL PROTECTION AGENCY ROOM G-099 1200 PENNSYLVANIA AV NW OFFICE OF POLLUTION PREV AND TOXICS DOCUMENT CONTROL OFFICE (7407) <b>WASHINGTON DC 20004-2403</b></p>	<p style="text-align: right;"><b>1 LBS</b></p> <p style="text-align: right;"><b>1 OF 1</b></p> <p style="text-align: center; font-size: 2em;"><b>MD 201 9-80</b></p> 	<p style="text-align: center; font-size: 2em;"><b>UPS NEXT DAY AIR SAVER 1P</b></p> <p>TRACKING #: 1Z 464 696 13 9961 3913</p> 	<p style="text-align: center;">BILLING: P/P</p>  <p style="text-align: right; font-size: 0.8em;">US 9.5.14.0 WPD260 66.0A 04/2007</p>
---	--	---	--

**DOW CORNING CORPORATION**  
**HEALTH & ENVIRONMENTAL SCIENCES**  
**TECHNICAL REPORT**

---

Report No.: 2003-I0000-52915

Title: *In Vivo* Percutaneous Absorption of <sup>14</sup>C-Decamethylcyclopentasiloxane in the Rat

Study No.: 9823-101

Test Article: <sup>14</sup>C-Decamethylcyclopentasiloxane (<sup>14</sup>C- D<sub>5</sub>)

Study Director: Marina L. Jovanovic, M.S.  
Associate Toxicology Specialist

Sponsor: Dow Corning Corporation

HES Management: Steven D. Crofoot, M.S.  
Team Leader, Toxicology  
Health and Environmental Sciences

Testing Facility: Dow Corning Corporation  
Health and Environmental Sciences  
2200 W. Salzburg Road  
Auburn, MI 48611

Study Completion Date: November 04, 2003

Security Statement: **Dow Corning Internal.** This report may be reproduced and shared with any Dow Corning employee. Distribution outside the Corporation must be approved by the Director of Health and Environmental Sciences. When this INTERNAL report is no longer needed, it may be placed in office waste baskets for destruction.

**2003© Dow Corning Corporation**

All rights reserved. No part of this work may be reproduced or transmitted in any form by any means, electronic or mechanical, including but not limited to photocopying or recording, or by any information storage or retrieval system without permission in writing from Dow Corning Corporation.

**TABLE OF CONTENTS**

<b>ABSTRACT</b> .....	4
<b>APPROVAL SIGNATURES</b> .....	5
<b>STUDY INFORMATION</b> .....	6
<b>GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT</b> .....	7
<b>QUALITY ASSURANCE STATEMENT</b> .....	8
<b>OBJECTIVE</b> .....	9
<b>INTRODUCTION</b> .....	9
<b>TEST SYSTEM INFORMATION</b> .....	10
<b>JUSTIFICATION FOR SELECTION OF TEST SYSTEM</b> .....	10
<b>METHOD OF RANDOMIZATION</b> .....	10
<b>HOUSING AND MAINTENANCE</b> .....	11
<i>A. Animal Receipt and Quarantine/Acclimation</i> .....	11
<i>B. Animal Housing</i> .....	11
<i>C. Environmental Conditions</i> .....	11
<i>D. Basal Diet and Drinking Water</i> .....	11
<i>E. Drinking Water</i> .....	11
<b>ANIMAL WELFARE ACT COMPLIANCE</b> .....	12
<b>TEST ARTICLE INFORMATION</b> .....	12
<b>EXPERIMENTAL DESIGN</b> .....	14
<b>ROUTE AND RATIONALE OF TEST ARTICLE ADMINISTRATION</b> .....	14
<b>DOSING SOLUTION</b> .....	14
<b>ORGANIZATION OF TEST SYSTEM GROUPS</b> .....	14
<b>TREATMENT REGIMEN AND KEY EVENTS</b> .....	14
<i>A. Animal preparation</i> .....	14
<i>B. Roth-Style Glass Metabolism Cage Set-up</i> .....	15
<i>C. Treatment Regimen</i> .....	15
<b>METHOD OF EUTHANASIA</b> .....	16
<b>TEST SYSTEM OBSERVATION</b> .....	16
<i>A. Mortality/Morbidity/Daily Observations</i> .....	16
<b>PARAMETERS MEASURED</b> .....	16
<b>SAMPLE COLLECTION</b> .....	16
<b>SAMPLE PROCESSING AND ANALYSIS</b> .....	18
<b>SAMPLE IDENTIFICATION AND STORAGE</b> .....	19
<b>DATA ANALYSIS</b> .....	19
<i>A. Parameters evaluated</i> .....	19
<b>STATISTICAL METHODS</b> .....	19
<b>DEVIATIONS</b> .....	20
<b>RESULTS AND DISCUSSION</b> .....	20
<b>CONCLUSIONS</b> .....	22
<b>ARCHIVE</b> .....	22
<b>REFERENCES</b> .....	22

**LIST OF TABLES**

Table I. Organization of Test Groups.....	24
Table II. Individual Dosing Data .....	25
Table III. Sample Collection Time Points.....	26

Table IV. Overall Mass Balance of Radioactivity in Female Fischer 344 Rats Following Dermal Application of Neat <sup>14</sup>C-D<sub>5</sub>.....27

Table V. Radioactivity Found on the Skin Surface of Female Fischer 344 Rats Following Dermal Application of Neat <sup>14</sup>C-D<sub>5</sub>.....28

Table VI. *In vivo* Disposition of Absorbed Radioactivity in Female Fischer 344 Rats Following Dermal Application of Neat <sup>14</sup>C-D<sub>5</sub> .....29

Table VII. Radioactivity Analysis of the Charcoal Tubes, KOH and Charcoal Baskets .....30

**LIST OF FIGURES**

Figure 1. Comparison of Escaped Volatiles in Dead Animals with Expired Volatiles in Live Animals .....31

Figure 2. Cumulative Dose Recovered in the Charcoal Tubes Over 24 Hours of Dermal Exposure to Neat <sup>14</sup>C-D<sub>5</sub>.....32

Figure 3. Disposition of Neat <sup>14</sup>C-D<sub>5</sub> Following Dermal Application.....33

Figure 4. Disposition of the Absorbed Dose 24 hr Post Application of Neat <sup>14</sup>C-D<sub>5</sub> and Six Days After Skin Has Been Washed.....34

Figure 5. Possible Fate of Residual <sup>14</sup>C-D<sub>5</sub> after Washing Skin 24 hr Post Exposure.....35

Figure 6. Skin depot (Application area = 2.5 cm<sup>2</sup>) Used for Test Article Application .....36

**LIST OF APPENDICES**

**Appendix A:** Radioactivity Spreadsheets and Parent Quantitation of D<sub>5</sub> in Expired Volatiles .....A1

**Appendix B:** Statistical Analysis.....B1

**ABSTRACT**

The percutaneous absorption of neat  $^{14}\text{C}$ -decamethylcyclopentasiloxane ( $^{14}\text{C-D}_5$ ) was evaluated in female Fischer 344 rats when applied topically at  $10.9 \text{ mg/cm}^2$  of skin. Four animals per group were exposed for 6 or 24 hr. Two control animals were euthanized at the 24 hr time point. In order to differentiate expired air from  $^{14}\text{C-D}_5$  that has escaped from the skin depot, an additional group of four euthanized rats (no expired air) was included in the study design. An additional 24 hr exposure group (Wash Group, N=4) was added to evaluate disposition of the residual  $\text{D}_5$  following a soap and water wash. During exposure, rats were housed in Roth-style metabolism cages to enable collection of urine, feces, and expired or escaped volatiles associated with the test article. Rats in the Wash Group were removed from the metabolism cages after 24 hr of exposure, dose sites were washed, charcoal baskets were replaced, and the animals were returned to the metabolism cages for continued collection of excreta and expired volatiles for a total 168 hr (24 hr of exposure + 144 hr post exposure). All rats were exposed in a semi-occluded manner using an aluminum skin depot with a charcoal basket for collection of volatilized test article. At the termination of exposure at 24 hr or at 168 hr post exposure, rats were euthanized by  $\text{CO}_2$  asphyxiation, the charcoal baskets were removed and extracted, skin was washed, tape stripped, excised, and solubilized in 35% TEAH. Remaining carcasses were also solubilized in 35% TEAH. Radioactivity content in each sample was measured by liquid scintillation counting. Total radioactivity in charcoal tubes was compared to the amount of unchanged  $\text{D}_5$  determined by GC-MS analysis.

Data suggested that the amount of radioactivity found in the expired volatile (charcoal tubes) and  $\text{CO}_2$  (KOH) traps could be attributed to the leakage from the dosing chamber and could be excluded from the total absorption. The majority of the test article that escaped from the dosing chamber was trapped in the charcoal tubes within 6 hours. Most of the test article evaporated from the skin surface and was trapped in an activated charcoal basket placed above the exposure site. The percent dose absorbed was determined as the amount of radioactivity in carcasses, feces, urine, skin dosing sites and cage rinses. Absorption of  $^{14}\text{C-D}_5$  ( $\pm$  standard error of the mean) in the Wash Group after 168 hr ( $0.089 \pm 0.0302\%$  of applied dose) was significantly lower ( $p < 0.05$ ) than that seen after 24 hr of exposure ( $0.243 \pm 0.0259\%$  of applied dose). These results demonstrated that the portion of  $\text{D}_5$  that remained in the skin after 24 hr of exposure, and was considered part of the absorbed dose, migrated to the skin surface and continued to evaporate, significantly decreasing apparent absorption of the  $\text{D}_5$ .



**STUDY INFORMATION**

Study Initiation Date: 03/24/2003

Experimental Start Date: 04/03/2003

Experimental Termination Date: 04/23/2003

Study Completion Date: 11/04/2003

Study Director: Marina L. Jovanovic, M.S.  
Associate Toxicology Specialist

Sponsor: Dow Corning Corporation  
2200 W. Salzburg Rd.  
Auburn, MI 48611

Management: Steven D. Crofoot  
Team Leader, Toxicology  
Health and Environmental Sciences

Roy A. Campbell  
Team Leader, Analytical Chemistry  
Health and Environmental Sciences

Key Study Personnel: James W. Crissman, D.V.M., Ph.D., D.A.C.V.P.  
Joan McMahon, Study coordinator  
Jeremy Durham, Bioanalytical support  
Debra McNett, Analytical specialist  
Joseph M. Tobin, Supervisor

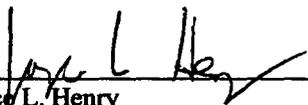


**QUALITY ASSURANCE STATEMENT**Title: *In Vivo* Percutaneous Absorption of <sup>14</sup>C-Decamethylcyclopentasiloxane in the Rat

Study Number: 9823-101

This study has been audited by the Dow Corning Corporation Health and Environmental Sciences Quality Assurance Unit according to approved Standard Operating Procedures to assure that the raw data are accurately reflected within this final report. The following are the inspection dates and the dates inspection findings were reported.

<u>Dates of Inspection</u>	<u>Phase Inspected</u>	<u>Findings Reported to Study Director</u>	<u>Findings Reported to Management</u>
10-14 Mar 03	Draft Protocol Review	17 Mar 03	19 Mar 03
02 Apr 03	Skin Depot Attachment	02 Apr 03	08 Apr 03
08 Apr 03	120-hour Sample Collection	08 Apr 03	10 Apr 03
14 Apr 03	Sample Processing	14 Apr 03	16 Apr 03
16-18, 29-31 Jul and 01-17 Aug 03	Draft Final Report and Associated Raw Data Review	18 Aug 03	10 Sep 03
17 Sep 03	Wet Specimen Verification	17 Sep 03	23 Sep 03

  
 Joyce L. Henry  
 Manager, Quality Assurance  
 Dow Corning Corporation  
 Health & Environmental Sciences

29 Oct 03  
 Date

**OBJECTIVE**

The objective of this study was to evaluate the percutaneous absorption of  $^{14}\text{C}$ -decamethylcyclopentasiloxane ( $^{14}\text{C}\text{-D}_5$ ) when applied neat to intact rat skin under conditions of semi-occlusion based on the current Environmental Protection Agency guidelines, OPPTS 870.7600.

**INTRODUCTION**

Decamethylcyclopentasiloxane is an ingredient in some consumer products including products that are applied to the skin. Therefore, it was important to provide data on the absorption of  $\text{D}_5$  following dermal application. In addition, this study was intended to imitate typical applications where personal care products are topically applied once a day and removed by a soap and water wash 24 hours later. The experimental design was such to allow determination of the fate of residual  $\text{D}_5$  after the skin had been washed at 24 hr post-exposure.

Due to the unique chemical and physical characteristics of  $\text{D}_5$  such as hydrophobicity, unusually low surface energy, and volatility, difficulties may arise when using the typical methodology to test this material. A previous study (HES study No. 7660) showed that  $\text{D}_5$  can migrate out of the skin depot and evaporate, in which case it was most likely trapped in the charcoal tubes of the exhaust system leading to a falsely high value attributed to the expired volatiles. This consequently would give a falsely high level of absorption. In order to differentiate expired  $\text{D}_5$  from  $\text{D}_5$  that had escaped from the skin depot, the experimental design included a group of euthanized (no expired air) animals. Escaped radioactivity was trapped in the same charcoal tubes that were used to trap expired volatiles. Comparison of expired volatiles (live animals) to escaped volatiles (euthanized animals) was used to understand the source of radioactivity found in expired volatiles traps.

**TEST SYSTEM INFORMATION**

Species:	Albino rat
Strain:	CDF <sup>®</sup> Fischer 344
Source:	The Charles River Breeding Laboratories, Inc. Raleigh, NC
Age:	11 weeks at experimental start
Body weight:	Females: 151-165 g at experimental start
Sex and number used on study:	18 females (nulliparous and non-pregnant)
Number of groups:	5 (Table I)
Quarantine/acclimation:	7 days
Identification method:	
Upon receipt:	Individual cage labels displaying a temporary quarantine (Q) number
Permanent identification:	Eartags and individual cage labels

**JUSTIFICATION FOR SELECTION OF TEST SYSTEM**

This species and strain of animal is recognized as appropriate for toxicity studies and is recommended in the EPA test guidelines. Fischer 344 female rats have previously been used in pharmacokinetic and metabolism studies of various silicone materials, and data obtained in those studies can be used as historical data. The choice of a single sex allowed for consistency and comparison between studies. The number of animals used was selected to provide adequate statistical power.

**METHOD OF RANDOMIZATION**

Extra animals were ordered and acclimated for randomization and substitution. After release from quarantine, the skin depot was attached to the back of the rat. Rats were then randomized by weight stratification into test groups using a table of random numbers generated by MicroSoft<sup>™</sup> Excel 2000. Three spare rats (Q0587, Q0588 and Q0590) were reserved for substitution if needed. This was considered necessary because of possible detachment of the skin depot. All animals were within  $\pm 20\%$  of the mean body weight for the group to which they were assigned. Animals not used on the study were returned to the Animal Resources group.

## HOUSING AND MAINTENANCE

### A. Animal Receipt and Quarantine/Acclimation

All animals received from Charles River Laboratories were judged to be in good health and suitable as test animals. The attending veterinarian examined all animals before release from quarantine and this was documented in the study records.

### B. Animal Housing

Animals were individually housed in suspended wire-mesh cages (7"x10"x7") during the quarantine/acclimation period. The cages were elevated above Bed-O'Cobs® (Alf-a'cobs) bedding and were subjected to routine cleaning. Upon release from quarantine animals were transferred to individual Roth-style glass metabolism cages and allowed to acclimate overnight to this experimental environment prior to dosing. After dosing, animals were housed in individual Roth-style glass metabolism cages for up to seven days.

### C. Environmental Conditions

Animals were housed in an environmentally controlled animal room (12-hour fluorescent-light/dark cycle, 64-79°F, 30-70% humidity, 10-15 air changes per hour). Temperature and humidity were recorded continuously and monitored twice a day on weekdays and once a day during weekends. The light cycle was interrupted periodically for sample collection, and those interruptions were documented in the data.

### D. Basal Diet

Certified Rodent Diet #5002, PMI® International Inc. (Lot number NOV 1502 1B) was offered *ad libitum*. Periodic analysis of this lot of certified feed for the presence of heavy metals and pesticides was performed and provided by the manufacturer to ensure that none were present in concentrations that would be expected to affect the outcome of the study.

There were no contaminants in the feed identified at levels that would interfere with the integrity of the study. Documentation of study director review was placed in the study file.

### E. Drinking Water

Municipal water, further purified by reverse osmosis was available *ad libitum*. The water was supplied by an automatic watering system (Edstrom RO purification system) when rats were housed in wire-mesh cages and *via* water bottles in the Roth-style glass metabolism cages. Drinking water was monitored routinely and also analyzed on a semi-annual basis by an independent laboratory (Ann Arbor Technical Services, Ann Arbor, MI). Results of the most recent water analysis were reviewed by the study director to ensure that no contaminants were present in concentrations that would be expected to affect the outcome of the study.

There were no contaminants in the water identified at levels that would interfere with the integrity of the study. Documentation of study director review was placed in the study file.

### ANIMAL WELFARE ACT COMPLIANCE

This study complied with all applicable sections of the final rules of the animal Welfare Act regulations (9 CFR, Part 1, 2 and 3) and was approved by the Laboratory Animal Care and Use Committee (LACUC) before animals were ordered.

### TEST ARTICLE INFORMATION

Test article characterization was done in compliance with the EPA Toxic Substances Control Act (TSCA), and Good Laboratory Practice Regulations (40 CFR Part 792).

### TEST ARTICLE I

The characterization of the unlabeled test article (D<sub>5</sub>) identified below included a visual inspection, purity by gas chromatography (GC) with thermal conductivity detector (TCD) and GC with mass spectrometry (MS) to verify the identity of the major component as D<sub>5</sub>. Records of characterization are maintained in the HES Archive. Documentation of study director review is kept in the study files. Any remaining test article was disposed of by study personnel. This was documented in the study records.

- Identification: Decamethylcyclopentasiloxane (supplied as Dow Corning™ 1693 Fluid)
- Lot Number: LL014002
- CAS Number: 541-02-6
- Source: Dow Corning Corporation  
2200 W. Salzburg Road  
Auburn, MI 48611
- Physical Description: Colorless liquid, odorless liquid
- Chemical stability: Stable (as specified in MSDS)
- Purity: 99.44 % by GC-TCD
- Solubility: Soluble in toluene, hexane, acetone, ethanol, tetrahydrofuran etc. (Angelotti, 1991; Varaprath *et al.*, 1998)
- Chemical characterization: HES Study No. 8824,

TIS Report No. 1997-I0000-43682

- Expiration Date: March 04, 2004
- Storage conditions: Room temperature (as specified in MDMS)
- Archive: A sample is retained in the HES Test Article Archives, Dow Corning Corporation, Auburn, MI 48611.

## TEST ARTICLE II

Chemical identity and radiochemical purity of the labeled test article ( $^{14}\text{C-D}_5$ ) was determined using gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) with a radioactivity flow-through detector (RAD). Records of characterization are maintained in the HES Archive. Documentation of study director review was placed in the study records. Any remaining test article was disposed of by study personnel. This was documented in the study records.

- Identification:  $^{14}\text{C-Decamethylcyclpentasiloxane } (^{14}\text{C-D}_5)$
- Reference Number: 18704-33
- Source: Dow Corning Corporation  
2200 W. Salzburg Road  
Auburn, MI 48611
- Physical Description: Colorless, odorless liquid
- Chemical stability: Stable (as specified in MSDS)
- Radiochemical Purity: 99.77 %
- Specific activity: 5.758 mCi/g
- Solubility: Soluble in hexane, heptane, toluene, tetrahydrofuran etc. (Angelotti, 1991; Varaprath *et al.*, 1998)
- Chemical characterization: HES study No. 9822-101  
TIS Report No. 2003-I0000-52822
- Expiration Date: March 31, 2005

- Storage Conditions: Freezer (as specified in MDMS)
- Archive: A sample is retained in the HES Test Article Archives, Dow Corning Corporation, Auburn, MI 48611.

## EXPERIMENTAL DESIGN

### ROUTE AND RATIONALE OF TEST ARTICLE ADMINISTRATION

Test article was applied to the surface of intact rat skin under conditions of semi-occlusion. The dermal route is an accepted method of administration of test article in pharmacokinetics and metabolism studies, and is a known route of human exposure to this test article.

### DOSING SOLUTION

Neat dosing solution was prepared three days prior to dosing by mixing appropriate volumes of unlabeled D<sub>5</sub> (Test article I), Lot No. LL014002 and <sup>14</sup>C-D<sub>5</sub>, Reference No. 18704-33 (Test article II). The radioactivity specific activity of 1.19 mCi/g of dosing solution provided approximately 32 µCi per animal at the average dose of 10.9 mg D<sub>5</sub>/cm<sup>2</sup> of exposed skin (Table II). Specific activity of dosing solution was measured by liquid scintillation analysis on the day of preparation as well as the day of dosing prior to experimental initiation. The specific activity results on the day of preparation and the day of exposure differed by 0.43%. This difference was calculated as a relative range between the two measurements. Radiochemical purity for the dosing test article was verified to be 100% on the day of preparation using HPLC with radiometric detection.

Documentation of these analyses is maintained in the study records. The dosing solution was stored in a -20°C freezer.

### ORGANIZATION OF TEST SYSTEM GROUPS

This study consisted of four exposure groups and one control group (Table I). In groups 2 and 4, skin was exposed for 6 and 24 hr, respectively, and in group 5 (Wash group), skin was exposed for 24 hr, then washed and the rats monitored for additional six days. Animals in group 3 were euthanized immediately prior to dosing. Skin of euthanized rats was exposed to <sup>14</sup>C-D<sub>5</sub> for 24hr. Animals in the control group 1 were not dosed.

### TREATMENT REGIMEN AND KEY EVENTS

#### A. Animal preparation

All the rats were weighed and prepared one day prior to dosing. All animals, including extras that were ordered for randomization and substitution, were prepared by clipping the hair from the dorsal surface of each animal. The attachment of the skin depot and administration of the dose followed a procedure that was developed for measuring percutaneous absorption of volatile chemicals (Susten et al., 1986). Animals were removed individually from the cages and anesthetized with isoflurane.

The skin depot was attached to the back of the rat with cyanoacrylate glue (Superglue gel, 3M, MN). The glue was allowed to dry (generally 3-5 min). Upon visual inspection additional glue was added as necessary. Animals with the skin depot attached were weighed, randomized, eartagged and placed into the individual Roth-style glass metabolism cages for the overnight acclimation.

#### B. Roth-Style Glass Metabolism Cage Set-up

Eighteen Roth-style glass metabolism cages were utilized for housing animals during the experiment. Each cage was operated at target flow rates and conditions for a minimum of 24 hours prior to housing of animals. During this time the system was evaluated for leaks by monitoring flow rates using flow meters and if necessary, appropriate actions were taken to assure the leaks were corrected and the system was properly sealed. In addition, evaluation of Roth-style glass metabolism system operation and integrity, plus the trapping of volatile D<sub>5</sub> from the cages onto charcoal tubes was assessed in a separate study prior to the experimental start (HES Study No. 9549).

Roth-style glass metabolism cages were operated in a manner that allowed adequate and uninterrupted airflow. Connections between parts were made using Tygon<sup>®</sup> tubing. Teflon<sup>®</sup> tubing was used for connections leading from the exhaust side of the chamber.

The room air was drawn through the cages using a vacuum pump. The airflow rate through each chamber was monitored using a calibrated flow meter and was maintained at 600 ml per minute. The room air entering the system was passed through a series of Drierite<sup>®</sup> and Ascerite<sup>®</sup> canisters designed to remove H<sub>2</sub>O and CO<sub>2</sub>, respectively. The temperature within the cage was monitored using a calibrated thermometer and recorded once in the A.M. and once in the P.M. every day during animal housing in the metabolism cages. The temperature within the cages housing live animals was maintained between 70 and 73°F. A previous method development experiment showed that temperature at the skin surface of the live rat, inside the dosing chamber, was approximately 93°F (HES Study No. 9818). Metabolism cages housing euthanized animals were heated using heating pads wrapped around the cage in order to provide similar temperature at the skin surface of the dead animals. Recorded temperatures inside the metabolism cages housing dead animals (84 and 100°F) were anticipated to provide temperature at the skin surface comparable to the temperature of the live animals.

Glass tubes containing charcoal were used for trapping expired or escaped volatiles. Urine and feces were collected over dry ice and CO<sub>2</sub> was collected in 4N potassium hydroxide (KOH) traps (gas towers).

#### C. Treatment Regimen

On the day of dosing all the animals were inspected for possible detachment of the skin depot. Animals in group 3 were euthanized by CO<sub>2</sub> asphyxiation immediately prior to the application of test article. Rat number D0572 was replaced with a spare rat number Q0590 because of detachment of the skin depot after the CO<sub>2</sub> euthanasia. All animals, except control animals in Group 1, were dosed using a Hamilton<sup>®</sup> gastight syringe. A single dose was applied to the skin surface defined by the attached skin depot (Figure 6). The skin area being dosed was approximately 2.5 cm<sup>2</sup>. The charcoal

basket with plastic cap was installed immediately and secured with an open aluminum cap wrapped with a Teflon™ tape. A hole previously made in the plastic cap of the charcoal baskets allowed air to circulate providing semi-occluded conditions. Immediately after dosing, both live and dead animals were placed into individual Roth-style glass metabolism cages for a maximum of 24 hr except for animals in group 5 (Table I). Animals in group 5 (Wash group) were removed from the cages 24 hr post-dose, the exposed area was washed, the charcoal basket was replaced with a new one, and animals were returned to the cages for an additional 6 days.

Animals in the control group were treated exactly the same as animals in the exposure groups except they were not dosed.

#### METHOD OF EUTHANASIA

All animals were euthanized by CO<sub>2</sub> asphyxiation at the appropriate time points (0 hr in group 3; 6 hr in group 2; 24 hr in groups 1 and 4; and 168 hr post-dosing in group 5).

#### TEST SYSTEM OBSERVATION

##### A. Mortality/Morbidity/Daily Observations

All animals were observed at least twice daily in their cages for mortality, morbidity, and moribundity by study personnel throughout the duration of the in-life phase of the study.

#### PARAMETERS MEASURED

1. Radioactivity content in expired/escaped volatiles (charcoal tubes), CO<sub>2</sub>, feces, urine, skin depot, charcoal basket, skin washes, excised exposure site and remaining carcasses.
2. The concentration of unchanged D<sub>5</sub> trapped in charcoal tubes (expired/escaped volatiles).

#### SAMPLE COLLECTION

Immediately after dosing both live and dead animals were placed in individual Roth-style glass metabolism cages for collection of excreta (Table III). Animals in the control group were placed in the Roth-style glass metabolism cages at the same time.

##### *Urine*

The Roth-style glass metabolism cages were modified to allow direct collection of urine into glass jars. Appropriate jars were pre-weighed and attached to the cage. While collecting, each jar was maintained on dry ice. At the appropriate time point, the collection jars were removed from the cage, capped, and maintained in a -80°C freezer until processing. Even if no sample was available at the targeted collection time, the jar was collected and maintained.

##### *Feces*

The Roth-style glass metabolism cages were modified to allow direct collection of feces into glass jars. Jars were pre-weighed and attached to the cage. While collecting, each jar was maintained on dry ice. At the appropriate time point, the jars were removed from the cage, capped, and maintained in a

-80°C freezer until processing. Even if no sample was available at the targeted collection time, the jar was collected and maintained.

#### *Expired/ Escaped Volatiles*

Glass tubes containing charcoal (Anasorb<sup>®</sup>) were used for trapping expired or escaped volatiles. Glass tubes were supplied closed at each end and were opened by etching and subsequent breaking of each end. Each tube was then attached in-line on the exhaust side of the cage. One tube was used per cage, per collection interval. At the appropriate time point, the charcoal tubes were removed, capped, and transferred to a -20°C freezer until processing.

#### *Carbon Dioxide (CO<sub>2</sub>)*

Cage exhaust air was passed through a glass gas trap filled with 134-190g of 4N KOH. Each gas trap was filled with KOH prior to initiation of collections. At the appropriate collection interval, KOH was collected into pre-weighed glass jars. Jars with KOH were capped, and re-weighed. Each sample was maintained refrigerated until radioactivity analysis.

#### *Test Article Removal*

Animals in groups 1, 2 and 4 were removed from the cages and euthanized by CO<sub>2</sub> asphyxiation at the termination of the exposure (6 and 24 hr). Animals in Group 3 were euthanized prior to dosing and placed in metabolism cages for 24 hr. Animals in the Wash group (Group 5) were euthanized at 168 hr post-dose. Charcoal baskets and Teflon<sup>™</sup> tape were removed from the skin depot chamber and placed in toluene for extraction. After the charcoal baskets were removed, the exposure area was wiped with a dry cotton swab, washed three times with a cotton swab soaked with 1% soap solution, followed by one dry wipe with a cotton swab, three washes with a cotton swab soaked with 70% ethanol and one dry swab to remove moisture. All the swabs were combined and extracted in toluene for radioactivity analysis. Following skin washes, the skin depot was removed and placed in toluene for extraction. The exposure area was dried with a cotton-tipped applicator prior to tape stripping. The exposure site was tape-stripped 10 times using adhesive tape (e.g. D-Squame<sup>®</sup>) to remove the *stratum corneum* from the skin surface. The tape was extracted in toluene together with all the swabs and analyzed for <sup>14</sup>C content. The exposure site was then excised, digested in a 35% aqueous solution of tetraethylammonium hydroxide (TEAH) and analyzed for <sup>14</sup>C content by liquid scintillation counting. The dorsal skin surface surrounding the dosing site was wiped with dry cotton swabs, which were analyzed separately for radioactivity content. All toluene extracts were kept refrigerated until processing.

In addition, animals in the "Wash group" were removed from the cages after 24 hr of exposure and the exposure area was wiped with a dry cotton swab, washed three times with a cotton swab soaked with 1% soap solution, and wiped with a dry cotton swab to remove residual moisture. All cotton swabs collected were combined and collected in toluene for radioactivity analysis. The charcoal baskets were removed and new charcoal baskets with a punched plastic cap were installed immediately after the washes were performed. Those animals were then returned to the metabolism cages and further monitored for six additional days.

#### *Roth-Style Glass Metabolism Cage Rinse*

Each cage was rinsed with tetrahydrofuran (THF) to remove fecal residues following removal of the animal. The rinses were collected in the pre-weighed jars. The cage rinses were maintained refrigerated until analysis for radioactivity content.

### SAMPLE PROCESSING AND ANALYSIS

The radioactivity of all samples collected was quantified by liquid scintillation analysis. Each sample was counted for at least 5 min or a 2 sigma % value of two, whichever came first. All counts were converted to absolute radioactivity (disintegrations per minute, DPM) by automatic quench correction. Results were corrected for matrix background radioactivity that was determined by using animals in the control groups.

Urine, KOH, and cage wash samples were weighed following collection and aliquots were directly analyzed for radioactivity content using liquid scintillation counter (LSC).

Feces were thawed, weighed, and homogenized with reverse osmosis water at 3:1 water to sample ratio (v:w). Aliquots of the fecal homogenate were solubilized using 35%TEAH, then decolorized with 30% hydrogen peroxide and neutralized by 6N hydrochloric acid (HCl) followed by LSC analysis for radioactivity content.

Charcoal tubes, that were used to trap expired volatiles, were desorbed with toluene (main and back-up portion combined). The charcoal tubes were removed from -20°C freezer and allowed to equilibrate to 5±4°C. The tubes were broken and the contents were placed in pre-weighed glass vials containing approximately 15 ml of a solution of toluene and tetrakis(trimethylsiloxy)silane (M<sub>4</sub>Q) as internal standard (ISTD). The charcoal samples were allowed to desorb in toluene for at least 24 hours. Duplicate aliquots of the toluene were taken for total radioactivity analysis by LSC. In addition, aliquots of the charcoal tube extracts were taken for analysis by GC-MS for determination of parent D<sub>5</sub> concentrations according to the method "Procedure for Determination of D<sub>5</sub> in Expired Volatiles (Charcoal Tubes)". This method was validated prior to this study and results from this validation can be found in the DC report No. 2001-I0000-50469. A copy of this method is kept in the study file. Instrumentation and GC-MS methodology is documented and included in the study file. Quality control (QC) samples were also prepared at the same time as the charcoal tube samples were processed as a check of the extraction and analysis and were prepared by spiking varying amounts of D<sub>5</sub> into control charcoal tubes and processed and analyzed in the same manner as the study samples. Parent D<sub>5</sub> was quantified in the charcoal tube samples and QC samples by comparing the extracts of these samples to calibration curves generated from toluene solvent standards containing M<sub>4</sub>Q and varying amounts of D<sub>5</sub>.

Charcoal baskets (main, back-up portion of charcoal, metal basket, plastic lid) and Teflon™ tape were extracted in toluene and aliquots analyzed by LSC. Toluene extraction efficiency was determined to be 100% in the spiking experiment. The skin depot was separately extracted in toluene and aliquots analyzed by LSC. Aliquots of extracts were counted after addition of the scintillation cocktail. All cotton swabs, except dry swabs of the dorsal skin, collected at each termination time point (6, 24 and 168 hr post-dose) were combined with tape strips, extracted in toluene and analyzed by LSC. Dry swabs of the dorsal skin surrounding exposure site were separately extracted in toluene and analyzed for radioactivity content. Also washes of the dosing site in the "Wash group" performed 24 hr post-dosing were analyzed separately.

Excised skin (application site) was digested in 35% TEAH. Aliquots of solubilized skin were taken, neutralized with 6N HCl, and analyzed for <sup>14</sup>C content after addition of scintillation cocktail. The carcasses were also solubilized with 35% TEAH and analyzed for radioactivity content by LSC.

### SAMPLE IDENTIFICATION AND STORAGE

Samples were stored under the following conditions:

KOH, Cage rinses	Refrigerator ( $5 \pm 4^{\circ}\text{C}$ )
Charcoal tubes	-20°C freezer
Toluene extracts	Refrigerator ( $5 \pm 4^{\circ}\text{C}$ )
Urine, Feces	-80°C freezer
Solubilized carcass and skin	Room temperature

### DATA ANALYSIS

#### A. Parameters evaluated

The radioactivity content in the samples collected during the study were expressed in terms of  $\mu\text{g D}_5$  equivalents, and as percent of total radioactivity recovered relative to the amount of the applied dose (percent of the applied dose).

*Escaped vs Expired volatiles (Dead vs Live animal).* The radioactivity found in the charcoal tubes and KOH traps for over 24 hr (Group 4 and Group 5 at 24 hr) was compared to the radioactivity found in the charcoal tubes and KOH traps in dead animals group (Group 3) to evaluate if trapped radioactivity was the result of leakage from the dosing chamber. In addition, the content of unchanged  $\text{D}_5$  in expired/escaped volatiles was expressed as  $\mu\text{g D}_5$  trapped in the charcoal tubes per hour ( $\mu\text{g D}_5/\text{hr}$ ).

*Absorption.* The radioactivity found in the carcass, excised skin (exposure site), urine and feces was considered to represent the absorbed portion of the applied dose. The absorbed dose was expressed in terms of  $\mu\text{g D}_5$  equivalents per  $\text{cm}^2$  of exposed skin, and as percent of the applied radioactivity.

*Volatilized  $\text{D}_5$ .* The radioactivity found in the charcoal baskets and Teflon™ tape represented volatilized portion of the applied dose.

*Skin Surface.* Radioactivity found in skin washes, *stratum corneum* (adhesive tape) and skin depot (dosing chamber) represented the non-absorbed portion of the applied dose found on the skin surface.

### STATISTICAL METHODS

Numerical data obtained during the conduct of the study were processed and analyzed using Microsoft Excel™ 2000 and subjected to calculation of parameters indicated above. This included group mean values and standard error of the mean, where appropriate.

*Escaped vs expired volatiles.* Radioactivity trapped in charcoal tubes and KOH traps for live and dead animals was compared using the t-test to determine if trapped volatiles were the result of leakage from the dosing chamber.

*Absorption.* Any outlier observation was identified using the statistical Q-test (Mitschele, 1991) in the percent absorbed dose data. This method flagged a suspected outlier with 90-95% confidence. Animal

number D0576 was flagged as an outlier and excluded from further statistical analysis. These data showed that animal number D0576 had a significantly higher percent dose absorbed than remaining observations in the same group. The F-test was used to test for variances for any treatment ( $p < 0.05$ ). The comparison of percent absorbed dose at 24 h and 168 h (Wash group) was carried out using the t-test.

In all cases, the statistic test was calculated to maintain an overall Type I error rate of 5 % ( $\alpha = 0.05$ ).

#### DEVIATIONS

1. A deviation occurred in the protocol with regard to the section XI. Experimental Design. All animals were weighed before and after attachment of skin depot, and animals in group 2 were weighed at terminal time point (6 hr). Protocol only specified that body weights would be taken after attachment of skin depot. This was considered to have no impact on the study outcome.
2. A deviation occurred in the protocol with regard to the section V. Test System. All animals were permanently identified by ear tags except animal number Q0590. This animal was identified by Q number and individual cage label. This was considered to have no impact on the study outcome.
3. A deviation occurred in the protocol with regard to the section XI.I. Sample Processing and Analysis. Six instead of 5 ml of scintillation cocktail was used to analyze charcoal tube extracts for radioactivity content. This was considered to have no impact on the study outcome.

#### **RESULTS AND DISCUSSION**

An *in vivo* method for measuring the percutaneous absorption of  $^{14}\text{C-D}_5$  in Fischer female rats was used in this study. The method utilized an aluminum skin depot device (dosing chamber) supplied with a charcoal basket, which served to capture the test article normally lost from the application site by volatilization (Figure 6). The skin depot was glued to the back of the rat and an average dose of 10.9 mg  $\text{D}_5/\text{cm}^2$  was applied to the intact skin of the rat (Table II) for duration of 6 and 24 hours. The "Wash group" was used to determine the fate of residual  $^{14}\text{C-D}_5$  after the skin had been washed at 24 hr post exposure. All rats were housed in Roth-style glass metabolism cages to enable direct determination of absorption from the radioactivity in excised exposed skin, carcass, urine and feces. An additional group of four euthanized rats was included in the study design because of the anticipated escape of  $^{14}\text{C-D}_5$  from the skin depot device (HES studies number 9818 and 7660). This group was added in order to differentiate expired volatiles from  $\text{D}_5$  that had escaped from the dosing chamber and trapped in the charcoal tubes (Table I and III).

Mass balance data (Table IV) showed that most (66-92%) of the test article volatilized from the skin surface and was trapped in an activated charcoal basket placed above the exposure site for all four groups. The total dose recovered was  $\geq 89\%$  in all groups. After 6 hr of exposure (Group 2) there was still a significant amount of  $\text{D}_5$  remaining on the skin surface (23.730% of applied dose) and in the excised skin (5.548% of applied dose) (Tables IV and V). These data indicate a significant amount of  $\text{D}_5$  still remained on the skin surface after only 6hr of exposure, which could not be completely removed by washing procedure and could contribute to the falsely elevated results in the excised skin. Therefore, it was considered that the 6hr data would not provide reliable data for understanding absorption of  $\text{D}_5$ .

When comparing the 24 hr groups (live vs dead), a significant amount of radioactivity was trapped in the charcoal tubes in both the live and dead animals with considerable variability (Figures 1 and 2). In addition, most of the test article that escaped from the dosing chamber was trapped in the charcoal tubes within 6 hours (Figure 2). Statistical analysis showed that the level of expired volatiles ( $8.747 \pm 8.4123\%$  of applied dose) and  $\text{CO}_2$  ( $0.008 \pm 0.0064\%$  of applied dose) in live animals after 24 hr of dermal exposure was not significantly different ( $p > 0.05$ ) from escaped volatiles ( $7.745 \pm 5.5673\%$  of applied dose) or  $\text{CO}_2$  ( $0.000 \pm 0.0001\%$ ) found in dead animals (Appendix B). Also, the level of expired volatiles in the "Wash group" after 24 hr of dermal exposure ( $7.005 \pm 4.1557\%$  of applied dose) was not significantly different from escaped volatiles ( $7.745 \pm 5.5673\%$  of applied dose) found in dead animals (Table VII). Radioactivity found in the KOH traps of dead animals ( $0.000 \pm 0.0001\%$  of applied dose) was not statistically different from KOH traps in the "Wash group" after 24hr of dermal exposure ( $0.003 \pm 0.0025\%$  of applied dose). Therefore the radioactivity in the expired volatiles and  $\text{CO}_2$  traps were attributed to leakage from the skin depot.

After 24 hr of exposure (Group 4), almost all of the applied dose had volatilized and only 0.165% of applied dose was found on the skin surface including  $\text{D}_5$  that was recovered from the skin depot. Less than 0.1% of applied dose was found in carcass, urine and feces (Figure 4). The average percent of applied dose being absorbed over the 24 hr exposure and recovered from urine, feces, excised application site and carcass was 0.243% of applied dose (Table VI). The majority of absorbed dose (0.175% of applied dose) was found in the skin application site after 24 hr of exposure.

As indicted above, radioactivity found in charcoal tubes and KOH traps was attributed to "escaped"  $\text{D}_5$  from the dosing chamber and excluded from the total absorption (Table VII, Figure 1). In addition, comparison of total radioactivity in charcoal tubes and the amount of parent  $\text{D}_5$  determined by GC-MS showed that all of the radioactivity could be attributed to the unchanged  $\text{D}_5$  (Appendix A).

A particularly high fraction of the applied dose was trapped in the charcoal tubes (55.90%) for animal number DO576 in Group 4 (individual animal data are presented in Appendix A). This animal was identified as an outlier based on experimental and statistical considerations using Q-test (Mitschele, 1991) for comparison of absorption data within the treatment group and it was removed from data set (Appendix B). The increased level of absorption in this animal could be caused by possible ingestion of some material that was deposited on the pelt as a result of the leakage from the dosing chamber.

Further, this study was designed to allow for the determination of the fate of the portion of the test article that was considered absorbed after the 24 hr exposure period. At the end of the 24 hr exposure, animals in the "Wash Group" were removed from the glass metabolism cages and the charcoal baskets above exposure sites were removed and replaced with new ones after the skin (application site) had been washed with 1% soap solution. Rats were then returned to the metabolism cages and were followed for six additional days. At 168 hr post-dosing (i.e. 144 hr after the application site was washed), the average total dose recovery was 95.93% of applied dose (Table IV and Figure 3). The absorbed dose in the "Wash Group" ( $0.089\% \pm 0.0302\%$  of applied dose) was significantly lower than that seen after 24 hr of exposure ( $0.243\% \pm 0.0259\%$  of applied dose) as shown in Figure 4 and Table VI. The percent of dose that remained in the skin application site after a 24 hr-wash and an additional six days (0.033%) was significantly lower than that found after 24 hr of exposure (0.175% of applied dose). The  $\text{D}_5$  was also found on the skin surface and in the charcoal basket that was placed above exposure site after the skin

had been washed (Figure 5, Appendix A) indicating further evaporation of D<sub>5</sub> over the additional six days. These data suggest that the majority of D<sub>5</sub> that remained in the skin and skin depot after the skin had been washed at 24 hr (approximately 0.3% of applied dose) continued to evaporate and was trapped in the charcoal basket placed above the exposure site (approximately 0.2%). According to the definition (EPA Guidelines), the quantity absorbed is the portion of the dose that enters the systemic compartment of the organism. However, our results indicated that most of D<sub>5</sub> that remained in the skin after 24 hr (and would be considered part of absorbed dose) was not available for systemic distribution but was localized in the epidermis, and was able to migrate to the skin surface and continue to evaporate. Therefore, the portion of D<sub>5</sub> that was found in the skin after 24 hr exposure should be considered "absorbable" rather than truly absorbed and systemically available. For that reason, considering the total amount of D<sub>5</sub> that remains in the skin after 24 hr exposure to be part of absorbed dose may be an overestimate of D<sub>5</sub> absorption.

## CONCLUSIONS

All of radioactivity found in the expired volatiles (charcoal tubes) can be attributed to the parent D<sub>5</sub> based on comparison of LSC and GC-MS analysis. Statistical analysis showed that the level of expired volatiles and CO<sub>2</sub> (live animals) was not significantly different from escaped volatiles and CO<sub>2</sub> (dead animals) indicating that the amount of radioactivity found in expired volatiles and CO<sub>2</sub> traps could be attributed to the leakage from the dosing chamber and could be excluded from the total absorption.

The portion of D<sub>5</sub> that remained in the skin (without *stratum corneum*) and could be considered part of the absorbed dose, actually migrated to the skin surface and continued to evaporate, significantly decreasing the apparent absorption of D<sub>5</sub> to 0.089 % of applied dose.

## ARCHIVE

Protocol, amendments and deviations, study authorization form, raw data, correspondence and final report, at minimum, are retained in the HES archives, Dow Corning Corporation, Auburn, MI 48611.

## REFERENCES

1. Susten A. S., Dames B.L. and Niemeier R.W. 1986. *In vivo* percutaneous absorption studies of volatile solvents in hairless mice. I. Description of a Skin -Depot. *J. Appl. Toxicology*, 6(1): 43-46.
2. Susten A.S., Niemeier R.W. and Simon S.D. 1990. *In vivo* percutaneous absorption studies of volatile organic solvents in hairless mice II. Toluene, Ethylbenzene and Aniline. *J. Appl. Toxicology* 10: 217-225.
3. HES Study No. 8824. Characterization of decamethylcyclopentasiloxane. DC Report No. 1997-I0000-43682.
4. Angelloti, N.C. 1991. In *The Analytical Chemistry of Silicones*; A.L. Smith, ed.; Analysis of Polymers, Mixtures, and Compositions (John Wiley & Sons, Inc. New York, NY), p:49.

5. Varaprath S., K.L. Salyers, K.P. Plotzke and S. Nanavati. 1998. Extraction of Octamethylcyclotetrasiloxane and its Metabolites from Biological Matrices. *Analytical Biochemistry*, 256: 14-22.
6. HES Study No. 9549. Non-Regulated Study: Evaluation of Roth-style metabolism cage system operation and integrity, and trapping efficiency spiked with radiolabeled  $^{14}\text{C}$ -octamethylcyclotetrasiloxane ( $^{14}\text{C-D}_4$ ) and  $^{14}\text{C}$ -decamethylcyclopentasiloxane ( $^{14}\text{C-D}_5$ ). DC Report No. 2001-I0000-49884.
7. HES Study No. 9822-101. Characterization of  $^{14}\text{C}$ -decamethylcyclopentasiloxane. DC Report No. 2003-I0000-52822.
8. HES Study No. 9818-101. Non-Regulated Study: Evaluation of the Method for Determination of Decamethylcyclopentasiloxane ( $^{14}\text{C-D}_5$ ) *In Vivo* Percutaneous Absorption in the Rat. DC Report No. 2003-I0000-52694.
9. HES Study No. 7660. *In vitro* Percutaneous Absorption of  $^{14}\text{C}$ -Decamethylcyclopentasiloxane ( $\text{D}_5$ ) in the Rat. DC Report No. 1996-I0000-41225.
10. HES Study No. 9105. Absorption, Distribution, Metabolism, and Excretion (ADME) Study of  $^{14}\text{C}$ -Decamethylcyclopentasiloxane ( $\text{D}_5$ ) in the Rat Following a Single Nose-only Vapor Inhalation Exposure to  $^{14}\text{C-D}_5$  at Two Dose Levels. DC Report No. 2001-I0000-50469.
11. Mitschele J. 1991. Small Sample Statistics, *J. Chem. Ed.*, 68:470-473.

**Table I. Organization of Test Groups**

<b>Group ID</b>	<b>Number of animals /Sex/ Group</b>	<b>Treatment</b>	<b>Targeted Dose (mg <sup>14</sup>C- D<sub>5</sub>/cm<sup>2</sup> of skin)</b>	<b>Exposure duration (hr)</b>
1	2 Females (Controls)	None	0	24
2	4 Females	Neat <sup>14</sup> C-D <sub>5</sub>	10	6
3 <sup>1)</sup>	4 Females (Dead)	Neat <sup>14</sup> C-D <sub>5</sub>	10	24
4	4 Females	Neat <sup>14</sup> C-D <sub>5</sub>	10	24
5 <sup>2)</sup> ("Wash Group")	4 Females	Neat <sup>14</sup> C-D <sub>5</sub>	10	24

<sup>1)</sup> Animals euthanized immediately prior to dosing

<sup>2)</sup> "Wash group" = Skin exposed for 24 hr, then washed and rats monitored for additional six days in metabolism cages

Table II. Individual Dosing Data

Animal ID	Group ID	Dose/cm <sup>2</sup> of Skin (mg <sup>14</sup> C-D <sub>2</sub> /cm <sup>2</sup> )	Average Dose (mg <sup>14</sup> C-D <sub>2</sub> /cm <sup>2</sup> )	Dose/Rat (mg <sup>14</sup> C-D <sub>2</sub> )	Dose/Rat (μCi)
D0568	2	10.7	10.8	26.8	31.9
D0569	2	11.0		27.4	32.6
D0570	2	10.8		27.0	32.1
D0571	2	10.9		27.2	32.4
Q0590	3 (Dead Group)	10.3	10.3	25.8	30.7
D0573	3 (Dead Group)	10.5		26.2	31.2
D0574	3 (Dead Group)	9.9		24.8	29.5
D0575	3 (Dead Group)	10.3		25.8	30.7
D0576	4	10.8	11.7	27.0	32.1
D0577	4	10.8		27.0	32.1
D0578	4	13.2		33.0	39.3
D0579	4	12.1		30.2	35.9
D0580	5 (Wash Group)	11.1	10.7	27.7	33.0
D0581	5 (Wash Group)	11.7		29.2	34.7
D0582	5 (Wash Group)	10.8		27.0	32.1
D0583	5 (Wash Group)	9.0		22.6	26.9

Average Dose = 10.9 mg of <sup>14</sup>C-D<sub>2</sub>/cm<sup>2</sup> of skin

**Table III. Sample Collection Time Points**

<b>Group ID</b>	<b>Collection duration (hr)</b>	<b>Expired or escaped volatiles (hr)</b>	<b>Urine (hr)</b>	<b>Feces (hr)</b>	<b>CO<sub>2</sub> (hr)</b>
1 (control)	24	24	24	24	24
2	6	1,2,4,6	6	6	6
3 (Dead)	24	24	N/A	N/A	24
4	24	1,2,4,6,12,24	6,12,24	6,12,24	6,12,24
5 ("Wash group")	168	1,2,4,6,12,24 and every 24 hr for next 6 days	6,12,24 and every 24 hr for next 6 days	6,12,24 and every 24 hr for next 6 days	6,12,24 and every 24 hr for next 6 days

Table IV. Overall Mass Balance of Radioactivity in Female Fischer 344 Rats Following Dermal Application of Neat  $^{14}\text{C-D}_5$ Average Percent of Applied Dose  $\pm$  Standard Error of the Mean

Group No.	Time point (hr)	Charcoal Basket	Charcoal Tube	KOH	Skin Surface <sup>2)</sup>	Excised Skin	Urine	Feces + Cage Rinse	Carcass	Total Recovery
2	6	58.779 $\pm$ 2.3055	0.196 $\pm$ 0.0405	0.000 $\pm$ 0.0000	23.730 $\pm$ 1.6605	5.548 $\pm$ 0.5050	0.000 $\pm$ 0.0000	0.001 $\pm$ 0.0001	0.270 $\pm$ 0.1158	88.523 $\pm$ 0.5779
3	24 (Dead)	86.446 $\pm$ 4.7913	7.745 $\pm$ 5.5673	0.000 $\pm$ 0.0001	0.386 $\pm$ 0.2209	0.254 $\pm$ 0.0606	N.A.	N.A.	0.055 $\pm$ 0.0353	94.887 $\pm$ 1.6349
4	24 (Live)	80.434 $\pm$ 9.7133	8.747 $\pm$ 8.4123	0.008 $\pm$ 0.0064	0.165 $\pm$ 0.0994	0.175 $\pm$ 0.0235	0.007 $\pm$ 0.0050	0.005 $\pm$ 0.0043	0.056 $\pm$ 0.0292	89.597 $\pm$ 2.0831
5	168 <sup>1)</sup>	88.753 $\pm$ 6.7023	7.022 $\pm$ 4.1673	0.012 $\pm$ 0.0034	0.048 $\pm$ 0.0130	0.033 $\pm$ 0.0132	0.024 $\pm$ 0.0065	0.008 $\pm$ 0.0059	0.025 $\pm$ 0.0098	95.925 $\pm$ 5.5392

<sup>1)</sup> "Wash group" = Skin exposed for 24 hr, then washed and rats monitored for additional six days in metabolism cages<sup>2)</sup> Skin surface = Swabs + Tape strips + Skin depot

**Table V. Radioactivity Found on the Skin Surface of Female Fischer 344 Rats Following Dermal Application of Neat <sup>14</sup>C-D<sub>5</sub>****Percent of Applied Dose ± Standard Error of the Mean**

Group No.	Time point (hr)	Average Dose (mg <sup>14</sup> C-D <sub>5</sub> /cm <sup>2</sup> )	N	Swabs and Tape	Skin Depot	Skin Surface <sup>2)</sup>
2	6	10.8	4	15.813 ± 1.9720	7.916 ± 1.4116	23.730 ± 1.6605
3	24 (Dead)	10.3	4	0.035 ± 0.0125	0.351 ± 0.2231	0.386 ± 0.2209
4	24	12.0	3	0.013 ± 0.0005	0.152 ± 0.0990	0.165 ± 0.0994
5	168 <sup>1)</sup>	10.7	4	0.015 ± 0.0036	0.033 ± 0.0124	0.048 ± 0.0130

<sup>1)</sup> "Wash group" = Skin exposed for 24 hr, then washed and rats monitored for additional six days in metabolism cages<sup>2)</sup> Skin surface = Swabs + Tape strips + Skin depot

Table VI. *In vivo* Disposition of Absorbed Radioactivity in Female Fischer 344 Rats Following Dermal Application of Neat <sup>14</sup>C-D<sub>5</sub>

Average Percent of Applied Dose ± Standard Error of the Mean

Group No.	Time point (hr)	N	Excised Skin	Urine	Feces+ Cage Rinse	Carcass	Absorbed <sup>2)</sup>
2	6	4	5.548 ± 0.5050	0.000 ± 0.0000	0.001 ± 0.0001	0.270 ± 0.1158	5.818 ± 0.4879
4	24	3	0.175 ± 0.0235	0.007 ± 0.0050	0.005 ± 0.0043	0.056 ± 0.0292	0.243 ± 0.0259
5	168 <sup>1)</sup>	4	0.033 ± 0.0132	0.024 ± 0.0065	0.008 ± 0.0059	0.025 ± 0.0098	0.089 ± 0.0302

<sup>1)</sup> "Wash group" = Skin exposed for 24 hr, then washed and rats monitored for additional six days in metabolism cages<sup>2)</sup> Absorbed dose = Excised Skin + Carcass + Urine + Feces + Cage rinse

Table VII. Radioactivity Analysis of the Charcoal Tubes, KOH and Charcoal Baskets

Average Percent of Applied Dose  $\pm$  Standard Error of the Mean

Time point	Charcoal tubes <sup>2)</sup>	KOH <sup>3)</sup>	Charcoal Baskets <sup>4)</sup>	Volatilized and Escaped <sup>5)</sup>
24 hr (Dead)	7.745 $\pm$ 5.5673	0.000 $\pm$ 0.0001	86.446 $\pm$ 4.7913	94.192 $\pm$ 1.6096
24 hr (Live)	8.747 $\pm$ 8.4123	0.008 $\pm$ 0.0064	80.434 $\pm$ 9.7133	89.189 $\pm$ 2.1195
"Wash group" at 24 hr	7.005 $\pm$ 4.1557	0.003 $\pm$ 0.0025	88.533 $\pm$ 6.7308	95.541 $\pm$ 5.6146
"Wash group" at 168 hr <sup>1)</sup>	7.022 $\pm$ 4.1673	0.012 $\pm$ 0.0034	88.753 $\pm$ 6.7023	95.788 $\pm$ 5.5740

1) "Wash group" = Skin exposed for 24 hr, then washed and rats monitored for additional six days in metabolism cages

2) Charcoal tubes were used to collect expired/escaped volatiles

3) KOH traps were used to collect CO<sub>2</sub>

4) Charcoal baskets above the dosing site were used to collect volatilized D<sub>5</sub>

5) Volatilized and escaped in live animals = Charcoal baskets + Charcoal tubes + KOH

Volatilized and escaped in dead animals = Charcoal baskets + Charcoal tubes + KOH + Cage rinse

Figure 1. Comparison of Escaped Volatiles in Dead Animals with Expired Volatiles in Live Animals

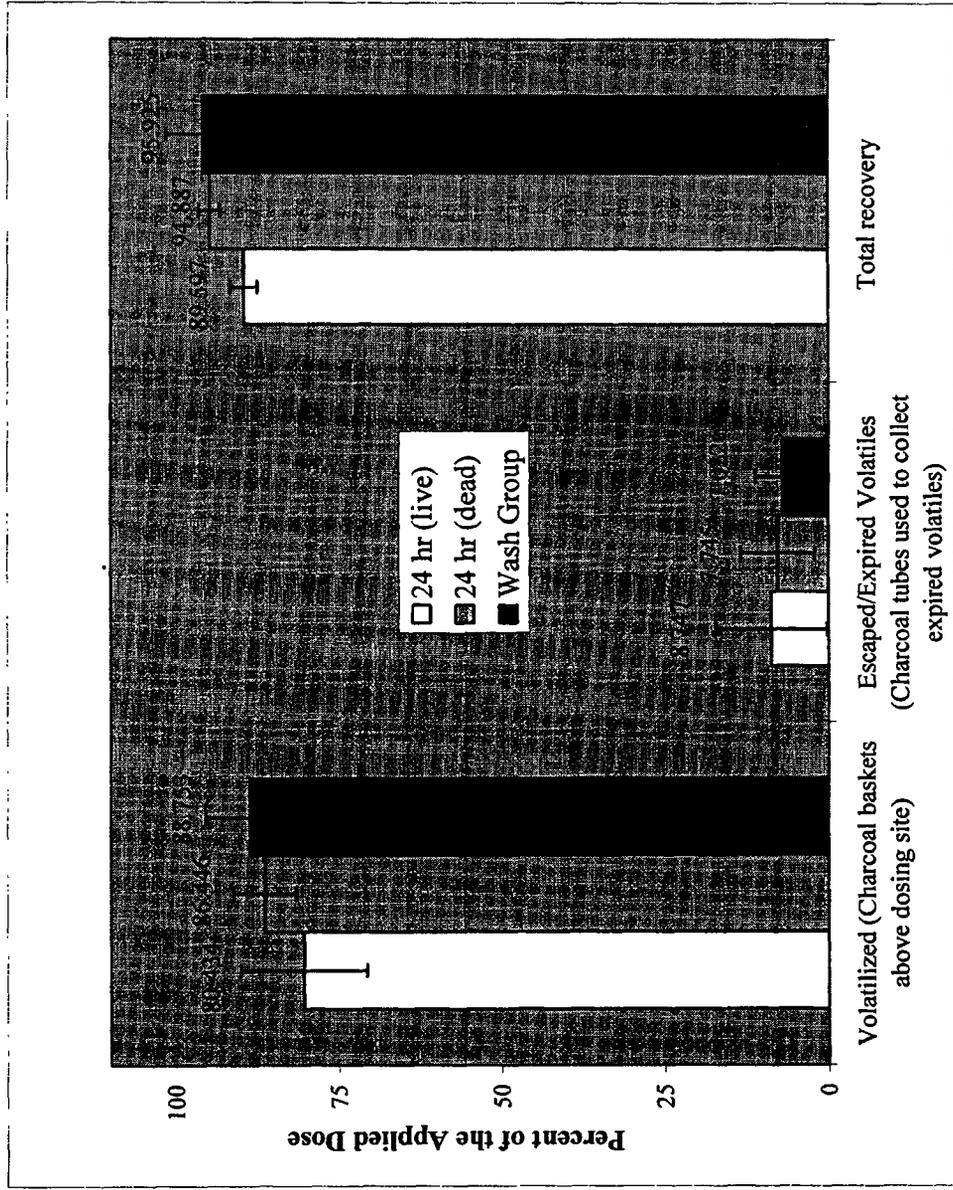


Figure 2. Cumulative Dose Recovered in the Charcoal Tubes Over 24 Hours of Dermal Exposure to Neat <sup>14</sup>C-D<sub>5</sub>

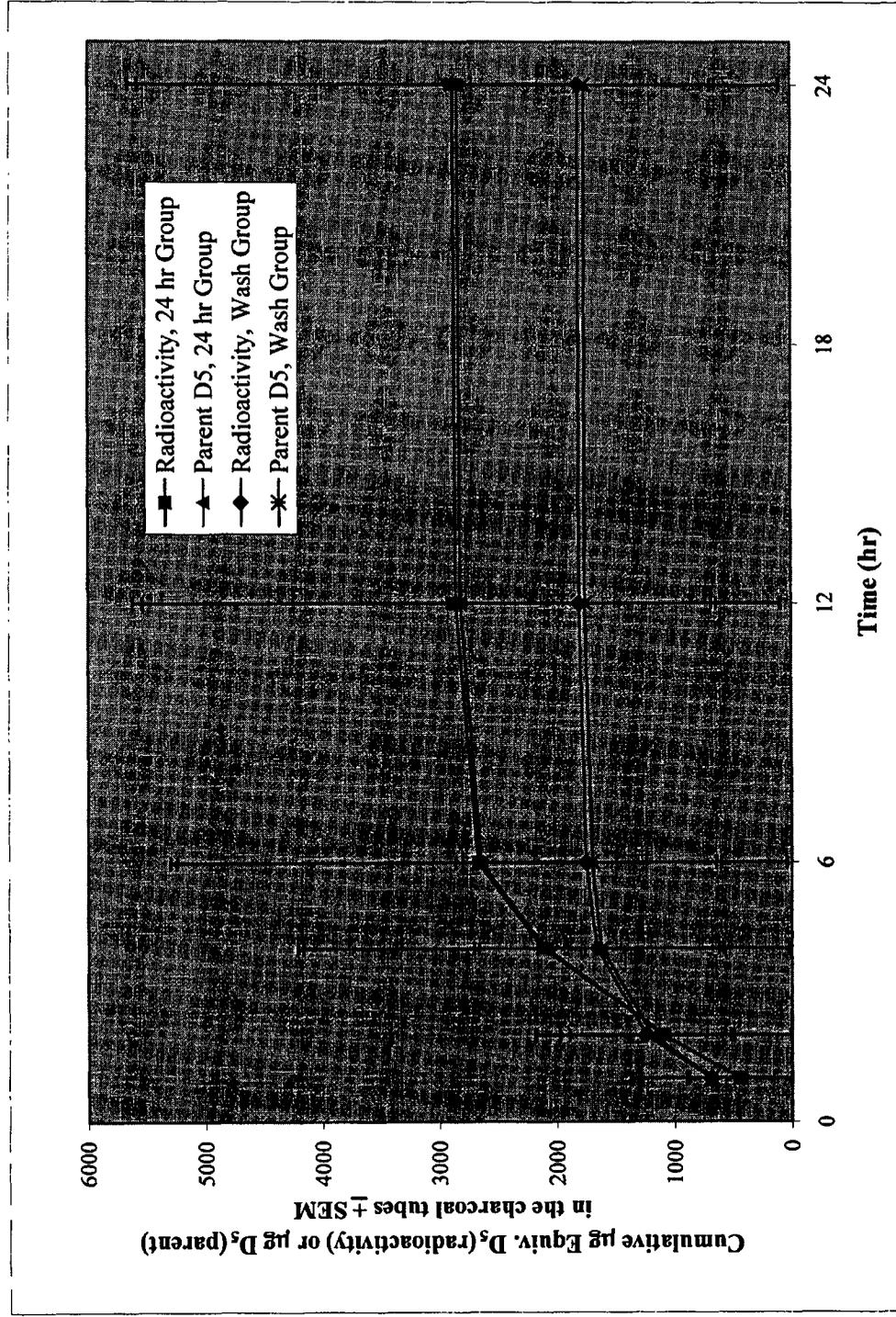


Figure 3. Disposition of Neat <sup>14</sup>C-D<sub>5</sub> Following Dermal Application

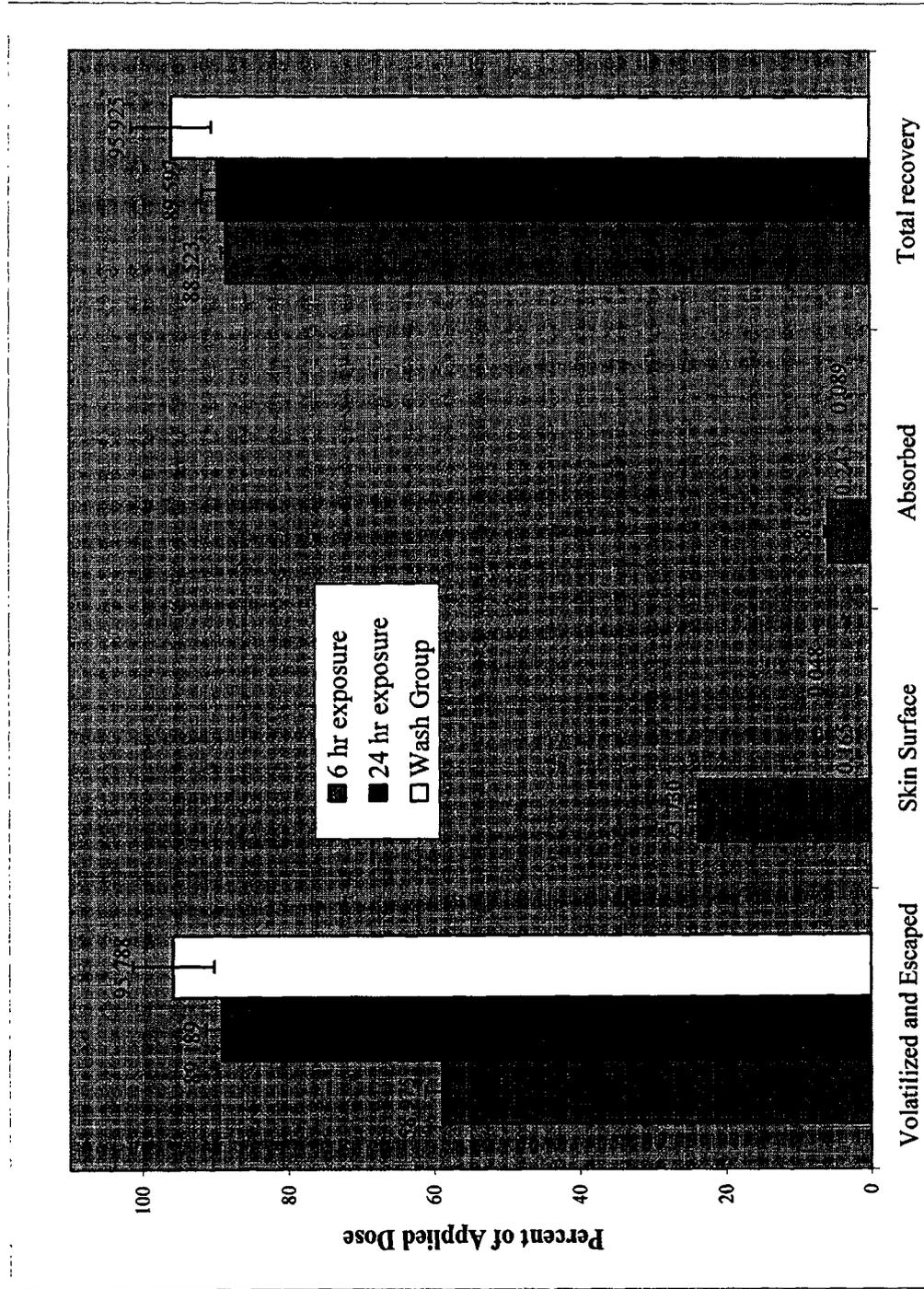
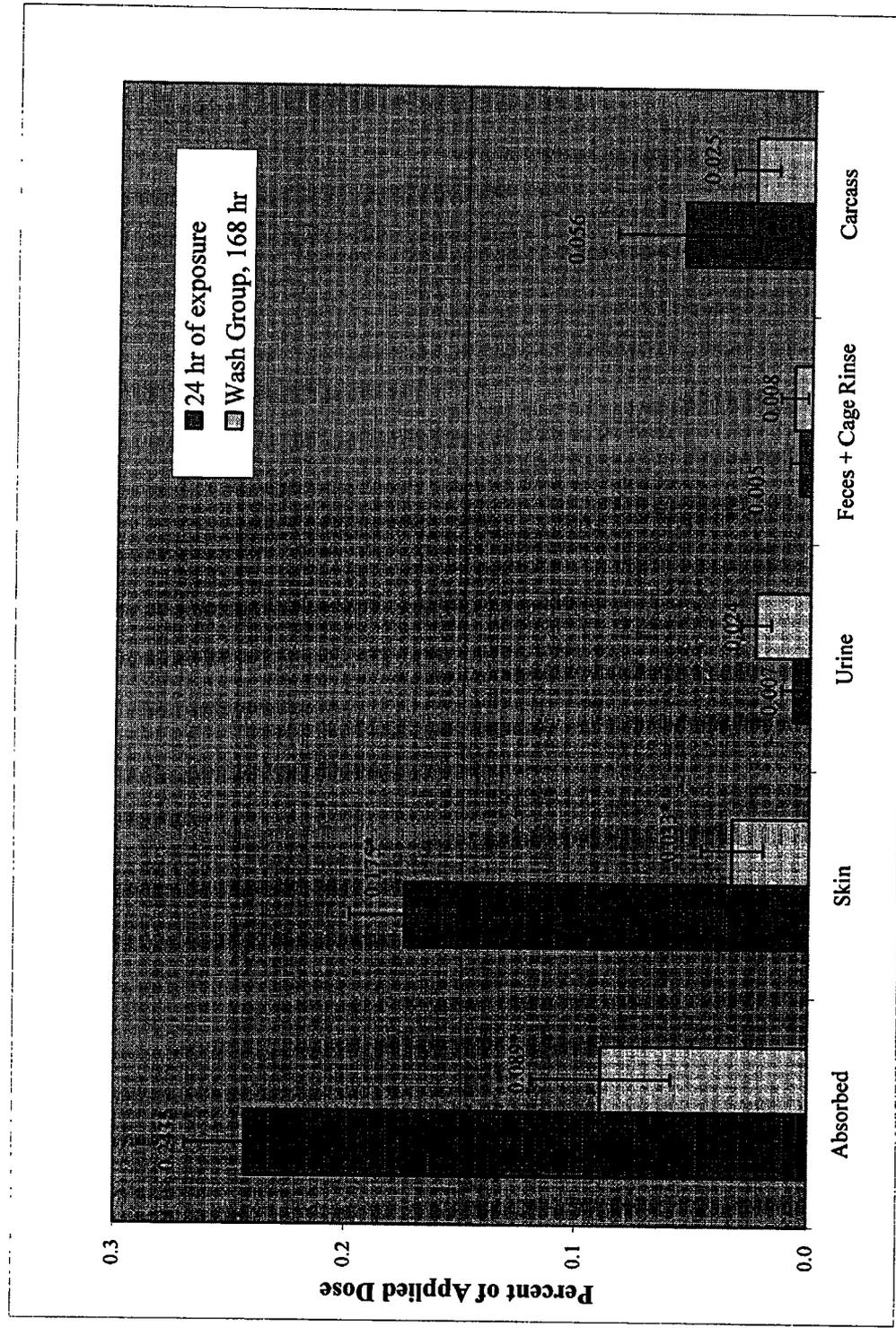


Figure 4. Disposition of the Absorbed Dose 24 hr Post Application of Neat <sup>14</sup>C-D<sub>5</sub> and Six Days After Skin Has Been Washed



\* Statistically different at  $p=0.05$

Figure 5. Possible Fate of Residual <sup>14</sup>C-D<sub>5</sub> after Washing Skin 24 hr Post Exposure

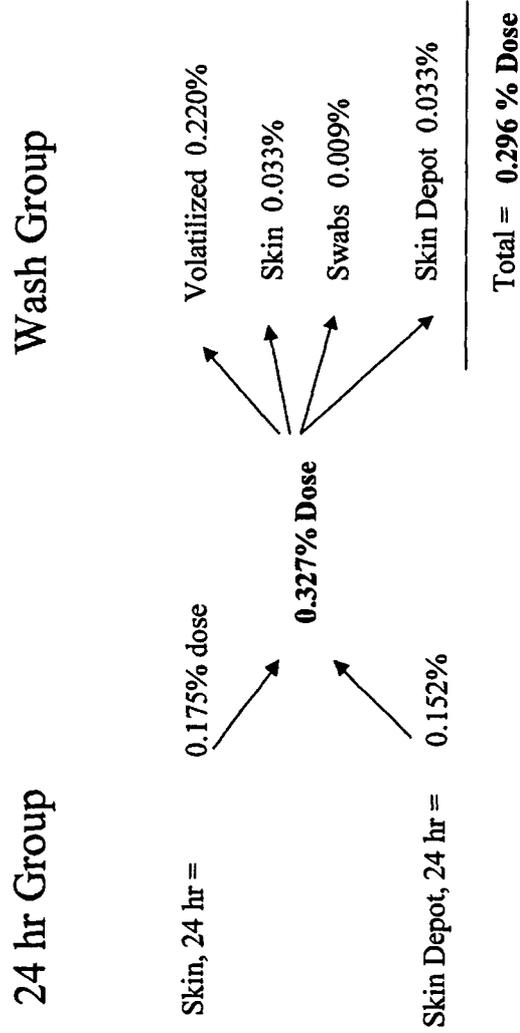
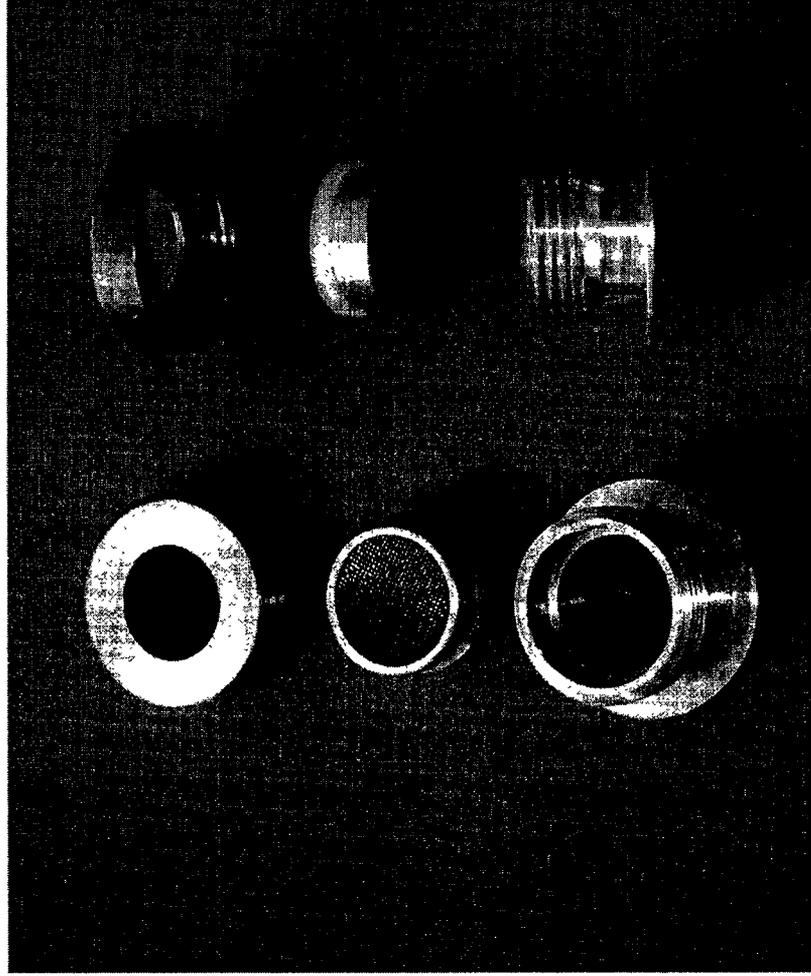


Figure 6. Skin depot (Application area = 2.5 cm<sup>2</sup>) Used for Test Article Application



## **APPENDIX A**

### **Radioactivity Spreadsheets and Parent Quantitation of D<sub>5</sub> in Expired Volatiles**

#### **Content:**

- Definitions, Calculations and Abbreviations
- Summary spreadsheets (Microsoft™ Excel 2000)

### *Definitions, Calculations and Abbreviations*

#### ***Definitions***

##### **Percutaneous absorption**

represents the amount of topically applied test article that is systemically available including amount found in the skin and amount that penetrated through the skin (urine, feces, cage rinse, carcass)

##### **Volatilized radioactivity**

represents radioactivity trapped in the charcoal baskets placed above the application site

##### **Escaped Radioactivity**

represents radioactivity trapped in the charcoal tubes and KOH traps

##### **Skin surface**

represents radioactivity found in swabs (skin washes), tape strips (*stratum corneum*) and skin depot

#### ***Calculations***

**1. Radioactivity**

$$1 \mu\text{Ci} = 2.22 \times 10^6 \text{ DPMs}$$

**2. Specific activity of dosing solution**

$$\text{DPM/mg of dosing solution} / 2.22 \times 10^6 (\text{DPM}/\mu\text{Ci}) = \mu\text{Ci/mg in dosing solution}$$

**3. Dose (DPMs):**

$$\text{S.A. of dosing solution (DPMs/mg)} \times \text{dose (mg)} = \text{DPMs}$$

**4. Dose ( $\mu\text{Ci}$ )**

$$\text{S.A. of dosing solution } (\mu\text{Ci/mg}) \times \text{dose (mg)} = \mu\text{Ci}$$

**5. Dose (mg of  $D_2/\text{cm}^2$ )**

$$\text{Dose (mg)} / 2.5 \text{ cm}^2$$

**6. Matrix Background**

Average DPM/g of control samples

**7. Radioactivity concentration in the samples (DPM/g)**

Aliquot DPM/Aliquot Wt.(g) - matrix background (DPM/g)

**8. Total DPMs in sample**

$$\text{Sample radioactivity conc. (DPM/g)} \times \text{Sample wt. (g)} = \text{DPMs}$$

**10. Percent of Applied Dose**

Sample Total DPMs

$$\frac{\text{Sample Total DPMs}}{\text{Dose (DPMs)}} \times 100 = \% \text{ Dose}$$

**11. Average Percent of Applied Dose**

Percent dose for the group of test systems.

**12. Cumulative Percent Dose**

Percent of applied dose collected in excreta added to the percent of the applied dose of the previous timepoint to get the total percent recovery in urine, feces, KOH and charcoal tubes over duration of exposure.

**13. Percent of Recovered Dose**

% of Applied Dose in a Sample

$$\frac{\text{Total Recovered Dose (\%)}}{\text{Total Recovered Dose (\%)}} \times 100 = \% \text{ of Total Recovered Dose}$$

**14. Percent of Absorbed Dose**

% of Applied Dose in a Sample

----- X 100 = % of Absorbed Dose

Total Absorbed Dose (%)

Sample: Urine, Feces, Excised Skin, Carcass

**15.  $\mu\text{g}$  Equivalent  $D_5$ /g of sample**

Sample radioactivity DPM corrected for controls / Sample weight (g) / Specific activity of dosing solution (DPM/mg) X 1000 ( $\mu\text{g}/\text{mg}$ )

Sample: Urine, Feces, Toluene Extracts, Excised Skin

**16.  $\mu\text{g}$  Equivalent  $D_5$  in the toluene extracts (e.g. charcoal tubes)**

Sample radioactivity DPM corrected for controls / Specific activity of dosing solution (DPM/mg) X 1000 ( $\mu\text{g}/\text{mg}$ )

or

$\mu\text{g}$  Equivalent  $D_5$ /g of sample X toluene weight (g)

**17.  $\mu\text{g}$  Equivalent  $D_5$  in charcoal tubes/hr**

$\mu\text{g}$  Equivalent  $D_5$  in toluene extract of charcoal tube corrected for controls / time interval

Time interval (hr) = Time between charcoal tube collections = Time of charcoal tube in use

**18. GC-MS data analysis**

Calculations for the calibration of the GC/MS and calculations of  $D_5$  concentrations in charcoal tube samples can be found in the method "Procedure for determination of  $D_5$  in expired volatiles (Charcoal tubes)". Copy of this method is kept in the study file.

**19.  $\mu\text{g}$  Parent  $D_5$  trapped in charcoal tubes/hr**

$\mu\text{g}$   $D_5$  (based on GC-MS data)/hr =  $\mu\text{g}$   $D_5$ /g of toluene corrected for controls X toluene weight (g)/time interval

**20.  $\mu\text{g}$  Equivalent  $D_5$ /cm<sup>2</sup> of skin**

% Dose Absorbed x Dose (mg/cm<sup>2</sup>) X 1000 ( $\mu\text{g}/\text{mg}$ )/100

or

Absorbed  $\mu\text{g}$  Equivalent  $D_5$  /2.5 cm<sup>2</sup> of skin

**21. Reanalysis Criteria (%RR)**

The %RR is derived by calculating the difference between two values (DPM/g) and expressing this difference as a percentage of the mean for the two values (aliquots). Samples which fail to meet the following criteria should be re-analyzed in duplicate:

- A) For DPM/g between 0 and 299 the %RR must be less than or equal to 20. (F1)
- B) For DPM/g between 300 and 999 the %RR must be less than or equal to 15. (F2)
- C) For DPM/g greater than 1000, the %RR must be less than 10. (F3)

An exception to the above criteria is when the % dose recovered in the sample is less than or equal to 0.1%, then no re-analysis of a sample is required, failure to meet criteria 1-3 notwithstanding.

All calculations for determining radioactivity present in the samples were performed using Excel spreadsheets version 2000 in full precision mode. Italicized samples were excluded as outliers based on statistical analysis and were not used in summarizing the data.

**Abbreviations:**

DPM = Disintegrations per minute  
Sol'n. = Solution  
DPM 1 = 1<sup>st</sup> Aliquot  
DPM 2 = 2<sup>nd</sup> Aliquot  
Wt. = Weight  
Eq./Equiv. = Equivalent  
Homog. = Homogenate  
GC = Gas chromatography  
MS = Mass Spectrometry  
N.A. = Not applicable  
N. D. = Not determined  
No. = Number  
Hr = Hour  
F= flag for non-uniformity of replicate samples  
µg Eq. = µg Equiv.= microgram equivalents of D<sub>5</sub>  
Cumul. = Cumulative

Avg. = Average  
S.A.= Specific Activity  
Rep. = Replicate  
LSC = Liquid Scintillation Counter  
St.Dev., S.D. = Standard Deviation  
SEM = Standard error of the mean  
TEAH = Tetraethylammonium hydroxide  
THF = Tetrahydrofuran  
Bkg. = Background  
N.S. = No sample  
B.W. = Body weight  
LOQ = Limit of quantification  
BLQ = Below limit of quantification  
HPLC= High performance liquid chromatography  
°C= Degree Celsius  
Conc. = Concentration

CHARCOAL TUBES

Animal ID	Group	Time Pt.	No.	Average Radioactivity µg Eq D5/hr	Radioactivity µg Eq D5/hr	SD	SEM	µg Eq. D5 per time interval	Parent µg D5/hr	Average Parent µg D5/hr	SD	SEM	Parent µg D5 per time point	% Dose in expired volatiles	Cumulative % Dose in expired volatiles	Average Cumulative % Dose in expired volatiles	SD	SEM
D0568	2	1hr	2	8.268					8.652					0.031	0.031			
D0569	2	1hr	2	5.836					6.275					0.021	0.021			
D0570	2	1hr	2	1.779					2.682					0.007	0.007			
D0571	2	1hr	2	4.292		2.7231	1.3616	5.044	5.053	5.666	2.4877	1.2438	5.666	0.016	0.016	0.019	0.0102	0.0051
<b>D0576</b>	<b>4</b>	<b>1hr</b>	<b>4</b>	<b>4316.638</b>					<b>3830.349</b>					<b>15.988</b>	<b>15.988</b>	<i>excluded</i>		
D0577	4	1hr	4	2.412					3.352					0.009	0.009			
D0578	4	1hr	4	1343.378					1314.867					4.071	4.071			
D0579	4	1hr	4	4.321		773.6564	446.6707	450.037	5.075	441.098	756.7065	436.8847	441.098	0.014	0.014	1.365	2.3436	1.3531
<b>D0580</b>	<b>5</b>	<b>1hr</b>	<b>5</b>	<b>2754.289</b>					<b>2734.637</b>					<b>9.943</b>	<b>9.943</b>			
D0581	5	1hr	5	3.059					4.070					0.010	0.010			
D0582	5	1hr	5	0.323					0.000					0.001	0.001			
D0583	5	1hr	5	2.233		1376.2089	688.1044	689.976	3.190	685.474	1366.1096	683.0548	685.474	0.010	0.010	2.491	4.9680	2.4840
<b>D0588</b>	<b>2</b>	<b>2hr</b>	<b>2</b>	<b>12.520</b>					<b>12.415</b>					<b>0.047</b>	<b>0.078</b>			
D0569	2	2hr	2	8.525					9.286					0.031	0.052			
D0570	2	2hr	2	3.557					4.519					0.013	0.020			
D0571	2	2hr	2	6.607		3.7518	1.8759	7.802	7.340	8.390	3.3211	1.6606	8.390	0.024	0.040	0.047	0.0242	0.0121

Animal ID	Time Pt.	Group No.	Average Radioactivity µg Eq D5/hr	Average Radioactivity µg Eq D5/hr	µg Eq. D5 per time interval	Parent µg D5/hr	Average Parent µg D5/hr	SD	SEM	Parent µg D5 per time point	% Dose in expired volatiles	% Dose in expired volatiles	% Dose in expired volatiles	SD	SEM
D0576	2hr	4	4252.226	4181.647							31.737	15.749	31.737	excluded	
D0577	2hr	4	4.951	5.794							0.027	0.018	0.027		
D0578	2hr	4	1937.015	1952.794							9.941	5.870	9.941		
D0579	2hr	4	7.503	7.993	649.823	649.823	655.527	1114.7415	643.5963	655.527	0.039	0.025	0.039	3.336	5.7200 3.3025
D0580	2hr	5	4.204	5.131							9.958	0.015	9.958		
D0581	2hr	5	6.606	7.089							0.033	0.023	0.033		
D0582	2hr	5	4.094	4.667							0.016	0.015	0.016		
D0583	2hr	5	2233.929	2028.648	562.208	562.208	511.384	1011.5101	505.7550	511.384	9.895	9.885	9.895	4.976	5.7168 2.8584
D0568	4hr	2	12.706	12.549							0.172	0.095	0.172		
D0569	4hr	2	9.911	9.602							0.125	0.072	0.125		
D0570	4hr	2	4.202	4.575							0.051	0.031	0.051		
D0571	4hr	2	8.014	8.202	17.416	17.416	8.732	3.3109	1.6554	17.464	0.099	0.059	0.099	0.112	0.0507 0.0254
D0576	4hr	4	2413.087	2494.591							49.611	17.875	49.611	excluded	
D0577	4hr	4	5.967	6.254							0.071	0.044	0.071		
D0578	4hr	4	1512.578	1481.995							19.108	9.167	19.108		
D0579	4hr	4	9.415	10.118	101.8640	101.8640	499.456	850.9064	491.2710	998.911	0.102	0.062	0.102	6.427	10.9819 6.3404

Animal ID	Time Pt.	Group No.	Radioactivity $\mu\text{g Eq DS/hr}$	Average Radioactivity $\mu\text{g Eq DS/hr}$	$\mu\text{g Eq. DS per time interval}$	Parent $\mu\text{g DS/hr}$	Average Parent $\mu\text{g DS/hr}$	SEM	SD	SEM	Parent $\mu\text{g DS per time point}$	% Dose in expired volatiles	% Dose in expired volatiles	% Dose in expired volatiles	SD	SEM
D0580	4hr	5	802.368			833.708						5.793	15.752			
D0581	4hr	5	7.717			8.121						0.053	0.086			
D0582	4hr	5	5.088			7.562						0.038	0.054			
D0583	4hr	5	4.756	204.982	409.964	5.267	213.665	199.1298	398.2597	206.6822	427.329	0.042	9.937	6.457	7.7479	3.8739
D0568	6hr	2	15.669			15.769						0.117	0.289			
D0569	6hr	2	11.269			11.160						0.082	0.207			
D0570	6hr	2	5.502			5.660						0.041	0.092			
D0571	6hr	2	13.325	11.441	22.883	13.320	11.477	2.1741	4.3482	2.1556	22.954	0.098	0.197	0.196	0.0811	0.0405
D0576	6hr	4	702.145			709.853						5.201	54.812	excluded		
D0577	6hr	4	6.896			6.922						0.051	0.123			
D0578	6hr	4	809.275			805.573						4.905	24.012			
D0579	6hr	4	11.029	275.734	551.467	10.872	274.456	266.7736	462.0654	265.5612	548.912	0.073	0.175	8.103	13.7778	7.9546
D0580	6hr	5	157.953			135.016						1.140	16.892			
D0581	6hr	5	9.553			9.602						0.065	0.151			
D0582	6hr	5	5.811			5.967						0.043	0.097			
D0583	6hr	5	6.365	44.921	89.841	6.516	39.275	37.6866	75.3733	31.9237	78.550	0.056	9.993	6.783	8.1890	4.0945

Animal ID	Time Pt.	Group No.	Average Radioactivity $\mu\text{g Eq DS/hr}$	Average Radioactivity $\mu\text{g Eq DS/hr}$	$\mu\text{g Eq DS}$ per time interval	Parent $\mu\text{g DS/hr}$	Average Parent $\mu\text{g DS/hr}$	SEM	SD	Parent $\mu\text{g DS}$ per time point	% Dose in expired volatiles	% Dose in expired volatiles	% Dose in expired volatiles	SD	SEM
D0576	12hr	4	35.630			34.871					0.792	55.604	excluded		
D0577	12hr	4	7.138			7.403					0.159	0.281			
D0578	12hr	4	75.841			68.754					1.379	25.391			
D0579	12hr	4	9.395	30.792	184.749	9.541	28.566	17.4100	34.8200	171.396	0.187	0.361	8.678	14.4743	7.2372
D0580	12hr	5	17.256			16.963					0.374	17.266			
D0581	12hr	5	8.331			8.466					0.171	0.323			
D0582	12hr	5	4.546			4.500					0.101	0.198			
D0583	12hr	5	5.655	8.947	53.683	6.033	8.990	2.7803	5.5606	53.943	0.150	10.143	6.982	8.2890	4.1445
Q0590	24hr	3	68.938			69.793					6.413	6.413			
D0573	24hr	3	3.388			3.327					0.310	0.310			
D0574	24hr	3	3.825			3.842					0.370	0.370			
D0575	24hr	3	256.765	83.229	1997.488	246.739	80.925	57.4321	114.8641	1942.201	23.885	23.885	7.745	11.1346	5.5673
D0576	24hr	4	6.604			6.375					0.293	55.898	excluded		
D0577	24hr	4	0.248			0.316					0.011	0.292			
D0578	24hr	4	4.960			4.959					0.180	25.572			
D0579	24hr	4	0.414	1.874	22.488	0.495	1.923	1.3153	2.6306	23.078	0.016	0.378	8.747	14.5705	8.4123

Animal ID	Time Pt.	Group No.	Radioactivity µg Eq DS/hr	Average Radioactivity µg Eq DS/hr	µg Eq. DS per time interval	Parent µg DS/hr	Average Parent µg DS/hr	SEM	SD	SEM	Parent µg D5 per time point	% Dose in expired volatiles	% Dose in expired volatiles	% Dose in expired volatiles	SD	SEM
D0580	24hr	5	1.465			1.404						0.063	17.329			
D0581	24hr	5	0.251			0.293						0.010	0.333			
D0582	24hr	5	0.187			0.269						0.008	0.206			
D0583	24hr	5	0.169	0.518	6.216	0.241	0.552	0.2844	0.5689	0.2844	6.620	0.009	10.152	7.005	8.3114	4.1557
D0580	48hr	5	0.314			0.344						0.027	17.357			
D0581	48hr	5	0.018			0.009						0.001	0.334			
D0582	48hr	5	0.021			0.008						0.002	0.208			
D0583	48hr	5	0.014	0.092	2.201	0.000	0.090	0.0848	0.1696	0.0848	2.164	0.002	10.154	7.013	8.3219	4.1610
D0580	72hr	5	0.133			0.179						0.012	17.368			
D0581	72hr	5	0.007			0.015						0.001	0.335			
D0582	72hr	5	0.009			0.002						0.001	0.209			
D0583	72hr	5	0.004	0.038	0.915	0.000	0.049	0.0436	0.0872	0.0436	1.174	0.000	10.154	7.017	8.3264	4.1632
D0580	96hr	5	0.074			0.111						0.006	17.375			
D0581	96hr	5	0.003			0.040						0.000	0.335			
D0582	96hr	5	0.005			0.002						0.000	0.210			
D0583	96hr	5	0.003	0.021	0.507	0.000	0.038	0.0260	0.0520	0.0260	0.918	0.000	10.154	7.018	8.3289	4.1644

Animal ID	Time Pt.	Group No.	Radioactivity $\mu\text{g Eq D}_5/\text{hr}$	Average Radioactivity $\mu\text{g Eq D}_5/\text{hr}$	$\mu\text{g Eq. D}_5$ per time interval	Parent $\mu\text{g D}_5/\text{hr}$	Average Parent $\mu\text{g D}_5/\text{hr}$	SEM	SD	SEM	Parent $\mu\text{g D}_5$ per time point	% Dose in expired volatiles	% Dose in expired volatiles	SEM	SD	SEM
D0580	120hr	5	0.071			0.069						0.006	17.381			
D0581	120hr	5	0.003			0.000						0.000	0.335			
D0582	120hr	5	0.003			0.000						0.000	0.210			
D0583	120hr	5	0.001	0.019	0.467	0.000	0.017	0.0173	0.0345	0.0173	0.414	0.000	10.154	7.020	8.3313	4.1657
D0580	144hr	5	0.055			0.053						0.005	17.386			
D0581	144hr	5	0.000			0.000						0.000	0.335			
D0582	144hr	5	0.002			0.000						0.000	0.210			
D0583	144hr	5	0.002	0.015	0.355	0.000	0.013	0.0134	0.0267	0.0134	0.321	0.000	10.155	7.021	8.3333	4.1666
D0580	168hr	5	0.042			0.094						0.004	17.389			
D0581	168hr	5	0.000			0.026						0.000	0.335			
D0582	168hr	5	0.004			0.000						0.000	0.210			
D0583	168hr	5	0.000	0.012	0.277	0.000	0.030	0.0221	0.0442	0.0221	0.719	0.000	10.155	7.022	8.3347	4.1673

Cumulative  $\mu\text{g Eq. D}_5$  (radioactivity) <sup>1)</sup>

Group 2	53.15
Group 3	1997.488
Group 4	2877.204
Group 5	1816.610

Cumulative  $\mu\text{g D}_5$  (parent) <sup>2)</sup>

Group 2	54.47
Group 3	1942.201
Group 4	2838.922
Group 5	1769.011

<sup>1)</sup> Radioactivity  $\mu\text{g Eq. D}_5$  in charcoal tubes per time interval added to  $\mu\text{g Eq. D}_5$  of the previous time interval to get cumulative amount of  $\text{D}_5$  over duration of exposure  
<sup>2)</sup> Parent  $\mu\text{g D}_5$  in charcoal tubes per time interval added to  $\mu\text{g parent D}_5$  of the previous time interval to get cumulative amount of  $\text{D}_5$  over duration of exposure

## KOH

Animal ID	Time Point	Group ID	Radioactivity µg Eq D5/g of KOH	Average Radioactivity µg Eq D5/g of KOH	SD	SEM	% Dose Recovered in KOH	Cumulative % Dose Recovered in KOH	Average Cumulative % Dose Recovered per Group	SD	SEM
D0568	6hr	2	0.000	0.000			0.000	0.000			
D0569	6hr	2	0.000	0.000			0.000	0.000			
D0570	6hr	2	0.000	0.000			0.000	0.000			
D0571	6hr	2	0.000	0.000	0.0001	0.0000	0.000	0.000	0.000	0.0000	0.0000
<b>D0576</b>	<b>6hr</b>	<b>4</b>	<b>0.000</b>				<b>0.000</b>	<b>0.000</b>	<b>excluded</b>		
D0577	6hr	4	0.001				0.000	0.000			
D0578	6hr	4	0.000				0.000	0.000			
D0579	6hr	4	0.001	0.000	0.0004	0.0002	0.000	0.000	0.000	0.0002	0.0001
D0580	6hr	5	0.000				0.000	0.000			
D0581	6hr	5	0.014				0.009	0.009			
D0582	6hr	5	0.000				0.000	0.000			
D0583	6hr	5	0.000	0.004	0.0072	0.0036	0.000	0.000	0.002	0.0043	0.0021
<b>D0576</b>	<b>12hr</b>	<b>4</b>	<b>0.000</b>				<b>0.000</b>	<b>0.000</b>	<b>excluded</b>		
D0577	12hr	4	0.007				0.004	0.004			
D0578	12hr	4	0.003				0.001	0.001			

Microsoft™ Excel 2000

Animal ID	Time Point	Group ID	Radioactivity		Average Radioactivity		% Dose Recovered in KOH	Cumulative % Dose Recovered in KOH	Average Cumulative % Dose Recovered per Group	SEM	SD
			µg Eq D5/g of KOH	µg Eq D5/g of KOH	µg Eq D5/g of KOH	µg Eq D5/g of KOH					
D0579	12hr	4	0.002	0.004	0.0029	0.0016	0.001	0.001	0.002	0.0018	0.0010
D0580	12hr	5	0.000	0.000	0.000	0.000	0.000	0.000			
D0581	12hr	5	0.000	0.000	0.000	0.000	0.000	0.009			
D0582	12hr	5	0.000	0.000	0.000	0.000	0.000	0.000			
D0583	12hr	5	0.000	0.000	0.0002	0.0001	0.000	0.000	0.002	0.0044	0.0022
Q0590	24hr	3 (dead)	0.001	0.001	0.0003	0.0002	0.000	0.000			
D0573	24hr	3 (dead)	0.001	0.001	0.000	0.000	0.000	0.000			
D0574	24hr	3 (dead)	0.000	0.000	0.000	0.000	0.000	0.000			
D0575	24hr	3 (dead)	0.001	0.001	0.0003	0.0002	0.000	0.000	0.000	0.0002	0.0001
<b>D0576</b>	<b>24hr</b>	<b>4</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>0.000</b>	<b>excluded</b>		
D0577	24hr	4	0.025	0.016	0.021	0.001	0.021				
D0578	24hr	4	0.000	0.000	0.000	0.001	0.001				
D0579	24hr	4	0.000	0.008	0.0143	0.0082	0.001	0.008	0.008	0.0111	0.0064
D0580	24hr	5	0.000	0.000	0.000	0.000	0.000				
D0581	24hr	5	0.003	0.002	0.011	0.001	0.011				
D0582	24hr	5	0.003	0.002	0.002	0.002	0.002				

Animal ID	Time Point	Group ID	Radioactivity µg Eq D5/g of KOH	Average Radioactivity µg Eq D5/g of KOH	SD	SEM	% Dose Recovered in KOH	Cumulative % Dose Recovered in KOH	Average Cumulative % Dose Recovered per Group	SD	SEM
D0583	24hr	5	0.000	0.002	0.0017	0.0008	0.000	0.000	0.003	0.0050	0.0025
D0580	48hr	5	0.000	0.000			0.000	0.000			
D0581	48hr	5	0.001				0.001	0.011			
D0582	48hr	5	0.003				0.002	0.004			
D0583	48hr	5	0.002	0.002	0.0014	0.0007	0.002	0.002	0.004	0.0048	0.0024
D0580	72hr	5	0.001				0.000	0.000			
D0581	72hr	5	0.000				0.000	0.011			
D0582	72hr	5	0.002				0.001	0.005			
D0583	72hr	5	0.002	0.001	0.0008	0.0004	0.001	0.004	0.005	0.0046	0.0023
D0580	96hr	5	0.004				0.002	0.003			
D0581	96hr	5	0.002				0.001	0.013			
D0582	96hr	5	0.001				0.001	0.006			
D0583	96hr	5	0.002	0.002	0.0013	0.0006	0.002	0.005	0.007	0.0043	0.0021
D0580	120hr	5	0.002				0.001	0.004			
D0581	120hr	5	0.005				0.003	0.016			

Animal ID	Time Point	Group ID	Radioactivity µg Eq D5/g of KOH	Average Radioactivity µg Eq D5/g of KOH	SD	SEM	% Dose Recovered in KOH	Cumulative % Dose Recovered in KOH	Average Cumulative % Dose Recovered per Group	SD	SEM
D0582	120hr	5	0.002	0.002	0.0020	0.0010	0.001	0.007	0.008	0.0052	0.0026
D0583	120hr	5	0.000	0.002	0.0020	0.0010	0.000	0.005	0.008	0.0052	0.0026
D0580	144hr	5	0.000	0.000	0.0000	0.0000	0.000	0.004	0.008	0.0052	0.0026
D0581	144hr	5	0.005	0.005	0.0003	0.0003	0.003	0.019	0.008	0.0052	0.0026
D0582	144hr	5	0.002	0.002	0.0001	0.0001	0.001	0.008	0.008	0.0052	0.0026
D0583	144hr	5	0.006	0.003	0.0026	0.0013	0.005	0.010	0.010	0.0061	0.0030
D0580	168hr	5	0.001	0.001	0.0001	0.0001	0.001	0.005	0.012	0.0068	0.0034
D0581	168hr	5	0.003	0.003	0.0002	0.0002	0.002	0.020	0.012	0.0068	0.0034
D0582	168hr	5	0.001	0.001	0.0001	0.0001	0.001	0.009	0.012	0.0068	0.0034
D0583	168hr	5	0.007	0.003	0.0028	0.0014	0.006	0.015	0.012	0.0068	0.0034

## CHARCOAL BASKET

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq D5/g of extract	Average Radioactivity $\mu\text{g}$ Eq D5/g of extract	SD	SEM	% Dose Volatilized	Average % Dose Volatilized per Group	SD	SEM
D0568	6hr	2	963.395				62.1282			
D0569	6hr	2	834.963				54.7248			
D0570	6hr	2	836.290				54.8953			
D0571	6hr	2	977.386	903.008	78.0173	39.0087	63.3669	58.779	4.6111	2.3055
Q0590	24hr	3 (dead)	1375.091				89.2978			
D0573	24hr	3 (dead)	1364.268				89.1278			
D0574	24hr	3 (dead)	1294.504				94.7436			
D0575	24hr	3 (dead)	1075.866	1277.432	139.0422	69.5211	72.6147	86.446	9.5826	4.7913
<b>D0576</b>	<b>24hr</b>	<b>4</b>	<b>349.416</b>				<b>34.0702</b>	<b>excluded</b>		
D0577	24hr	4	1292.616				93.1114			
D0578	24hr	4	1176.129				61.3472			
D0579	24hr	4	1500.853	1323.199	164.5080	94.9787	86.8427	80.434	16.8240	9.7133
D0580	24hr wash	5	1134.294				69.8930			
D0581	24hr wash	5	1647.618				92.7603			
D0582	24hr wash	5	1594.231				89.6353			
D0583	24hr wash	5	1344.983	1430.282	237.3456	118.6728	101.8414	88.533	13.4617	6.7308

Animal ID	Time Point	Group ID	Average		SD	SEM	% Dose Volatilized	Average % Dose Volatilized per Group	SD	SEM
			Radioactivity $\mu\text{g}$ Eq D5/g of extract	Radioactivity $\mu\text{g}$ Eq D5/g of extract						
D0580	24-168hr	5	2.763	2.763	0.2659					
D0581	24-168hr	5	1.083	1.083	0.0990					
D0582	24-168hr	5	3.899	3.899	0.3823					
D0583	24-168hr	5	1.149	1.149	0.1346	0.6804	0.220	0.1296	0.0648	
D0580	168hr <sup>1)</sup>	5	1137.057	1137.057	70.1589					
D0581	168hr	5	1648.702	1648.702	92.8594					
D0582	168hr	5	1598.130	1598.130	90.0177					
D0583	168hr	5	1346.132	1346.132	101.9760	118.6459	88.753	13.4046	6.7023	

<sup>1)</sup>Total volatilized in "Wash group"= Charcoal basket collected at 24 hr + Charcoal basket used for collection from 24 to 168hr

**EXCISED SKIN - APPLICATION SITE**

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g Eq D5/cm}^2$ of skin	Average Radioactivity $\mu\text{g Eq D5/cm}^2$ of skin	SD	SEM	% Dose Recovered in Skin Application Site	Average % Dose Recovered per Group	SD	SEM
D0568	6hr	2	459.20				4.284			
D0569	6hr	2	738.99				6.743			
D0570	6hr	2	616.51				5.708			
D0571	6hr	2	593.72	602.102	114.6525	57.3263	5.457	5.548	1.0099	0.5050
Q0590	24hr	3 (dead)	25.24				0.245			
D0573	24hr	3 (dead)	44.89				0.428			
D0574	24hr	3 (dead)	17.80				0.179			
D0575	24hr	3 (dead)	16.96	26.220	12.9892	6.4946	0.164	0.254	0.1212	0.0606
<b>D0576</b>	<b>24hr</b>	<b>4</b>	<b>10.35</b>				<b>0.096</b>	<b>excluded</b>		
D0577	24hr	4	23.66				0.219			
D0578	24hr	4	18.33				0.139			
D0579	24hr	4	20.13	20.707	2.7107	1.5650	0.167	0.175	0.0407	0.0235
D0580	168hr	5	4.38				0.040			
D0581	168hr	5	1.67				0.014			
D0582	168hr	5	7.33				0.068			
D0583	168hr	5	1.00	3.595	2.8882	1.4441	0.011	0.033	0.0264	0.0132

## CARCASS

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq DS/g carcass	Average Radioactivity $\mu\text{g}$ Eq DS/g carcass	SD	SEM	% Dose Recovered in Carcass	Average % Dose Recovered per Group	SD	SEM
D0568	6hr	2	0.478				0.253			
D0569	6hr	2	0.293				0.151			
D0570	6hr	2	0.144				0.075			
D0571	6hr	2	1.163	0.520	0.4504	0.2252	0.600	0.270	0.2316	0.1158
Q0590	24hr	3 (dead)	0.272				0.159			
D0573	24hr	3 (dead)	0.015				0.009			
D0574	24hr	3 (dead)	0.028				0.016			
D0575	24hr	3 (dead)	0.070	0.097	0.1194	0.0597	0.037	0.055	0.0705	0.0353
<b>D0576</b>	<b>24hr</b>	<b>4</b>	<b>0.918</b>				<b>0.467</b>	<b>excluded</b>		
D0577	24hr	4	0.055				0.028			
D0578	24hr	4	0.276				0.114			
D0579	24hr	4	0.054	0.128	0.1279	0.0739	0.025	0.056	0.0505	0.0292
D0580	168hr	5	0.110				0.053			
D0581	168hr	5	0.031				0.015			
D0582	168hr	5	0.043				0.023			
D0583	168hr	5	0.013	0.049	0.0420	0.0210	0.008	0.025	0.0197	0.0098

SKIN SURFACE		Animal ID	Time Point	Group ID	% Dose Recovered in		% Dose Recovered in skin depot extracts	% Dose Recovered on the skin surface	Average % Dose Recovered per		SEM
					swabs and tape strips				Group	SD	
D0568	6hr	2	17.083	5.700	22.784						
D0569	6hr	2	14.689	11.733	26.422						
D0570	6hr	2	20.419	5.860	26.279						
D0571	6hr	2	11.062	8.373	19.435		23.730	3.3211	1.6605		
Q0590	24hr	3 (dead)	0.024	1.016	1.041						
D0573	24hr	3 (dead)	0.070	0.151	0.221						
D0574	24hr	3 (dead)	0.031	0.178	0.209						
D0575	24hr	3 (dead)	0.012	0.060	0.072		0.386	0.4417	0.2209		
<b>D0576</b>	<b>24hr</b>	<b>4</b>	<b>0.007</b>	<b>0.060</b>	<b>0.067</b>	<b>excluded</b>					
D0577	24hr	4	0.012	0.076	0.088						
D0578	24hr	4	0.014	0.349	0.362						
D0579	24hr	4	0.013	0.032	0.045		0.165	0.1721	0.0994		
D0580	168hr	5	0.013	0.066	0.079						
D0581	168hr	5	0.009	0.030	0.039						
D0582	168hr	5	0.025	0.030	0.055						
D0583	168hr	5	0.011	0.006	0.018		0.048	0.0260	0.0130		







Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ radioactivity $\mu\text{g}$ Eq		Average		% Dose Recovered in Feces	Cumulative % Dose Recovered in Feces	Average Cumulative % Dose Recovered per Group	SD	SEM
			D5/g of Feces	D5/g of Feces	D5/g of Feces	D5/g of Feces					
D0580	168hr	5	0.070	0.000	0.001	0.025	0.000	0.000	0.008	0.0118	0.0059
D0581	168hr	5	0.000	0.000	0.000	0.001	0.000	0.000			
D0582	168hr	5	0.000	0.000	0.000	0.004	0.000	0.000			
D0583	168hr	5	0.000	0.018	0.0350	0.0175	0.000	0.000			

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq D5/g THF	Average Radioactivity $\mu\text{g}$ Eq D5/g THF	SEM	SD	% Dose Recovered in Cage Rinses	Average % Dose Recovered per Group	SD	SEM
D0568	6hr	2	0.008				0.001			
D0569	6hr	2	0.008				0.001			
D0570	6hr	2	0.003				0.000			
D0571	6hr	2	0.009	0.007	0.0014	0.0029	0.001	0.001	0.0002	0.0001
Q0590	24hr	3	0.004				0.000			
D0573	24hr	3	0.000				0.000			
D0574	24hr	3	0.002				0.000			
D0575	24hr	3	0.013	0.005	0.0028	0.0055	0.002	0.001	0.0012	0.0006
<b>D0576</b>	<b>24hr</b>	<b>4</b>	<b>0.020</b>				<b>0.003</b>	<i>excluded</i>		
D0577	24hr	4	0.005				0.001			
D0578	24hr	4	0.011				0.001			
D0579	24hr	4	0.005	0.007	0.0021	0.0036	0.001	0.001	0.0003	0.0002
D0580	168hr	5	0.000				0.000			
D0581	168h	5	0.000				0.000			
D0582	168hr	5	0.000				0.000			
D0583	168hr	5	0.000	0.000	0.0000	0.0000	0.000	0.000	0.0000	0.0000

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq DS/g of Urine	Average Radioactivity $\mu\text{g}$ Eq DS/g of Urine	SD	SEM	% Dose Recovered in Urine	Cumulative % Dose Recovered in Urine	Average Cumulative % Dose Recovered per Group	SD	SEM
D0568	6hr	2	0.009				0.000	0.000			
D0569	6hr	2	No sample				No sample	No sample			
D0570	6hr	2	0.014				0.000	0.000			
D0571	6hr	2	0.027	0.017	0.0096	0.0055	0.000	0.000	0.000	0.0000	0.0000
<b>D0576</b>	<b>6hr</b>	<b>4</b>	<b>3.172</b>				<b>0.020</b>	<b>0.020</b>	<b>excluded</b>		
D0577	6hr	4	0.010				0.000	0.000			
D0578	6hr	4	0.119				0.000	0.000			
D0579	6hr	4	0.008	0.046	0.0632	0.0365	0.000	0.000	0.000	0.0000	0.0000
D0579	6hr	4	0.008	0.046	0.0632	0.0365	0.000	0.000	0.000	0.0000	0.0000
D0580	6hr	5	0.072				0.000	0.000			
D0581	6hr	5	0.012				0.000	0.000			
D0582	6hr	5	0.021				0.000	0.000			
D0583	6hr	5	0.009	0.028	0.0295	0.0147	0.000	0.000	0.000	0.0000	0.0000
<b>D0576</b>	<b>12hr</b>	<b>4</b>	<b>11.049</b>				<b>0.042</b>	<b>0.062</b>	<b>excluded</b>		
D0577	12hr	4	0.080				0.000	0.000			
D0578	12hr	4	1.112				0.006	0.006			
D0579	12hr	4	0.070	0.421	0.5984	0.3455	0.000	0.000	0.002	0.0031	0.0018

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq D5/g of Urine	Average Radioactivity $\mu\text{g}$ Eq D5/g of Urine	SD	SEM	% Dose Recovered in Urine	Cumulative % Dose Recovered in Urine	Average Cumulative % Dose Recovered per Group	SD	SEM
D0580	12hr	5	0.545				0.005	0.005			
D0581	12hr	5	0.046				0.000	0.000			
D0582	12hr	5	Lost sample				Lost sample	0.000			
D0583	12hr	5	0.046	0.213	0.2878	0.1459	0.000	0.000	0.001	0.0023	0.0011
D0583	12hr	5									
D0576	24hr	4	7.905				0.087	0.149	<i>excluded</i>		
D0577	24hr	4	0.217				0.002	0.003			
D0578	24hr	4	1.612				0.012	0.017			
D0579	24hr	4	0.192	0.674	0.8124	0.4691	0.002	0.002	0.007	0.0087	0.0050
D0580	24hr	5	1.279				0.012	0.017			
D0581	24hr	5	0.176				0.002	0.002			
D0582	24hr	5	0.207				0.002	0.003			
D0583	24hr	5	0.165	0.457	0.5486	0.2743	0.002	0.002	0.006	0.0071	0.0035
D0580	48hr	5	0.938				0.009	0.025			
D0581	48hr	5	0.152				0.003	0.005			
D0582	48hr	5	0.287				0.006	0.008			
D0583	48hr	5	0.215	0.398	0.3641	0.1821	0.003	0.006	0.011	0.0097	0.0048

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq D5/g of Urine	Average Radioactivity $\mu\text{g}$ Eq D5/g of Urine	SD	SEM	% Dose Recovered in Urine	Cumulative % Dose Recovered in Urine	Average Cumulative % Dose Recovered per Group	SD	SEM
D0580	72hr	5	0.594				0.006	0.032			
D0581	72hr	5	0.107				0.003	0.008			
D0582	72hr	5	0.243				0.006	0.014			
D0583	72hr	5	0.115	0.265	0.2280	0.1140	0.002	0.008	0.015	0.0112	0.0056
D0580	96hr	5	0.629				0.003	0.035			
D0581	96hr	5	0.090				0.002	0.010			
D0582	96hr	5	0.158				0.004	0.018			
D0583	96hr	5	0.108	0.246	0.2567	0.1283	0.002	0.010	0.018	0.0116	0.0058
D0580	120hr	5	0.371				0.003	0.038			
D0581	120hr	5	0.071				0.001	0.012			
D0582	120hr	5	0.124				0.003	0.021			
D0583	120hr	5	0.076	0.161	0.1425	0.0713	0.001	0.011	0.020	0.0124	0.0062
D0580	144hr	5	0.177				0.002	0.040			
D0581	144hr	5	0.061				0.002	0.014			
D0582	144hr	5	0.089				0.002	0.023			
D0583	144hr	5	0.056	0.096	0.0563	0.0282	0.001	0.012	0.022	0.0125	0.0062

Animal ID	Time Point	Group ID	Radioactivity $\mu\text{g}$ Eq D5/g of Urine	Average Radioactivity $\mu\text{g}$ Eq D5/g of Urine	SEM	% Dose Recovered in Urine	Cumulative % Dose Recovered in Urine	Average Cumulative % Dose Recovered per Group	SD	SEM
D0580	168hr	5	0.183			0.002	0.041			
D0581	168hr	5	0.036			0.001	0.015			
D0582	168hr	5	0.053			0.002	0.025			
D0583	168hr	5	0.040	0.078	0.0351	0.001	0.013	0.024	0.0130	0.0065

Volatilized and escaped radioactivity = Radioactivity trapped in the charcoal tubes, KOH and charcoal baskets

Animal ID	Group ID	Group Description	%Dose Volatilized (Charcoal basket)	%Dose Escaped volatiles (Charcoal Tubes)	%Dose CO <sub>2</sub> (KOH traps)	%Dose Cage Rinse <sup>2)</sup>	%Dose Volatilized + Escaped <sup>1)</sup>	Average %Dose Volatilized + Escaped	SEM
D0568	2	6 hr group	62.128	0.289	0.000	N.A.	62.418		
D0569	2	6 hr group	54.725	0.207	0.000	N.A.	54.932		
D0570	2	6 hr group	54.895	0.092	0.000	N.A.	54.987		
D0571	2	6 hr group	63.367	0.197	0.000	N.A.	63.564	58.975	2.3303
Q0590	3	group	89.298	6.413	0.000	0.000	95.711		
D0573	3	group	89.128	0.310	0.000	0.000	89.439		
D0574	3	group	94.744	0.370	0.000	0.000	95.114		
D0575	3	group	72.615	23.885	0.000	0.002	96.503	94.192	1.6096
D0576 <sup>1)</sup>	4	24 hr group	34.070	55.898	0.000	N.A.	89.968	excluded	
D0577	4	24 hr group	93.111	0.292	0.021	N.A.	93.424		
D0578	4	24 hr group	61.347	25.572	0.001	N.A.	86.920		
D0579	4	24 hr group	86.843	0.378	0.001	N.A.	87.222	89.189	2.1195
D0580	5	Wash group at 24 hr	69.893	17.329	0.000	N.A.	87.222		
D0581	5	Wash group at 24 hr	92.760	0.333	0.011	N.A.	93.104		
D0582	5	Wash group at 24 hr	89.635	0.206	0.002	N.A.	89.844		
D0583	5	Wash group at 24 hr	101.841	10.152	0.000	N.A.	111.994	95.541	5.6146
D0580	5	Wash group at 168 hr	70.159	17.389	0.005	N.A.	87.553		
D0581	5	Wash group at 168 hr	92.859	0.335	0.020	N.A.	93.215		
D0582	5	Wash group at 168 hr	90.018	0.210	0.009	N.A.	90.237		
D0583	5	Wash group at 168 hr	101.976	10.155	0.015	N.A.	112.146	95.788	5.5740

<sup>1)</sup> Animal = D0576 was excluded as an outlier based on Q-test

<sup>2)</sup> Cage rinse in dead animal group is part of escaped radioactivity

% Dose Recovered on the Skin Surface = Swabs, Tape strips and Skin depot

Animal ID	Group ID	Group Description	% Dose Swabs and tape strips	Average % Dose Swabs and Tape	SEM	Dose Skin Depo	Average % Dose in Skin Depot	SEM	% Dose on Skin surface	Average % Dose on Skin surface	SEM	N
D0566	1	Control										
D0567	1	Control										
D0568	2	6 hr group	17.083			5.700			22.784			
D0569	2	6 hr group	14.689			11.733			26.422			
D0570	2	6 hr group	20.419			5.860			26.279			
D0571	2	6 hr group	11.062	15.813	1.9720	8.373	7.916	1.4116	19.435	23.730	1.6605	4
Q0590	3	group	0.024			1.016			1.041			
D0573	3	group	0.070			0.151			0.221			
D0574	3	group	0.031			0.178			0.209			
D0575	3	group	0.012	0.035	0.0125	0.060	0.351	0.2231	0.072	0.386	0.2209	4
D0576 <sup>1)</sup>	4	24 hr group	0.007			0.060			0.067	excluded		
D0577	4	24 hr group	0.012			0.076			0.088			
D0578	4	24 hr group	0.014			0.349			0.362			
D0579	4	24 hr group	0.013	0.013	0.0005	0.032	0.152	0.0990	0.045	0.165	0.0994	3
D0580	5	Wash group <sup>2)</sup>	0.013			0.066			0.079			
D0581	5	Wash group	0.009			0.030			0.039			
D0582	5	Wash group	0.025			0.030			0.055			
D0583	5	Wash group	0.011	0.015	0.0036	0.006	0.033	0.0124	0.018	0.048	0.0130	4

<sup>1)</sup> Animal = D0576 was excluded as an outlier based on Q-test

<sup>2)</sup> Total skin surface in Wash group over 168hr = Swabs at 24 hr+swabs and tape strips at 168hr + skin depot

% Total Absorbed = Excised skin application site, Urine, Feces, Carcass, Cage rinse

Animal ID	Group ID	Group Description	%Dose Excised skin (application site)	%Dose Carcass	%Dose Urine	%Dose Feces	%Dose Cage rinse	%Dose Feces + Cage rinse	%Dose Absorbed	Average % Dose Absorbed	SEM	µg Equiv.D <sub>50</sub> /cm <sup>2</sup> of skin
D0568	2	6 hr group	4.284	0.253	0.000	No sample	0.001	0.001	4.538			
D0569	2	6 hr group	6.743	0.151	0.000	0.000	0.001	0.001	6.894			
D0570	2	6 hr group	5.708	0.075	0.000	No sample	0.000	0.000	5.784			
D0571	2	6 hr group	5.457	0.600	0.000	0.000	0.001	0.001	6.058	5.818	0.4879	630.718
Q0590	3	group	0.245	0.159	NA	NA	NA <sup>1)</sup>	NA	0.404			
D0573	3	group	0.428	0.009	NA	NA	NA	NA	0.437			
D0574	3	group	0.179	0.016	NA	NA	NA	NA	0.195			
D0575	3	group	0.164	0.037	NA	NA	NA	NA	0.201	0.309	0.0645	31.742
D0576 <sup>1)</sup>	4	24 hr group	0.096	0.467	0.149	0.157	0.003	0.159	0.871	excluded		
D0577	4	24 hr group	0.219	0.028	0.003	0.000	0.001	0.001	0.250			
D0578	4	24 hr group	0.139	0.114	0.017	0.013	0.001	0.014	0.284			
D0579	4	24 hr group	0.167	0.025	0.002	0.001	0.001	0.001	0.195	0.243	0.0259	29.242
D0580	5	68hr Wash group	0.040	0.053	0.041	0.025	0.000	0.025	0.159			
D0581	5	68hr Wash group	0.014	0.015	0.015	0.001	0.000	0.001	0.046			
D0582	5	68hr Wash group	0.068	0.023	0.025	0.004	0.000	0.004	0.120			
D0583	5	68hr Wash group	0.011	0.008	0.013	0.000	0.000	0.000	0.033	0.089	0.0302	9.511

<sup>1)</sup> Animal = D0576 was excluded as an outlier based on Q-test

<sup>2)</sup> Cage rinse is not considered part of absorbed dose in dead animals

% Total Mass Balance

Animal ID	Group ID	Group Description	%Dose Volatilized + Escaped <sup>1)</sup>	%Dose Volatilized + Escaped <sup>1)</sup>	SEM	%Dose Skin surface <sup>2)</sup>	Average %Dose Skin Surface	%Dose Absorbed <sup>3)</sup>	Average %Dose Absorbed	Total %Dose recovered	Average Total recovery <sup>4)</sup>	SEM
D0568	2	6 hr group	62.418			22.784		4.538		89.739		
D0569	2	6 hr group	54.932			26.422		6.894		88.248		
D0570	2	6 hr group	54.987			26.279		5.784		87.050		
D0571	2	6 hr group	63.564	58.975	2.3303	19.435	23.730	6.058	5.818	89.056	88.523	0.5779
D0590	3	group	95.711			1.041		0.404		97.156		
D0573	3	group	89.439			0.221		0.437		90.096		
D0574	3	group	95.114			0.209		0.195		95.519		
D0575	3	group	96.503	94.192	1.6096	0.072	0.386	0.201	0.309	96.776	94.887	1.6347
D0576	4	24 hr group	89.968	excluded		0.067		0.871		90.906	excluded	
D0577	4	24 hr group	93.424			0.088		0.250		93.763		
D0578	4	24 hr group	86.920			0.362		0.284		87.567		
D0579	4	24 hr group	87.222	89.189	2.1195	0.045	0.165	0.195	0.243	87.462	89.597	2.0831
D0580	5	Wash group (168 hr)	87.553			0.079		0.159		87.791		
D0581	5	Wash group (168 hr)	93.215			0.039		0.046		93.300		
D0582	5	Wash group (168 hr)	90.237			0.055		0.120		90.412		
D0583	5	Wash group (168 hr)	112.146	95.788	5.5740	0.018	0.048	0.033	0.089	112.196	95.925	5.5392

<sup>1)</sup> Volatilized + Escaped volatiles in live animals = Charcoal basket + Charcoal tube+KOH traps  
Volatilized + Escaped Volatiles in dead animals = Charcoal basket + Charcoal tube + KOH traps + Cage rinse

<sup>2)</sup> Skin surface = Swabs + Tape strips + Skin Depot

<sup>3)</sup> % Absorbed in live animals = Urine+feces+cage rinse+carcass+ skin application site

<sup>4)</sup> % Absorbed in dead animals = Carcass+ skin application site

<sup>5)</sup> % Total dose recovered = Volatilized/escaped volatiles+ skin surface + absorbed

**MASS BALANCE SUMMARY DATA EXPRESSED AS % OF THE APPLIED DOSE ± SEM**

Group Number	Group Description	N	Average escaped (charcoal tubes) (%)	SEM	Average Volatilized (Charcoal basket)	SEM	Average KOH (%)	SEM	Volatilized and escaped <sup>1)</sup>	SEM	Average Skin Surface <sup>2)</sup>	SEM	Average Absorbed (%)	SEM	Total recovery (%)	SEM
2	6hr	4	0.196	0.0405	58.779	2.3055	0.000	0.0000	58.975	2.3003	23.730	1.6605	5.818	0.4879	88.523	0.5779
3	Dead Group	4	7.745	5.5673	86.446	4.7913	0.000	0.0001	94.192	1.6096	0.386	0.2209	0.309	0.0645	94.887	1.6347
4	24hr	3	8.747	8.4123	80.434	9.7133	0.008	0.0064	89.189	2.1195	0.165	0.0994	0.243	0.0259	89.597	2.0831
5	Wash Group (24hr)	4	7.005	4.1557	88.533	6.7308	0.003	0.0025	95.541	5.6146	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
5	Wash Group (168hr)	4	7.022	4.1673	88.753	6.7023	0.012	0.0034	95.788	5.5740	0.048	0.0130	0.089	0.0302	95.925	5.5392

<sup>1)</sup> Volatilized and escaped = Charcoal baskets + Charcoal tubes + KOH  
<sup>2)</sup> Skin surface= swabs + tape+ skin depot

**DISPOSITION OF THE ABSORBED DOSE EXPRESSED AS % OF THE APPLIED DOSE ± SEM**

Group Number	Group Description	Average Skin (%)	SEM	Average Urine (%)	SEM	Average Feces + Cage Rinse (%)	SEM	Average Carcass (%)	SEM	Average Absorbed (%)	SEM
2	6hr	5.348	0.5050	0.000	0.0000	0.001	0.0001	0.270	0.1158	5.818	0.4879
3	Dead Group	0.254	0.0606	NA	NA	NA	NA	0.055	0.0353	0.309	0.0645
4	24hr	0.175	0.0235	0.007	0.0050	0.005	0.0043	0.056	0.0292	0.243	0.0259
5	Wash Group	0.033	0.0132	0.024	0.0065	0.008	0.0059	0.025	0.0098	0.089	0.0302

**DISTRIBUTION OF THE RECOVERED DOSE (% OF TOTAL RECOVERED DOSE)**

Group Number	Group Description	Average escaped (charcoal tubes) (%)	SEM	Average KOH (%)	SEM	Average Skin Surface (%)	SEM	Average Absorbed (%)	SEM	Sum (%)
2	6hr	0.222	66.399	0.000	66.621	26.806	6.573	100		100
4	24hr	9.763	89.775	0.009	99.544	0.184	0.271	100		100
5	Wash Group (168hr)	7.321	92.524	0.0129	99.857	0.0497	0.093	100		100

Volatilized and escaped = Charcoal baskets + Charcoal tubes + KOH

**DISTRIBUTION OF THE ABSORBED DOSE (% OF TOTAL ABSORBED DOSE)**

Group Number	Group Description	Average Skin (%)	SEM	Average Urine (%)	SEM	Average Feces + Cage Rinse (%)	SEM	Average Carcass (%)	SEM	Sum (%)
2	6hr	95.350	0.001	0.011	0.001	4.638	0.001	100		100
4	24hr	71.917	3.061	2.136	22.886	100		100		100
5	Wash Group (168hr)	37.171	26.336	8.670	27.824	100		100		100

**Possible Fate of Residual <sup>14</sup>C-D<sub>2</sub> after Washing Skin 24 hr Post Exposure**

Volatilized 24-168hr	0.1417 %
Recovered at 168 hr	0.1192 %
	<b>0.2609 %</b>
	0.2205 %
	0.009 %
	<b>0.2296 %</b>

<b>"24 hr Group"</b>	
Skin at 24hr	0.1749 %
Depot at 24 hr	0.1522 %
	<b>0.3271 %</b>
<b>"Wash Group"</b>	
Skin at 168 hr	0.033 %
Depot at 168 hr	0.033 %
Swabs at 168 hr	0.009 %
Volatilized	0.220 %
	<b>0.2958 %</b>

## **APPENDIX B**

### **Statistical Analysis**

#### **Content:**

- Q – Test for Elimination of Outliers;
- t-test for comparison of expired and escaped volatiles (Charcoal tubes for live vs. charcoal tubes for dead animals KOH traps for live vs. dead animals);
- t-test for comparison of percent absorbed dose at 24 h post-dose and in “Wash group” (skin exposed for 24 hr, then washed and rats monitored for additional six days in metabolism cages).



Animal ID	Group Number	Group Description	%Dose				%Dose Carcass	%Dose Urine	%Dose Feces	%Dose Cage rinse	%Dose Absorbed
			Expired volatiles	Skin (application site)							
D0580	5	Wash group	17.389	0.040	0.053	0.041	0.025	0.000	0.159		
D0581	5	Wash group	0.335	0.014	0.015	0.015	0.001	0.000	0.046		
D0582	5	Wash group	0.210	0.068	0.023	0.025	0.004	0.000	0.120		
D0583	5	Wash group	10.155	0.011	0.008	0.013	0.000	0.000	0.033		

Q-test for #D0580 to be rejected as outlier based on % dose absorbed

Q-test for % Total Absorbed

Q = 0.3105 <90% confidence

Also: Q-test for % Urine

Q = 0.590 <90% confidence

Q-test for % Feces

Q = 0.859 95% confidence

Q-test for % Carcass

Q = 0.658 <90% confidence

#D0580 can not be rejected as outlier based on Q-test

Comparison of % dose recovered in charcoal tubes between dead and live animals

ESCAPED vs EXPIRED VOLATILES

CHARCOAL TUBES		Animal ID (Group 3)		Animal ID (Group 4)		Animal ID (Group 5)	
Animal ID (Group 2)	%Dose in Expired volatiles (6hr)	Animal ID (Group 3)	%Dose in Escaped volatiles (24 hr, dead)	Animal ID (Group 4)	%Dose in Expired volatiles (24hr, live)	Animal ID (Group 5)	%Dose in Expired volatiles (24 hr, live in wash group)
D0568	0.2893	Q0590	6.4128	D0576	55.8976	D0580	17.3294
D0569	0.2070	D0573	0.3103	D0577	0.2922	D0581	0.3329
D0570	0.0916	D0574	0.3702	D0578	25.5717	D0582	0.2064
D0571	0.1970	D0575	23.8851	D0579	0.3776	D0583	10.1521
Average SEM	0.1962 0.0405	Average SEM	7.7446 5.5673	Average (N=3) SEM	8.7472 8.4123	N=4 SEM	7.0052 4.1557

Dead vs live 24hr groups (Group 3 vs Group 4), D0576 excluded

NO DIFFERENCE between escaped and expired volatiles

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	7.74459428	8.747188742
Variance	123.9798592	212.3008245
Observations	4	3
df	3	2
F	0.583981996	
P(F<=f) one-ta	0.319077516	
F Critical one-	0.104688702	

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	7.74459428	8.747188742
Variance	123.9798592	212.3008245
Observations	4	3
Pooled Variance	159.3082453	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.104003457	
P(T<=t) one-tail	0.46060478	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.921209561	P >= 0.05
t Critical two-tail	2.570577635	Not Different

**Dead vs live groups (Group 3 vs Group 5 at 24hr)**

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	7.74459428	7.005250617
Variance	123.9798592	69.07867806
Observations	4	4
df	3	3
F	1.794763053	
P(F<=f) one-ta	0.321431312	
F Critical one-	9.276618584	

**NO DIFFERENCE between escaped and expired volatiles**

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	7.74459428	7.005201217
Variance	123.9798592	69.07865286
Observations	4	4
Pooled Variance	96.52925602	
Hypothesized Mean Difference	0	
df	6	
t Stat	0.106429223	
P(T<=t) one-tail	0.459355537	
t Critical one-tail	1.943180905	
P(T<=t) two-tail	0.918711075	P >= 0.05
t Critical two-tail	2.446913641	Not Different

**CONCLUSION:**

There was no statistically significant difference between escaped and expired volatiles based on comparison of dead vs live animals

**Comparison of % dose recovered in KOH traps between dead and live animals**

Dead vs live 24 hr groups (Group 3 vs Group 4), D0576 excluded

Animal ID (Group 3)	Cumulative % dose in KOH	Animal ID (Group 4)	Cumulative % dose in KOH
Q0590	0.0003	D0577	0.0206
D0573	0.0004	D0578	0.0014
D0574	0.0000	D0579	0.0013
D0575	0.0004		
Average	0.0003	Average	0.0078
SEM	0.0001	SEM	0.0064

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	0.000298811	0.007768054
Variance	4.1307E-08	0.000123734
Observations	4	3
df	3	2
F	0.000333838	
P(F<=f) one-ta	1.11973E-05	
F Critical one-	0.104688702	

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.000298811	0.007768054
Variance	4.1307E-08	0.000123734
Observations	4	3
Pooled Variance	4.95183E-05	
Hypothesized M	0	
df	5	
t Stat	-1.38974601	
P(T<=t) one-tail	0.111654039	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.223308077	P >= 0.05
t Critical two-tail	2.570577635	

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	Variable 2
Mean	0.000298811	0.007768054
Variance	4.1307E-08	0.000123734
Observations	4	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.162890454	
P(T<=t) one-tail	0.182432207	
t Critical one-tail	2.91998731	
P(T<=t) two-tail	0.364864414	P >= 0.05
t Critical two-tail	4.302655725	

Dead vs Live (Group 3 vs Wash Group at 24 hr)

Animal ID (Group 3)	%Dose in KOH (dead, 24 hr)	Animal ID (Group 5)	%Dose in KOH (24 hr, live in Wash group)
Q0590	0.0003	D0580	0.0000
D0573	0.0004	D0581	0.0106
D0574	0.0000	D0582	0.0021
D0575	0.0004	D0583	0.0002
Average	0.0003	Average	0.0032
SEM	0.0001	SEM	0.0025

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	0.000298811	0.003228543
Variance	4.1307E-08	2.47813E-05
Observations	4	4
df	3	3
F	0.001666861	
P(F<=f) one-tail	0.000115185	
F Critical one-	0.107798215	

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	Variable 2
Mean	0.000298811	0.003228543
Variance	4.1307E-08	2.47813E-05
Observations	4	4
Hypothesized Mean Difference	0	
df	0	3
t Stat	-1.176072365	
P(T<=t) one-tail	0.162198408	
t Critical one-tail	2.353363016	
P(T<=t) two-tail	0.324396817	P<=0.05
t Critical two-tail	3.182449291	

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.000298811	0.003228543
Variance	4.1307E-08	2.47813E-05
Observations	4	4
Pooled Variance	1.24113E-05	
Hypothesized M	0	
df	6	
t Stat	-1.17607236	
P(T<=t) one-tail	0.142055527	
t Critical one-tai	1.943180905	
P(T<=t) two-tail	0.284111054	P>=0.05
t Critical two-tai	2.446913641	

CONCLUSION:

There was no statistically significant difference in % dose recoveries in KOH traps between dead and live animals

OVERALL CONCLUSION:

There was no statistically significant difference in % dose recoveries in the expired/escaped volatile and CO2 traps between dead and live animals indicating that radioactivity in charcoal tubes and KOH traps could be attributed to a leakage from the dosing chamber.

T-TEST for COMPARISON of D<sub>5</sub> ABSORPTION AFTER DERMAL APPLICATION

ABSORPTION at 24 hr vs 168hr

Animal ID	T=24 hr		T=168 hr	
	%Dose Absorbed	Animal ID	%Dose Absorbed	Animal ID
D0577	0.2503	D0580	0.1591	
D0578	0.2840	D0581	0.0458	
D0579	0.1952	D0582	0.1198	
Average:	0.2431	D0583	0.0325	
		Average:	0.0893	

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	0.243140109	0.089302736
Variance	0.002010106	0.003636494
Observations	3	4
df	2	3
F	0.5527593	
P(F<=f) one-ta	0.375359922	
F Critical one-	0.052180482	

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.243140109	0.089302736
Variance	0.002010106	0.003636494
Observations	3	4
Pooled Variance	0.002985939	
Hypothesized Mean Difference	0	
df	5	
t Stat	3.686065158	
P(T<=t) one-tail	0.007101825	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.014203651	Different 24hr>168hr
t Critical two-tail	2.570577635	

CONCLUSION:

Percent dose ABSORBED at 24hr was significantly higher than in Wash group at 168 hr.

**SKIN (Application site) 24 hr vs 168 hr**

Animal ID	T= 24 hr %Dose Skin application site	T= 168 hr %Dose Skin application site	Animal ID
D0577	0.2191	0.0395	D0580
D0578	0.1389	0.0143	D0581
D0579	0.1667	0.0679	D0582
Average:	0.1749	0.0111	D0583
		Average:	0.0332

**F-Test Two-Sample for Variances**

	Variable 1	Variable 2
Mean	0.174859256	0.033194299
Variance	0.001658541	0.000696972
Observations	3	4
df	2	3
F	2.379638164	
P(F<=f) one-ta	0.24040863	
F Critical one-	9.552081792	

**t-Test: Two-Sample Assuming Equal Variances**

	Variable 1	Variable 2
Mean	0.174859256	0.033194299
Variance	0.001658541	0.000696972
Observations	3	4
Pooled Variance	0.001081599	
Hypothesized Mean Difference	0	
df	5	
t Stat	5.639892153	
P(T<=t) one-tail	0.001215441	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.002430882	
t Critical two-tail	2.570577635	

**CONCLUSION:**

Percent dose recovered in SKIN APPLICATION SITE at 24 hr was significantly higher than in Wash group at 168 hr.

**CARCASS**

Animal ID	T=24 hr		T=168 hr	
	%Dose Carcass	Animal ID	%Dose Carcass	Animal ID
D0577	0.0279	D0580	0.0528	
D0578	0.1139	D0581	0.0154	
D0579	0.0251	D0582	0.0232	
Average:	0.0556	D0583	0.0079	
		Average:	0.0248	

**F-Test Two-Sample for Variances**

	Variable 1	Variable 2
Mean	0.055644383	0.024847156
Variance	0.002550851	0.000387197
Observations	3	4
df	2	3
F	6.587988165	
P(F<=f) one-tail	0.07986861	
F Critical one-	9.552081792	

**t-Test: Two-Sample Assuming Equal Variances**

	Variable 1	Variable 2
Mean	0.055644383	0.024847156
Variance	0.002550851	0.000387197
Observations	3	4
Pooled Variance	0.001252659	
Hypothesized Mean Difference	0	
df	5	
t Stat	1.139296613	
P(T<=t) one-tail	0.153100719	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.306201437	Not Different
t Critical two-tail	2.570577635	

**CONCLUSION:**

Percent dose recovered in CARCASS at 24 hr was not significantly different from the Wash group at 168 hr.

**URINE**

Animal ID	T= 24 hr		T= 168 hr	
	%Dose	Urine	%Dose	Urine
D0577	0.0027	D0580	0.0414	
D0578	0.0174	D0581	0.0148	
D0579	0.0022	D0582	0.0247	
Average:	0.0074	D0583	0.0132	
		Average:	0.0235	

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	0.007442847	0.023518947
Variance	7.4962E-05	0.000167934
Observations	3	4
df	2	3
F	0.446376759	
P(F<=f) one-tail	0.323455258	
F Critical one-tail	0.052180482	

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.007442847	0.023518947
Variance	7.4962E-05	0.000167934
Observations	3	4
Pooled Variance	0.000130745	
Hypothesized Mean Difference	0	
df	5	
t Stat	-1.84081068	
P(T<=t) one-tail	0.062508598	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.125017197	Not Different
t Critical two-tail	2.570577635	

**CONCLUSION:**

Cumulative percent dose recovered in URINE over 24 hr was not significantly different from the Wash group at 168 hr.

**FECES**

Animal ID	T= 24 hr		T= 168 hr	
	% Dose Feces	168hr Animal ID	%Dose Feces	
D0577	0.0001	D0580	0.0253	
D0578	0.0126	D0581	0.0013	
D0579	0.0006	D0582	0.0039	
Average:	0.0044	D0583	0.0004	
		Average:	0.0077	

F-Test Two-Sample for Variances

	Variable 1	Variable 2
Mean	0.004424963	0.007738874
Variance	5.00412E-05	0.000139476
Observations	3	4
df	2	3
F	0.358779821	
P(F<=f) one-ta	0.275071849	
F Critical one-	0.052180482	

t-Test: Two-Sample Assuming Equal Variances

	Variable 1	Variable 2
Mean	0.004424963	0.007738874
Variance	5.00412E-05	0.000139476
Observations	3	4
Pooled Variance	0.000103702	
Hypothesized Mean Difference	0	
df	5	
t Stat	-0.426077422	
P(T<=t) one-tail	0.343885488	
t Critical one-tail	2.015049176	
P(T<=t) two-tail	0.687770975	Not Different
t Critical two-tail	2.570577635	

**CONCLUSION:**

Cumulative percent dose recovered in FECES over 24 hr was not significantly different from the Wash group at 168 hr