

# Final Report

## METABOLISM AND PHARMACOKINETICS OF DIISOPROPYL ETHER IN MALE AND FEMALE RATS: PILOT STUDY

### SUBMITTED TO:

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*\*RTI International is a trade name of Research Triangle Institute.*

## SUMMARY

This study evaluated the pharmacokinetics and disposition of diisopropyl ether (DIPE) in male and Fischer 344 rats following nose only exposure. Three separate studies were conducted.

In Study A, nose-only inhalation exposure of jugular-vein cannulated male and female Fischer 344 rats to 3600 ppm DIPE for 6 hours (actual concentration  $3643 \pm 177$  ppm) was conducted, and serial blood samples were obtained up to 24 h following exposure. Headspace GC-MS analysis of DIPE, isopropanol and acetone was conducted on the blood samples. DIPE was less than the limit of quantitation (LOQ) in preexposure samples. DIPE accumulated rapidly during the 6 hours of exposure, and rapidly declined after the exposure. There was little difference in blood levels at peak concentration between males and females. All samples were below LOQ by 16 hr. In the male rats, isopropanol was below LOQ in preexposure samples, all three samples collected at 5 min, and in one of the three collected at 10 min. The concentration of isopropanol rose steadily during the exposure, and then declined gradually. In male rats, blood concentrations of isopropanol reached peak concentrations at approximately twice those of female rats. All samples were below LOQ by 16 hr. In the female rats, isopropanol was below LOQ in preexposure samples, and all samples collected at 5, 10, and 15 min. The concentration of isopropanol rose steadily during the exposure, and then declined gradually. In male rats, blood concentrations of isopropanol peaked at approximately twice those of female rats. All samples were below LOQ by 16 hr. In male and female rats, acetone was above LOQ in 5 of the 6 preexposure samples. Acetone levels in both male and female rats rose steadily during the exposure, and in males continued to rise following exposure until 8 hr following the initiation of exposure. In females, the acetone levels were approximately the same between 6 and 8 hours following the exposure, and then declined rapidly reaching preexposure levels by 16 hrs. The concentration achieved in males exceeded the range of the calibration curve, and for samples obtained between 6 hours and 10 hours are estimated by linear extrapolation of the calibration curve. The peak concentrations of acetone achieved in male rats were approximately 50% higher than those in female rats, and for isopropanol the peak concentrations in males were almost twice those in females. Male rats reached preexposure levels by 24 hours. Non-compartmental pharmacokinetic analysis of the mean concentrations of each analyte was conducted using WinNonlin. Pharmacokinetic analysis of DIPE indicated a half life of 0.92 h in male rats, and 0.76 h in female rats. Isopropanol had an apparent half life of 1.16 and 1.23 h in male and female rats, respectively. Acetone had an apparent half life of 2.31 and 2.32 h in male and female rats, respectively.

In Study B, four male Fischer 344 rats were exposed to [2-<sup>14</sup>C] diisopropyl ether in a nose-only inhalation system at a target concentration of 3600 ppm for 6 hours (actual concentration  $3529 \pm 40$  ppm). Following exposure, the 4 rats were euthanized immediately and the radioactivity in the carcass was determined by digestion and scintillation counting (Group 2, Study B). In Study C, four male Fischer 344 rats were exposed to a mixture of <sup>13</sup>C<sub>6</sub> and [2-<sup>14</sup>C] diisopropyl ether in a nose-only inhalation system at a target concentration of 3600 ppm for 6 hours (actual concentration  $3334 \pm 99$  ppm, actual exposure time

5 hours and 40 minutes). The rats were transferred to all-glass metabolism cages, and urine, feces, exhaled CO<sub>2</sub> and exhaled volatiles were collected for seven days (Group 3, Study C). After seven days, the rats were euthanized and the radioactivity remaining in the carcass was determined by digestion and scintillation counting. Radioactivity was eliminated primarily as exhaled volatiles (70 % of the recovered radioactivity). Approximately 20 % was recovered as <sup>14</sup>CO<sub>2</sub>. Small amounts of radioactivity were recovered in urine (5% of the recovered radioactivity). A lesser amount was recovered (1%) in feces, and 2.6% in carcass at 7 days.

Based on the recovered radioactivity, the estimated dose in the rats in Group 2 was  $373 \pm 8$  mg/kg, and in Group 2 was  $318 \pm 8$  mg/kg. Elimination of radioactivity was rapid, with 93.7% and 95.5% of the radioactivity recovered within 24 h and 48 h, respectively, following the end of exposure.

The objectives of the study to develop a system for inhalation exposure to DIPE and to develop analytical methods for the analysis of DIPE, acetone and isopropanol were achieved. Additional objectives achieved were characterisation of the kinetics of DIPE, acetone and isopropanol in blood from rats exposed to 3600 ppm DIPE by inhalation for 6 hours, and analysis of the disposition of radioactivity conducted following exposure to [2-<sup>14</sup>C] DIPE, and <sup>13</sup>C<sub>6</sub> and [2-<sup>14</sup>C] DIPE. The objective of characterizing urinary metabolites was not pursued because of the low excretion of radioactivity in urine.



### Quality Assurance Statement

**Study Title:** Metabolism and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats: Pilot Study

**Sponsor:** Section 211(b) Research Group, American Petroleum Institute

**Protocol Number:** RTI-934

**Study Code:** Rt05-934

This study was audited by the Regulatory, Quality, and Records Management – Quality Assurance Unit and the results of the inspections and audits were reported to the Study Director and management as identified below.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Audit	August 9, 10, 14, 2006	August 14, 2006
Protocol Audit – Revised Protocol	February 15, 2007	February 15, 2007
Protocol Amendment 1	September 24, 2007	September 24, 2007
Inhalation Exposure and Blood Collection Inspection	September 27, 2007	September 27, 2007
Protocol Amendment 2	February 20, 2008	February 20, 2008
Data and Report Audit – Validation	October 26-December 16, 2011	December 16, 2011
Data and Report Audit – Pilot Study	April 29-May 3, May 6-10, 14, 16-17, 20, 2013	May 21, 2013
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## Laboratory GLP Compliance Statement

This study was carried out in compliance with the EPA Good Laboratory Practices (GLP) Standards for Inhalation Exposure Health Effects Testing, 40 CFR part 79, subpart F § 79.60.

The prestudy exposure system method development was completed at CIIT Centers for Health Research and was not conducted under GLPs.

The method of synthesis of the test chemical diisopropyl ether and [2-<sup>14</sup>C]-diisopropyl ether, or their location was not available at the time of conduct of the study. The suppliers of diisopropyl ether have been unable to provide information on the methods of synthesis.

There were no significant deviations (Appendix A) that would affect the integrity or quality of the study or the interpretation of the results. Specimens, as applicable, raw data, copies of the inhalation data, and the final report generated as a result of this study are archived at RTI International.



5-31-2017

Timothy R. Fennell, Ph.D.

Date

Study Director

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## 1.0 INTRODUCTION

The purpose of this pilot study was to develop and validate procedures to be used to evaluate the adsorption, distribution, metabolism, excretion and pharmacokinetics of diisopropyl ether (DIPE) in rats. DIPE is used as an oxygenate additive to gasoline.

## 2.0 OBJECTIVES AND PROTOCOL

The objectives of this pilot study were to:

### Study A

- 1) Collect blood from unexposed (control) male rats (n=10).
- 2) Develop a gas chromatography (GC) method for the analysis of the concentration of DIPE, isopropanol, and acetone in blood.
- 3) Develop a nose-only exposure system for exposure of male and female rats to DIPE, and conduct exposure of cannulated rats to unlabelled DIPE (n=6; 3 backup unexposed).
- 4) Collect blood from rats exposed to unlabelled DIPE, and measure the concentration of DIPE, isopropanol, and acetone in blood.

### Study B

- 5) Conduct a nose-only exposure of male rats to  $^{14}\text{C}$  DIPE/DIPE (n=4; 1 backup unexposed).
- 6) Analyze the amount of  $^{14}\text{C}$  in whole body digests from rats exposed to  $^{14}\text{C}$  DIPE/DIPE.

### Study C

- 7) Conduct a nose-only exposure of male rats to  $^{14}\text{C}$  DIPE/(U- $^{13}\text{C}_6$ ) DIPE (n=4; 2 backup unexposed).
- 8) Collect excreta from rats exposed to  $^{14}\text{C}$  DIPE/(U- $^{13}\text{C}_6$ ) DIPE.
- 9) Analyze  $^{14}\text{C}$  in whole-body digests, urine, feces, exhaled  $\text{CO}_2$ , and exhaled volatiles in rats exposed to  $^{14}\text{C}$  DIPE/(U- $^{13}\text{C}_6$ ) DIPE.
- 10) Develop a method for analysis of metabolites in urine using HPLC and NMR.

Copies of the approved protocol and protocol amendments for these studies are included in Appendix A. It should be noted that some of these objectives listed above were involved method development (development of a GC method for analysis, development of a nose only inhalation exposure system) and were not conducted according to Good Laboratory Practice.

### 3.0 MATERIALS AND METHODS

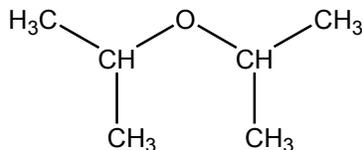
#### 3.1 Test Substance

*NAME:* Diisopropyl ether (DIPE; CAS No. 108-20-3)

*MOLECULAR FORMULA:* C<sub>6</sub>H<sub>14</sub>O

*MOLECULAR WEIGHT:* 102.18

*STRUCTURE:*



##### 3.1.1 Nonradiolabeled diisopropyl ether

*SOURCE OF NON-LABELED TEST SUBSTANCE:* The non-labeled DIPE was purchased from Sigma-Aldrich, Milwaukee, WI. A certificate of analysis from the vendor indicated purity of 99.6% by GLC, with 6.6 PPM BHT

*PRODUCT NUMBER:* 398276

*LOT NUMBER:* 03658JC

The unlabeled test material was characterized at RTI by gas chromatographic (GC) analysis for purity and stability, and for identify by <sup>1</sup>H and <sup>13</sup>C Nuclear Magnetic Resonance (NMR) spectroscopy and by GC-mass spectroscopy. Details of the analysis are included in Appendix B.

##### 3.1.2 (U-<sup>13</sup>C<sub>6</sub>)-labeled diisopropyl ether

*SOURCE OF <sup>13</sup>C-LABELED TEST SUBSTANCE:* (U-<sup>13</sup>C<sub>6</sub>) Substituted DIPE was obtained from ISOTEC, Miamisburg, OH. A certificate of analysis from the vendor indicated purity of 95.9%.

*PRODUCT NUMBERS:* ISOTEC Number T83-03014

*ALDRICH Number 632384*

*LOT NUMBER:* ST1187

The (U-<sup>13</sup>C<sub>6</sub>)-labeled diisopropyl ether was characterized at RTI by gas chromatography-mass spectrometry analysis for identity, and by GC-FID with a purity of 94.9%.

##### 3.1.2 [2-<sup>14</sup>C]-labeled diisopropyl ether

*Position of radiolabel:* [2-<sup>14</sup>C]-

*Source:* American Radiolabeled Chemicals, Inc. St. Louis MO, prepared as a custom synthesis

<i>Specific Activity:</i>	2.1 mCi/mmol
<i>Product No.:</i>	ARC-3099
<i>Lot No.:</i>	070626
<i>Identity:</i>	The identity of [2- <sup>14</sup> C]- <i>diisopropyl ether</i> was confirmed by chromatographic comparison with unlabeled DIPE using HPLC coupled with radioactivity detection.
<i>Purity:</i>	The radiochemical purity of [2- <sup>14</sup> C]- <i>diisopropyl ether</i> was determined chromatographically to be 97.23 %, using the HPLC system described in Appendix C.

The labeled test material was characterized at RTI by HPLC coupled with radioactivity detection for purity and stability, and by HPLC coupled with radioactivity detection and refractive index detection. Details of the analysis are included in Appendix C.

### 3.2 Reference Substances

Isopropanol. 2-propanol, product number 34863, lot number 05761JC was obtained from Sigma-Aldrich. The purity specified by the Vendor's certificate of analysis was 99.98%. This material was assigned a chemical receipt number of DPK-B-0005. Identity of the material was verified by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, and by gas chromatography coupled with mass spectrometry.

Acetone, product number 34850, lot number 53250 was obtained from Sigma-Aldrich. Purity from the Vendor Certificate of Analysis was 99.9%. This material was assigned a chemical receipt number of DPK-B-004. Identity of the material was verified by <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy, and by gas chromatography coupled with mass spectrometry.

### 3.3 Test System

**Source:** Male Fischer 344 rats were purchased from Charles River (Kingston, NY). Animal weights at the time they were used in studies are shown in Table 1.

**Diet:** Animals were fed Certified Purina Rodent Chow #5002 and were furnished tap water *ad libitum*. The analysis of each feed batch for nutrient levels and possible contaminants was performed by the supplier, examined by the Study Director, and maintained in the study records. The feed was stored at approximately 60–70 °F, and the period of use did not exceed six months from the milling date. The source of the water was the City of Durham, NC. Approximately once a year, the City of Durham provides analyses of the drinking water for potential contaminants. Documentation of these analyses were inspected by the Study Director and maintained in the study records. In addition, samples of water were collected before and after the study for analysis of tertiary butanol, tertiary amyl methyl ether, ethyl tertiary butyl ether, diisopropyl ether, and methyl tertiary butyl ether. The samples were sent to Kiff Analytical (Davis, CA 95616) for analysis. All of the analytes were below the reporting limit for the method in the samples obtained before and after the study (tertiary butanol 1 µg/L, tertiary amyl methyl ether 0.1 µg/L, ethyl tertiary butyl ether 0.1 µg/L, diisopropyl ether 0.1 µg/L, and methyl tertiary butyl ether 0.1 µg/L).

**Identification:** Individual ear tags were used to uniquely identify animals used.

**Housing:** Rats were housed (maximum of three per cage) in polycarbonate cages with stainless steel bar lids accommodating a water bottle until they were used in an experiment. Cage sizes are 19" x 10.5" x 8" high (143 sq. in. floor space). Contact bedding was Sani-Chips hardwood chips (P. J. Murphy Forest Products Co.; Montville, NJ). Cannulated rats used in Study A were housed individually in cages with dimensions of 9 ¼" x 10.5" x 8 ¼" high (approximately 76.3 sq. in. floor space)

Following exposure in Study C, 4 male rats were housed in individual glass metabolism chambers for 7 days for collection of urine, feces, exhaled CO<sub>2</sub>, and exhaled volatiles. Two unexposed male rats were housed in individual glass metabolism chambers for 2 days for collection of urine, feces, exhaled CO<sub>2</sub>, and exhaled volatiles for determination of radioactivity background.

**Quarantine:** Rats were quarantined for a minimum of one week before use on a study, with the exception of cannulated rats which were quarantined for 1-2 days. A veterinarian or qualified designee examined the animals prior to their release from quarantine.

**Randomization:** The ear tag numbers of animals were assigned in numerical order to sequential values using a series of computer generated numbers as described in SOP DPK-HUS-001 Assignment of Animals into Groups, using the procedure for Assignment of Animals within a Single Group or Multiple Groups without Regard to Weight Mean and Range. Four groups were prepared: Study A (n=9 for males and females), Study B (n=4), Study C (n=4).

**Environmental:** Temperature and relative humidity in RTI animal rooms were continuously monitored, controlled, and recorded using an automated system (Siebe/Barber-Colman Network 8000 System with Revision 4.4.1 for Signal® software [Siebe Environmental Controls (SEC)/Barber-Colman Company; Loves Park, IL]). The target environmental ranges were 64–79 °F (18 °C - 26 °C) for temperature and 30–70% relative humidity, with a 12-h light cycle per day.

**Euthanasia:** At the end of the in-life phase, the rats were euthanized by overexposure to carbon dioxide.

### 3.4 Test Chemical Preparation and Analysis

For inhalation exposure to unlabeled DIPE (Study A), an appropriate amount of unlabeled DIPE was prepared for generation of the exposure atmosphere, calculated based on the exposure concentration, the duration of exposure, the number of animals, and the flow rate of air through the exposure tower.

For inhalation exposure to [2-<sup>14</sup>C]DIPE/DIPE (Study B), <sup>14</sup>C DIPE was added to an appropriate amount of unlabeled DIPE for generation of the exposure atmosphere. The amount of labeled and unlabeled DIPE required was calculated based on the exposure concentration, the duration of exposure, the number of animals, and the flow rate of air through the exposure tower.

For inhalation exposure to [2-<sup>14</sup>C]DIPE/(U-<sup>13</sup>C<sub>6</sub>)DIPE (Study C), <sup>14</sup>C DIPE was added to an appropriate amount of (U-<sup>13</sup>C<sub>6</sub>)DIPE for generation of the exposure atmosphere. The amount of labeled

and (U-<sup>13</sup>C<sub>6</sub>)DIPE required was calculated based on the exposure concentration, the duration of exposure, the number of animals, and the flow rate of air through the exposure tower.

For inhalation exposure to [2-<sup>14</sup>C]DIPE/DIPE, [2-<sup>14</sup>C]DIPE was weighed into a tared flask with a Teflon faced screw cap. The weight of the labeled chemical added was recorded. Unlabeled DIPE was added, and the weight added was recorded. The nominal specific activity of the DIPE mixture was then calculated. This was verified by weighing an aliquot into a sealed flask, recording the weight added, and adding solvent, and recording the weight added. Aliquots of the solution of DIPE were placed in scintillation vials, and the weight added was recorded. The amount of solution added was calculated based on the density of the solvent. Ultima Gold™ scintillation cocktail (Perkin Elmer) was added to the scintillation vials, and the amount of radioactivity added was determined by liquid scintillation spectroscopy (LSS). The specific activity of the labeled DIPE was then calculated from the data obtained.

A procedure analogous to that described above was followed for the preparation of [2-<sup>14</sup>C]DIPE/(U-<sup>13</sup>C<sub>6</sub>)DIPE.

The exposure atmosphere concentration was monitored using a calibrated Miran IR detector. The concentration of DIPE was monitored continuously at the inlet to the tower, and at the outlet. The stability of DIPE under the conditions of administration was monitored by sampling the inlet of the exposure tower during the exposure and at the end of the exposure. Samples of the exposure atmosphere were collected using a 1ml syringe, and bubbled through water for analysis by HPLC, and through methanol for analysis by GC-MS.

### 3.5 Inhalation Exposure

A single nose-only inhalation exposure to DIPE was conducted with a total of 8 male and 8 female rats for Study A on September 27, 2007. A single nose-only inhalation exposure to [2-<sup>14</sup>C]DIPE/DIPE was conducted with a total of 4 male rats for Study B on October 3, 2007. A single nose-only inhalation exposure to [2-<sup>14</sup>C]DIPE/(U-<sup>13</sup>C<sub>6</sub>)DIPE was conducted with a total of 4 male rats for Study C on October 4, 2007. For each study, the DIPE preparation was drawn into a clean syringe. The generation system consisted of a syringe containing DIPE with a syringe pump to deliver the chemical to the air supply of the exposure chamber. The DIPE exposure atmosphere was generated by pumping liquid DIPE into the air stream flowing into a Cannon nose-only exposure system. The syringe pump and clean air flow rate were set such that a target concentration of 3600 ppm was achieved. The exposure air flow rate delivered to each animal was at least 1.5 times the animal minute ventilation rate. The air supply was controlled with an electronic mass flow meter to maintain a total air flow of at least 12 air changes per hour. The generation and delivery system were placed in a chemical hood to contain any DIPE that might leak from the system.

The exposure system (see Appendix E and Appendix F) consisted of a Cannon flow-past nose-only exposure system containing 52 exposure ports. This chamber is a dynamic non-rebreathing system that allows for the simultaneous exposure of up to 51 animals. The chamber is cylindrical in shape, and is constructed in stainless steel. Unused ports were plugged with solid metal rods to reduce consumption

of the exposure material. The Cannon nose-only chamber operated on a push-pull basis. The sampling lines and main exposure atmosphere delivery lines were Teflon. The incoming air for the exposure system was filtered to eliminate the possibility of contamination in the air supply. To the extent possible, the air supply temperature and relative humidity were maintained between 64 to 79 degrees Fahrenheit and 30 to 70 %, respectively. The chamber exhaust flow was adjusted to maintain a slight negative pressure during the exposure to prevent DIPE from entering the laboratory area. The chamber exhaust was filtered through a disposable charcoal filter and disposed at the end of the exposure. Closed nose-only tubes were used to hold the test animals during inhalation exposures. The inhalation system was strategically placed in a chemical hood to prevent any DIPE from entering the laboratory.

### **3.5.1 Exposure Analysis**

Prior to the inhalation exposure of rats, the performance of the inhalation system was verified to ensure that the test atmosphere could be generated on the nose only tower to achieve the test concentration over the duration of the exposure ( $\leq 10\%$  difference between actual and target concentration), and that the port to port variability was within accepted limits ( $\leq 10\%$  difference in measurement taken at multiple locations on the tower). A report describing the preexposure characterization of the inhalation system is attached to this report as Appendix E, and reports characterizing the exposures of rats is attached as Appendix F.

## **3.6 Collection of Samples**

### **3.6.1 Blood Sample Collection**

For Study A, blood samples were collected by jugular vein cannula prior to dosing from each rat (time = 0), and from three rats of each sex, at approximately 5 min, 10 min, 15 min, 30 min, and at approximately 1, 2, 4 and 6 hr after the exposure initiation. At the end of the 6 hr exposure, all rats were removed from the inhalation exposure tower, and placed in cages. Blood was drawn from three rats at approximately 375, 390, 420, 440, 480, 600, 960, and 1440 min after the beginning of the exposure (at approximately 15, 30, 60, 80 min, and 2 hr, 4 hr, 10 hr, and 18 hr after the end of the exposure). To facilitate the withdrawal of blood samples, animals were stagger-started on the exposure tower. At each time point approximately 100  $\mu$ L of blood was withdrawn using a heparinized syringe.

Blood samples were drawn from the jugular vein cannula into a heparinized 1 ml syringe, and the sample was immediately placed in a preweighed headspace vial. The amount of blood was approximately 100  $\mu$ L. The vial was immediately crimped and weighed, and the weight of the blood aliquot was determined. After the last sample has been collected from each animal, the rats were euthanized by exposure to CO<sub>2</sub>. The blood samples were maintained on ice, until placed in the headspace autosampler for analysis. All blood samples were analyzed by GC-MS within 24 hours of collection.

### 3.6.2 Excreta

In Study C, 4 rats (CM-01, CM-02, CM-03, and CM-04) were placed in all-glass metabolism cages for the separate collection of urine, feces, exhaled volatiles, and exhaled CO<sub>2</sub>. Urine was collected over dry ice at 0–8, 8–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure. Feces were collected over dry ice at 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure. Exhaled volatile organics were collected on a series of two charcoal traps (Product Number 226-16, SKC Sorbent Tube, Anasorb CSC, Coconut Charcoal, 10 X 110 mm size, 2-section, 200/800 mg sorbent, SKC Inc, Eighty Four, PA) at 0–1, 1–3, 3–6, 6–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure. Expired <sup>14</sup>CO<sub>2</sub> was collected in 1.0 N KOH at 0–1, 1–3, 3–6, 6–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure. At the end of excreta collection, the cages were rinsed with water and with ethanol, and combined. The rinses were analyzed for total radioactivity. The weight of urine and/or feces collected for each sample interval was measured. Urine and feces were analyzed for total radioactivity. Excreta not assayed within a day of collection were stored at approximately -20 °C in the dark.

### 3.6.3 Carcass

For Study B, the amount of <sup>14</sup>C retained was determined by placing each rat in the nose-only restraint tube in a Tedlar gas bag. The bag was sealed, and CO<sub>2</sub> was pumped into the bag to euthanize the rat. The gas from the gas bag was then forced through a charcoal filter trap, to determine the amount of exhaled <sup>14</sup>C, and the carcass was digested with 2N ethanolic NaOH. After sample digestion, the amount of radioactivity in the carcass was determined by LSS. Any feces in the nose-only restraint tube were collected and the amount of radioactivity in the feces sample was determined by LSS. The nose-only tube was rinsed with water, and the amount of <sup>14</sup>C contained in the rinse was determined by LSS.

For Studies B and C, the carcasses were digested with 2N ethanolic NaOH. After sample digestion, the amount of radioactivity in the carcass was determined by LSS.

### 3.6.4 Tissues

For Study C, tissues were obtained following sacrifice from a single rat (CM-02):

femur	cecum + large intestine + rectum
skin (hair shaved)	small intestine
subcutaneous fat	liver
abdominal fat	muscle (gastrocnemius)
brain	heart
testes (epididymis removed)	kidneys
stomach contents	small intestine contents
stomach	large intestine + cecum contents

lungs  
residual carcass

spleen

The harvested tissues were weighed and analyzed for radiochemical ( $^{14}\text{C}$ ) content by LSS. The remaining carcass was analyzed for total radioactivity. Tissues were prepared as described in section 3.8.2 below.

### **3.7 Analysis of Blood Samples for DIPE, Isopropanol, and Acetone**

A GC-MS method was developed for quantitating DIPE, isopropanol and acetone using blood from control male Fischer 344 rats. The methods development involved the generation of a standard curve for analysis of DIPE, isopropanol and acetone in the headspace of airtight vials containing a known volume of control blood and a known concentration of each analyte. The stability of DIPE, isopropanol and acetone was evaluated under storage conditions that were used in this study during this method development. This included analysis of DIPE, isopropanol and acetone immediately after spiking in control blood, after storage at room temperature (8 hr), or at approximately  $4^{\circ}\text{C}$  (16 hr). The concentration of DIPE, isopropanol and acetone in the headspace of vials containing a known volume of blood from rats administered DIPE by inhalation was determined by comparison to the standard curve. A detailed description of the analytical method is contained in Appendix C.

The method was applied to the analysis of samples obtained from jugular vein cannulated rats exposed to DIPE by inhalation. Blood samples were obtained from cannulas (or in the cases where samples could not be obtained via the cannula, from the tail vein) as described in section 3.6.1. Samples were transferred immediately to headspace vials, crimp sealed, and analyzed as described in Appendix C. Vials and caps were weighed prior to addition of blood, and after addition of blood, and the weight of blood added was calculated. Internal standard solution (10  $\mu\text{L}$ ) containing MTBE (50  $\mu\text{g}/\text{mL}$ ) and 1-propanol (500  $\mu\text{g}/\text{mL}$ ) was added using a 10- $\mu\text{L}$  microsyringe. After analysis by headspace GC-MS, the concentration of each analyte was corrected for the weight of blood collected. The method is described in detail in Appendix C.

### **3.8 Analysis of Samples for Total Radioactivity**

Ultima Gold™ scintillation cocktail was used in all determinations of radiochemical content. Radioactivity was determined using a Packard 1900CA Liquid Scintillation Counter. Weights of samples collected were recorded, and aliquots were prepared by weight for scintillation counting.

#### **3.8.1 Excreta**

Duplicate aliquots of urine (approximately 0.05 g) were analyzed directly (without solubilization or bleaching) for radiochemical content. Duplicate aliquots of urine were weighed into scintillation vials containing scintillation cocktail. Feces were homogenized with an approximately equal mass of water.

The weight of the feces homogenate was determined, and duplicate homogenate aliquots were weighed into scintillation vials. After solubilization of the homogenate aliquots with Soluene-350™ (about 2 ml per sample), scintillation cocktail was added to the vials, and the samples were analyzed for total radioactivity by LSS. Samples were bleached (by adding approximately 125 µl of 70% perchloric acid, and then approximately 0.3 ml of 30% H<sub>2</sub>O<sub>2</sub>) prior to addition of scintillation cocktail.

### **3.8.2 Tissues and Carcass**

Carcasses were analyzed for total radioactivity following solubilization in 2N ethanolic NaOH. Duplicate samples of the solubilized carcass were analyzed. Tissues were analyzed for total radioactivity following solubilization in Soluene-350™ (about 2 mL per tissue sample). Liver was homogenized, and duplicate aliquots of the homogenate solubilized. Other tissues were solubilized in their entirety or after being divided into multiple pieces. Solubilized tissue samples were bleached (by adding approximately 125 µl of 70% perchloric acid, and then approximately 0.3 ml of 30% H<sub>2</sub>O<sub>2</sub>) prior to addition of scintillation cocktail and analysis by LSS.

### **3.8.3 Exhaled Breath Traps**

Aliquots of 1.0 N KOH from the exhaled breath trap for CO<sub>2</sub> were analyzed by LSS after addition of scintillation cocktail.

### **3.8.4 Exhaled Volatiles Traps**

Each charcoal trap was extracted by elution with dimethylformamide (DMF) to determine absorbed radioactivity. For a single extraction, charcoal was transferred to a centrifuge tube and weighed. DMF (8.0 ml for samples of VOC Trap 1 from 0 – 24 h, and 4.0 ml for samples collected following 24 h) was added, and the sample weighed. After vortexing for approximately 30 seconds, the samples were centrifuged, and the supernatant was removed. The DMF was removed following centrifugation and radioactivity was determined in duplicate aliquots of the DMF extract by LSS.

## **3.9 Data Collection and Reporting**

Study data was collected and reported in the Debra™ system version 5.5.10.75. This includes data for pot weights, sample weights, homogenate weights and aliquot weights, and scintillation counting data. Calculations of sample data are described in Appendix G, and were reported with the Debra™ system. Individual values for sample weights and radioactivity determinations are presented in Appendix H. Data for radioactivity where the sample aliquot dpm values were below the background for the sample were considered to be less than the limit of detection, and are reported as 0 dpm per aliquot. Data for all samples that were above the limit of detection are reported, and included in calculations. However,

where the sample aliquot dpm values were less than three times the background for the sample, the sample has been flagged with an asterisk in the Summary Tables and appendix tables.

## **4.0 RESULTS**

### **4.1 Analysis of Radiolabeled Test Chemical Identity and Purity**

Information on the identity, purity, and stability of the test chemical is included in Appendix B. Prior to study initiation, the test chemical purity was determined by GC to be  $99.38 \pm 0.16$  %. Following completion of the study, test chemical purity was determined to be  $99.63 \pm 0.006$  %. The radiochemical purity of 2-<sup>14</sup>C DIPE specified by the vendor was 96.83 % by HPLC, and was verified by HPLC at RTI prior to the inhalation exposure of rats as  $97.23 \pm 0.04$ % (Appendix C). A sample of the exposure atmosphere was taken from the tower inlet to measure purity of the radiolabeled test chemical during the exposure. However, the recovery of <sup>14</sup>C from the atmosphere sample was insufficient to determine radiochemical purity. The purity of the (U-<sup>13</sup>C<sub>6</sub>) DIPE specified by the vendor was 95.9 % and was verified by NMR spectroscopy, and by GC as  $95.17 \pm 0.03$  %. Analysis of the reference substances acetone and isopropanol is reported in Appendix C.

### **4.2 Test Chemical Formulation**

For Study A, unlabeled DIPE was used for generating the exposure atmosphere. For Study B, the test chemical was prepared by mixing unlabeled DIPE with 2-<sup>14</sup>C labeled DIPE, and used without further dilution. The specific activity of the formulation is reported in Table 3, and was measured as 0.4503 μCi/mg. For Study C, the test chemical was prepared by mixing (U-<sup>13</sup>C<sub>6</sub>) DIPE with 2-<sup>14</sup>C labeled DIPE, and used without further dilution. The specific activity of the formulation is reported in Table 4, and was measured as 0.4241 μCi/mg.

### **4.3 Inhalation exposure**

The body weights of the animals used during the course of Studies A, B and C are shown in Table 1. The exposure concentration data for each six-hour exposure of rats to a target concentration of 3600 ppm DIPE is shown in Table 2, 3, and 4. The details of the inhalation exposure system are described in Appendix E, Inhalation Summary Report for Setup and Evaluation of Inhalation System at RTI International and the details of the actual exposure are reported in Appendix F, Inhalation Reports: DIPE, <sup>14</sup>C-DIPE/DIPE and <sup>14</sup>C-DIPE/<sup>13</sup>C-DIPE Nose-Only Inhalation Exposure at RTI International. The actual exposure concentration was  $3643 \pm 177$  ppm for Study A (Table 2),  $3529 \pm 40$  ppm for Study B (Table 3),  $3334 \pm 99$  ppm for Study C (Table 4). The exposure duration for Study C was less than 6 hours, with the limited supply of <sup>13</sup>C DIPE providing an exposure duration of 5 hours and 40 minutes. Analyses of samples of the exposure atmosphere are reported in Appendix F, Analysis of Exposure Atmospheres: Diisopropyl Ether. GC-MS analysis of samples of the exposure atmosphere sampled during Studies A, B and C confirmed the presence of DIPE or <sup>13</sup>C<sub>6</sub>-DIPE in the exposure atmosphere near the beginning and end of the exposures, verifying the stability of DIPE in the inhalation system. With

the exception of one sample that showed a small peak containing acetonitrile, that is thought to have arisen from contamination of a sampling syringe, there were no significant impurity peaks suggesting that the DIPE is stable in the inhalation system. Radioactivity from air samples collected during the course of the inhalation exposures for Studies B and C chromatographed with the expected retention time of DIPE on HPLC. However, the levels of radioactivity recovered were insufficient to reliably establish purity.

#### **4.4 DIPE, Acetone and Isopropanol in Blood During and Following Exposure**

DIPE, isopropanol and acetone were determined by headspace GC-MS analysis in blood samples obtained from jugular vein cannulated male and female rats (Study A, Group 1, rats AM-01 – AM-08, AF-01 – AF-08). The concentrations determined are presented in Tables 5 - 9 and in Figures 1 - 4. DIPE was less than the limit of quantitation (LOQ) in preexposure samples. DIPE accumulated rapidly during the 6 hours of exposure, and rapidly declined after the exposure. There was little difference in blood levels at peak concentration between males and females. All samples were below LOQ by 16 hr. In the male rats, blood concentrations of isopropanol were below LOQ in preexposure samples, all three samples collected at 5 min, and in one of the three collected at 10 min. The concentration of isopropanol rose steadily during the exposure, and then declined gradually. In male rats, blood concentrations of isopropanol reached peak concentrations at approximately twice those of female rats. All samples were below LOQ by 16 hr. In the female rats, isopropanol blood concentrations were below LOQ in preexposure samples, and all samples collected at 5, 10, and 15 min. The concentration of isopropanol rose steadily during the exposure, and then declined gradually. In male and female rats, acetone was above LOQ in 5 of the 6 preexposure samples. Acetone levels in both male and female rats rose steadily during the exposure. In males and females, the acetone levels were approximately the same between 6 and 8 hours following the exposure, and then declined rapidly reaching preexposure levels by 16 hrs. The concentration achieved in males exceeded the range of the calibration curve, and for some samples obtained between 4 hours and 10 hours, concentrations are estimated by linear extrapolation of the calibration curve. The peak concentrations of acetone achieved in male rats were approximately 50% higher than those in female rats. Male rats reached preexposure levels by 24 hours.

Noncompartmental pharmacokinetic analysis was conducted using the mean concentration of DIPE, isopropanol or acetone rather than individual values. With the alternating sampling of the animals during and following exposure, the use of mean values with all of the data points provides the best pharmacokinetic analysis. With noncompartmental analysis, the elimination rate constant and half life are determined from the rate of decline of blood concentration following the end of the exposure. Semi-log plots of concentration vs. time for DIPE in blood are shown in Figure 5. Pharmacokinetic analysis of DIPE indicated a half life of 0.92 h in male rats, and 0.76 h in female rats (Table 8). Isopropanol had an apparent half life of 1.16 and 1.23 h in male and female rats, respectively (Table 8 and Figure 6). Acetone had an apparent half life of 2.31 and 2.34 h in male and female rats, respectively (Table 8 and

Figure 7). Acetone achieved the highest levels in blood (C<sub>max</sub> values of 319 and 211 µg/mL in male and female rats, respectively), compared with DIPE (C<sub>max</sub> values of 56 and 66 µg/mL in male and female rats, respectively), and isopropanol (C<sub>max</sub> values of 66 and 36 µg/mL in male and female rats, respectively). The peak concentrations of acetone achieved in male rats were approximately 50% higher than those in female rats, and for isopropanol the peak concentrations in males were almost twice those in females. T<sub>max</sub> values for DIPE were at 4 h in male rats and 6 h in female rats. The T<sub>max</sub> values for acetone and isopropanol occurred at between 6 and 6.5 h, suggesting that the metabolism of DIPE to isopropanol and acetone occurs rapidly. Acetone achieved the highest area under the curve (AUC<sub>0-∞</sub> values of 3208 and 1735 µg·hr/mL in male and female rats, respectively) compared with isopropanol (AUC<sub>0-∞</sub> values of 339 and 156 µg·hr/mL in male and female rats, respectively) and DIPE (AUC<sub>0-∞</sub> values of 312 and 363 µg·hr/mL in male and female rats, respectively)

#### 4.5 Radioactivity Retained Following Exposure

Four rats were euthanized immediately following inhalation exposure to [2-<sup>14</sup>C]DIPE/DIPE (Study B, Group 1, rats BM-01, BM-02, BM-03, and BM-04). The amount of radioactivity retained in the carcass was determined by solubilization followed by scintillation counting. The total radioactivity retained and the estimated dose in mg/kg are presented in Table 9. The amount of radioactivity retained in Group B ranged from approximately 33 to 34 µCi. The estimated dose was 373 ± 8 mg/kg. This estimate of dose included radioactivity recovered in carcass, a VOC trap that was used to trap any exhaled volatiles while the rat was in the transfer bag, the nose only tube wash, and feces recovered from the nose only tube. The vast majority (98.6%) of the radioactivity was recovered in the carcass (Table 10).

#### 4.6 Excretion of Radioactivity

Four rats were placed in metabolism cages for collection of urine and feces and exhaled breath immediately following the inhalation exposure to [2-<sup>14</sup>C]DIPE/DIPE (Study C, Group 3, Rats CM-01, CM-02, CM-03, and CM-04). Transfer of the rats from the inhalation tower to the metabolism cage was conducted with the rat retained in the nose-only tube contained within a polyethylene bag. Following transfer of the rat to the metabolism cage, radioactivity retained in the bag was recovered by washing with water. The nose-only tube was washed and any feces were collected for digestion and scintillation counting. The rats were maintained in the metabolism cages for 7 days following the end of the exposure. The exhaled breath traps from one rat were not analyzed, but were saved to provide an opportunity for analysis at a later date, and are not included in calculations of recovery. The total recovery of radioactivity from all of the samples collected from rats in Group 3, Study C is presented in Table 9, and ranged from approximately 25 to 28 µCi (with the exception of rat CM-04, where exhaled breath traps were saved for possible future analysis). The estimated dose was 318 ± 8 mg/kg (excluding CM-04, Table 9).

The amount of radioactivity in urine, feces, exhaled VOCs, and CO<sub>2</sub> expressed as a percentage of the total radioactivity recovered is presented in Table 11. Exhalation of volatile material was the primary route of elimination, with 71% of the recovered radioactivity trapped in the Exhaled VOC Traps.

Exhaled  $^{14}\text{CO}_2$  accounted for approximately 20.2 % of the recovered radioactivity. Approximately 5 % and 1% were excreted in urine and feces, respectively.

Radioactivity in urine was excreted primarily in the first 24 hours (Table 12), with 3% and 1.5% at 12 and 24 hours, respectively. 0.2 % or less of the radioactivity was recovered in urine at 48, 72, 96, 120, 144, and 168 hours. In feces (Table 13), approximately 0.6 % of the recovered activity was found in the 0-24 h samples, which contained the largest amount of fecal radioactivity.

Radioactivity in the first  $\text{CO}_2$  trap was excreted primarily in the first 24 hours (Table 14). Little radioactivity was recovered in the second  $\text{CO}_2$  trap (Table 15). Many of the samples collected contained levels of radioactivity that were below 3 times background.

The majority of the radioactivity retained on the charcoal traps for exhaled volatiles was retained on the first charcoal trap of two in series in the first 24 hours (Table 16). Each of the Exhaled VOC Trap 1 samples collected between 0 and 24 hours contained a substantial portion of the dose. Very little of the dose was trapped in the VOC Trap 2 samples (Table 17), with the exception of the 6-24 h samples for rats CM-01 and CM-02.

The cumulative excretion of radioactivity (Table 18) shows that approximately 94% of the radioactivity was recovered within 24 h following exposure, and 95% of the activity was recovered by 48 hours following exposure.

Tissue recovery was evaluated for one rat at 7 days following exposure (Table 19). Liver contained approximately 0.12 % of the recovered radioactivity. All other tissues collected contained lower percentages of the dose.

## 5.0 DISCUSSION

The objectives of the study were accomplished. An inhalation exposure system was developed, and used to conduct the exposures to 3600 ppm DIPE. A headspace GC-MS method for analysis of DIPE, isopropanol, and acetone was developed, and applied to samples from male and female rats exposed to DIPE. The disposition of  $^{14}\text{C}$  DIPE was evaluated in male rats, and determined immediately after exposure, and up to 7 days following exposure. The extent of excretion of metabolites in urine was less than 5% of the dose, so the objective of determining the identity of metabolites in urine was modified.

Comparison of the exposure of rats to 3600 ppm [ $^{14}\text{C}$ ]DIPE/DIPE euthanized immediately following exposure ( $373 \pm 8$  mg/kg) with that of rats euthanized following 7 days of collection of excreta and exhaled breath ( $318 \pm 8$  mg/kg) yielded values that were very similar. When corrected for the exposure concentration and duration a similar recovery per ppm.hr was found with Study B ( $3529$  ppm x  $6$  hr =  $21174$  ppm.hr,  $372.99$  mg/kg/  $21174$  ppm.hr =  $0.017615$  mg/kg/ppm.hr) and Study C ( $3334$  ppm x  $5.66$  hr =  $18870$  ppm.hr,  $317.89$  mg/kg/ $18870$  ppm.hr =  $0.016846$  mg/kg/ppm.hr). Since the recovery of Study C was 95.6% of Study B when corrected for exposure concentration and duration, this suggests

that there were no significant losses of radioactivity from the various sample types during processing of radioactivity.

Radioactivity was rapidly excreted by exhalation as volatile components (71% of the recovered radioactivity) and as  $^{14}\text{CO}_2$  (20.2% of the recovered radioactivity). The cumulative excretion of radioactivity was 94% and 95% within 24 and 48 hours, respectively, of the end of the exposure. At seven days, only 0.6% was recovered in the carcass.

DIPE in blood was readily detected during and after exposure. DIPE levels fell rapidly following the end of the exposure, reaching the limit of quantitation by 10 hours after initiation of exposure. Acetone and isopropanol were detected in blood, increasing with the duration of exposure and decreasing following the end of the exposure. The acetone levels were substantially higher than those of DIPE and isopropanol. The conversion of DIPE to isopropanol and acetone is consistent with the high recovery of radioactivity in the exhaled breath volatiles and as  $^{14}\text{CO}_2$ .

## 6.0 REFERENCES

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## 7.0 RECORDS AND REPORTS

The following will be maintained in the record:

- a) Protocol and any amendments
- b) Animal receipt records
- c) Quarantine records
- d) Temperature and humidity records for the treatment rooms
- e) Animal research facility room logs
- f) Feed and water analysis for contaminants
- g) Randomization records
- h) Test chemical receipt, storage, and use records
- i) Balance calibration log references
- j) Correspondences
- k) All other raw data and documentation

## 8.0 STORAGE OF RECORDS AND BIOLOGICAL SAMPLES

A copy of the final report and the records for this study, including all raw data, will be retained in the RTI Archives, under the responsibility of the RTI Quality Assurance Unit, for the length of time specified in the appropriate regulations.

Biological specimens that remain stable for reanalysis will be stored at RTI in a secured area by the RTI project number for up to 10 years after the date of submission of the final report.

The Sponsor will be notified in writing when the RTI retention time has expired.

## 9.0 REGULATORY COMPLIANCE

These studies were performed in compliance with the EPA Good Laboratory Practices (GLP) Standards for Inhalation Exposure Health Effects Testing, 40 CFR part 79, subpart F § 79.60. Deviations from the approved protocol are documented in Appendix A.

## 10.0 STUDY PARTICIPANTS

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**Table 1. Male and Female Fischer 344 Rats Used on the Study**

<b>Group</b>	<b>Subject</b>	<b>Species</b>	<b>Strain</b>	<b>Gender</b>	<b>Body Wt<sup>a</sup></b>
Group 1 Study A	AM-01	Rat	Fischer 344	Male	185.10 g
Group 1 Study A	AM-02	Rat	Fischer 344	Male	172.51 g
Group 1 Study A	AM -03	Rat	Fischer 344	Male	191.92 g
Group 1 Study A	AM-04	Rat	Fischer 344	Male	185.81 g
Group 1 Study A	AM-05	Rat	Fischer 344	Male	163.70 g
Group 1 Study A	AM-06	Rat	Fischer 344	Male	173.98 g
Group 1 Study A	AM -07	Rat	Fischer 344	Male	164.56 g
Group 1 Study A	AM-08	Rat	Fischer 344	Male	177.08 g
Group 1 Study A	AF-01	Rat	Fischer 344	Female	117.23 g
Group 1 Study A	AF-02	Rat	Fischer 344	Female	122.97 g
Group 1 Study A	AF-03	Rat	Fischer 344	Female	112.56 g
Group 1 Study A	AF-04	Rat	Fischer 344	Female	114.36 g
Group 1 Study A	AF-05	Rat	Fischer 344	Female	118.86 g
Group 1 Study A	AF-06	Rat	Fischer 344	Female	119.86 g
Group 1 Study A	AF-07	Rat	Fischer 344	Female	116.93 g
Group 1 Study A	AF-08	Rat	Fischer 344	Female	112.15 g
Group 2 Study B	BM-01	Rat	Fischer 344	Male	200.58 g
Group 2 Study B	BM-02	Rat	Fischer 344	Male	203.03 g
Group 2 Study B	BM-03	Rat	Fischer 344	Male	202.66 g
Group 2 Study B	BM-04	Rat	Fischer 344	Male	197.58 g
Group 3 Study C	CM-01	Rat	Fischer 344	Male	188.27 g
Group 3 Study C	CM-02	Rat	Fischer 344	Male	187.41 g
Group 3 Study C	CM-03	Rat	Fischer 344	Male	208.47 g
Group 3 Study C	CM-04	Rat	Fischer 344	Male	190.91 g
Group 4 Study C BKGb	CB-01		Fischer 344	Male	197.34 g
Group 4 Study C BKGb	CB-02		Fischer 344	Male	200.99 g

<sup>a</sup>Body weight on the day of exposure

<sup>b</sup>Animals used in Group 4 were not exposed to [2-<sup>14</sup>C]DIPE/DIPE, and provided samples for determination of background only.

**Table 2. Exposure Conditions and Concentration of DIPE, Study A.**

	Target (ppm)	3600
	Number of Open Ports	17
<hr/>		
ACC - Inlet	<b>Mean</b>	<b>3643</b>
(ppm)	Std Dev	177
	No. of Data Points	14
ACC - Exhaust	<b>Mean</b>	<b>3470</b>
(ppm)	Std Dev	48
	No. of Data Points	14
Exposure	<b>Mean</b>	<b>76.3</b>
Temperature	Std Dev	2.2
(°F)	No. of Data Points	14
Exposure	<b>Mean</b>	<b>37</b>
Relative Humidity	Std Dev	3
(%)	No. of Data Points	14
Exposure	<b>Mean</b>	<b>0.0</b>
Static Pressure	Std Dev	0.0
(in H <sub>2</sub> O)	No. of Data Points	14
<hr/>		

<sup>1</sup>ACC Analytical Chamber Concentration

**Table 3. Exposure Conditions and Concentration of [2-<sup>14</sup>C]DIPE/DIPE, Study B.**Analysis results

Treatment name: DIPE 3600 ppm Nose-Only Inhalation  
 Dpm/g of dose prep: 999562646.6 dpm/g  
 Molecular weight: 102.18  
 S/A comp/dose  $\mu$ Ci: 0.4503  $\mu$ Ci/mg

Exposure Summary

	Target (ppm)	3600
	Number of Open Ports	5
ACC <sup>1</sup> - Inlet (ppm)	<b>Daily mean</b>	<b>3529</b>
	Std Dev	40
	No. of Data Points	13
ACC - Exhaust (ppm)	<b>Daily mean</b>	<b>3357</b>
	Std Dev	130
	No. of Data Points	13
Exposure Temperature (°F)	<b>Daily mean</b>	<b>72.3</b>
	Std Dev	0.2
	No. of Data Points	13
Exposure Relative Humidity (%)	<b>Daily mean</b>	<b>42</b>
	Std Dev	0
	No. of Data Points	13
Exposure Static Pressure (in H <sub>2</sub> O)	<b>Daily mean</b>	<b>-0.15</b>
	Std Dev	0.0
	No. of Data Points	13

<sup>1</sup>ACC Analytical Chamber Concentration

**Table 4. Exposure Conditions and Concentration of [2-<sup>14</sup>C]DIPE/<sup>13</sup>CDIPE, Study C.**Analysis results

Treatment name:	DIPE 3600 ppm Nose-Only Inhalation
Dpm/g of dose prep:	941599268.6 dpm/g
Molecular weight:	108.18
S/A comp/dose $\mu$ Ci:	0.4241 $\mu$ Ci/mg

Exposure Summary

	Target (ppm)	3600
	Number of Open Ports	5
<hr/>		
ACC <sup>1</sup> - Inlet (ppm)	<b>Daily mean</b>	<b>3334</b>
	Std Dev	99
	No. of Data Points	13
ACC - Exhaust (ppm)	<b>Daily mean</b>	<b>1650</b>
	Std Dev	113
	No. of Data Points	12
Exposure Temperature (°F)	<b>Daily mean</b>	<b>72.2</b>
	Std Dev	0.2
	No. of Data Points	12
Exposure Relative Humidity (%)	<b>Daily mean</b>	<b>42</b>
	Std Dev	0
	No. of Data Points	12
Exposure Static Pressure (in H <sub>2</sub> O)	<b>Daily mean</b>	<b>-0.15</b>
	Std Dev	0.03
	No. of Data Points	12
<hr/>		

<sup>1</sup>ACC Analytical Chamber Concentration

**Table 5. Concentration of DIPE in blood from male and female rats exposed to 3600 ppm DIPE.**

Time (hr)	Male Rats		Female Rats	
	DIPE <sup>a</sup> µg/mL in blood		DIPE <sup>a</sup> µg/mL in blood	
	Mean	SD	Mean	SD
0.0833	19.298	5.1557	20.219	2.8453
0.1667	25.213	5.4138	29.693	4.3215
0.25	31.753	2.6903	31.596	4.0032
0.5	37.948	10.504	42.048	0.3598
1	44.554	6.6781	47.609	4.4577
2	48.719	7.9949	60.344	3.5563
4	55.708	2.4736	60.562	6.0246
6	47.557	17.696	65.61	5.5047
6.25	17.939	5.8199	18.672	1.1612
6.5	10.337	1.8071	12.058	2.5825
7	7.8988	0.6505	6.1304	0.8475
7.33	3.8405	1.3364	4.4085	0.606
8	2.2091	0.2765	1.9226	0.4641
10	0.5031	0.2859	0.387	0.0259
16	<LOQ <sup>b</sup>	<LOQ	<LOQ	<LOQ
24	<LOQ	<LOQ	<LOQ	<LOQ

<sup>a</sup> Values are mean ± SD of 3 animals.

<sup>b</sup> Less than the limit of quantitation.

**Table 6. Concentration of Isopropanol in blood from male and female rats exposed to 3600 ppm DIPE.**

Time (hr)	Male Rats		Female Rats	
	Isopropanol <sup>a</sup> µg/mL in blood		Isopropanol <sup>a</sup> µg/mL in blood	
	Mean	SD	Mean	SD
0.0833	<LOQ <sup>b</sup>	<LOQ	<LOQ	<LOQ
0.1667	2.655	0.3136	<LOQ	<LOQ
0.25	3.3402	0.2985	<LOQ	<LOQ
0.5	5.8764	1.0548	3.3292	0.1971
1	11.665	0.6519	5.1457	0.7126
2	22.367	4.2463	10.752	0.4521
4	42.303	3.8943	21.477	5.0914
6	66.182	8.3302	31.609	1.4026
6.25	63.386	6.378	35.708	4.836
6.5	63.394	7.4217	25.59	1.9423
7	50.948	6.6648	25.368	4.4118
7.33	43.933	5.1632	15.16	1.6226
8	31.884	3.6357	11.813	2.648
10	9.1135	0.6807	3.4767	0.1157
16	<LOQ	<LOQ	<LOQ	<LOQ
24	<LOQ	<LOQ	<LOQ	<LOQ

<sup>a</sup> Values are mean ± SD of 3 animals.

<sup>b</sup> Less than the limit of quantitation.

**Table 7. Concentration of Acetone in blood from male and female rats exposed to 3600 ppm DIPE.**

Time (hr)	Male Rats		Female Rats	
	Acetone <sup>a</sup> µg/mL in blood		Acetone <sup>a</sup> µg/mL in blood	
	Mean	SD	Mean	SD
0.0833	5.3986	0.9902	4.3126	2.0263
0.1667	8.6662	1.5401	5.0105	0.7406
0.25	12.545	2.4086	8.5253	1.8353
0.5	25.403	3.4346	16.779	4.5256
1	50.448	4.9173	36.046	1.9947
2	98.814	5.3419	70.829	5.0068
4	203.31	16.378	147.54	25.297
6	288.42	76.855	209.39	10.934
6.25	298.9	19.328	211.33	8.349
6.5	319.38	15.137	190.35	18.436
7	312.47	22.028	208.43	18.258
7.33	315.15	19.22	197.69	22.746
8	301.87	16.539	199.75	40.378
10	230.07	3.7206	115.47	4.5947
16	66.649	10.736	1.8999	0.0564
24	1.646	0.2375	2.7144 <sup>b</sup>	n/a

<sup>a</sup> Values are mean ± SD of 3 animals.

<sup>b</sup>One animal, with two animals with measurement less than the limit of quantitation.

**Table 8. Pharmacokinetic Analysis of DIPE, Isopropanol and Acetone in male and female rats exposed to 3600 ppm DIPE.**

	Units	DIPE	DIPE	Isopropanol	Isopropanol	Acetone	Acetone
		Male	Female	Male	Female	Male	Female
Cmax	µg /mL	55.7082	65.6099	66.1823	35.7084	319.3774	211.3271
Tmax	hr	4	6	6	6.25	6.5	6.25
Half Life	hr	0.9165	0.7587	1.1598	1.2261	2.3107	2.3365
Lambda z	1/hr	0.7563	0.9136	0.5976	0.5653	0.3000	0.2967
AUCall	µg·hr/mL	311.6541	363.2418	338.858	156.0429	3208.097	1734.658

**Table 9. Total Recovery<sup>a</sup> of Radioactivity following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/DIPE**

<b>Rat</b>	<b>Total DPM Recovered</b>	<b>Total <math>\mu</math>Ci</b>	<b>Dose (mg)<sup>c</sup></b>	<b>Bodyweight (g)</b>	<b>Dose (mg/kg)</b>	<b>Mean Dose (mg/kg)<sup>d</sup></b>	<b>SD<sup>e</sup></b>
<b>BM-01</b>	77199927	34.747	77.23	200.58	385.1	372.99	8.33
<b>BM-02</b>	74997744	33.7828	75.03	203.03	369.6		
<b>BM-03</b>	74152179	33.4019	74.18	202.66	366.1		
<b>BM-04</b>	73329601	33.0314	73.36	197.58	371.3		
<b>CM-01</b>	55226373	24.8767	55.25	188.27	311.5	317.89	7.67
<b>CM-02</b>	57600304	25.9461	57.63	187.41	326.4		
<b>CM-03</b>	61974740	27.9165	62.00	208.47	315.7		
<b>CM-04<sup>b</sup></b>	15313501	6.8980	15.32	190.91	85.2		

<sup>a</sup> Total recovery calculated from the total DPM recovered in all samples

<sup>b</sup> Total recovery for CM-04 does not include the analysis of exhaled breath traps, which have been saved for other possible analysis.

<sup>c</sup>Dose calculated from the total DPM recovered/specific activity

<sup>d</sup>Mean dose mg/kg indicates the mean of 4 rats for BM and the mean of 3 rats for CM.

<sup>e</sup> SD indicates the standard deviation of the dose in mg/kg of 4 rats for BM and of 3 rats for CM

**Table 10. Recovery of Radioactivity in Carcass following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/DIPE (Group 2)**

	<b>Group 2 Study B</b>					
<b>Sample</b>	BM-01	BM-02	BM-03	BM-04	Mean	SD
<b>Nose Only Tube Rinse</b>	0.22	0.17	0.24	0.16	0.199	0.037
<b>Carcass Digest</b>	98.20	97.75	99.53	98.73	98.552	0.764
<b>Exhaled VOC</b>	0.63	1.17	0.00	0.52	0.773	0.350
<b>Exposure Urine</b>	N.S.	N.S.	N.S.	N.S.	0.000	0.000
<b>Exposure Feces</b>	0.95	0.91	0.23	0.38	0.617	0.365
<b>Exp. Urine Sample 2</b>	N.S.	N.S.	N.S.	0.21	0.052	0.105
<b>Total</b>	100.00	100.00	100.00	100.00	100.000	0.000

<sup>a</sup>N.S. indicates no sample.

\* Value is below 3 x background for the sample.

**Table 11. Recovery of Radioactivity in Excreta and Carcass following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

	Group 3 Study C <sup>a</sup>				
	Male	Male	Male		
Sample	CM-01	CM-02	CM-03	Mean	SD
Urine	5.5382	4.2344	4.9174	4.897	0.652
Feces	1.0493	0.8501	1.4376	1.112	0.299
CO2 Trap 1	17.5640	17.6930	19.5450	18.267	1.108
CO2 Trap 2	2.6872	1.1723	2.0266	1.962	0.760
Exhaled VOC Trap 1	64.7990	69.4660	68.0450	67.436	2.392
Exhaled VOC Trap 2	5.4380	3.8925	0.5220	3.284	2.514
Exposure Urine	N.S.	N.S.	N.S.	0.000	0.000
Exposure Feces	0.0198	N.S.	0.1172	0.046	0.063
Nose Only Tube Rinse	0.2170	0.1873	0.3494	0.251	0.086
Carcass Digest	2.6486	2.1383	3.0034	2.597	0.435
Cage Rinse	*0.0394	*0.0392	*0.0366	0.038	0.002
<b>Total</b>	100.00	100.00	100.00	100.000	0.000

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

<sup>b</sup>N.S. indicates no sample.

\* Value is below 3 x background for the sample.

**Table 12. Recovery of Radioactivity in Urine following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

		<b>Group 3 Study C<sup>a</sup></b>				
		<b>Male</b>	<b>Male</b>	<b>Male</b>		
		<b>CM-01</b>	<b>CM-02</b>	<b>CM-03</b>	<b>Mean</b>	<b>SD</b>
<b>Urine</b>	0 - 8 h	3.0834	2.9938	2.9543	3.011	0.066
<b>Urine</b>	8 h - 24 h	2.0143	0.8914	1.6701	1.525	0.575
<b>Urine</b>	24 h - 48 h	0.2756	0.1731	0.1519	0.200	0.066
<b>Urine</b>	48 h - 72 h	0.0774	0.0797	0.0588	0.072	0.011
<b>Urine</b>	72 h - 96 h	0.0374	0.0448	0.0346	0.039	0.005
<b>Urine</b>	96 h - 120 h	0.0243	0.0231	0.0194	0.022	0.003
<b>Urine</b>	120 h - 144 h	0.0138	0.0141	0.0180	0.015	0.002
<b>Urine</b>	144 h - 168 h	0.0120	0.0144	0.0103	0.012	0.002
<b>Subtotal</b>		5.538	4.234	4.917	4.897	0.652

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

\* Value is below 3 x background for the sample.

**Table 13. Recovery of Radioactivity in Feces following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

		Group 3 Study C <sup>a</sup>				
		Male	Male	Male		
		CM-01	CM-02	CM-03	Mean	SD
<b>Feces</b>	<b>0 h - 24 h</b>	0.5510	0.3695	0.8500	0.590	0.243
<b>Feces</b>	<b>24 h - 48 h</b>	0.1480	0.1845	0.2471	0.193	0.050
<b>Feces</b>	<b>48 h - 72 h</b>	0.1816	0.1262	0.0861	0.131	0.048
<b>Feces</b>	<b>72 h - 96 h</b>	0.0606	0.0490	0.1303	0.080	0.044
<b>Feces</b>	<b>96 h - 120 h</b>	0.0366	0.0521	0.0494	0.046	0.008
<b>Feces</b>	<b>120 h - 144 h</b>	0.0431	0.0343	0.0126	0.030	0.016
<b>Feces</b>	<b>144 h - 168 h</b>	0.0284	0.0345	0.0620	0.042	0.018
<b>Subtotal</b>		1.049	0.850	1.438	1.112	0.299

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

\* Value is below 3 x background for the sample.

**Table 14. Recovery of Radioactivity in CO2 Trap 1 following exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

		Group 3 Study C <sup>a</sup>				
		Male	Male	Male		
		CM-01	CM-02	CM-03	Mean	SD
CO2 Trap 1	0-1 h	0.4472	0.4165	0.3813	0.415	0.033
CO2 Trap 1	1-3 h	*0.0174	1.1806	1.0756	0.758	0.643
CO2 Trap 1	3-6 h	3.0727	3.5843	3.5934	3.417	0.298
CO2 Trap 1	6-24 h	12.2670	11.2350	12.2830	11.928	0.600
CO2 Trap 1	24-48 h	0.8406	0.6149	1.1934	0.883	0.292
CO2 Trap 1	48-72 h	0.4159	0.2429	0.3996	0.353	0.096
CO2 Trap 1	72-96 h	0.1860	0.1479	0.2059	0.180	0.029
CO2 Trap 1	96-120 h	0.1496	0.1047	0.1822	0.146	0.039
CO2 Trap 1	120-144 h	0.1002	0.1007	0.1460	0.116	0.026
CO2 Trap 1	144-168 h	*0.0678	*0.0658	0.0841	0.073	0.010
<b>Subtotal</b>		17.564	17.693	19.545	18.267	1.108

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

\* Value is below 3 x background for the sample.

**Table 15. Recovery of Radioactivity in CO2 Trap 2 following exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

		Group 3 Study C <sup>a</sup>				
		Male	Male	Male		
		CM-01	CM-02	CM-03	Mean	SD
CO2 Trap 2	0-1 h	*0.0006	*0.0021	*0.0019	0.002	0.001
CO2 Trap 2	1-3 h	1.2647	*0.0112	*0.0089	0.428	0.724
CO2 Trap 2	3-6 h	*0.0134	*0.0216	0.0273	0.021	0.007
CO2 Trap 2	6-24 h	0.9221	0.5623	1.2530	0.912	0.345
CO2 Trap 2	24-48 h	0.2068	0.2278	0.4163	0.284	0.115
CO2 Trap 2	48-72 h	0.1094	0.1368	0.1339	0.127	0.015
CO2 Trap 2	72-96 h	0.0584	0.0769	0.0691	0.068	0.009
CO2 Trap 2	96-120 h	0.0383	0.0673	0.0367	0.047	0.017
CO2 Trap 2	120-144 h	*0.0402	*0.0372	*0.0437	0.040	0.003
CO2 Trap 2	144-168 h	*0.0332	*0.0290	*0.0357	0.033	0.003
<b>Subtotal</b>		2.687	1.172	2.027	1.962	0.760

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

\* Value is below 3 x background for the sample.

**Table 16. Recovery of Radioactivity in Exhaled VOC Trap 1 following exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

		Group 3 Study C <sup>a</sup>				
		Male	Male	Male		
		CM-01	CM-02	CM-03	Mean	SD
Exhaled VOC Trap 1	0-1 h	29.4100	34.3340	28.5230	30.756	3.131
Exhaled VOC Trap 1	1-3 h	18.2220	21.0790	19.8230	19.708	1.432
Exhaled VOC Trap 1	3-6 h	12.9700	12.7320	13.9460	13.216	0.643
Exhaled VOC Trap 1	6-24 h	4.1053	1.2148	5.6436	3.655	2.249
Exhaled VOC Trap 1	24-48 h	0.0488	0.0364	0.0668	0.051	0.015
Exhaled VOC Trap 1	48-72 h	0.0191	0.0258	0.0183	0.021	0.004
Exhaled VOC Trap 1	72-96 h	0.0090	0.0190	0.0098	0.013	0.006
Exhaled VOC Trap 1	96-120 h	*0.0062	0.0103	*0.0063	0.008	0.002
Exhaled VOC Trap 1	120-144 h	*0.0046	*0.0081	*0.0046	0.006	0.002
Exhaled VOC Trap 1	144-168 h	*0.0038	*0.0059	*0.0039	0.005	0.001
<b>Subtotal</b>		64.799	69.466	68.045	67.436	2.392

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

\* Value is below 3 x background for the sample.

**Table 17. Recovery of Radioactivity in Exhaled VOC Trap 2 following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

		<b>Group 3 Study C<sup>a</sup></b>				
		<b>Male</b>	<b>Male</b>	<b>Male</b>		
		<b>CM-01</b>	<b>CM-02</b>	<b>CM-03</b>	<b>Mean</b>	<b>SD</b>
<b>Exhaled VOC Trap 2</b>	<b>0-1 h</b>	*0.0017	*0.0030	*0.0028	0.003	0.001
<b>Exhaled VOC Trap 2</b>	<b>1-3 h</b>	*0.0033	*0.0040	*0.0039	0.004	0.000
<b>Exhaled VOC Trap 2</b>	<b>3-6 h</b>	*0.0013	*0.0058	*0.0048	0.004	0.002
<b>Exhaled VOC Trap 2</b>	<b>6-24 h</b>	5.4265	3.8723	0.0000	3.100	2.795
<b>Exhaled VOC Trap 2</b>	<b>24-48 h</b>	*0.0032	*0.0050	0.5099	0.173	0.292
<b>Exhaled VOC Trap 2</b>	<b>48-72 h</b>	*0.0007	*0.0007	*0.0003	0.001	0.000
<b>Exhaled VOC Trap 2</b>	<b>72-96 h</b>	*0.0006	*0.0005	0.0000	0.000	0.000
<b>Exhaled VOC Trap 2</b>	<b>96-120 h</b>	*0.0005	0.0003	0.0000	0.000	0.000
<b>Exhaled VOC Trap 2</b>	<b>120-144 h</b>	0.0001	*0.0006	0.0000	0.000	0.000
<b>Exhaled VOC Trap 2</b>	<b>144-168 h</b>	0.0002	*0.0002	0.0004	0.000	0.000
<b>Subtotal</b>		5.438	3.893	0.522	3.284	2.514

<sup>a</sup> CM-04 is excluded from the table, since exhaled breath traps were saved for possible future analysis.

\* Value is below 3 x background for the sample.

**Table 18. Cumulative Recovery of Radioactivity in Excreta and Carcass following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

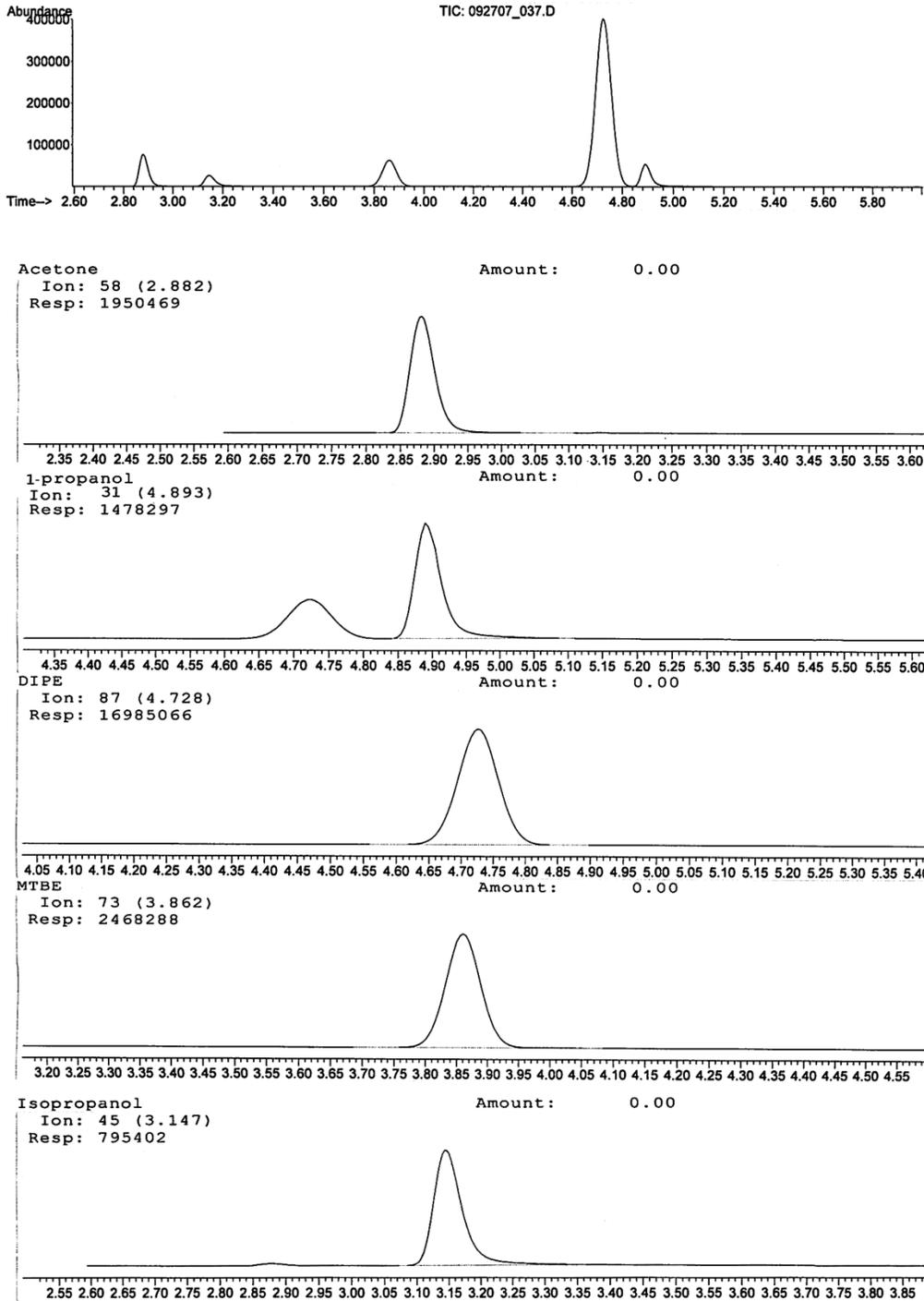
<b>Timepoint</b>	<b>CM-01</b>	<b>CM-02</b>	<b>CM-03</b>	<b>Mean</b>	<b>SD</b>
<b>0 h</b>	0.2368	0.1873	0.4667	0.297	0.149
<b>1 h</b>	30.0960	34.9430	29.3760	31.472	3.028
<b>3 h</b>	49.6040	57.2180	50.2870	52.370	4.213
<b>6 h</b>	65.6610	73.5620	67.8580	69.027	4.078
<b>8 h</b>	68.7440	76.5560	70.8130	72.037	4.047
<b>24 h</b>	94.0300	94.7010	92.5130	93.748	1.121
<b>48 h</b>	95.5530	95.9430	95.0980	95.531	0.423
<b>72 h</b>	96.3570	96.5550	95.7950	96.236	0.394
<b>96 h</b>	96.7090	96.8930	96.2450	96.616	0.334
<b>120 h</b>	96.9650	97.1510	96.5390	96.885	0.314
<b>144 h</b>	97.1670	97.3460	96.7640	97.092	0.298
<b>168 h</b>	100.0000	100.0000	100.0000	100.000	0.000

**Table 19. Recovery of Radioactivity in Tissues from Rat CM-02 following Exposure to 3600 ppm [2-<sup>14</sup>C]DIPE/<sup>13</sup>C<sub>6</sub>DIPE (Group 3)**

<b>Timepoint</b>	<b>Tissue</b>	<b>% of Dose in Tissue</b>
CM-02	Blood	0.001
CM-02	Spleen	0.005
CM-02	Liver	0.119
CM-02	Kidney	0.024
CM-02	Brain	0.014
CM-02	Heart	0.007
CM-02	Lung	0.012
CM-02	Stomach	0.011
CM-02	Small Intestine	0.026
CM-02	Femur	0.006
CM-02	Testes	0.026
CM-02	Cecum+Lg Int+Rect	0.019
CM-02	Adip (Abdominal)	0.006
CM-02	Cage Rinse	0.039
CM-02	Skin (Neck)	0.005
CM-02	Adipose (subcut)	0.006
CM-02	Stomach Contents	0.001
CM-02	Muscle Gastrocnemius	0.004
CM-02	Small Intestine Conents	0.019

**Figure 1. Headspace GC-MS analysis of DIPE, acetone and isopropanol in blood.**

Selected ion monitoring with total ion chromatogram (top) and (in order) acetone, 1-propanol (internal standard), DIPE, MTBE (internal standard) and isopropanol.



**Figure 2. Concentration of DIPE in male and female rat blood during and following exposure to 3600 ppm DIPE for 6 hours.**

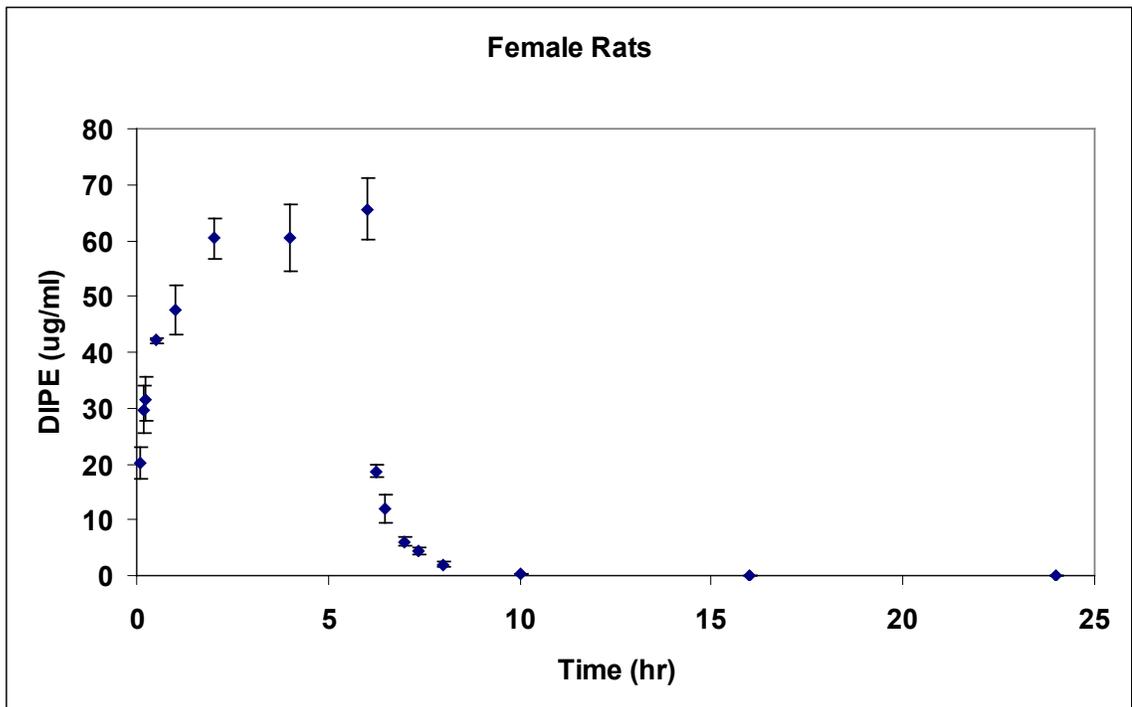
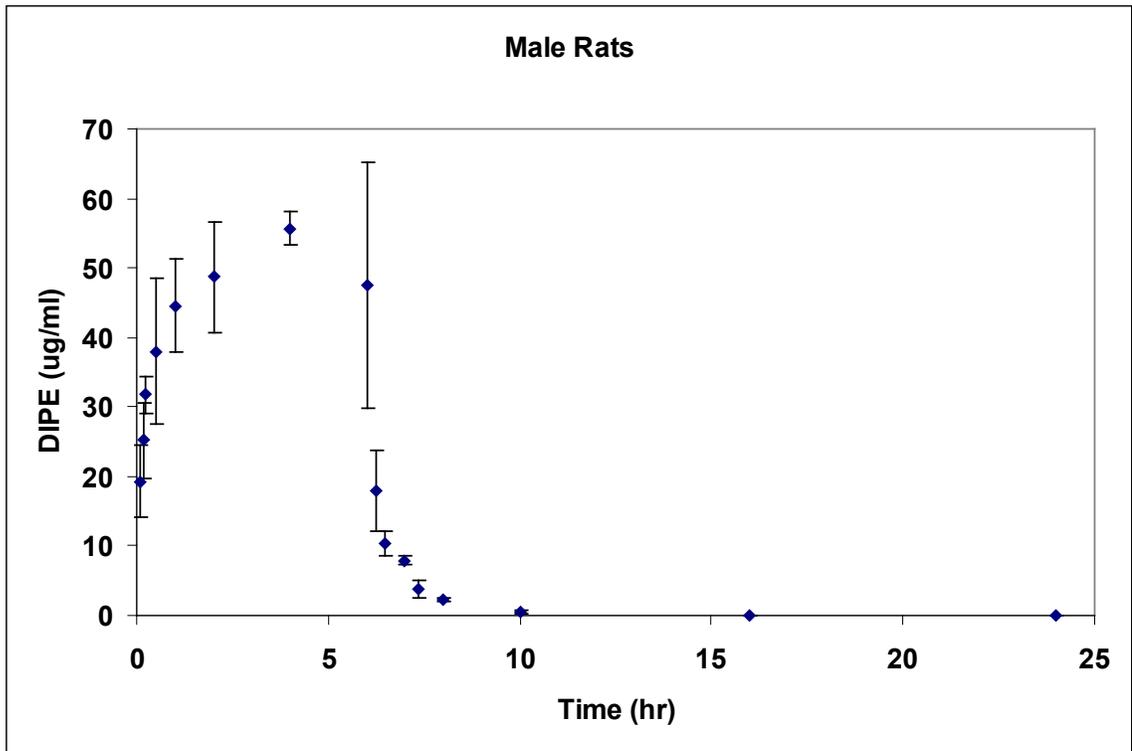
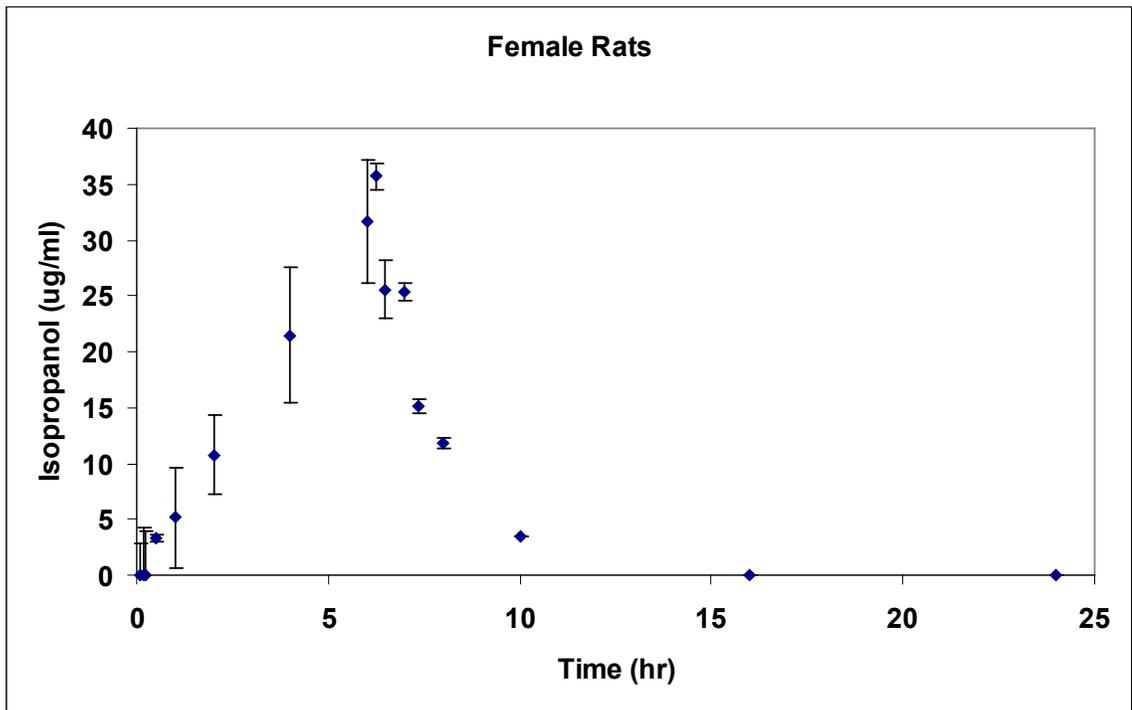
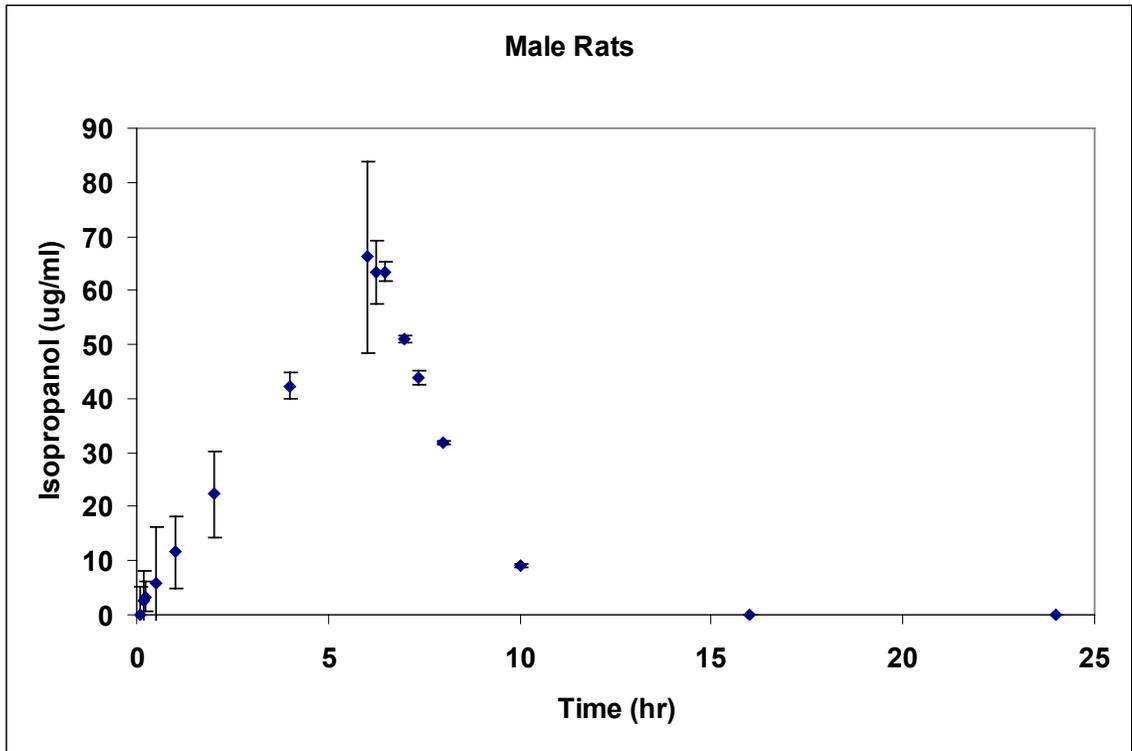
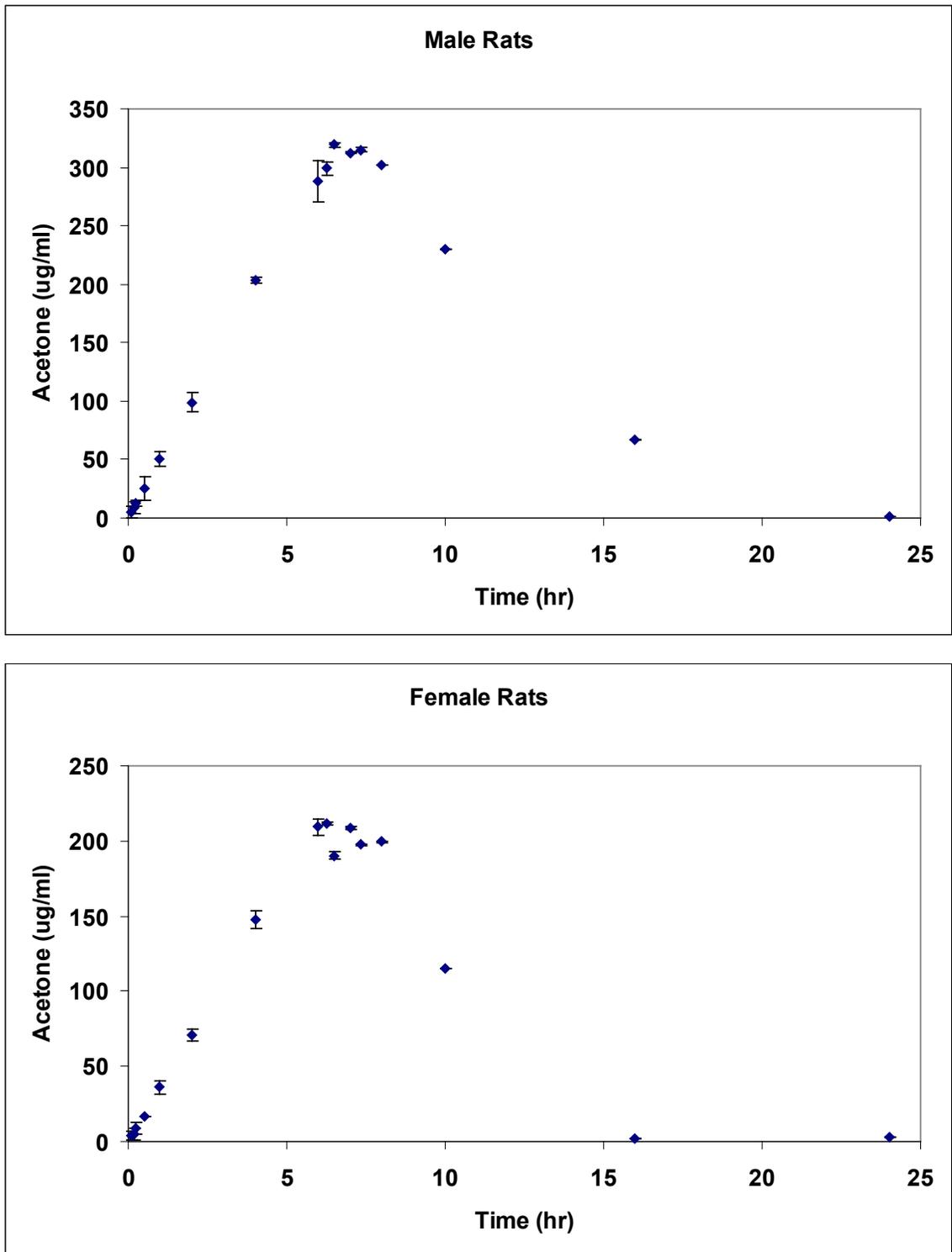


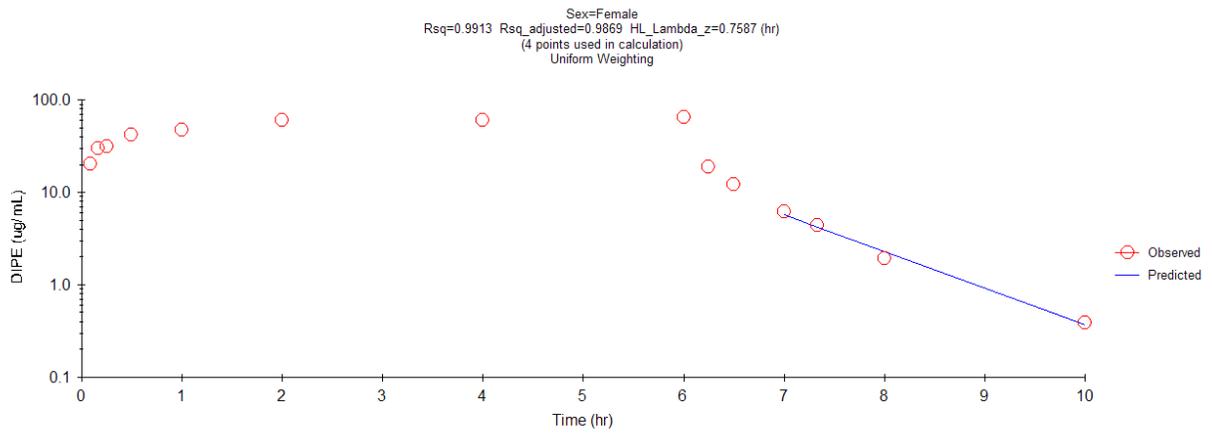
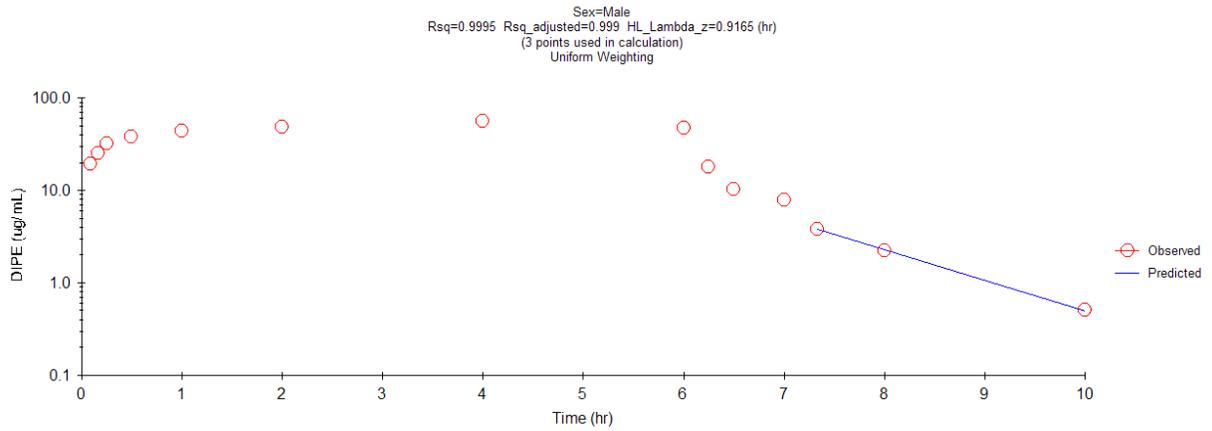
Figure 3. Concentration of Isopropanol in male and female rat blood during and following exposure to 3600 ppm DIPE for 6 hours.



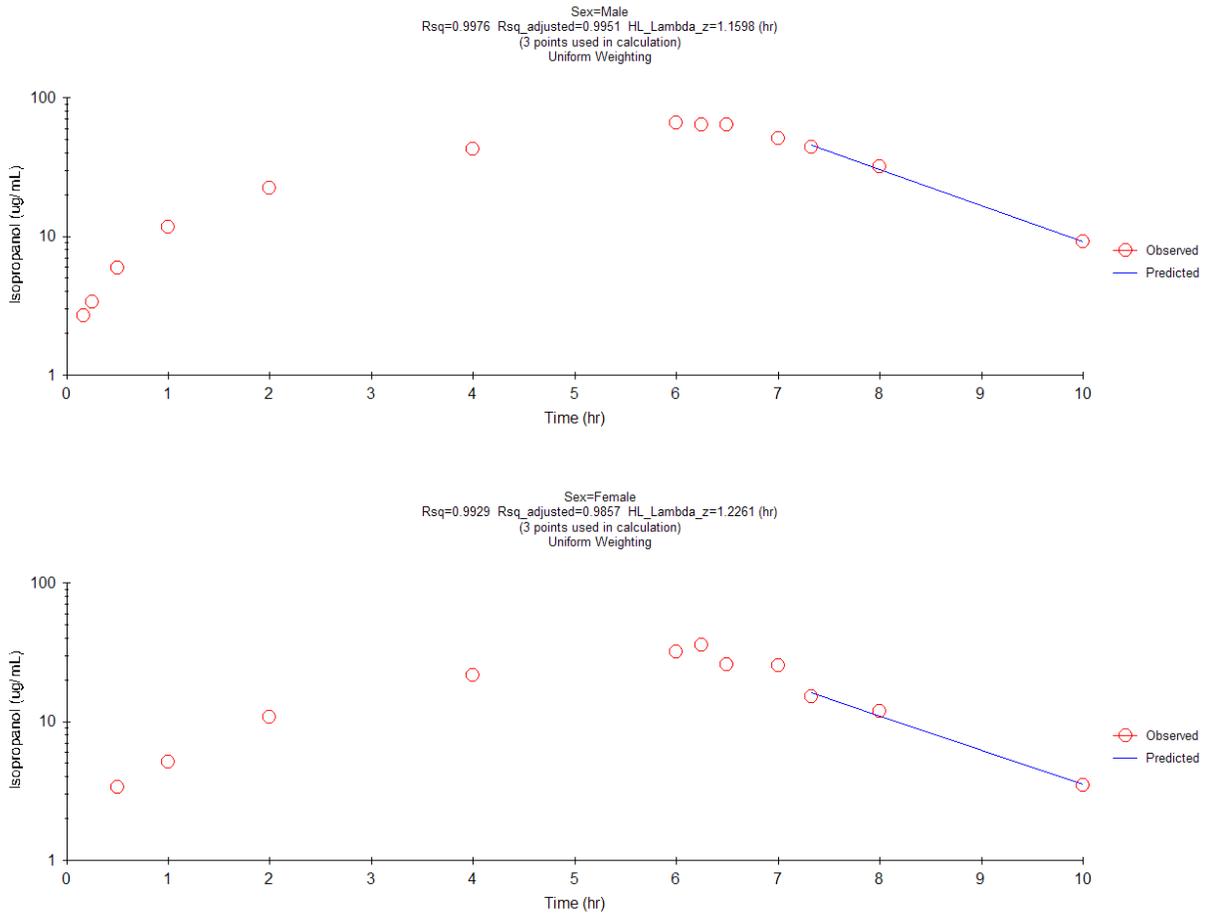
**Figure 4. Concentration of Acetone in male and female blood during and following exposure to 3600 ppm DIPE for 6 hours.**



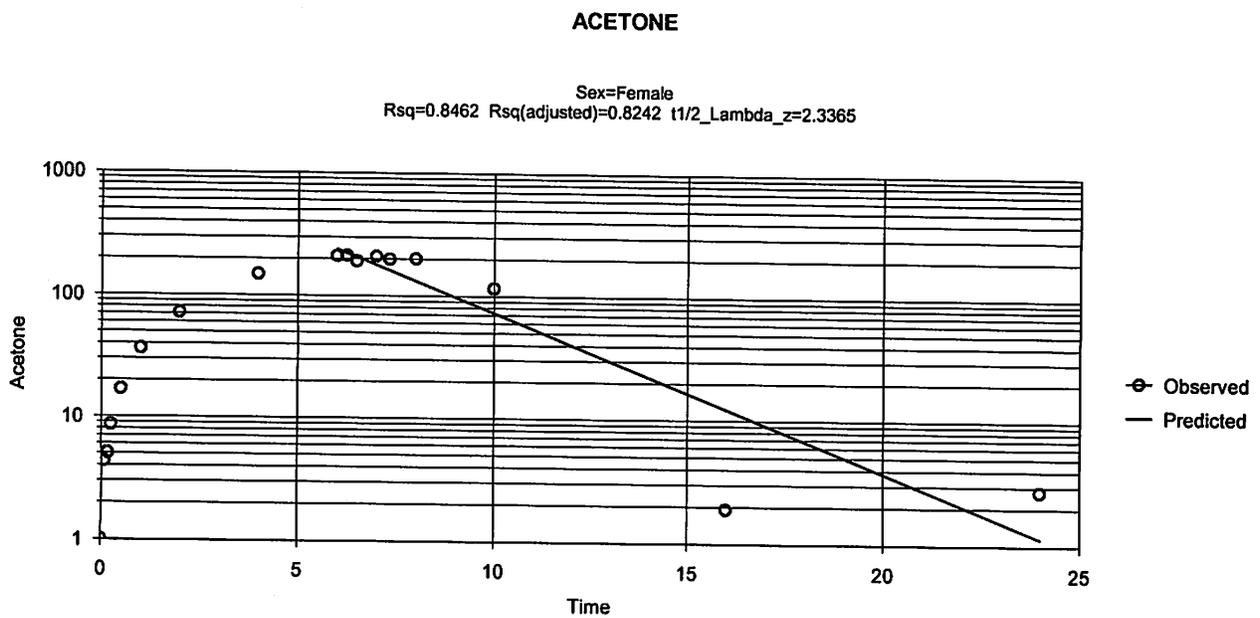
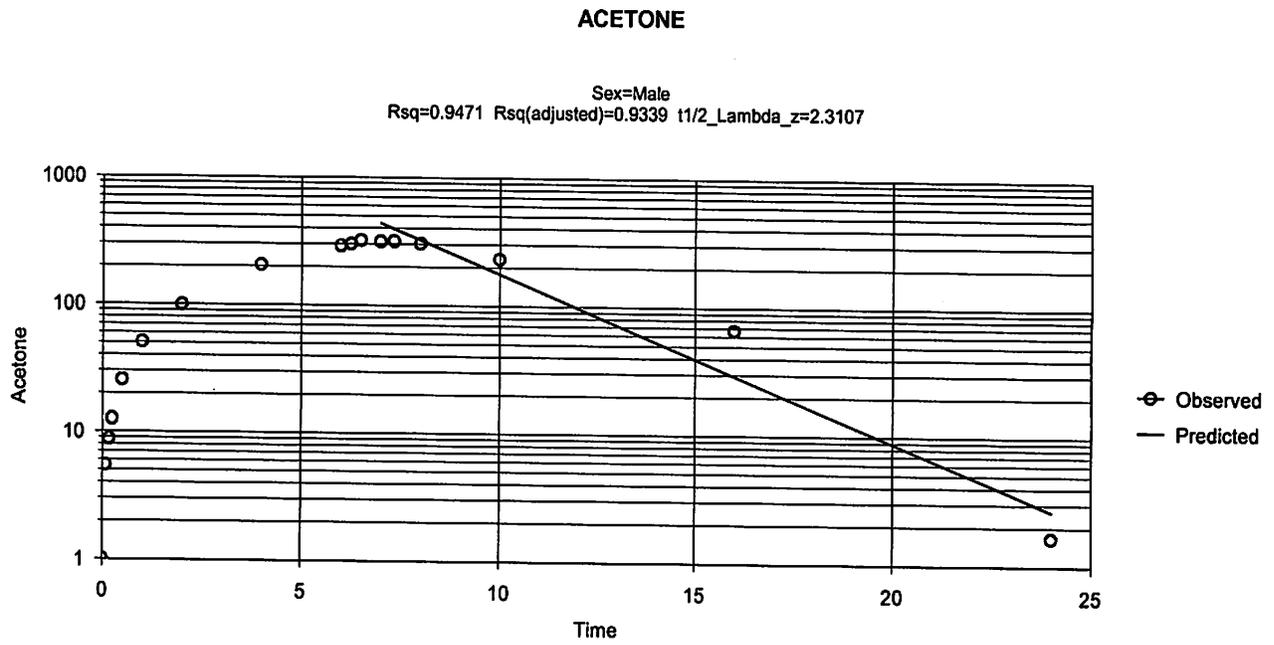
**Figure 5. Noncompartmental analysis of DIPE in male (top) and female (bottom) rat blood during and following exposure to 3600 ppm DIPE for 6 hours.**

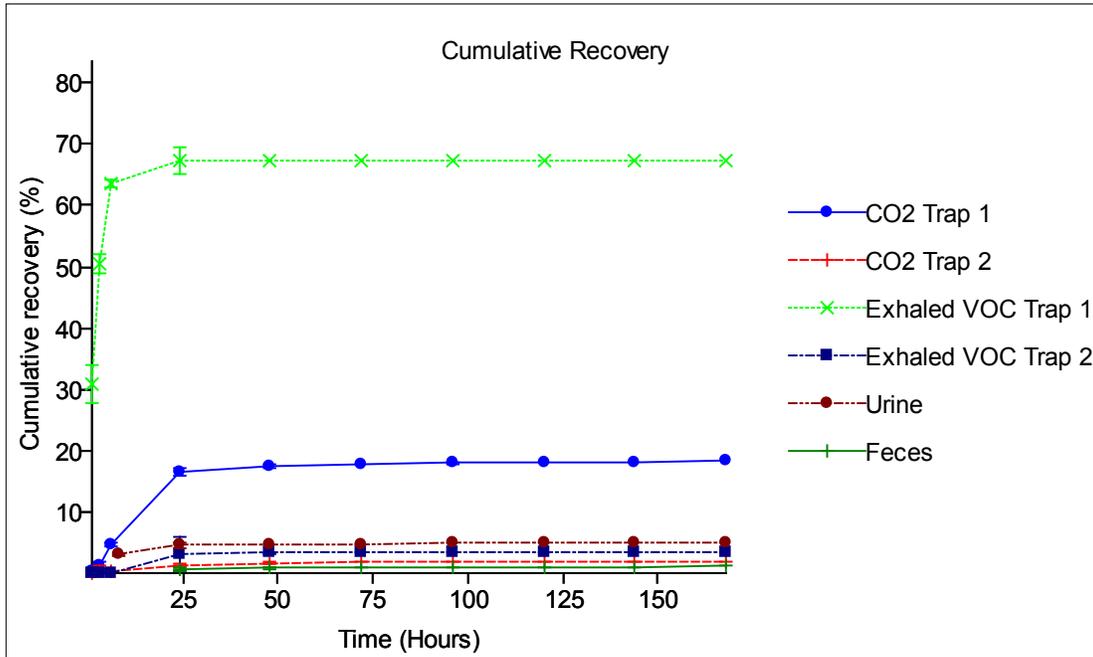


**Figure 6. Noncompartmental analysis of isopropanol in male (top) and female (bottom) rat blood during and following exposure to 3600 ppm DIPE for 6 hours.**



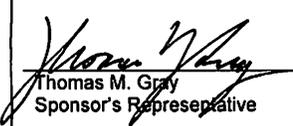
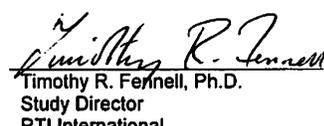
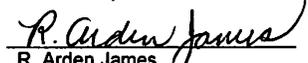
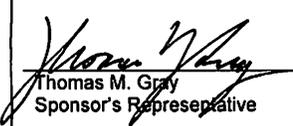
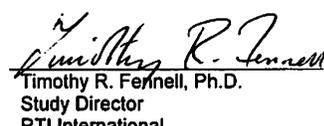
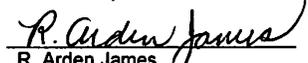
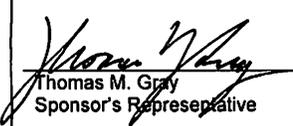
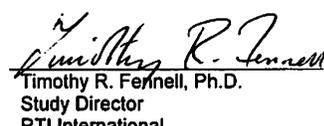
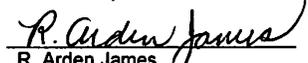
**Figure 7. Noncompartmental analysis of acetone in male (top) and female (bottom) rat blood during and following exposure to 3600 ppm DIPE for 6 hours.**



**Figure 8. Cumulative Excretion of Radioactivity in Urine and Feces, CO<sub>2</sub> and Exhaled VOC Traps.**

## **Appendix A**

### **Approved Study Protocol, Amendments, and Deviations**

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<p><b>TITLE:</b> METABOLISM AND PHARMACOKINETICS OF DIISOPROPYL ETHER IN MALE AND FEMALE RATS: PILOT STUDY</p> <p><b>SPONSOR:</b> Section 211(b) Research Group American Petroleum Institute 1220 L Street NW Washington, DC 20005</p> <p><b>TESTING FACILITY:</b> RTI International* Science and Engineering 3040 Cornwallis Road Post Office Box 12194 Research Triangle Park, NC 27709</p> <p><b>RTI PROJECT NO.:</b> 0209408.001</p> <p><b>RTI Study Code:</b> Rt05-934</p> <p><b>RTI STUDY DIRECTOR:</b> Timothy R. Fennell</p> <p><b>PROPOSED EXPERIMENTAL START DATE:</b> April 2nd, 2007</p> <p><b>PROPOSED EXPERIMENTAL TERMINATION DATE:</b> To be added by Amendment</p> <p><b>AMENDMENTS:</b></p> <table border="1" style="width: 100%; border-collapse: collapse; margin-left: 20px;"> <thead> <tr> <th style="width: 10%;">No.</th> <th style="width: 15%;">Date</th> <th style="width: 55%;">Section</th> <th style="width: 20%;">Pages</th> </tr> </thead> <tbody> <tr><td style="text-align: center;">1</td><td></td><td></td><td></td></tr> <tr><td style="text-align: center;">2</td><td></td><td></td><td></td></tr> <tr><td style="text-align: center;">3</td><td></td><td></td><td></td></tr> <tr><td style="text-align: center;">4</td><td></td><td></td><td></td></tr> </tbody> </table>			No.	Date	Section	Pages	1				2				3				4			
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<table style="width: 100%; border: none;"> <tr> <td style="width: 35%; vertical-align: bottom;">                   Thomas M. Gray                  Sponsor's Representative             </td> <td style="width: 15%; vertical-align: bottom; text-align: center;"> <u>2/22/07</u>                  Date             </td> <td style="width: 35%; vertical-align: bottom;">                   Timothy R. Fennell, Ph.D.                  Study Director                  RTI International             </td> <td style="width: 15%; vertical-align: bottom; text-align: center;"> <u>2-23-07</u>                  Date             </td> </tr> <tr> <td style="vertical-align: bottom;">                   R. Arden James                  Principal Investigator                  CIIT at The Hamner Institutes                  for Health Sciences             </td> <td style="vertical-align: bottom; text-align: center;"> <u>02/23/2007</u>                  Date             </td> <td colspan="2"></td> </tr> </table>			 Thomas M. Gray Sponsor's Representative	<u>2/22/07</u> Date	 Timothy R. Fennell, Ph.D. Study Director RTI International	<u>2-23-07</u> Date	 R. Arden James Principal Investigator CIIT at The Hamner Institutes for Health Sciences	<u>02/23/2007</u> Date														
 Thomas M. Gray Sponsor's Representative	<u>2/22/07</u> Date	 Timothy R. Fennell, Ph.D. Study Director RTI International	<u>2-23-07</u> Date																			
 R. Arden James Principal Investigator CIIT at The Hamner Institutes for Health Sciences	<u>02/23/2007</u> Date																					
<p><small>*RTI International is a trade name of Research Triangle Institute.</small></p>																						

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<p><b>1.0 OBJECTIVES</b></p> <p>The objectives of this pilot study are to:</p> <p><b>Study A</b></p> <ol style="list-style-type: none"> <li>1) Collect blood from unexposed (control) male rats (n=10).</li> <li>2) Develop a gas chromatography (GC) method for the analysis of the concentration of DIPE, isopropanol, and acetone in blood.</li> <li>3) Develop a nose-only exposure system for exposure of male and female rats to DIPE, and conduct exposure of cannulated rats to unlabelled DIPE (n=6; 3 backup unexposed).</li> <li>4) Collect blood from rats exposed to unlabelled DIPE, and measure the concentration of DIPE, isopropanol, and acetone in blood.</li> </ol> <p><b>Study B</b></p> <ol style="list-style-type: none"> <li>5) Conduct a nose-only exposure of male rats to <sup>14</sup>C DIPE/DIPE (n=4; 1 backup unexposed).</li> <li>6) Analyze the amount of <sup>14</sup>C in whole body digests from rats exposed to <sup>14</sup>C DIPE/DIPE.</li> </ol> <p><b>Study C</b></p> <ol style="list-style-type: none"> <li>7) Conduct a nose-only exposure of male rats to <sup>14</sup>C DIPE/(U-<sup>13</sup>C<sub>6</sub>) DIPE (n=4; 2 backup unexposed).</li> <li>8) Collect excreta from rats exposed to <sup>14</sup>C DIPE/(U-<sup>13</sup>C<sub>6</sub>) DIPE.</li> <li>9) Analyze <sup>14</sup>C in whole-body digests, urine, feces, exhaled CO<sub>2</sub>, and exhaled volatiles in rats exposed to <sup>14</sup>C DIPE/(U-<sup>13</sup>C<sub>6</sub>) DIPE.</li> <li>10) Develop a method for analysis of metabolites in urine using HPLC and NMR.</li> </ol> <p><b>2.0 STUDY ORGANIZATION</b></p> <p>Testing Facility: RTI International Science and Engineering 3040 Cornwallis Road PO Box 12194 Research Triangle Park, NC 27709</p> <p>For the purposes of this study, the terms "RTI", "RTI International", and "Research Triangle Institute" are synonymous.</p> <p>Test Facility Management: Alan Staple, Vice President Health Sciences Science and Engineering RTI International</p>		

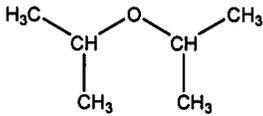
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<p style="text-align: right;">Phone: (919) 485 5674</p> <p>Study Director: Timothy R. Fennell, Ph.D. Health Sciences Science and Engineering RTI International Phone: (919) 485 2781 FAX: (919) 541 6499 Email: fennell@rti.org</p> <p>The Study Director has overall responsibility for the conduct of the entire study and will sign the final report to indicate acceptance of responsibility for the validity of the data.</p> <p>Lead Quality Assurance Contact: Celia Keller, M.S. Science and Engineering QA Unit RTI International Phone: (919) 541 7272 Email: cdk@rti.org</p> <p>The lead Quality Assurance contact has overall responsibility for quality assurance for the entire study.</p> <p>RTI Study Personnel Timothy Fennell, Ph.D. – Study Director Susan Sumner, Ph.D., NMR – Spectroscopist Norman Gaudette, B.S. – Research Chemist Rodney Snyder, M.S. – Research Chemist Yan Hong, M.S. – Research Chemist Jem Scott-Emuakpor, DVM – Veterinarian Melody Gower – Biologist</p> <p>Other personnel will be used as required. A full list of study participants will be included in the study report.</p> <p>Test Site for the development of the inhalation exposure system:</p>		

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<p style="text-align: right;">CIIT at The Hamner Institutes for Health Sciences 6 Davis Drive P.O. Box 12137 Research Triangle Park NC 27709</p>		
<p>For the purposes of this study, the terms "CIIT at The Hamner Institutes for Health Sciences", and "CIIT" are synonymous.</p>		
CIIT Principal Investigator	<p>R. Arden James CIIT Phone (919) 558 1279 Fax (919) 558 1300 Email: <a href="mailto:james@ciit.org">james@ciit.org</a></p>	
CIIT Quality Assurance Contact	<p>Patricia O'Brien Pomerleau CIIT Phone (919) 558 1341 Fax (919) 558 1300 Email: <a href="mailto:pomerleau@ciit.org">pomerleau@ciit.org</a></p>	
CIIT Study Personnel	<p>R. Arden James, Principal Investigator Brian A. Wong, Ph.D. – Senior Research Investigator Kay C. Roberts, A.S. – Research Associate Marianne W. Marshall, B.A. – Research Associate Carl U. Parkinson, LATg – Research Associate</p>	
<p><b>3.0 STUDY DESIGN</b></p>		
<p>All work will be conducted at RTI International, except for the development of the inhalation exposure system, which will be conducted at CIIT.</p>		
<p>Prior to the exposure of rats to DIPE, an inhalation exposure system will be set up and evaluated for the nose-only exposure of rats to a single exposure of DIPE. The exposure system will consist of a</p>		

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<p>Cannon nose-only inhalation tower, which is designed for nose-only inhalation exposure of rodents without rebreathing (Cannon et al., 1983). The exposure atmosphere generation will be designed to safely generate a reproducible and stable atmosphere for exposure of rodents to DIPE for 6 hrs. The concentration of DIPE on the exposure tower will be monitored using a Miran infrared analyzer (Foxboro, MA) or by GC. The system used will be documented in the raw data and will be described in the final report. Prior to the exposure of animals, a report documenting the setup and performance of the exposure system will be prepared by the CIIT PI, and provided to the Sponsor for review. This report will be included in an Appendix to the final report. For verification of performance of the nose-only exposure system, a test atmosphere will be generated with unlabeled DIPE at the exposure concentration to be used in the DIPE pilot studies, with no animals on the exposure tower. The test exposure will be run for approximately 6 hours, with sampling of air for DIPE measurement at strategic locations to document the exposure concentrations in the exposure tower. The criteria that will indicate successful performance of the system are agreement (<math>\leq 10\%</math> difference) between target and actual concentration on the tower, and agreement (<math>\leq 10\%</math> difference) in concentration measurements taken at multiple locations on the tower. The stability of DIPE concentrations during the exposure will be monitored by comparison of the DIPE concentration measurements at the beginning and end of the exposures.</p> <p><b>Study A</b></p> <p>For the pharmacokinetic analysis of DIPE, a GC method will be developed for the quantitation of DIPE, isopropanol and acetone in blood. To provide rat blood for development of the method, up to thirty male rats will be sacrificed for the collection of control blood. The rats will be euthanized under CO<sub>2</sub> as needed on the study, and exsanguinated by cardiac puncture.</p> <p>For the analysis of blood concentrations, all time-point blood samples from DIPE exposures will be placed in glass crimp seal vials and sealed. Control blood samples (collected under CO<sub>2</sub>) will be used to develop a GC method for analyzing the concentration of DIPE in whole blood. Once the method is verified, blood samples will be used to prepare a standard curve. Aliquots of standards will be frozen at approximately -20°C or below to provide sets of standard curve solutions for the definitive pharmacokinetic studies. Details of the method will be recorded in the raw data. The method will be validated for quantitation of DIPE, isopropanol and acetone.</p> <p>Six male rats and six female rats will be cannulated with jugular vein cannulas up to 4 days prior to exposure and the cannulas will be kept patent. Three additional male rats and three additional female rats will be cannulated and kept on hand in the event that a cannula fails.</p> <p>Six cannulated male rats and six cannulated female rats will be exposed by nose-only inhalation exposure to unlabelled DIPE at 3600 ppm. In addition, two cannulated male and female rats will be</p>		

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<p>placed on the nose-only tower in the event that a cannula fails. Blood (100 <math>\mu</math>L (See Sec. 8.5.1)) will be removed through the cannula from three rats at approximately 5 min, 10 min, 15 min, 30 min, and at approximately 1, 2, 4 and 6 hr after the exposure initiation. At the end of the approximate 6 hr exposure, all rats will be removed from the inhalation exposure tower, and placed in individual cages. Blood will be drawn at approximately 375, 390, 420, 440, 480, 600, 960, and 1440 min after the beginning of the exposure. To facilitate the withdrawal of blood samples, animals will be stagger-started on the exposure tower.</p> <p>The blood samples collected from rats exposed to 3600 ppm DIPE will be analyzed using the above method to verify that the method is sufficiently sensitive for the definitive studies.</p> <p>Any spare rats will be used in training sessions, other studies, or sacrificed and documented in the study files.</p> <p><b>Study B</b></p> <p>Four male F344 rats will be exposed for approximately 6 hr to 3600 ppm <math>^{14}</math>C DIPE/DIPE via nose only inhalation. At the end of exposure, the four rats will be immediately euthanized by CO<sub>2</sub> asphyxiation and exsanguinated. Radioactivity will be determined for the whole body digest. The dose administered will be determined by the total radioactivity measured in the whole body digest. The unexposed rat will be euthanized by CO<sub>2</sub> asphyxiation for collection of a carcass sample that will be used for the determination of background radioactivity.</p> <p><b>Study C</b></p> <p>Four male F344 rats will be exposed for approximately 6 hr to a mixture of 3600 ppm (U-<math>^{13}</math>C<sub>6</sub>) and <math>^{14}</math>C DIPE via nose only inhalation. They will be transferred to glass metabolism cages for the collection of urine, feces, expired volatiles, and expired CO<sub>2</sub>. Urine and feces will be collected over dry ice approximately every 24 hr for up to 7 days or until 90% of the dose is eliminated. Expired volatiles and CO<sub>2</sub> will be collected at approximately 1, 3, 6, and 24 hr after the end of exposure, and at approximately every 24 hr thereafter until 90% of the dose is excreted (or up to 7 days). The radioactivity in these samples will be determined via scintillation counting. The two unexposed rats will be used for the collection of urine, feces, and tissue samples that will be used for determination of background radioactivity. Urine and feces will be collected from the unexposed rats for a period of 48 hr, at which point they will be euthanized for collection of tissues.</p> <p>After 90% of the dose is eliminated or at 7 days, the rats will be sacrificed. The radioactivity will be measured in the carcass via scintillation counting after whole body digestion. All urine, feces, and</p>		

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<p>expired volatile trap samples will be stored at approximately -20°C or below until analyzed. CO<sub>2</sub> trap samples will be stored at room temperature.</p> <p>Carbon 13 NMR spectra will be acquired on an aliquot of urine after addition of D<sub>2</sub>O. The total volume needed for NMR analysis is approximately 800 µL. The amount of D<sub>2</sub>O in the 800 µL will be 15% of the 800 µL volume when urine volumes are sufficient. NMR spectra will be interpreted for the assignment of metabolites. An HPLC system with radioactivity detection will be developed and used to separate urinary metabolites. Fractions corresponding to the radioactivity peaks will be collected. NMR spectra acquired on the individual fractions will enable characterization of the HPLC metabolite profile for DIPE.</p> <p><b>4.0 JUSTIFICATIONS</b></p> <p><b>4.1 Animal Species</b></p> <p>The present studies are designed to evaluate the pharmacokinetics, distribution, and excretion of a chemical entity to provide information that will be used for safety assessments to humans. No <i>in vitro</i> techniques are available that allow for adequate determination of pharmacokinetics, distribution, and excretion of chemicals by mammals. Fischer 344 rats are an established animal species and strain for toxicological testing, and pharmacokinetic studies.</p> <p><b>4.2 Numbers of Animals</b></p> <p>The numbers of animals used in this study are considered acceptable to develop the analytical procedures, and to evaluate the appropriateness of the exposure generation system for further study.</p> <p><b>4.3 Routes of Administration and Dose Levels</b></p> <p>The route of administration is an expected potential exposure route in humans (inhalation) and has been used in toxicity and safety assessment studies. The exposure concentration is similar to the mid range concentration from an inhalation study conducted on the subchronic toxicity of DIPE (Dalbey and Feuston, 1996), and is expected to be without significant toxicity.</p> <p><b>5.0 REGULATORY COMPLIANCE</b></p> <p>This study will be carried out in compliance with the EPA Good Laboratory Practices (GLP) Standards for Inhalation Exposure Health Effects Testing, 40 CFR part 79, subpart F § 79.60. The pre-study exposure system method development will be completed at CIIT and will not be conducted under GLPs.</p> <p>The Quality Assurance Unit at the testing facility will prepare and sign a QA Statement to be included in the final report. It will specify the phases of the study that were inspected, the dates on which</p>		

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<p>inspections were made, and the dates on which results of the inspections were reported to the Study Director and the Study Director's management.</p> <p>The Quality Assurance Unit at the test site will prepare and sign a QA Statement to be included in the CIIT report on the inhalation exposure. It will specify: the phases of the study that were inspected by CIIT Quality Assurance Unit; the dates on which inspections were made; the dates on which the results of the inspections were reported to the CIIT Principal Investigator and the Principal Investigator's Management; and the dates on which the results of the inspections were reported to the Study Director and the Study Director's Management.</p> <p><b>6.0 TEST SUBSTANCE</b></p> <p><b>NAME:</b> Diisopropyl ether (DIPE; CAS No. 108-20-3)</p> <p><b>MOLECULAR FORMULA:</b> C<sub>6</sub>H<sub>14</sub>O</p> <p><b>MOLECULAR WEIGHT:</b> 102.18</p> <p><b>STRUCTURE:</b></p> <div style="text-align: center;">  </div> <p><b>SOURCE OF NON-LABELED TEST SUBSTANCE:</b> The non-labeled DIPE was purchased from Sigma-Aldrich, Milwaukee, WI. A certificate of analysis from the vendor indicated purity of 99.6% by GLC, with 6.6 PPM BHT.</p> <p><b>PRODUCT NUMBER:</b> 398276</p> <p><b>LOT NUMBER:</b> 03658JC</p> <p><b>SOURCE OF <sup>13</sup>C-LABELED TEST SUBSTANCE:</b> (U-<sup>13</sup>C<sub>6</sub>) Substituted DIPE was obtained from ISOTEC, Miamisburg, OH. The amount to be ordered was determined in consultation with the inhalation staff from CIIT who will conduct the exposures. A certificate of analysis from the vendor indicated purity of 95.9%.</p> <p><b>PRODUCT NUMBERS:</b> ISOTEC Number T83-03014 ALDRICH Number 632384</p> <p><b>LOT NUMBER:</b> ST1187</p> <p><b>SOURCE OF RADIOLABELED TEST SUBSTANCE:</b> [2-<sup>14</sup>C]-labeled DIPE will be obtained by custom synthesis from a source to be added by amendment. The target specific activity will be 5-10 mCi/mmol.</p>		

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<p><i>LOT NUMBER(S)</i>: To be listed in the final report.</p> <p><i>IDENTITY AND PURITY</i>: The identity of the unlabeled DIPE will be confirmed at RTI by <math>^1\text{H}</math> and <math>^{13}\text{C}</math> nuclear magnetic resonance (NMR) spectroscopy, and by mass spectrometry. The purity of the test chemical will be determined by GC with flame ionization detector (FID), or by GC-MS. The identity of (<math>\text{U-}^{13}\text{C}_6</math>) DIPE will be confirmed by <math>^1\text{H}</math> and <math>^{13}\text{C}</math> NMR spectroscopy, and purity will be determined by GC with FID detection, or by GC-MS. The identity of [<math>2\text{-}^{14}\text{C}</math>] DIPE will be confirmed by <math>^1\text{H}</math> NMR spectroscopy, and by coelution of radioactivity with the unlabeled DIPE standard on HPLC with radioactivity detection. The chemical and radiochemical purity of the test substance will be verified by RTI using GC methods based upon those available in the literature, or developed by RTI. For safety concerns in working with DIPE, which can form explosive peroxides on storage, the unlabeled DIPE will be monitored semi-quantitatively for buildup of peroxides using commercially available peroxide test strips. If the levels of peroxide exceed 80 ppm, the DIPE will be disposed of immediately in accordance with RTI safety standards, and an additional batch will be procured.</p> <p><i>STORAGE CONDITIONS</i>: [<math>2\text{-}^{14}\text{C}</math>]DIPE and (<math>\text{U-}^{13}\text{C}_6</math>) DIPE will be stored in the dark at approximately <math>-20^\circ\text{C}</math>. Nonradiolabeled DIPE will be stored in the dark at room temperature.</p> <p><i>STABILITY</i>: DIPE presents particular problems with storage, since the recommended length of storage to avoid the buildup of peroxides is 3 months. At the conclusion of the study, all unused DIPE will be disposed of. A sample of the unlabeled DIPE will be analyzed before the start of this study, periodically throughout the study, and after the study to confirm stability. Radiochemical purity of the [<math>2\text{-}^{14}\text{C}</math>]- DIPE will be confirmed by HPLC before and after the animal exposures, and after the study to confirm stability. A sample of the (<math>\text{U-}^{13}\text{C}_6</math>) DIPE will be analyzed before and after the animal exposures and after the study to confirm stability.</p> <p><b>7.0 ANIMALS</b></p> <ol style="list-style-type: none"> <li>1. Species and Strains: Fischer 344 rats</li> <li>2. Approximate Age: 8-9 weeks old at time of exposure</li> <li>3. Approximate Weight: 200 g</li> <li>4. Number/Sex: <ul style="list-style-type: none"> <li>Study A: 19 Males rats and 9 female rats</li> <li>Study B: 5 Male rats</li> <li>Study C: 6 Male rats</li> </ul> </li> </ol> <p>At least 39 rats will be ordered to ensure enough animals for the completion of the studies.</p>		

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<p>5. Sources: Charles River Laboratories, Inc. (Kingston, NY) will be the primary source of animals. In the event that suitable animals cannot be provided from the primary source, acceptable alternate sources are Charles River Laboratories, Inc. (Portage, MI) and Hartan (Indianapolis, IN). The source(s) of all animals will be documented in the raw data, and included in the Final Report.</p> <p><b>7.1 Husbandry</b> Research Triangle Institute is accredited by AAALAC International. Animal procedures detailed in this protocol are in accordance with the Animal Welfare Act, "Guide for the Care and Use of Laboratory Animals" (NRC, 1996), and the Office of Laboratory Animal Welfare (NIH). All animal procedures will be reviewed by RTI's Institutional Animal Care and Use Committee (IACUC) before initiation of the studies. In the opinion of the Sponsor and Study Director, the study does not unnecessarily duplicate any previous work.</p> <p><b>7.1.1 Identification</b> Rats will be identified by individual eartags. Metabolism cages will be individually coded by number and color that are related to dose and treatment groups. All individual animal data will be referenced to either eartag number or to treatment group and animal number or to both.</p> <p><b>7.1.2 Quarantine</b> Uncannulated animals will be quarantined for a minimum of seven days before use on a study. Animals will be examined by a veterinarian prior to their release from quarantine, and only animals determined to be in good health as indicated by body weight gain and the absence of clinical signs will be used. During the quarantine period and prior to initiation of the experiments detailed in Section 8.0, rats will be housed (maximum of 3 per cage) in polycarbonate cages with stainless steel bar lids accommodating a water bottle. Cage sizes are approximately 19" x 10 1/2" x 8" high (ca.143 sq. in. floor space). Contact bedding will be Sani-Chips (P.J. Murphy Forest Products Corp, Montville, NJ). Cannulated animals will be housed individually, and will be used within 1-2 days of arrival at RTI.</p> <p><b>7.1.3 Feed and Water</b> Animals will be provided Certified Purina Rodent Chow (5002) <i>ad libitum</i>, except during the periods of inhalation exposure. Water will be provided <i>ad libitum</i> except during the period of inhalation exposure. The source of the water is the City of Durham, NC. The analysis of water and analysis of the rodent chow for chemical composition and possible chemical contamination will be provided by the suppliers and maintained in the Study Records. In addition, approximately once per year, RTI conducts an analysis of drinking water contaminants using an outside laboratory. Samples of water will be collected for analysis for tertiary butyl alcohol, tertiary amyl methyl ether, ethyl tertiary butyl ether,</p>		

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<p>diisopropyl ether, and methyl tertiary butyl ether. The samples will be sent to Kiff Analytical (Davis, CA) for analysis. It is anticipated that contaminant levels will be below those permitted in the certified feed and will not affect the design, conduct, or conclusions of this study. It is anticipated that contaminant levels measured in water will not affect the design, conduct, or conclusions of this study.</p> <p><b>7.1.4 Environmental</b></p> <p>Air circulation will be 100% fresh air. Room temperature will be maintained at 64–79°F and relative humidity at 30–70% and monitored at least once a day. Light/darkness will be cycled at 12-h intervals. Any deviations from these conditions shall be included in the study records. Environmental parameters will be recorded automatically using a computerized HVAC Monitoring and Control System.</p> <p><b>7.1.5 Acclimation and Housing during Studies</b></p> <p>During the course of Study C, rats will be placed in glass Roth-type metabolism cages for the collection of urine and feces. There will be no acclimation period.</p> <p><b>7.2 Randomization and Assignment of Animals to Treatment Groups</b></p> <p>Animals designated as blood donors for method development will not be randomized. Animals will be specifically purchased for either Study A or for Study B and C, and will not be assigned randomly to specific study groups. For the cannulated rats within Study A, and the rats in Studies B and C, animals will each be assigned an eartag number, and then assigned unique study designations using a series of computer generated numbers as described in SOP DPK-HUS-001 <i>Assignment of Animals into Groups</i>, using the procedure for Assignment of Animals within a Single Group or Multiple Groups without Regard to Weight Mean and Range.</p> <p><b>7.3 Body Weights</b></p> <p>Individual body weights will be measured during the quarantine period, the day of exposure, and at sacrifice.</p> <p><b>7.4 Found Dead/Moribund Animals</b></p> <p>The Study Director or the veterinarian with the approval of the Study Director will authorize euthanasia of animals with life-threatening clinical signs that indicate that they are unlikely to survive until the next scheduled observation. The time of death will be estimated as precisely as possible and recorded.</p> <p><b>7.5 Euthanasia</b></p> <p>Rats will be euthanized with CO<sub>2</sub> exposure.</p>		

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<p><b>8.0 STUDY PROCEDURES</b></p> <p><b>8.1 Cannulation of Rats for Blood Collection</b> Rats with indwelling jugular vein cannulae will be purchased from Charles River Laboratories, Inc. (Kingston, NY). Animals will be cannulated by the vendor, and shipped to RTI the day after surgery. The rats will be exposed to DIPE 1 to 2 days after arrival at RTI. Cannula patency will be maintained with daily flushings with heparinized saline (sterile saline containing 20 IU/mL of sodium heparin).</p> <p><b>8.2 Test Chemical Preparation and Analysis</b> For inhalation exposure to unlabeled DIPE (Study A), an exposure atmosphere will be generated using unlabeled DIPE. For exposure to labeled DIPE (Studies B and C), the amount of material required will be calculated based on the exposure concentration, the duration of exposure, the number of animals, and the flow rate of air through the exposure tower. For inhalation exposure to [2-<sup>14</sup>C]DIPE/DIPE (Study B), [2-<sup>14</sup>C]DIPE will be weighed into a tared flask with a Teflon faced screw cap. The weight of the labeled chemical added will be recorded. Unlabeled DIPE will be added, and the weight added will be recorded. The nominal specific activity of the DIPE mixture will then be calculated. This will be verified by weighing an aliquot into a sealed flask, recording the weight added, and adding solvent, and recording the weight added. Aliquots of the solution of DIPE will be placed in scintillation vials, and the weight added will be recorded. The amount of solution added will be calculated based on the density of the solvent. Ultima Gold™ scintillation cocktail (Perkin Elmer) will be added to the scintillation vials, and the amount of radioactivity added will be determined by liquid scintillation spectroscopy (LSS). The specific activity of the labeled DIPE will then be calculated from the data obtained. A mixture of <sup>14</sup>C/(U-<sup>13</sup>C<sub>6</sub>) DIPE will be prepared as described above. The specific activity of the <sup>14</sup>C/(U-<sup>13</sup>C<sub>6</sub>) DIPE will be calculated as described above. The exposure atmosphere concentration will be monitored using a calibrated analytical instrument (e.g., MIRAN IR spectrophotometer or GC). Concentrations of DIPE will be monitored at strategic locations to document the exposure concentrations. The stability of DIPE under the conditions of administration will be monitored by sampling the inlet of the exposure tower at the beginning and the end of the exposure. The samples will be analyzed by an analytical instrument (e.g. HPLC, GC) and the resulting chromatograms compared.</p> <p><b>8.3 Inhalation Exposure</b> The inhalation system including the generation and exposure systems will be constructed with materials that are chemically compatible with DIPE to minimize chemical losses. The generation system</p>		

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<p>will include a generator and delivery system to deliver a steady flow of DIPE to the exposure tower air supply at appropriate flow rates to maintain the target concentration of 3600 ppm. The generation system will consist of a syringe containing the chemical with a syringe pump to deliver the chemical to the air supply of the exposure chamber. The air supply will be controlled with an electronic mass flow meter to maintain a total air flow that will assure at least 12 air changes per hour. The generation and delivery system will be placed in a chemical hood to contain any DIPE that may leak from the system.</p> <p>The exposure system will be a flow-past nose only exposure system. The incoming air for the exposure system will be filtered to eliminate the possibility of contamination in the air supply. The air supply temperature and relative humidity will be maintained between 64 to 79 degrees Fahrenheit and 30 to 70%, respectively. The chamber exhaust flow will be adjusted to maintain a slight negative pressure during the exposure to prevent DIPE from entering the laboratory area. The chamber exhaust will be filtered through a disposable charcoal filter and disposed at the end of the exposure. Closed nose-only tubes will be used to hold the uncannulated test animals during inhalation exposures. Specially designed nose only tubes will be used to hold the cannulated test animals. The inhalation system will be strategically placed in front of a chemical hood to prevent any DIPE from entering the laboratory.</p> <p>All data necessary to recreate the inhalation exposure will be documented in a study notebook and will be reviewed by the CIIT Quality Assurance Unit.</p> <p><b>8.4 Ante mortem Observations and Functional Assessments</b></p> <p>Animals will be observed twice per day for mortality, morbidity, signs of toxicity, and for any acute distress that might be related to the test procedure or test substances. Animals exhibiting adverse reactions will be closely monitored. All signs of poor health or abnormal behavior will be recorded. Samples collected from dead or moribund animals will be included in the analysis if the animal was not moribund/dead at the time of collection. The Sponsor will be notified as soon as possible if it is anticipated that the sacrifice may affect the integrity of the study. If possible, an extra animal will be substituted for the animal removed from the study (see 12.0 Data from "Extra" Animals).</p> <p><b>8.5 Collection and Storage of Biological Samples</b></p> <p><b>8.5.1 Blood</b></p> <p>Except at sacrifice, blood will be sampled through indwelling jugular cannulae in Study A. For pharmacokinetic determinations, blood will be sampled from each animal pre-exposure and from three rats each at approximately 5 min, 10 min, 15 min, 30 min, and at approximately 1, 2, 4 and 6 hr after the exposure initiation. At the end of the approximate 6 hr exposure, all rats will be removed from the inhalation exposure tower, and placed in cages. Blood will be drawn from three rats at approximately 375, 390, 420, 440, 480, 600, 960, and 1440 min after the beginning of the exposure (at approximately</p>		

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<p>15, 30, 60, 80 min, and 2 hr, 4 hr, 10 hr, and 18 hr after the end of the exposure). To facilitate the withdrawal of blood samples, animals will be stagger-started on the exposure tower. At each time point approximately 100 <math>\mu</math>L of blood will be withdrawn using a heparinized syringe. The total volume of blood collected over the 24-h period will not be more than 20% of blood volume from each rat. Heparinized saline will be injected into each cannula as necessary to help prevent blood clots from forming in the cannula. If a vascular cannula is determined to no longer be patent before exposure on Day 1, that animal will not be used in the study. If the vascular cannula is determined not to be patent after exposure, the animal will be replaced by a designated "extra" animal if it is available. If an "extra" animal is not available, blood will be sampled from the tail vein for the remainder of the study and recorded appropriately. Each blood sample will be placed immediately in a preweighed headspace vial, and the vial will be capped with a crimp seal. The blood samples will be analyzed within 24 h of collection for DIPE, acetone and isopropanol by GC/MS.</p> <p><b>8.5.2 Excreta</b></p> <p>In Study C, urine will be collected over dry ice at 0–8, 8–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure or until 90% of the radioactivity has been eliminated. Feces will be collected over dry ice at 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure or until 90% of the radioactivity has been eliminated. Exhaled volatile organics will be collected on a series of two charcoal traps, and expired <math>^{14}\text{CO}_2</math> will be collected in 1.0 N KOH at 0–1, 1–3, 3–6, 6–8, 8–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after termination of exposure or until 90% of the radioactivity has been eliminated. At the end of excreta collection, the cage will be rinsed with water, and with ethanol. The rinses will be analyzed for total radioactivity as described for urine (Section 8.6.2). The weight of urine and/or feces collected for each sample interval will be measured. Urine and feces will be analyzed for total radioactivity. Excreta not assayed within a day of collection will be stored at approximately <math>-20^\circ\text{C}</math> in the dark.</p> <p><b>8.5.3 Carcass</b></p> <p>For Study B, the amount of <math>^{14}\text{C}</math> retained will be determined by placing each rat in the nose-only restraint tube in a Tedlar gas bag. The bag will be sealed, and <math>\text{CO}_2</math> will be pumped into the tube to euthanize the rat. The gas from the gas bag will then be forced through a charcoal filter trap, to determine the amount of exhaled <math>^{14}\text{C}</math>, and the carcass will be digested with 2N ethanolic NaOH. After digestion, the amount of radioactivity in the carcass will be determined by LSS of aliquots.</p> <p>For Study C, the carcasses of three rats will be digested with 2N ethanolic NaOH. After digestion, the amount of radioactivity in the carcass will be determined by LSS of aliquots.</p>		

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<p><b>8.5.3.1 Tissues</b></p> <p>In Study C, following sacrifice, selected tissues will be collected from one animal:</p> <table data-bbox="423 527 1060 869"> <tbody> <tr> <td>femur</td> <td>cecum + large intestine + rectum</td> </tr> <tr> <td>skin (hair shaved)</td> <td>small intestine</td> </tr> <tr> <td>subcutaneous fat</td> <td>liver</td> </tr> <tr> <td>abdominal fat</td> <td>muscle (gastrocnemius)</td> </tr> <tr> <td>brain</td> <td>heart</td> </tr> <tr> <td>testes (epididymis removed)</td> <td>kidneys</td> </tr> <tr> <td>stomach contents</td> <td>small intestine contents</td> </tr> <tr> <td>stomach</td> <td>large intestine + cecum contents</td> </tr> <tr> <td>lungs</td> <td>spleen</td> </tr> <tr> <td>residual carcass</td> <td></td> </tr> </tbody> </table> <p>The harvested tissues will be weighed and analyzed for radiochemical (<math>^{14}\text{C}</math>) content by LSS. The remaining carcass of the animal from which the tissues listed above have been taken will be analyzed for total radioactivity. Tissues will be prepared as described in section 8.6.3 below.</p> <p><b>8.6 Analysis of Biological Samples for Total Radioactivity</b></p> <p><b>8.6.1 Blood</b></p> <p>For Study C, whole blood samples will be assayed for total radioactivity (<math>^{14}\text{C}</math>) in duplicate by LSS. For LSS analysis, blood aliquots (approximately 50 <math>\mu\text{l}</math>) will be placed in tared vials containing approximately 1 mL Soluene-350 for solubilization and then weighed. After solubilization, samples will be bleached (by adding approximately 125 <math>\mu\text{l}</math> of 70% perchloric acid, and then adding approximately 0.3 ml of 30% <math>\text{H}_2\text{O}_2</math>) prior to addition of scintillation cocktail and analysis by LSS.</p> <p><b>8.6.2 Excreta</b></p> <p>For Study C, duplicate aliquots of urine will be analyzed directly (without solubilization or bleaching) for radiochemical content. Feces will be homogenized with an approximately equal mass of water. The weight of the feces homogenate will be determined, and duplicate homogenate aliquots will be weighed into scintillation vials. After solubilization of the homogenate aliquots with Soluene-350 (normally about 2 mL per sample), scintillation cocktail will be added to the vials, and the samples will be analyzed for total radioactivity by LSS. Samples may be bleached (by adding approximately 125 <math>\mu\text{l}</math> of 70% perchloric acid, and then adding approximately 0.3 ml of 30% <math>\text{H}_2\text{O}_2</math>) prior to addition of scintillation cocktail, if necessary. The requirement for bleaching will be determined by the intensity of the color of the</p>			femur	cecum + large intestine + rectum	skin (hair shaved)	small intestine	subcutaneous fat	liver	abdominal fat	muscle (gastrocnemius)	brain	heart	testes (epididymis removed)	kidneys	stomach contents	small intestine contents	stomach	large intestine + cecum contents	lungs	spleen	residual carcass	
femur	cecum + large intestine + rectum																					
skin (hair shaved)	small intestine																					
subcutaneous fat	liver																					
abdominal fat	muscle (gastrocnemius)																					
brain	heart																					
testes (epididymis removed)	kidneys																					
stomach contents	small intestine contents																					
stomach	large intestine + cecum contents																					
lungs	spleen																					
residual carcass																						

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<p>samples. Control samples of urine and feces will be collected from unexposed rats, and analyzed for radiochemical content to determine background counts.</p> <p><b>8.6.3 Tissues and Carcass</b> Tissues and carcass will be analyzed for total radioactivity following solubilization in Soluene-350 (normally about 2 mL per tissue sample) or 2N ethanolic sodium hydroxide. Duplicate samples of the solubilized carcass will be analyzed. Liver will be homogenized, and duplicate aliquots of the homogenate solubilized. Other tissues will be solubilized in their entirety or after being divided into multiple pieces. Solubilized tissue samples may be bleached (by adding approximately 125 µl of 70% perchloric acid, and then adding approximately 0.3 ml of 30% H<sub>2</sub>O<sub>2</sub>) prior to addition of scintillation cocktail and analysis by LSS. The requirement for bleaching will be determined by the intensity of the color of the samples. Dark tissues, including blood, liver, lung, heart, muscle and kidneys will require bleaching. The procedure used for individual samples will be recorded in the study records.</p> <p><b>8.6.4 Exhaled Breath Traps</b> Aliquots of 1.0 N KOH from the exhaled breath trap for CO<sub>2</sub> will be analyzed by LSS after addition of scintillation cocktail.</p> <p><b>8.6.5 Exhaled Volatiles Traps</b> Each charcoal trap will be extracted by elution with 4.0 ml of dimethylformamide (DMF). The radioactivity in the DMF wash will be measured by LSS of duplicate aliquots.</p> <p><b>8.7 Analysis of Blood Samples for DIPE and DIPE metabolites</b> A quantitative method for the analysis of DIPE, acetone, and isopropanol in blood will be developed by GC with headspace analysis. The method used will be documented in the raw data, and in the final report. After initial development of the method, it will be documented in a Project Specific Analytical Method. The accuracy, precision, range, and lower limit of quantitation (LLOQ) for the analytical method will be determined. Acceptance criteria will be based on those outlined in the US Food and Drug Administration's Guidance for Industry: Bioanalytical Method Validation (2001). The method will be used for the quantitative analysis of DIPE, acetone and isopropanol in blood collected in study A.</p> <p><b>8.8 Analysis of Urine Samples using <sup>13</sup>C NMR Spectroscopy</b> Urine samples collected from Study C will be selected for identification of urinary metabolites by <sup>13</sup>C NMR. The selection will be based on the concentration of radioactivity determined by LSS. Selected samples will be analyzed by <sup>13</sup>C NMR at 125 MHz, using a Varian Inova 500 MHz NMR spectrometer. Two-dimensional NMR spectra will be determined as appropriate to aid in identification of the metabolites</p>		

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<p>detected. <math>^{13}\text{C}</math> NMR spectra of isopropanol and acetone will be determined for aid in assignment of metabolites.</p> <p><b>8.9 Analysis of Urine Samples for Radioactive Metabolites</b></p> <p>An HPLC system with radioactivity detection will be set up for the separation of urinary metabolites of DIPE. It is expected that the metabolites will consist of isopropanol, and acetone. If possible, metabolites will be separated by HPLC, and collected for NMR analysis. However, since the metabolites must be concentrated prior to NMR analysis, volatility of metabolites may preclude this approach. Other techniques such as mass spectral analysis will be considered as necessary, and documented in the study record. Urine samples selected for analysis will contain <math>\geq 5\%</math> of the total radioactivity recovered.</p> <p><b>9.0 DATA COLLECTION</b></p> <p>The Debra™ laboratory information management system will be used for collection of body weights, animal observations, tissue and sample weights, and radioactivity data. Therefore, the raw data for these measurements will be the electronic data collected in Debra unless otherwise noted in the study records. The Debra system will be used to calculate and report radioactivity recovered in each aliquot analyzed, in each sample, and in each animal. The Debra system will be used to calculate and report summary data for tissues and excreta.</p> <p>Room temperature and humidity data will be collected using a computerized HVAC Monitoring and Control System. All other data, such as animal receipt and quarantine records, will be manually recorded unless noted otherwise in the study records.</p> <p><b>10.0 PHARMACOKINETIC CALCULATIONS</b></p> <p>Blood concentration data for DIPE and if applicable, acetone and isopropanol, will be presented graphically. Data obtained following the end of exposure (at 360 min and greater time points) will be used to derive terminal elimination kinetic data. Mean blood concentration-time data will be analyzed, as appropriate, by noncompartmental (model-independent) methods using the least-squares fitting program WinNonlin™ (Statistical Consulting Inc., Cary, NC). Pharmacokinetic analysis will not be conducted for individual animals, because of the rotating sampling schedule for the collection of blood.</p> <p>After the best-fit model is selected, the following pharmacokinetic parameters will be determined as appropriate: terminal elimination rate constant, terminal elimination half-life (<math>T_{1/2}</math>), area under the blood concentration-time curve extrapolated from time zero to infinity (AUC), maximum concentration achieved (<math>C_{\text{max}}</math>), time to maximum concentration (<math>T_{\text{max}}</math>).</p>		

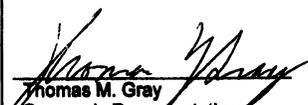
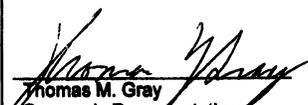
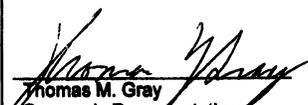
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<p>Occasionally, a data point (i.e., a concentration) that cannot be predicted by a PK method of analysis may be encountered. The following procedure will be used to evaluate such data points as outliers.</p> <ol style="list-style-type: none"> <li>1. Identify suspected outliers by visual inspection of the data. Types of data points that should be considered as suspected outliers include non-zero concentrations prior to dosing, an individual concentration that is much different from that predicted by the PK method of analysis (such as lone high concentration preceded and followed by much lower concentrations).</li> <li>2. Rule out physiological or other processes which may explain the suspected outlier. Certain processes, such as enterohepatic circulation or absorption from more than one site in the gastrointestinal tract, may result in unusual C-T profiles, which a PK method of analysis would be unable to approximate. Furthermore, non-zero concentrations at time zero may be possible if the analyte is present endogenously, or if some endogenous material interferes with the assay for the target analyte. If a concentration is deemed to be an outlier solely because it cannot be explained by PK methods of analysis, the possibility of some process which might explain it such as sample analysis should be considered.</li> <li>3. Once a concentration that is suspected as a PK outlier cannot be explained by PK methods of analysis or physiologic processes, the Grubbs method (Grubbs 1969) will be used to test whether it is an outlier.</li> <li>4. If a concentration cannot be explained by PK methods of analysis and is substantially different from that seen in the other animals at the same time (based on the above criteria), it is considered a PK outlier and will not be included in the calculation of PK parameters or mean concentrations. Reasons for exclusion will be documented in the raw data and in the final report.</li> </ol> <p><b>11.0 STATISTICAL ANALYSIS</b></p> <p>All blood concentration data, and tissue and excreta <sup>14</sup>C-content will be reported in tables as the mean ± standard deviation (SD). No statistical analysis will be conducted.</p> <p><b>12.0 DATA FROM "EXTRA" ANIMALS</b></p> <p>To better ensure that complete data from the required number of animals in Study A will be obtained, one or more additional animals, predesignated as "extras", will start the study as part of each treatment group. If a "core" study animal fails to complete the study due to loss of samples, misdosing,</p>		

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<p>morbidity, or other accidents, it will be replaced with a designated "extra" animal and data will cease to be obtained from the "core" animal. The "extra" animal will then become part of the "core" group and the original animal will be removed from the "core" group. All such substitutions will be documented as to reason and approved in writing by the Study Director. An "extra" animal may also be substituted for a "core" animal as described in Section 8.5.1. Terminal blood samples or blood samples obtained via the tail vein may be included in the analysis in the event that cannulas are no longer patent. Except for animals that become part of a "core" group, samples from "extra" animals will not normally be analyzed. However, all data obtained in the study will be reported. Data used to construct group means and to obtain pharmacokinetic parameters will consist of the data obtained from the "core" study animals (not the "extras") except in cases where an "extra" has taken the place of a "core" animal. In that case, the data from the "extra" animal will be used instead. Data from the "core" animal that was eliminated from the study will be used in these calculations, if the data are from samples collected prior to elimination.</p> <p><b>13.0 RECORDS AND REPORT</b></p> <p>The following will be maintained in the record:</p> <ol style="list-style-type: none"> <li>a. Protocol and any amendments</li> <li>b. Animal receipt records</li> <li>c. Quarantine records</li> <li>d. Temperature and humidity records for the treatment rooms</li> <li>e. Animal research facility room logs</li> <li>f. Feed and water analysis for contaminants</li> <li>g. Test chemical receipt, storage and use records</li> <li>h. Balance calibration log references</li> <li>i. Correspondences</li> <li>j. All other raw data and documentation.</li> </ol> <p>Results of the studies will be described in an audited draft report, which will be submitted to the Sponsor for approval. This report will include but not be limited to:</p> <ol style="list-style-type: none"> <li>a. Name and address of the facility performing the study, dates of study initiation and completion, and RTI study number.</li> <li>b. A copy of the signed, dated and approved protocol and all deviations and authorized amendments to the original protocol.</li> <li>c. A detailed description of all methods used.</li> <li>d. The lot number(s) of the test substances and details of the formulation of doses.</li> </ol>		

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<p>e. Animal information to include: supply source, species, strain or substrain, sex, individual animal weights (randomization through sacrifice), approximate age at initiation of dosing, and procedure used for individual animal identification and assignment to the treatment group.</p> <p>f. Tabulated individual results for blood, urine and tissues.</p> <p>g. Tabulated mean results for blood, urine, and tissues.</p> <p>h. Pharmacokinetic data. (No statistical analysis per Sec. 11.0)</p> <p>i. Graphical presentation of disposition data for radioactivity.</p> <p>j. Inhalation exposure report, which will include a description of the exposure system and the atmosphere generation system used, a description of the procedure for analysis of test chemical concentration, and the analysis data from each day of exposure, including a table of individual measurements of test chemical concentration at the exposure port, together with mean and standard deviation.</p> <p>k. A statement prepared and signed by the Quality Assurance Unit that specifies the dates audits and inspections were made and findings reported to the Study Director and to management.</p> <p>l. A compliance statement signed by the Study Director.</p> <p>Upon acceptance of the audited draft report by the Sponsor, a final report will be issued. Four (unbound) copies of the audited draft report and four copies (unbound) of the final report will be shipped to:</p> <p style="padding-left: 40px;">Derek Swick Regulatory Analysis and Scientific Affairs American Petroleum Institute 1220 L Street NW Washington, DC 20005 p: (202)682-8341 f: (202)682-8031</p> <p><b>14.0 MAINTENANCE OF RECORDS AND RAW DATA</b></p> <p>Records will be maintained in the laboratories of the study personnel while the studies are being conducted. Copies of raw data generated while conducting the study and any transformations, calculations or operations performed on the data will be recorded in the study file. All original study records, protocols, amendments, and the final report will be stored in the RTI International Archives. The applicable record retention requirements for this study are the Good Laboratory Practices (GLPs)</p>		

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<p>Standards for Inhalation Exposure Health Effects Testing, 40 CFR part 79, subpart F § 79.60. Facility data will be maintained in the archives of RTI International and CIIT, respectively. Documentation and raw data will be maintained in the Archives for a period of ten years following issuance of the final report. The storage location of biological samples will be documented in the final report. Chemical and biological samples, or aliquots thereof, will be maintained for a minimum of ten years following issuance of the final report, or for as long as the quality of the preparation affords evaluation, whichever is less. Wet specimens of blood, urine and feces will be disposed of after quality assurance verification (when the QAU assures that discarding the samples does not negatively impact the integrity of the study). The test substance, DIPE, is known to differ markedly in stability and quality during storage, producing dangerous explosive peroxides. Therefore, for reasons of safety, no samples of the test substance will be retained after the end of the study.</p> <p>Materials will be maintained in the RTI Archive for a period of one year after the signature of the final report as part of the initial study cost. At that point, the Sponsor will be contacted to determine the final disposition of these materials. The Sponsor may continue to store these materials in the RTI Archive, have RTI ship them to the Sponsor or an alternative archive facility, or have RTI dispose of them. The Sponsor will be responsible for all costs associated with the storage of these materials beyond 1 year from the issuance of the final report, and for any costs associated with the shipment of these materials to the Sponsor or to any other facility designated by the Sponsor.</p> <p><b>15.0 SAFETY PRECAUTIONS</b></p> <p>This study will be conducted in accordance with Nuclear Regulatory Commission (NRC) regulations, North Carolina License #032-0131-1.</p> <p>a. Precautions for laboratory personnel:</p> <p>All work will be done in well-ventilated areas properly designated for use of radiolabeled compounds. Work will be carried out in accordance with RTI safety standards for work with radiolabeled compounds.</p> <p>b. All radioactive wastes will be disposed of in accordance with standard RTI safety policies.</p> <p>DIPE is a volatile liquid that represents a severe peroxide forming hazard on exposure to air. Peroxides are potentially explosive. The container should be tightly closed when not in use. Storage under a nitrogen atmosphere is recommended to avoid the generation of peroxides. The unlabeled test substance should be tested for peroxides periodically after opening, using a peroxide test strip. Testing should be conducted at two months after opening a container, and monthly thereafter. If peroxide levels of greater than 80 ppm are detected, the material should be disposed of. Use personal protective equipment, including safety glasses, lab coat, and chemical resistant gloves.</p>		

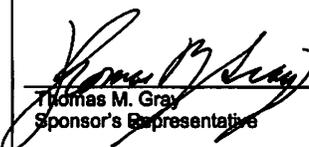
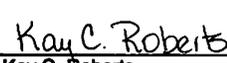
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<p>The inhalation exposure system will be placed in or in close proximity to a fume hood. The exposure generation system will be grounded.</p> <p><b>16.0 PROTOCOL AMENDMENTS AND DEVIATIONS</b></p> <p>This protocol may be amended by the Study Director with agreement of the Sponsor as the study progresses. Normally, a formal amendment will be prepared and signed by the Study Director and the Sponsor's Representative prior to the change. If instances arise where a change is urgent, the change may become effective upon approval by the Study Director. A notification of the urgent change will be sent to the Sponsor's Representative (email, facsimile, or telephone) as soon as feasible (no more than 24 h after the Study Director's approval). Subsequently, a formal protocol amendment will be prepared for approval by the Study Director and the Sponsor's Representative.</p> <p>Any deviations from the protocol that occur in the course of the conduct of the study will be documented. The cause for the deviation and its effect if any on the outcome of the study will be explained and the Study Director will sign the document.</p> <p><b>17.0 REFERENCES</b></p> <p>Cannon, W. C., Blanton, E. and McDonald, K. E. (1983). The flow-past chamber: an improved nose-only exposure system for rodents. <i>Am. Ind. Hyg. Assoc. J.</i> <b>44</b>, 923-928.</p> <p>Dalbey, W. and Feuston, M. (1996). Subchronic and developmental toxicity studies of vaporized diisopropyl ether in rats. <i>J Toxicol Environ Health</i> <b>49</b>, 29-43.</p> <p>Grubbs FE (1969). Procedure for detecting outlying observations in samples. <i>Technometrics</i>, <b>11</b>:1 21.</p> <p>National Research Council (1996). Guide for the Care and Use of Laboratory Animals. National Academy Press: Washington, DC.</p> <p>US Food and Drug Administration (2001). Guidance for Industry: Bioanalytical Method Validation.</p>		

<b>PROTOCOL</b>	<b>RTI INTERNATIONAL * POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709</b>	<b>RTI-934 Amendment No. 1 Page 1 of 4</b>								
<b>AMENDMENT 1</b>										
<b>TITLE: METABOLISM AND PHARMACOKINETICS OF DIISOPROPYL ETHER IN MALE AND FEMALE RATS: PILOT STUDY</b>										
<b>SPONSOR:</b>	Section 211(b) Research Group American Petroleum Institute 1220 L Street NW Washington, DC 20005									
<b>TESTING FACILITY:</b>	RTI International* Science and Engineering 3040 Cornwallis Road Post Office Box 12194 Research Triangle Park, NC 27709									
<b>RTI PROJECT NO.:</b>	0209408.001									
<b>RTI Study Code:</b>	R105-934									
<b>RTI STUDY DIRECTOR:</b>	Timothy R. Fennell									
<b>PROPOSED EXPERIMENTAL START DATE:</b>	September 27, 2007									
<b>PROPOSED EXPERIMENTAL TERMINATION DATE:</b>	October 12, 2007									
<b>APPROVED BY:</b>										
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; text-align: center; vertical-align: bottom;">   <u>Thomas M. Gray</u>                      Sponsor's Representative                 </td> <td style="width: 10%; text-align: center; vertical-align: bottom;"> <u>9/25/07</u>                      Date                 </td> <td style="width: 50%; text-align: center; vertical-align: bottom;">   <u>Timothy R. Fennell, Ph.D.</u>                      Study Director                      RTI International                 </td> <td style="width: 10%; text-align: center; vertical-align: bottom;"> <u>9/26/2007</u>                      Date                 </td> </tr> <tr> <td colspan="4" style="padding-top: 10px;">   <u>R. Arden James</u>                      Principal Investigator                      CIIT at The Hamner Institutes                      for Health Sciences                 </td> </tr> </table>			 <u>Thomas M. Gray</u> Sponsor's Representative	<u>9/25/07</u> Date	 <u>Timothy R. Fennell, Ph.D.</u> Study Director RTI International	<u>9/26/2007</u> Date	 <u>R. Arden James</u> Principal Investigator CIIT at The Hamner Institutes for Health Sciences			
 <u>Thomas M. Gray</u> Sponsor's Representative	<u>9/25/07</u> Date	 <u>Timothy R. Fennell, Ph.D.</u> Study Director RTI International	<u>9/26/2007</u> Date							
 <u>R. Arden James</u> Principal Investigator CIIT at The Hamner Institutes for Health Sciences										
<small>*RTI International is a tradename of Research Triangle Institute.</small>										

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<p><b>Protocol Change No.:</b> 1</p> <p><b>Change (Title Page, Page 1):</b></p> <p><b>PROPOSED EXPERIMENTAL START DATE:</b> April 2nd, 2007  <b>PROPOSED EXPERIMENTAL TERMINATION DATE:</b> To be added by Amendment</p> <p><b>To:</b></p> <p><b>PROPOSED EXPERIMENTAL START DATE:</b> September 27, 2007  <b>PROPOSED EXPERIMENTAL TERMINATION DATE:</b> October 12, 2007</p> <p><b>Reason for change:</b>  To include proposed experimental start date and experimental termination date as required by GLP regulations.</p> <p><b>Protocol Change No.:</b> 2</p> <p><b>Change (Section 3.0, Study Design, Study A, Page 7):</b>  Six male rats and six female rats will be cannulated with jugular vein cannulas up to 4 days prior to exposure and the cannulas will be kept patent. Three additional male rats and three additional female rats will be cannulated and kept on hand in the event that a cannula fails.</p> <p><b>To:</b></p> <p>Nine male rats and nine female rats will be cannulated with jugular vein cannulas by the vendor up to 7 days prior to exposure and the cannulas will be kept patent. The cannulated animals will have a truncated quarantine period of 1-2 days.</p> <p><b>Reason for change:</b>  To clarify the procedure for procurement of cannulated animals.</p> <p><b>Protocol Change No.:</b> 3</p> <p><b>Change (Section 3.0, Study Design, Study C, Page 8):</b>  Urine and feces will be collected over dry ice approximately every 24 hr for up to 7 days or until 90% of the dose is eliminated.</p> <p><b>To:</b></p> <p>Urine and feces will be collected over dry ice approximately at 8 hr (urine only) and then at every 24 hr after the end of exposure for up to 7 days or until 90% of the dose is eliminated.</p> <p><b>Reason for change:</b>  Clarification of urine collection times to be consistent with Section 8.5.2.</p>		

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<p><b>Protocol Change No.:</b> 4</p> <p><b>Change (Section 6.0, Test Substance, Pages 10 and 11):</b></p> <p><i>SOURCE OF RADIOLABELED TEST SUBSTANCE:</i> [2-<sup>14</sup>C]-labeled DIPE will be obtained by custom synthesis from a source to be added by amendment. The target specific activity will be 5-10 mCi/mmol.</p> <p><i>LOT NUMBER(S):</i> To be listed in the final report.</p> <p><b>To:</b></p> <p><i>SOURCE OF RADIOLABELED TEST SUBSTANCE:</i> [2-<sup>14</sup>C]-labeled DIPE was obtained by custom synthesis from American Radiolabeled Chemicals (St. Louis, MO). The specific activity is 2.1 mCi/mmol.</p> <p><i>PRODUCT NUMBER:</i> ARC3099</p> <p><i>LOT NUMBER(S):</i> 070626.</p> <p><b>Reason for change:</b></p> <p>To clarify the radiolabeled material to be used.</p> <p><b>Protocol Change No.:</b> 5</p> <p><b>Change (Section 7.1.2, Quarantine, Page 12):</b></p> <p>Cannulated animals will be housed individually, and will be used within 1-2 days of arrival at RTI.</p> <p><b>To:</b></p> <p>Cannulated animals will be housed individually in cages with dimensions of 9 ¼" x 8 ¼" x 8" (ca. 76.3 sq. in. floor space), and will be used within 1-2 days of arrival at RTI.</p> <p><b>Reason for change:</b></p> <p>To describe the cages that will be used for individually housing animals.</p> <p><b>Protocol Change No.:</b> 6</p> <p><b>Change (Section 8.5.2, Excreta, Page 16):</b></p> <p>Exhaled volatile organics will be collected on a series of two charcoal traps, and expired <sup>14</sup>CO<sub>2</sub> will be collected in 1.0 N KOH at 0-1, 1-3, 3-6, 6-8, 8-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours after termination of exposure or until 90% of the radioactivity has been eliminated.</p> <p><b>To:</b></p> <p>Exhaled volatile organics will be collected on a series of two charcoal traps, and expired <sup>14</sup>CO<sub>2</sub> will be collected in 1.0 N KOH at 0-1, 1-3, 3-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144, and 144-168 hours after termination of exposure or until 90% of the radioactivity has been eliminated.</p> <p><b>Reason for change:</b></p>		

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<p>Changed to be consistent with the timepoints indicated in Section 3.0, Study C, Page 8.</p> <p><b>Protocol Change No.:</b> 7</p> <p><b>Change (Section 8.6.5, Exhaled Volatiles Traps, Page 18):</b> Each charcoal trap will be extracted by elution with 4.0 ml of dimethylformamide (DMF).</p> <p><b>To:</b> Each charcoal trap will be extracted by elution with approximately 4.0 ml of dimethylformamide (DMF). Samples of the first charcoal trap obtained from 0-24 hr following exposure will be extracted with up to 8.0 ml.</p> <p><b>Reason for Change:</b> Higher levels of radioactivity are expected in the first trap between 0 and 24 h after exposure. The additional wash of the first charcoal trap will aid in recovery of radioactivity.</p> <p><b>Protocol Change No.:</b> 8</p> <p><b>Change (Section 9.0, Data Collection, Page 19):</b> The Debra™ laboratory information management system will be used for collection of body weights, animal observations, tissue and sample weights, and radioactivity data.</p> <p><b>To:</b> The Debra™ laboratory information management system will be used for collection of body weights, animal observations, exposure and sample collection times, tissue and sample weights, and radioactivity data.</p> <p><b>Reason for change:</b> Clarification of the collection of time data in the Debra™ system.</p>		

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<b>AMENDMENT 2</b>		
<b>TITLE: METABOLISM AND PHARMACOKINETICS OF DIISOPROPYL ETHER IN MALE AND FEMALE RATS: PILOT STUDY</b>		
<b>SPONSOR:</b>	Section 211(b) Research Group American Petroleum Institute 1220 L Street NW Washington, DC 20005	
<b>TESTING FACILITY:</b>	RTI International* Science and Engineering 3040 Cornwallis Road Post Office Box 12194 Research Triangle Park, NC 27709	
<b>RTI PROJECT NO.:</b>	0209408.001	
<b>RTI Study Code:</b>	Rt05-934	
<b>RTI STUDY DIRECTOR:</b>	Timothy R. Fennell	
<b>PROPOSED EXPERIMENTAL START DATE:</b>	September 27, 2007	
<b>PROPOSED EXPERIMENTAL TERMINATION DATE:</b>	October 12, 2007	
<b>APPROVED BY:</b>		
 Thomas M. Gray Sponsor's Representative	<u>9/3/08</u> Date	 Timothy R. Fennell, Ph.D. Study Director RTI International
 Kay C. Roberts Principal Investigator The Hamner Institutes for Health Sciences	<u>04-Sep-08</u> Date	<u>29-09-08</u> Date
<small>*RTI International is a trademark of Research Triangle Institute.</small>		

<b>PROTOCOL</b>	<b>RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709</b>	<b>RTI-935 Amendment No. 2 Page 2 of 4</b>												
<p><b>Protocol Change No.: 1</b></p> <p><b>Change (Title Page, Page 1):</b></p> <p><b>Principal Investigator:</b></p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 60%; border-bottom: 1px solid black; padding-bottom: 5px;"> <b>R. Arden James</b>                      Principal Investigator                      CIIT at the Hamner Institutes                      for Health Sciences                 </td> <td style="width: 40%; border-bottom: 1px solid black; padding-bottom: 5px; text-align: center;">                     Date                 </td> </tr> <tr> <td colspan="2" style="padding-top: 20px;"><b>To:</b></td> </tr> <tr> <td style="border-bottom: 1px solid black; padding-bottom: 5px;"> <b>Kay C. Roberts</b>                      Principal Investigator                      The Hamner Institutes                      for Health Sciences                 </td> <td style="border-bottom: 1px solid black; padding-bottom: 5px; text-align: center;">                     Date                 </td> </tr> </table> <p><b>Reason for change:</b></p> <p>R. Arden James is no longer employed by The Hamner Institutes for Health Sciences. The name "CIIT Centers for Health Research" has been changed to The Hamner Institutes for Health Sciences (The Hamner Institutes) and is globally changed in the protocol with this protocol amendment.</p> <p><b>Protocol Change No.: 2</b></p> <p><b>Change (Page 6):</b></p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 45%; vertical-align: top;">                 CIIT Principal Investigator             </td> <td style="width: 55%; vertical-align: top;"> <b>R. Arden James</b>                  CIIT                  Phone (919) 558 1279                  Fax (919) 558 1300                  Email: <a href="mailto:james@ciit.org">james@ciit.org</a> </td> </tr> <tr> <td colspan="2" style="padding-top: 20px;"><b>To:</b></td> </tr> <tr> <td style="vertical-align: top;">                 The Hamner Institutes                  Principal Investigator             </td> <td style="vertical-align: top;"> <b>Kay C. Roberts</b>                  CIIT                  Phone (919) 558 1306                  Fax (919) 558 1300                  Email: <a href="mailto:roberts@thehamner.org">roberts@thehamner.org</a> </td> </tr> </table>			<b>R. Arden James</b> Principal Investigator CIIT at the Hamner Institutes for Health Sciences	Date	<b>To:</b>		<b>Kay C. Roberts</b> Principal Investigator The Hamner Institutes for Health Sciences	Date	CIIT Principal Investigator	<b>R. Arden James</b> CIIT Phone (919) 558 1279 Fax (919) 558 1300 Email: <a href="mailto:james@ciit.org">james@ciit.org</a>	<b>To:</b>		The Hamner Institutes Principal Investigator	<b>Kay C. Roberts</b> CIIT Phone (919) 558 1306 Fax (919) 558 1300 Email: <a href="mailto:roberts@thehamner.org">roberts@thehamner.org</a>
<b>R. Arden James</b> Principal Investigator CIIT at the Hamner Institutes for Health Sciences	Date													
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CIIT Principal Investigator	<b>R. Arden James</b> CIIT Phone (919) 558 1279 Fax (919) 558 1300 Email: <a href="mailto:james@ciit.org">james@ciit.org</a>													
<b>To:</b>														
The Hamner Institutes Principal Investigator	<b>Kay C. Roberts</b> CIIT Phone (919) 558 1306 Fax (919) 558 1300 Email: <a href="mailto:roberts@thehamner.org">roberts@thehamner.org</a>													

PROTOCOL	RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-935 Amendment No. 2 Page 3 of 4				
<p><b>Reason for change:</b></p> <p>R. Arden James is no longer employed by CIIT Centers for Health Research.</p> <p><b>Protocol Change No.:</b> 3</p> <p><b>Change (Page 6):</b></p> <table border="0"> <tr> <td style="vertical-align: top;">CIIT Study Personnel</td> <td style="vertical-align: top;">R. Arden James, Principal Investigator Brian A. Wong, Ph.D. – Senior Research Investigator Kay C. Roberts, A.S. – Research Associate Marianne W. Marshall, B.A. – Research Associate Carl U. Parkinson, LATg – Research Associate</td> </tr> <tr> <td style="vertical-align: top;"><b>To:</b> The Hamner Institutes Study Personnel</td> <td style="vertical-align: top;">Kay C. Roberts, A.S., Principal Investigator Brian A. Wong, Ph.D. – Associate Investigator Marianne W. Marshall, B.A. – Research Associate Carl U. Parkinson, LATg – Research Associate</td> </tr> </table> <p><b>Reason for change:</b></p> <p>R. Arden James is no longer employed by CIIT Centers for Health Research.</p> <p><b>Protocol Change No.:</b> 4</p> <p><b>Change (Section 3.0, Page 9):</b></p> <p>Carbon 13 NMR spectra will be acquired on an aliquot of urine after addition of D<sub>2</sub>O. The total volume needed for NMR analysis is approximately 800 µL. The amount of D<sub>2</sub>O in the 800 µL will be 15% of the 800 µL volume when urine volumes are sufficient. NMR spectra will be interpreted for the assignment of metabolites. An HPLC system with radioactivity detection will be developed and used to separate urinary metabolites. Fractions corresponding to the radioactivity peaks will be collected. NMR spectra acquired on the individual fractions will enable characterization of the HPLC metabolite profile for DIPE.</p>			CIIT Study Personnel	R. Arden James, Principal Investigator Brian A. Wong, Ph.D. – Senior Research Investigator Kay C. Roberts, A.S. – Research Associate Marianne W. Marshall, B.A. – Research Associate Carl U. Parkinson, LATg – Research Associate	<b>To:</b> The Hamner Institutes Study Personnel	Kay C. Roberts, A.S., Principal Investigator Brian A. Wong, Ph.D. – Associate Investigator Marianne W. Marshall, B.A. – Research Associate Carl U. Parkinson, LATg – Research Associate
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<b>To:</b> The Hamner Institutes Study Personnel	Kay C. Roberts, A.S., Principal Investigator Brian A. Wong, Ph.D. – Associate Investigator Marianne W. Marshall, B.A. – Research Associate Carl U. Parkinson, LATg – Research Associate					

<b>PROTOCOL</b>	<b>RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709</b>	<b>RTI-935 Amendment No. 2 Page 4 of 4</b>
<p><b>To:</b></p> <p>No analysis will be conducted on urine samples. The elution and analysis of a set of VOC trap samples from a single animal will not be conducted; however, trap samples will be retained for possible future analysis. Disposition data will be provided and summarized for three of the animals from Study C.</p> <p><b>Reason for change:</b></p> <p>The amount of radioactivity excreted in urine was insufficient to warrant characterization of the small amount of material excreted by this route. Radioactivity was excreted largely by exhalation as volatile material trapped on the charcoal traps or as CO<sub>2</sub>. Retention of a set of traps will afford the capability to analyze these samples as necessary at a later time.</p>		

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**RTI Protocol No. RTI-934 (RTI Project No. 020908.001)**

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**Deviations from the Approved Protocol**

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The deviations listed below did not affect the results of the study.

**Deviation**

For Study C, animals received 3600 ppm nose-only inhalation exposures for ca. five hours and forty minutes instead of six hours as stated in the protocol. Actual exposure animal exposure lengths for individual animals are detailed in the study record.

**Reason for the Deviation**

Insufficient volume of 14C DIPE/13C DIPE formulation was available for a six-hour exposure.

**Deviation**

The protocol states (section 8.1) that: "Cannula patency will be maintained with daily flushings of heparinized saline (sterile saline containing 20 IU/mL of sodium heparin). Jugular cannulas will not be flushed with heparinized saline prior to use on exposure day.

**Reason for the Deviation**

The vendor (Charles River Laboratories) specified that the cannulas retain patency best if the heparinized solution used to lock the cannula is not disturbed until use (up through one week prior to use).

**Deviation**

Aliquots of the Study B and Study C inhalation formulations were transferred to a single vial (for each formulation) already containing 20 mL of dimethylformamide instead of an empty vial. Weights of )1 empty vial, 2) vial+DMF, and 3) weight of vial+DMF+formulation aliquot were recorded in the process. Aliquots of the DMF dilution were then transferred to vials already containing scintillation cocktail for LSS analysis instead of adding cocktail after the transfer.

**Reason for the Deviation**

At the time, concern about evaporation of the DIPE formulation during transfer to empty vials, thereby affecting quantitation results. Addition of DIPE formulation or the dilution to solvent was expected to minimize evaporation.

**Appendix B**  
**Test Chemical Analysis Report**

## Test Chemical Analysis Report

### Diisopropyl Ether

### RTI Reference 12322-04

**SUBMITTED TO:**

Section 211(b) Research Group  
American Petroleum Institute  
1220 L Street NW  
Washington, DC 20005

**TESTING FACILITY:**

RTI International\*  
3040 Cornwallis Road  
P.O. Box 12194  
Research Triangle Park, NC 27709-2194

  
\_\_\_\_\_  
Timothy R. Fennell, Ph.D.

5-29-2017  
\_\_\_\_\_  
Date

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04**

In November 2005, 1 liter of diisopropyl ether (DIPE) was purchased from Sigma Aldrich. The material was 99+% A.C.S. Reagent, Product Number 398276, Lot Number 03658JC, Formula Weight 102.18. Purity from the Vendor Certificate of Analysis was 99.6% by GLC, with 6.6 PPM BHT (butylated hydroxytoluene). No expiration date was indicated by the vendor.

RTI assigned this material a Test Article Number of 12322-04. RTI confirmed the identity of the material using nuclear magnetic resonance spectroscopy, and mass spectrometry. RTI confirmed the purity by gas chromatography.

**Nuclear Magnetic Resonance Spectroscopy.**

All NMR data were acquired on a 300 MHz Bruker spectrometer. The  $^1\text{H}$  NMR spectra were acquired with a relaxation delay of 30 sec, a 6173 Hz sweep width, and an 8  $\mu\text{sec}$  pulse. The sample was prepared in  $\text{CDCl}_3$  (deuteriochloroform). The  $^1\text{H}$ -decoupled  $^{13}\text{C}$  NMR spectrum was acquired with a relaxation delay of 2 sec, a sweep width of 23810 Hz, and a 5.5  $\mu\text{sec}$  pulse.

The  $^1\text{H}$  NMR spectrum of the sample contained two singlets at 1.119 and 1.099 ppm, and a multiplet at 3.622 ppm (Figure 1). The singlets at 1.119 and 1.099 ppm are attributed to the isopropyl  $\text{CH}_3$  groups. The multiplet at 3.622 ppm is attributed to the isopropyl CH groups. The ratio of integrals (6.0:1.0) is consistent with the expected ratio for 12 methyl protons, and 2 methyne protons.

The  $^{13}\text{C}$  NMR spectrum of the sample contained singlets at approximately 22.8 ppm and 68.3 ppm (Figure 2). The triplet at approximately 77.0 ppm is assigned to  $\text{CDCl}_3$ . The signal at 22.8 ppm is consistent with the isopropyl  $\text{CH}_3$  groups, and the signal at 68.3 ppm is consistent with the isopropyl CH groups.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of the sample are consistent with the structure of DIPE.

**GC Analysis**

Equipment: Agilent 6890 gas chromatograph equipped with a split-splitless injector and a flame ionization detector.  
Agilent 6890 autoinjector with controller  
Millenium data system.

Column DB-1, J&W Scientific 30m x 0.53 mm i.d., 3  $\mu\text{m}$  film thickness  
(J&W, Agilent Technologies, Wilmington, DE)

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04**

Injection port	split/splitless
Temperature	200 °C
Split ratio	100:1
Carrier gas	Helium
Flow rate	1 ml/min
Injection volume	1 µl

Initial temperature	35 °C
Initial time	1 min
Temperature rate	5 °C/min
Final temperature	220 °C
Final time	1 min

From May 25, 2006, analyses were conducted as described above with the following exceptions:

Split ratio	50:1
Flow rate	4 ml/min

Temperature rate	10 °C/min
Final time	2 min

Empower 2 has replaced Waters Millennium 32 Version 4.0 as the chromatography data system used for the analyses since March 7, 2007

Purity of the material was determined by injection of 3 1-µl samples onto the GC column. The initial purity determined was 99.63 %, with a standard deviation of 0.02 %. Figure 3 shows a typical chromatogram. The purity of the material measured on subsequent dates is presented in Table 1.

**GC-MS analysis**

Equipment: Agilent 6890 gas chromatograph equipped with a split-splitless injector and a flame ionization detector.  
Agilent 5973 Mass Selective Detector.

Column DB-624 30m x 0.32 mm i.d., 1.8 µm film thickness  
(J&W, Agilent technologies, Wilmington, DE)

Injection port	split/splitless
Temperature	150 °C
Split ratio	5:1

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04**

Carrier gas	Helium
Flow rate	1.7 ml/min
Injection volume	1 $\mu$ l
Initial temperature	30 $^{\circ}$ C
Initial time	3 min
Temperature rate	5 $^{\circ}$ C/min
Final temperature	80 $^{\circ}$ C
Final time	0
Ramp	100 $^{\circ}$ C/min
Final temperature	200 $^{\circ}$ C
Final time	1 min

**5973 MSD**

Mode	El mode
Scan	10-150 amu
Source temperature	230 $^{\circ}$ C
Quad temperature	150 $^{\circ}$ C
Transfer line	250 $^{\circ}$ C
Tune	Atune.u
Solvent delay	2.75 min

Identity was verified by GC-MS analysis. A sample of 10  $\mu$ l DIPE was dissolved in 20 ml of methanol, and 1  $\mu$ l was injected.

The total ion chromatogram showed a single peak at approximately 4.1 min (Figure 4, upper panel). The mass spectrum of this peak (Figure 4, lower panel) showed a molecular ion at  $m/z$  102, and major fragment ions at 87 (consistent with  $M-CH_3$ ), 59, and 45 (consistent with  $CH_3-CH=OH^+$ ). A library search indicated a match with the spectrum of diisopropyl ether.

**Peroxide check**

A check for the presence of peroxide was conducted by dipping a test stick (Quanfofix, peroxide 100, Macherey-Nagel) in to a sample of DIPE liquid for about 1 second, shaking off excess liquid, and reading the color at 5 seconds. A white color indicates the peroxide value at 0 mg/L. through storage period.

**Conclusion**

The NMR and mass spectral data of the material are consistent with the structure of DIPE. The initial purity of the material measured by GC with FID is was 99.63 %, with a standard deviation of 0.02 %.

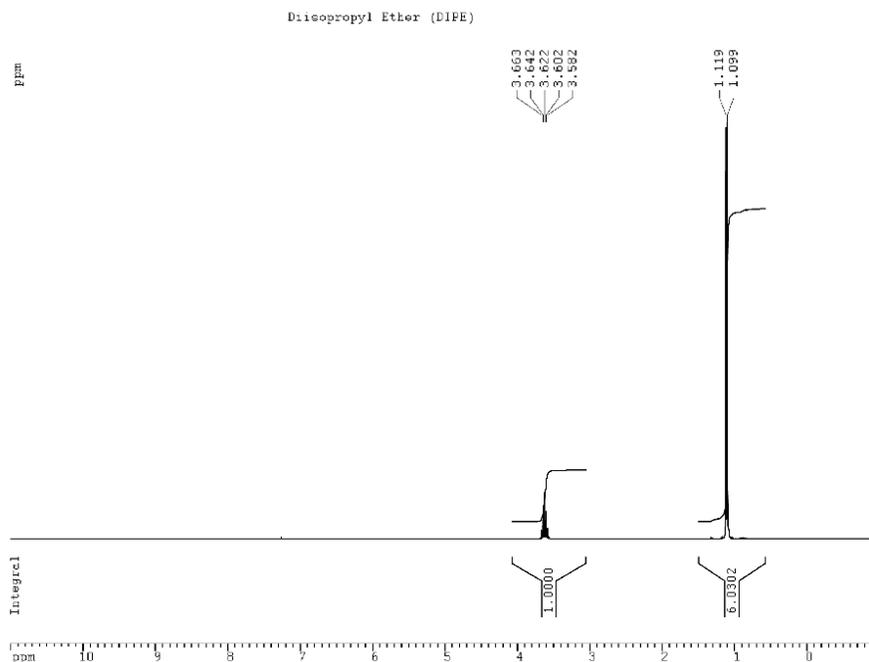
**Test Chemical Analysis Report**

**Diisopropyl Ether  
RTI Reference 12322-04**

Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

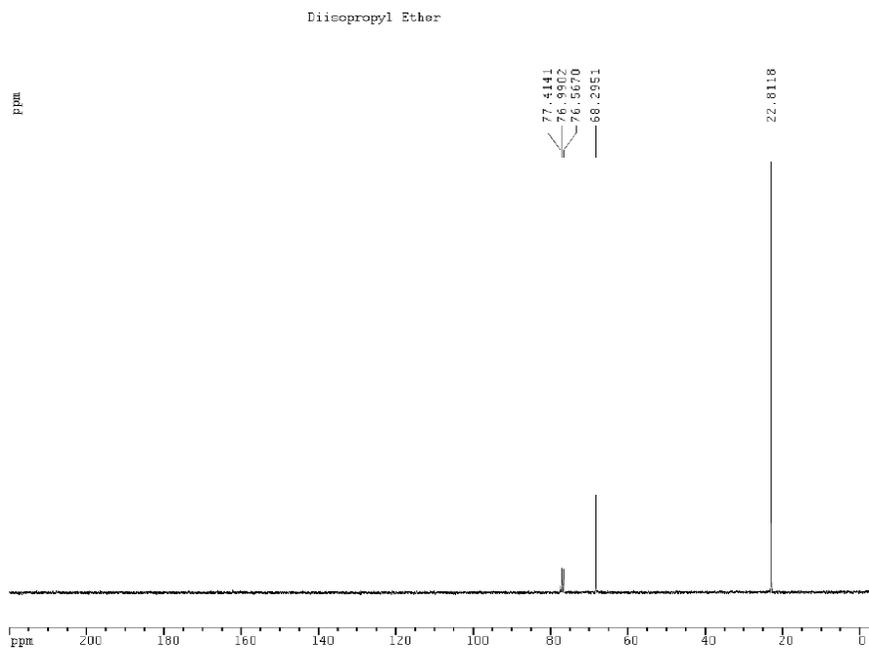
Figure 1. 300 MHz <sup>1</sup>H NMR of DIPE



Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

Figure 2. 75 MHz <sup>13</sup>C NMR of DIPE



Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

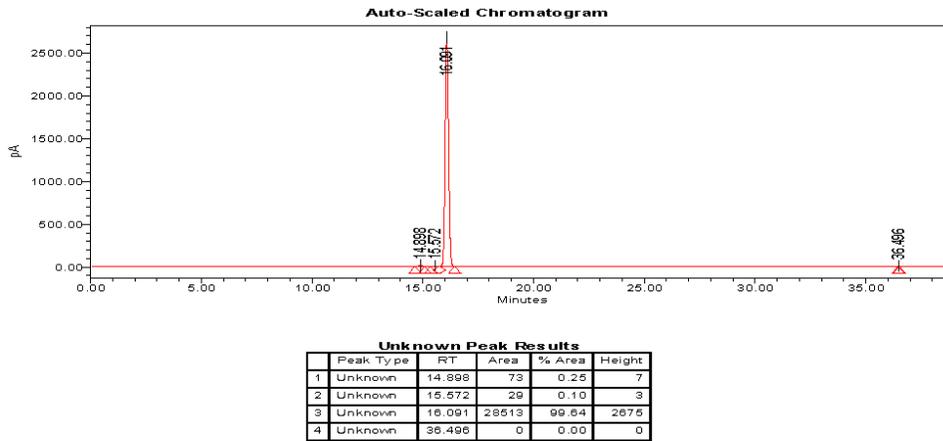


Figure 3. GC-FID Chromatogram of DIPE.

Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

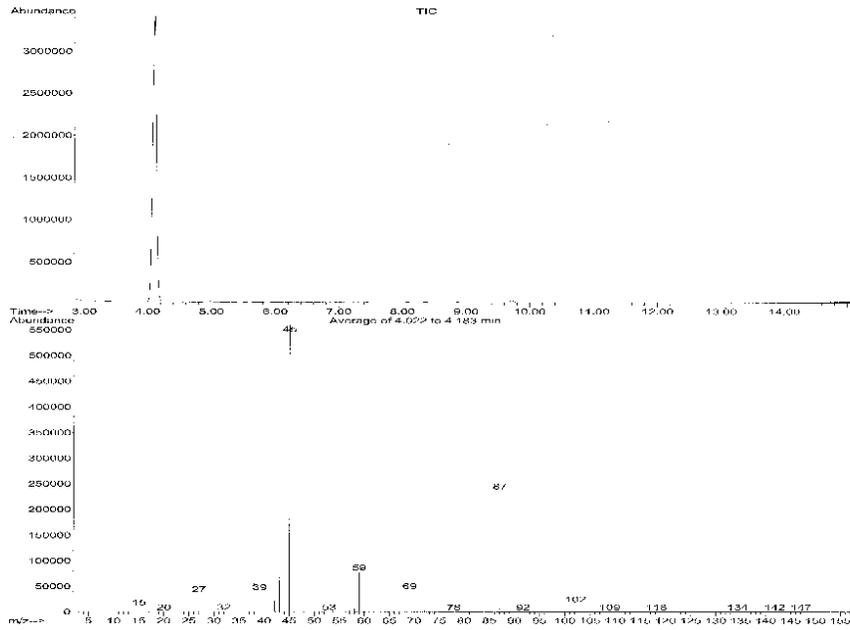


Figure 4. Total Ion Chromatogram (top) and Mass Spectrum (bottom) of DIPE

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04**

Table 1. Purity of DIPE determined by gas chromatography with flame ionization detection.

Date of Analysis	Purity (%) (Mean $\pm$ SD, 3 determinations)	Peroxide value (mg/L)
November, 14, 2005	99.63 $\pm$ 0.02	n/a
January 05, 2006	99.61 $\pm$ 0.03	n/a
March 03, 2006	99.62 $\pm$ 2.65	0
May 26, 2006	99.62 $\pm$ 0.01	0
August 23, 2006	99.61 $\pm$ 0.01	0
November, 29 2006	99.64 $\pm$ 0.01	0
March, 7, 2007	99.62 $\pm$ 0.01	0
September 19, 2007	99.38 $\pm$ 0.16	0
October 5, 2007	99.63 $\pm$ 0.006	0

## Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

Library Searched : C:\Database\Wiley275.L  
Quality : 91  
ID : Propane, 2,2'-oxybis- (CAS) \$\$ Isopropyl ether \$\$ Diisopropyl ether  
\$\$ Diisopropyl oxide \$\$ 2-Isopropoxypropane \$\$ Bis(isopropyl) ethe  
r \$\$ Diisopropyl ether \$\$ 2,2'-Oxybispropane \$\$ (iso-C<sub>3</sub>H<sub>7</sub>)<sub>2</sub>O \$\$ 1,1  
'-dimethyldiethyl ether \$\$ Ether, isopropyl \$\$ Ether 1

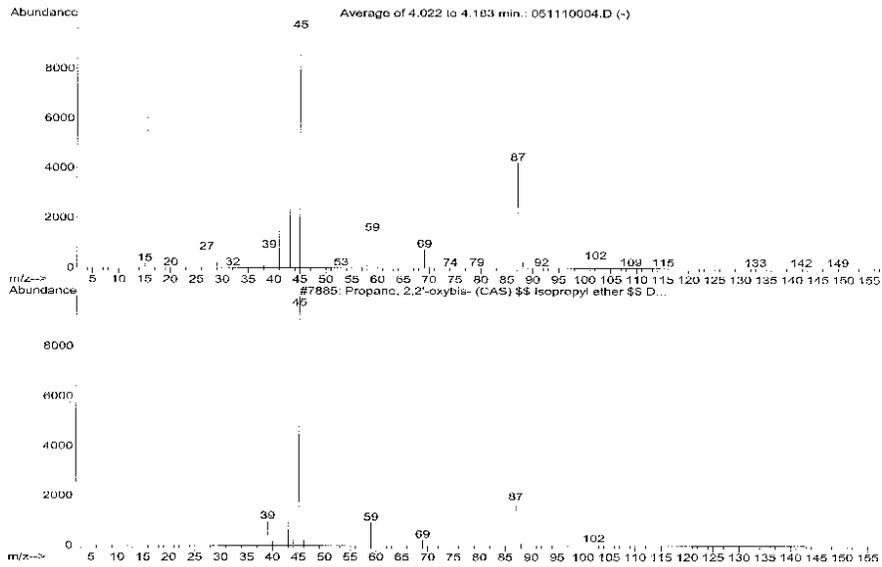


Figure 5. EI Mass Spectrum of DIPE (top) and Library Search (bottom)

## **Appendix C**

**Test Chemical Analysis Report: 2-<sup>14</sup>C Diisopropyl ether**

**Test Chemical Analysis Report: <sup>13</sup>C<sub>6</sub> Diisopropyl ether**

## Test Chemical Analysis Report

[2-<sup>14</sup>C] Diisopropyl Ether

RTI Reference 12322-106

**SUBMITTED TO:**

Section 211(b) Research Group  
American Petroleum Institute  
1220 L Street NW  
Washington, DC 20005

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Research Triangle Park, NC 27709-2194

  
Timothy R. Fennell, Ph.D.

05-29-2017  
Date

**Test Chemical Analysis Report****<sup>14</sup>C-Diisopropyl Ether  
RTI Reference 12322-106**

In July, 2007, 20 mCi of diisopropyl ether-2-<sup>14</sup>C (DIPE) was purchased from American Radiolabeled Chemicals (St. Louis, Mo) (received on July 2, 2007). The material was obtained as a custom synthesis. The specific activity indicated by the vendor is 2.1 mCi/mmol. The product number is ARC 3099. The lot number is 070626. Four vials containing approximately 5 mCi were designated vials A through D, and a fifth vial containing approximately 10  $\mu$ Ci was designated vial E.

Purity from the Vendor-supplied chromatogram was 96.83 % by HPLC. No expiration date was indicated by the Vendor.

RTI assigned this material Test Article Number of 12322-106. RTI confirmed the identity of the radiolabeled material by coelution on HPLC with unlabeled DIPE, (Test Article Number 12322-04), and by <sup>1</sup>H NMR spectroscopy. RTI confirmed the purity of the material by HPLC with radiochemical detection.

**HPLC Analysis**

For HPLC analysis a sample of the radiolabeled DIPE was prepared by dilution. A sample of 2-<sup>14</sup>C DIPE was prepared by transferring approximately 0.5  $\mu$ l to an autosampler vial containing approximately 250  $\mu$ l water and 250  $\mu$ l acetonitrile.

HPLC Analysis of <sup>14</sup>C-DIPE was conducted on a Waters Atlantis dC18 column, 4.6 mm i.d. x 25 cm, 5  $\mu$ m particle size. The mobile phase consisted of 75 % water and 25% acetonitrile, with a 20-minute linear gradient to 5% water, 95% acetonitrile. Chromatography was conducted using a system that consisted of 2X Waters 515 Pumps, a Waters 717 Plus Autoinjector, with a ABI 759A UV detector, and a  $\beta$ -RAM Model 3 radioactivity detector. The column flow rate was 1.0 ml/min, and 100% of the flow went to the radioactivity detector. A 500  $\mu$ l solid phase cell was used for detection. UV absorbance was monitored at 195 nm. After injection of a 10- $\mu$ l aliquot of the 2-<sup>14</sup>C DIPE solution, the HPLC effluent was collected in scintillation vials, and after addition of Ultima Gold scintillant, radioactivity in each of the fractions was determined by scintillation counting using a Packard 1900 CA Tricarb scintillation counter. For determination of recovery of radioactivity from the column, triplicate aliquots of the 2-<sup>14</sup>C DIPE solution were prepared for scintillation counting and were counted directly to determine the total amount of radioactivity injected on the column.

A single radioactive peak was observed at approximately 16 minutes (Figure 1). The purity was 97.23 %, (mean of three injections, Std. Dev. 0.04%). The recovery from the column was 104, 105, and 99.6% of the radioactivity injected, for each of three injections.

**Test Chemical Analysis Report****<sup>14</sup>C-Diisopropyl Ether  
RTI Reference 12322-106**

In Figure 2, the UV chromatograms of a blank injection (top), of 2-<sup>14</sup>C DIPE (middle), and a mixture of 2-<sup>14</sup>C DIPE and a reference standard of unlabeled DIPE (bottom) are shown. A peak not present in the blank injection was present at approximately 15.5 minutes in the sample with unlabeled DIPE. The retention times on the radiochromatogram of approximately 16 minutes, and the UV chromatogram of approximately 15.5 minutes are consistent with the retention delay between the UV detector (first in line following the HPLC column) and the radioactivity detector (following the UV detector).

**NMR Analysis****Nuclear Magnetic Resonance Spectroscopy.**

All NMR data were acquired on a 300 MHz Bruker spectrometer. The <sup>1</sup>H NMR spectra were acquired with a relaxation delay of 10 sec, a 6173 Hz sweep width, and an 8 µsec pulse. The sample was prepared in CDCl<sub>3</sub> (deuteriochloroform).

The <sup>1</sup>H NMR spectrum of the sample contained two singlets at 1.118 and 1.139 ppm, and a multiplet at 3.642 ppm (Figure 3). The singlets at 1.118 and 1.139 ppm are attributed to the isopropyl CH<sub>3</sub> groups. The multiplet at 3.642 ppm is attributed to the isopropyl CH groups. The ratio of integrals (6.3:1.0) is consistent with the expected ratio for 12 methyl protons, and 2 methyne protons.

The <sup>1</sup>H NMR data of the sample are consistent with the structure of DIPE.

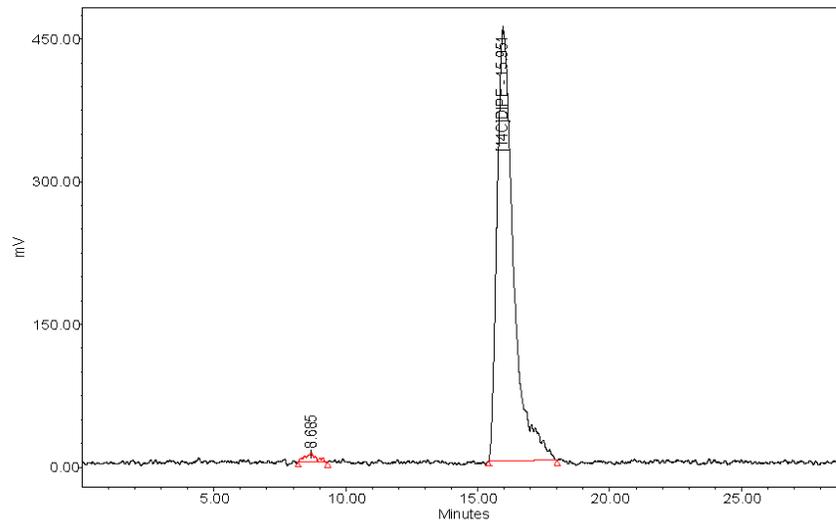
**Conclusion**

The test substance 12322-106 diisopropyl ether-2-<sup>14</sup>C obtained from American Radiolabeled Chemicals had HPLC characteristics that were consistent with diisopropyl ether. The purity of the test substance was 97.23% by HPLC with detection of radioactivity. This was similar to the Vendor-supplied purity of the test chemical of 96.83%, and indicates that the radiolabeled material is stable.

## Test Chemical Analysis Report

<sup>14</sup>C-Diisopropyl Ether  
RTI Reference 12322-106

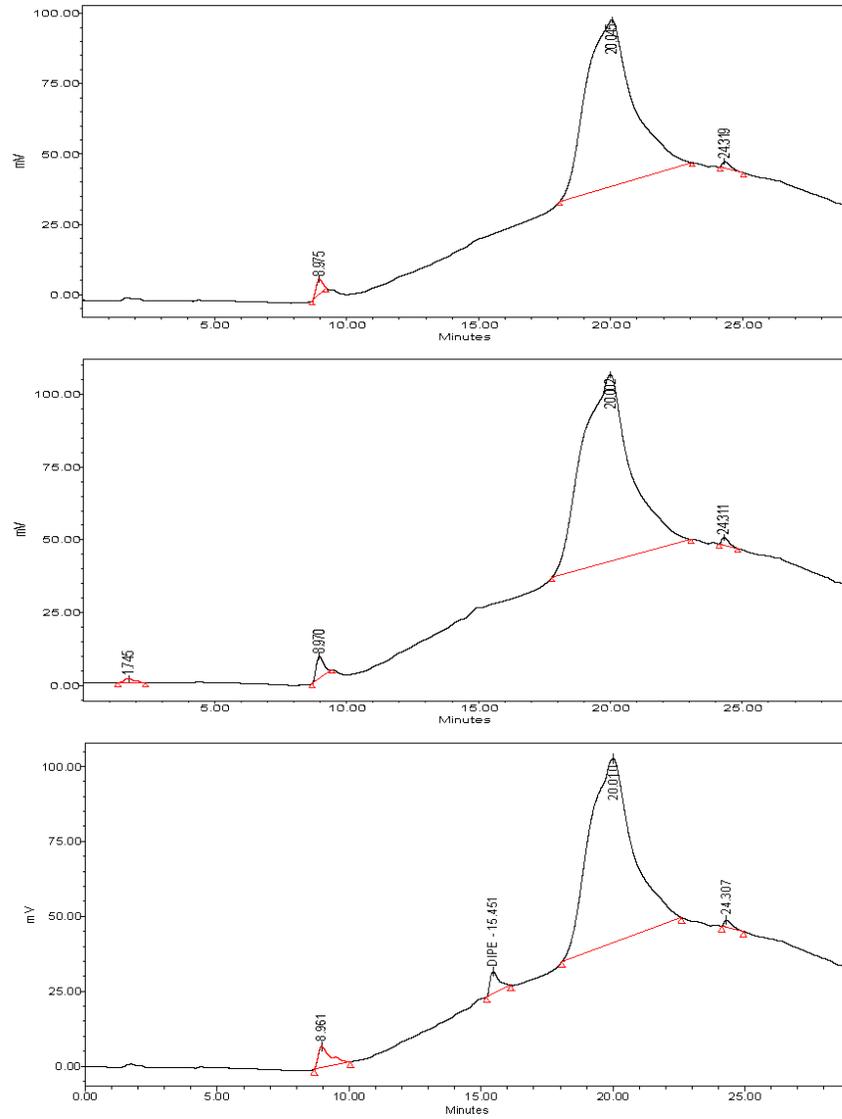
Figure 1. HPLC Radiochromatogram of 2-<sup>14</sup>C DIPE. (Analyzed by N. Gaudette on 08/03/07)



## Test Chemical Analysis Report

<sup>14</sup>C-Diisopropyl Ether  
RTI Reference 12322-106

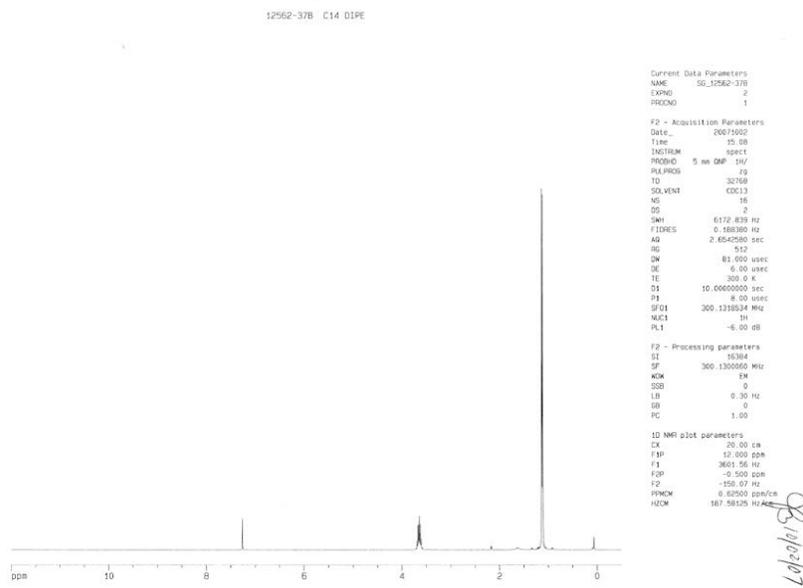
Figure 2. UV 195 nm Chromatograms of a Blank (top), 2-<sup>14</sup>C DIPE. (middle) and a mixture of 2-<sup>14</sup>C DIPE and Unlabeled DIPE Standard (bottom) (Analyzed by N. Gaudette on 08/03/07).



Test Chemical Analysis Report

<sup>14</sup>C-Diisopropyl Ether  
RTI Reference 12322-106

Figure 3. <sup>1</sup>H NMR of 2-<sup>14</sup>C DIPE. (Analyzed by J. Burgess on 10/02/07)



## Test Chemical Analysis Report

$^{13}\text{C}_6$ -Diisopropyl Ether

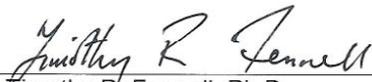
RTI Reference 12322-10

**SUBMITTED TO:**

Section 211(b) Research Group  
American Petroleum Institute  
1220 L Street NW  
Washington, DC 20005

**TESTING FACILITY:**

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Timothy R. Fennell, Ph.D.

05-29-2017  
Date

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-10**

In April 2006, 15 g of  $^{13}\text{C}_6$ -diisopropyl ether (DIPE) was purchased from ISOTEC (Miamisburg, OH). The material provided with identifying numbers, including an ISOTEC number T83-03014, an Aldrich number 632384, and a batch number ST1187. Purity from the Vendor Certificate of Analysis was 95.9 % by GC. Enrichment with  $^{13}\text{C}$  was 99.5 atom %. No expiration date was indicated by the vendor.

RTI assigned this material a Test Article Number of 12322-10. RTI confirmed the identity of the material using nuclear magnetic resonance spectroscopy, and mass spectrometry. RTI confirmed the purity by gas chromatography.

**Nuclear Magnetic Resonance Spectroscopy.**

All NMR data were acquired on a 300 MHz Bruker spectrometer. The  $^1\text{H}$  NMR spectra were acquired with a relaxation delay of 10 sec, a 6173 Hz sweep width, and an 8  $\mu\text{sec}$  pulse. The sample was prepared in  $\text{CDCl}_3$  (deuteriochloroform). The  $^1\text{H}$ -decoupled  $^{13}\text{C}$  NMR spectrum was acquired with a relaxation delay of 2 sec, a sweep width of 23810 Hz, and a 5.5  $\mu\text{sec}$  pulse.

The  $^1\text{H}$  NMR spectrum of the sample, as expected, was more complex than that obtained with natural abundance DIPE because of additional coupling of the proton signals to the enriched carbon atoms. The spectrum contained two multiplets at 1.3 and 0.9 ppm, and a pair of multiplets at 3.4 and 3.8 ppm (Figure 1). The multiplets at 1.3 and 0.9 ppm are attributed to the isopropyl  $\text{CH}_3$  groups, with coupling to the CH signal. The multiplets at 3.4 and 3.8 ppm are attributed to the isopropyl CH groups. The ratio of integrals (6.25:1.0) is consistent with the expected ratio for 12 methyl protons, and 2 methyne protons.

The  $^{13}\text{C}$  NMR spectrum of the sample was also more complex than that for natural abundance DIPE, because of the carbon-carbon coupling observed with adjacent labeled carbon atoms. Doublets were observed at approximately 22.6 and 23.1 ppm and a triplet was observed at approximately 68.3 ppm (Figure 2). The triplet at approximately 77.0 ppm is assigned to  $\text{CDCl}_3$ . The signals at 22.8 ppm are consistent with the isopropyl  $\text{CH}_3$  groups, and the signals at 68.3 ppm are consistent with the isopropyl CH groups.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of the sample are consistent with the structure of  $^{13}\text{C}_6$  DIPE.

**GC Analysis**

Equipment: Agilent 6890 gas chromatograph equipped with a split-splitless injector and a flame ionization detector.  
Agilent 6890 autoinjector with controller

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-10**

Millenium data system.

Column DB-1, J&W Scientific 30m x 0.53 mm i.d., 3 µm film thickness  
(J&W, Agilent Technologies, Wilmington, DE)

Injection port	split/splitless
Temperature	200 °C
Split ratio	100:1
Carrier gas	Helium
Flow rate	1 ml/min
Injection volume	1 µl

Initial temperature	35 °C
Initial time	1 min
Temperature rate	5 °C/min
Final temperature	220 °C
Final time	1 min

From August 23, 2006, analyses were conducted as described above with the following exceptions:

Split ratio	50:1
Flow rate	4 ml/min

Temperature rate	10 °C/min
Final time	2 min

Empower 2 has replaced Waters Millennium 32 Version 4.0 as the chromatography data system used for the analyses since March 7, 2007

Purity of the material was determined by injection of 3 1-µl samples onto the GC column. The initial purity determined was 95.17 %, with a standard deviation of 0.03 %. Figure 3 shows a typical chromatogram. A number of minor impurity peaks were detected in the chromatograms, but were not identified. The purity of the material measured after the conclusion of the exposure was  $95.25 \pm 0.08$  %.

**GC-MS analysis**

Equipment: Agilent 6890 gas chromatograph equipped with a split-splitless injector and a  
Agilent 5973 Mass Selective Detector.

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-10**

Column DB-624 30m x 0.32 mm i.d., 1.8 um film thickness  
(J&W, Agilent technologies, Wilmington, DE)

Injection port split/splitless  
Temperature 150 °C  
Split ratio 5:1  
Carrier gas Helium  
Flow rate 1.7 ml/min  
Injection volume 1 µl

Initial temperature 30 °C  
Initial time 3 min  
Temperature rate 5 °C/min  
Final temperature 80 °C  
Final time 0  
Ramp 100 °C/min  
Final temperature 200 °C  
Final time 1 min

**5973 MSD**

Mode EI mode  
Scan 10-150 amu  
Source temperature 230 °C  
Quad temperature 150 °C  
Transfer line 250 °C  
Tune Atune.u  
Solvent delay 2.75 min

Identity was verified by GC-MS analysis. A sample of 10 µl <sup>13</sup>C<sub>6</sub> DIPE was dissolved in 20 ml of methanol, and 1 µl was injected.

The total ion chromatogram showed a single peak at approximately 5.5 min (Figure 4, upper panel). The mass spectrum of this peak (Figure 4, lower panel) showed a molecular ion at *m/z* 108, consistent with the presence of 6 <sup>13</sup>C atoms, and major fragment ions at 92 (consistent with *M*-CH<sub>3</sub>), 62, and 47 (consistent with CH<sub>3</sub>-CH=OH<sup>+</sup>). A library match was not conducted because of the presence of the, but the mass spectrum was consistent with the addition of 6 <sup>13</sup>C atoms.

**Peroxide check**

A check for the presence of peroxide was conducted by dipping a test stick (Quanfofix, peroxide 100, Macherey-Nagel) in to a sample of DIPE liquid for about 1 second,

**Test Chemical Analysis Report****Diisopropyl Ether  
RTI Reference 12322-10**

shaking off excess liquid, and reading the color at 5 seconds. A white color indicates the peroxide value at 0 mg/L. through storage period.

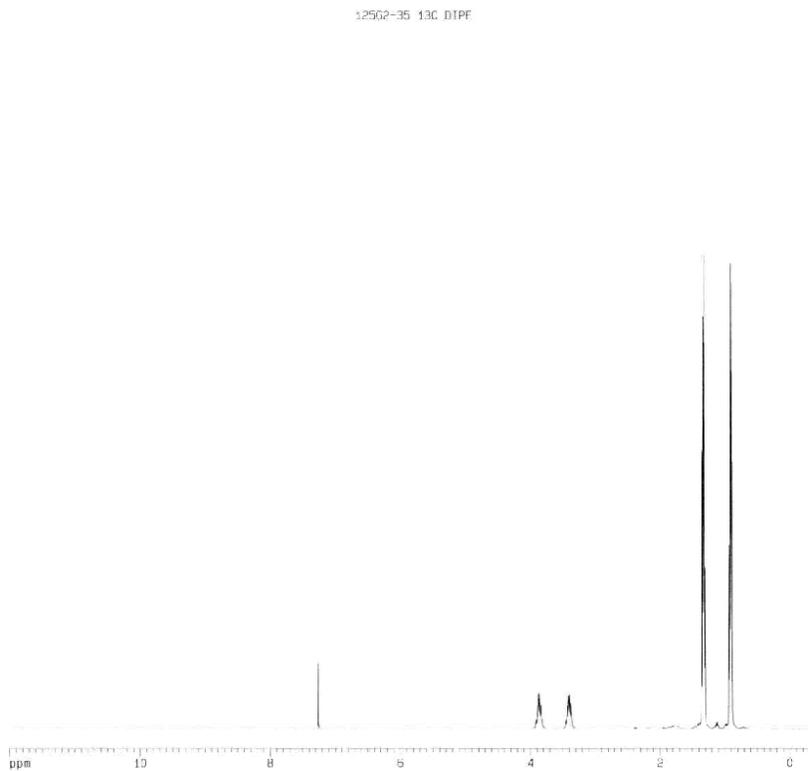
**Conclusion**

The NMR and mass spectral data of the material are consistent with the structure of <sup>13</sup>C<sub>6</sub>-DIPE. The initial purity of the material measured by GC with FID is was 95.17 %, with a standard deviation of 0.03 %. A second determination conducted after the inhalation exposure indicated a similar purity and that the material was stable.

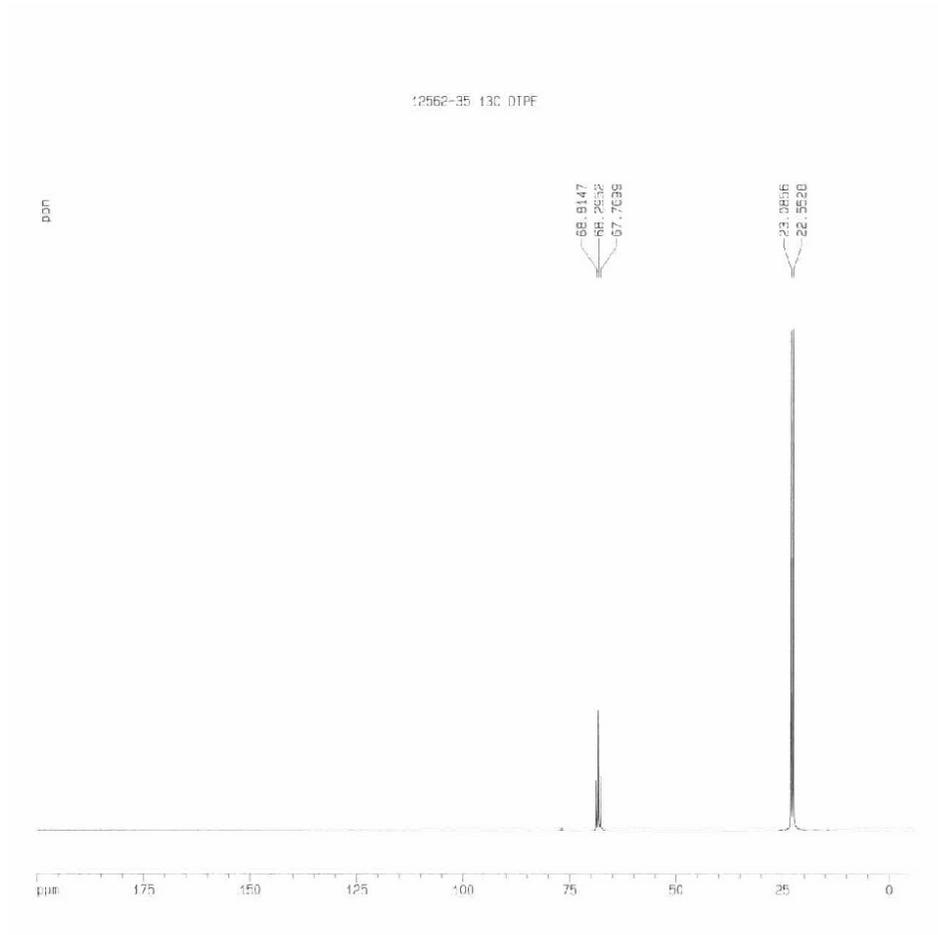
**Test Chemical Analysis Report**

**Diisopropyl Ether  
RTI Reference 12322-10**

Figure 1. 300 MHz <sup>1</sup>H NMR of <sup>13</sup>C<sub>6</sub>-DIPE



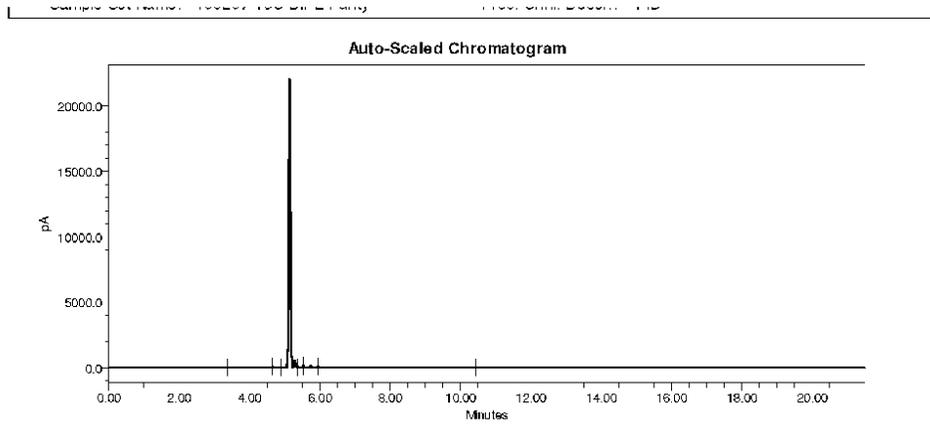
## Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-10Figure 2. 75 MHz  $^{13}\text{C}$  NMR of  $^{13}\text{C}_6$ -DIPE

Test Chemical Analysis Report

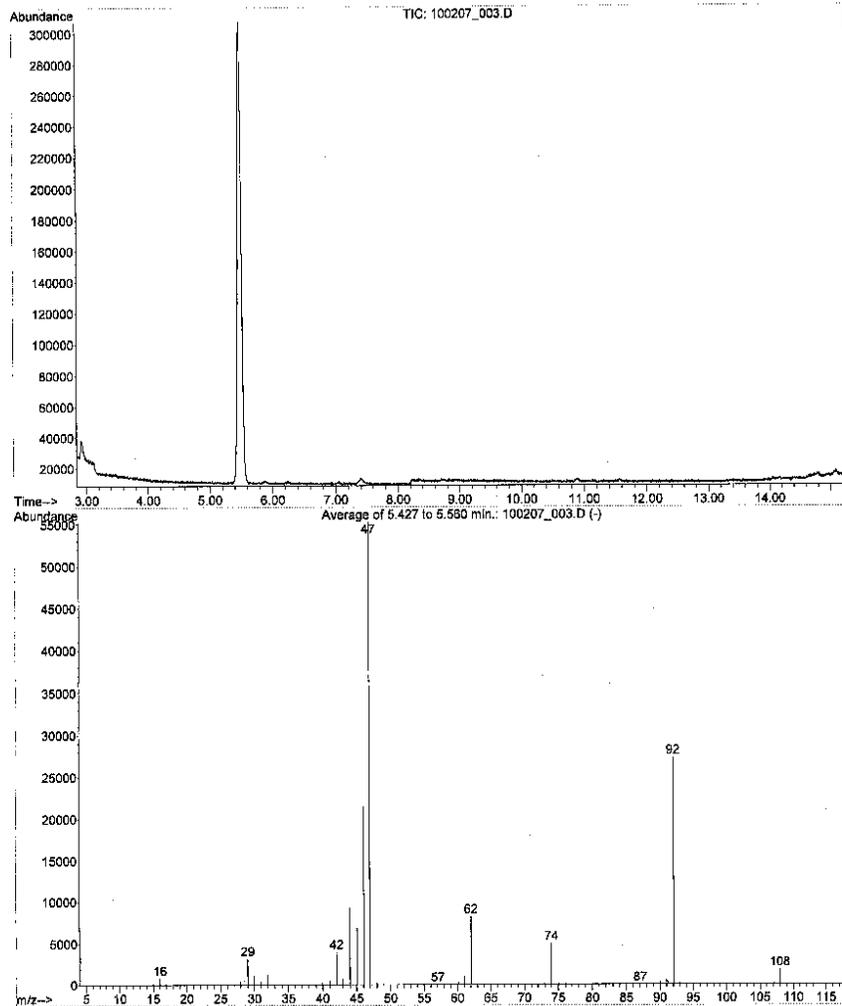
Diisopropyl Ether  
RTI Reference 12322-10

Figure 3. GC-FID Chromatogram of <sup>13</sup>C<sub>6</sub>-DIPE.



Unknown Peak Results					
Peak Type	RT	Area	% Area	Height	
1	Unknown n	3.248	78	0.10	35
2	Unknown n	3.372	55	0.07	23
3	Unknown n	4.642	288	0.37	85
4	Unknown n	4.892	48	0.06	17
5	Unknown n	4.960	160	0.21	51
6	Unknown n	5.136	73610	95.15	22029
7	Unknown n	5.289	1639	2.12	546
8	Unknown n	5.366	196	0.25	69
9	Unknown n	5.533	437	0.56	148
10	Unknown n	5.741	376	0.49	123
11	Unknown n	5.942	265	0.34	85
12	Unknown n	6.026	56	0.07	19
13	Unknown n	10.428	158	0.20	52

## Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-10Figure 4. Total Ion Chromatogram (top) and Mass Spectrum (bottom) of  $^{13}\text{C}_6$ -DIPE

## Test Chemical Analysis Report

Diisopropyl Ether  
RTI Reference 12322-10Table 1. Purity of  $^{13}\text{C}$ -DIPE determined by gas chromatography with flame ionization detection.

Date of Analysis	Purity (%) (Mean $\pm$ SD, 3 determinations)	Peroxide value (mg/L)
October 2, 2007	95.17 $\pm$ 0.03	n/a
October 5, 2007	95.25 $\pm$ 0.08	n/a

## **Appendix D**

### **Method Validation for Analysis of DIPE, Isopropanol and Acetone**

# Method Validation Report

## Analysis of Diisopropyl Ether in Blood

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**RTI PROTOCOL NO:** RTI-934

**RTI PROJECT NO.:** 0209408.001

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\*RTI International is a trade name of Research Triangle Institute.

## SUMMARY

The analytical method titled Project Specific Method for Analysis of Diisopropyl Ether in Blood Samples (AM-0209408.001) was validated in preparation for analyses of blood samples in order to determine concentrations of the compound diisopropyl ether (DIPE) present in the matrix following an inhalation exposure study. This validation established a Limit of Quantitation (LOQ), accuracy, and precision of the method. The method consists of GC/MS analysis of DIPE using mass-selective detector with injection of headspace from sample vials.

The validation procedure established a calibration range of 0.0887  $\mu\text{g/ml}$  to 244  $\mu\text{g/ml}$  for analysis of DIPE in the headspace of blood samples using two calibration curves. The low-concentration curve was defined by a linear regression ( $y=bx+a$ ) of slope 0.05121, intercept -0.00001467, and linear correlation coefficient of 0.995. The high-concentration curve was defined by the linear regression ( $y=bx+a$ ) of slope 0.0564, intercept -0.0307, and linear correlation coefficient of 0.997. Using the regression equation, calculated concentrations for at least 75% of calibration standards per curve were within the acceptance criteria of  $\pm 15\%$  (20% for the LOQ) of the nominal concentrations. Mean calculated concentrations for the replicate concentration points assayed at 0.451  $\mu\text{g/ml}$ , 4.88  $\mu\text{g/ml}$ , and 102  $\mu\text{g/ml}$  for determination of precision and accuracy ranged from 95.4% to 102% of nominal concentration. Precision around the mean for each of these replicate concentration points was calculated to be well within the acceptance criteria of 15% CV. Additionally, the mean values for each concentration were well within the acceptance criteria of 15% deviation from the nominal concentration. The assay's limit of quantitation was established at 0.0887  $\mu\text{g/ml}$ . Replicate measurements at this DIPE concentration varied around the mean value by within 11.0% (precision), and the mean value was 101% of the nominal concentration (accuracy). Both accuracy and precision values at the limit of quantitation which were within the  $\pm 20\%$  acceptance criteria.

Single calibration curves were constructed for quantitation of isopropanol and acetone in blood. For isopropanol, the calibration range encompassed concentrations of 2.28  $\mu\text{g/ml}$  to 242  $\mu\text{g/ml}$ . Slope and intercept values were 0.04305 and -0.0397, respectively for the isopropanol curve. The acetone calibration range was 4.66  $\mu\text{g/ml}$  to 237  $\mu\text{g/ml}$ . Slope and intercept values were 0.02336 and -0.004077, respectively. Linear correlation coefficients ( $r$ ) for both curves were 0.999.

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## 1.0 INTRODUCTION

The analytical method titled Project Specific Method for Analysis of Diisopropyl Ether in Blood Samples (AM-0209408.001) was validated in preparation for analyses of blood samples in order to determine concentrations of diisopropyl ether (DIPE) present in the matrix following an inhalation exposure study (RTI Protocol RTI-934, Metabolism and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats, Pilot Study). This validation established a Limit of Quantitation (LOQ), accuracy, and precision for the method and its calibration range. The method utilizes GC/MS analysis of headspace from vials containing the blood samples with mass-selective detection. Procedures conducted in the validation included preparation of calibration standards, analysis of standards and samples, regression analysis, comparison to acceptance criteria, and sample concentration calculation. This validation was conducted with a Validation Plan (Diisopropyl Ether Bioanalytical Method Validation, RTI Validation Plan). Criteria for acceptance of the validation (US Food and Drug Administration, 2001) were specified in the Validation Plan, and they are summarized in Table 1 below.

**Table 1.**  
**Method Validation Acceptance Criteria**

<b>Item</b>	<b>Acceptance Criteria</b>
<u>Accuracy</u>	
Limit of Quantitation (LLOQ)	± 20% of Nominal
Low Conc. (above the LLOQ).	± 15% of Nominal
Middle Conc.	± 15% of Nominal
High Conc.	± 15% of Nominal
<u>Precision</u>	
Limit of Quantitation (LLOQ)	± 20%
Low Conc (above LLOQ)	± 15% CV
Middle Conc:	± 15% CV
High Conc.	± 15% CV
<u>Calibration Curve</u>	
Linear Correlation Coefficient (r)	≥ 0.990

**Table 1 (Continued).**  
**Method Validation Acceptance Criteria**

Item	Acceptance Criteria
<u>Storage Stability in Blood</u>	
Low Conc. (above the LLOQ), (Room temp. for 8 h, Refri. temp. for 16 h and 24 h).	$\pm 15\%$ of Nominal $\pm 15\%$ CV (DIPE)
High Conc. (Room temp. for 8 h, Refri.temp. for 16 h and 24 h).	$\pm 15\%$ of Nominal $\pm 15\%$ CV (DIPE)
<u>Spiking Solution Concentration</u>	
Low Conc (Ice storage for 6 h, Freezer temp. for 7 days).	$\pm 15\%$ of Initial Conc. $\pm 15\%$ CV
Middle Conc: (Ice storage for 6 h, Freezer temp. for 7 days).	$\pm 15\%$ of Initial Conc. $\pm 15\%$ CV
<u>Int. Standard Storage Stability</u>	
Internal Standard Solution (Storage at -20 °C for 7 days, storage in ice for 6 h)	$\pm 10\%$ of Initial Analyte/Internal. Std Ratio.

The primary focus of this validation is the quantitation of DIPE in the blood matrix. However, the analytical method incorporated acetone and isopropanol in the calibration standards in order to measure amounts of these compounds in blood samples. These two analytes are both potential metabolites of DIPE, but may arise from other endogenous sources. Acetone is known to be present as an endogenous metabolite in blood with the potential for a high and variable background. The methodology for quantitation of acetone and isopropanol in the blood samples was validated to the extent possible. However, it was expected that that background levels of these compounds would vary significantly, and would constrain the limit of quantitation, accuracy, and precision of the method for acetone and isopropanol.

## 2.0 DIISOPROPYL ETHER TEST SUBSTANCE (DIPE)

The DIPE test substance was utilized as the analytical standard for preparation of calibration standards.

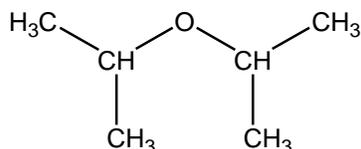
*NAME:* Diisopropyl Ethyl (DIPE; diisopropyl ether, diisopropyl oxide, 2-Isopropoxypropane)

*CAS No.:* 108-20-3

*MOLECULAR FORMULA:* C<sub>6</sub>H<sub>14</sub>O

*MOLECULAR WEIGHT:* 102.18

*STRUCTURE:*



*SOURCE OF TEST SUBSTANCE:* DIPE was purchased from Sigma-Aldrich, St. Louis, MO. (Product number 398276, specified purity 99.6%). A certificate of analysis was obtained from the vendor.

*LOT NUMBER(S):* 03658JC

*IDENTITY AND PURITY:* The identity of the unlabeled DIPE was confirmed at RTI by <sup>1</sup>H and <sup>13</sup>C NMR, and by mass spectrometry. The purity of the test chemical was determined by GC. Results of purity and identity determinations are contained in a separate test chemical analysis report..

*STORAGE CONDITIONS:* DIPE was stored in the dark at room temperature.

## 3.0 REAGENTS AND CHEMICALS

Diisopropyl Ether: Lot 03658JC, 99.6% purity, Sigma-Aldrich, St. Louis, MO. This test substance lot was used for preparation of all standards and QC samples. See Section 2.0 for a detailed description of the DIPE test article.

Methyl tertiary Butyl Ether (MTBE): Product number 650560, Batch 06646DE, 99.9%, HPLC, Sigma-Aldrich, St. Louis, MO. This compound serves as the internal standard for DIPE quantitation.

Acetone: Chromasolv for HPLC, Product number 34850, Lot number 53250, Sigma-Aldrich, St. Louis, MO.

Isopropanol: Chromasolv for HPLC, Product number 34863, Lot number 05761JC, 99.98%, Sigma-Aldrich, St. Louis, MO.

1-Propanol: Chromasolv for HPLC, 99.9% Aldrich, Product number 34871, Lot number 06061JC Sigma-Aldrich, St. Louis, MO. This compound serves as the internal standard for acetone and isopropanol.

N, N-Dimethylformamide (DMF): Product number 319937, Batch 01045AD, 99.8%, A.C.S reagent, Sigma-Aldrich, St. Louis, MO.

Distilled/Deionized (D/I) water

Helium: 99.996% purity, obtained from National Welders, Durham NC.

Blank Rat Blood: Obtained from male Fischer 344 rats via cardiac puncture using syringes containing with sodium heparin.

## **4.0 STOCK SOLUTIONS**

### **4.1 Multi-Analyte Stock Solutions**

Two aqueous multi-analyte stock solutions (Stock A and Stock B) containing DIPE, isopropanol, and acetone were prepared for use in producing spiking solutions for use in preparation of calibration standards. The multi-analyte stock solutions were prepared by combining 1-ml aliquots of single-analyte stock solutions. Prior to use, each single analyte solution was prepared in 10 ml of distilled/deionized water (acetone and isopropanol) or dimethylformamide (DMF, for DIPE). Just prior to addition of analyte, a volume of solvent equivalent to the volume of analyte about to be added was removed from the flask. The analyte was then added to the flask, the flask was capped, and the weight added to the flask was recorded. Flask contents were then mixed by hand and immediately cooled in ice. Table 2 includes the concentration of stock solutions and multi stock solutions used for the validation tasks.

Use of two stock solutions to prepare adjacent calibration points was intended to provide additional evidence of the method's accuracy. Alternating the use of these stocks for preparation of adjacent calibration standards demonstrated accuracy through quality of the linear regression.

## **5.0 INTERNAL STANDARD SOLUTION**

An internal standard solution consisting of MTBE at 50  $\mu\text{g/ml}$  concentration and 1-propanol at 500  $\mu\text{g/ml}$  was prepared for use in preparing calibration standards and samples for analysis. Aliquots of the internal standard solution were sealed in separate vials ensuring a fresh unopened aliquot was used for preparing each set of standards or samples. The internal standard solution aliquots were stored at approximately  $-20\text{ }^{\circ}\text{C}$ .

**Table 2.**  
**Stock Solutions Preparation**

Stock Solution		DIPE Conc. (mg/ml)	Isopropanol Conc. (mg/ml)	Acetone Conc. (mg/ml)	Validation tasks	
Single analyte stock Solutions	Stock A	31.21	30.92	29.85	1) DIPE standard curve in rat blood 2) Stability in blood	
	Stock B	31.83	30.78	30.29		
Multi analyte stock Solutions	Stock A	10.539	10.456	10.240		
	Stock B	10.860	10.340	10.359		
Single analyte stock Solutions	Stock A	30.99	30.84	30.71		Stability in solution
	Stock B	30.75	31.05	30.92		
Multi analyte stock Solutions	Stock A	10.581	10.450	10.407		
	Stock B	10.634	10.464	10.406		
Single analyte stock Solutions	Stock A	30.91	30.30	30.74	1) Stability in blood for 16 and 24 h stability 2) Stability in solution	
	Stock B	30.97	30.75	30.39		
Multi analyte stock Solutions	Stock A	10.699	10.236	10.312		
	Stock B	10.701	10.341	10.257		
Single analyte stock Solutions	Stock A	30.97	29.92	31.11	Stability in solution	
	Stock B	31.37	30.69	31.38		
Multi analyte stock Solutions	Stock A	10.666	10.144	10.448		
	Stock B	10.828	10.341	10.582		

The internal standard solution was prepared by diluting aliquots of two stock solutions each containing these compounds. Both stock solutions were prepared in 10-ml volumetric flasks containing distilled/deionized water. The MTBE stock solution was prepared by weighing 16 µl of MTBE into a 10-ml volumetric flask filled with distilled/deionized water. Prior to adding MTBE, an equivalent volume of distilled/deionized water was removed from the flask. The 1-propanol stock solution was prepared by weighing 130 µl of 1-propanol into a 10-ml volumetric flask filled with distilled/deionized water. Prior to adding 1-propanol, an equivalent volume of distilled/deionized water was removed from the flask. The

weight of MTBE and 1-propanol added to each flask was recorded. Concentrations of the MTBE and 1-propanol stock solutions were listed in Table 3.

**Table 3.**  
**Internal Standard Stock Solutions Preparation**

<b>MTBE Conc. (mg/ml)</b>	<b>1-propanol Conc. (mg/ml)</b>	<b>Validation tasks</b>
1.09	10.60	1) DIPE standard curve in rat blood 2) Stability in blood
1.10	10.54	1) DIPE standard curve in rat blood for 16 and 24 h stability 2) Stability in solution

## **6.0 PREPARATION OF CALIBRATION STANDARDS AND SAMPLES FOR ANALYSIS**

### **6.1 Calibration Spiking Solutions**

Eleven calibration spiking solutions shown in Table 4 were prepared for use in producing calibration standards. Each solution was prepared by combining distilled/deionized water and an aliquot of multi-analyte stock solution in vials cooled in wet ice during the procedure. Adjacent spiking solution concentrations were prepared from different stock solutions. Distilled/deionized water was also cooled in wet ice prior to and during use. Weights of distilled/deionized water added to each solution and the total solution weight were recorded. Dilution factors used to calculate actual analyte concentrations in each spiking standard solution were calculated by dividing the weight of added stock solution by the total weight of solution. Aliquots of each calibration spiking solution were removed and stored at approximately -20 °C for use in preparation of separate sets of calibration standards and samples.

**Table 4.**  
**Calibration Spiking Solution Preparation**

Spiking Standard Solution	Water (mg)	Stock or Spiking Solution Used	Total Solution Weight (g)	Dilution Factor	DIPE Conc. (µg/ml)	Isopropanol Conc. (µg/ml)	Acetone Conc. (µg/ml)
Spk. Std. A6	2.9408	Stock A	3.8252	0.231	2437	2417	2368
Spk. Std. B5 (HQC)	3.3754	Stock B	3.7238	0.0936	1016	967	969
Spk. Std. A5	3.0267	Spk. Std. A6	3.7584	0.195	474	471	461
Spk. Std. B4	3.0283	Spk. Std. B5	4.0093	0.245	249	237	237
Spk. Std. A4	2.9455	Spk. Std. A5	3.6739	0.198	94.1	93.3	91.4
Spk. Std. B3 (MQC)	3.0211	Spk. Std. B4	3.7597	0.196	48.8	46.5	46.6
Spk. Std. A3	3.0207	Spk. Std. A4	3.9973	0.244	23.0	22.8	22.3
Spk. Std. B2	3.0155	Spk. Std. B3	3.7548	0.197	9.62	9.16	9.17
Spk. Std. A2 (LQC)	3.0306	Spk. Std. A3	3.7709	0.196	4.51	4.48	4.38
Spk. Std. B1	3.0273	Spk. Std. B2	4.0159	0.246	2.37	2.25	2.26
Spk. Std. A1 (LLQC)	3.0143	Spk. Std. A2	3.7523	0.197	0.887	0.880	0.862

**Table 4 (Continued).****Calibration Spiking Solution Preparation**

<b>Spiking Standard Solution</b>	<b>Water (mg)</b>	<b>Stock or Spiking Solution Used</b>	<b>Total Solution Weight (g)</b>	<b>Dilution Factor</b>	<b>DIPE Conc. (µg/ml)</b>	<b>Isopropanol Conc. (µg/ml)</b>	<b>Acetone Conc. (µg/ml)</b>
Spk. Std. A6	2.8383	Stock A	3.7171	0.236	2502	2471	2460
Spk. Std. B5 (HQC)	3.2837	Stock B	3.6355	0.096	1019	1002	997
Spk. Std. A5	3.0386	Spk. Std. A6	3.7811	0.196	491	485	483
Spk. Std. B4	2.9464	Spk. Std. B5	3.9332	0.251	256	251	250
Spk. Std. A4	2.9401	Spk. Std. A5	3.6897	0.203	99.8	98.6	98.2
Spk. Std. B3 (MQC)	3.0149	Spk. Std. B4	3.7525	0.197	50.2	49.4	49.2
Spk. Std. A3	3.0254	Spk. Std. A4	4.0179	0.247	24.7	24.3	24.2
Spk. Std. B2	3.0232	Spk. Std. B3	3.7699	0.198	9.95	9.79	9.74
Spk. Std. A2 (LQC)	2.9669	Spk. Std. A3	3.7130	0.201	4.95	4.89	4.87
Spk. Std. B1	2.9449	Spk. Std. B2	3.9320	0.251	2.50	2.46	2.44
Spk. Std. A1 (LLQC)	2.9395	Spk. Std. A2	3.6875	0.203	1.00	0.992	0.988

**Table 4 (Continued).****Calibration Spiking Solution Preparation**

<b>Spiking Standard Solution</b>	<b>Water (mg)</b>	<b>Stock or Spiking Solution Used</b>	<b>Total Solution Weight (g)</b>	<b>Dilution Factor</b>	<b>DIPE Conc. (µg/ml)</b>	<b>Isopropanol Conc. (µg/ml)</b>	<b>Acetone Conc. (µg/ml)</b>
Spk. Std. A6	2.8639	Stock A	3.7403	0.234	2507	2398	2416
Spk. Std. B5 (HQC)	3.4609	Stock B	3.8083	0.091	976	943	936
Spk. Std. A5	3.0502	Spk. Std. A6	3.7834	0.194	486	465	468
Spk. Std. B4	3.0289	Spk. Std. B5	4.0089	0.244	239	231	229
Spk. Std. A4	3.0227	Spk. Std. A5	3.7607	0.196	95.3	91.2	91.9
Spk. Std. B3 (MQC)	3.0306	Spk. Std. B4	3.7691	0.196	46.8	45.2	44.8
Spk. Std. A3	3.0241	Spk. Std. A4	4.0061	0.245	23.4	22.4	22.5
Spk. Std. B2	3.0271	Spk. Std. B3	3.7693	0.197	9.21	8.90	8.82
Spk. Std. A2 (LQC)	3.0192	Spk. Std. A3	3.7617	0.197	4.61	4.41	4.45
Spk. Std. B1	3.0302	Spk. Std. B2	4.0245	0.247	2.27	2.20	2.18
Spk. Std. A1 (LLQC)	3.0279	Spk. Std. A2	3.7654	0.196	0.903	0.864	0.871

**Table 4 (Continued).****Calibration Spiking Solution Preparation**

Spiking Standard Solution	Water (mg)	Stock or Spiking Solution Used	Total Solution Weight (g)	Dilution Factor	DIPE Conc. (µg/ml)	Isopropanol Conc. (µg/ml)	Acetone Conc. (µg/ml)
Spk. Std. A6	2.8555	Stock A	3.7255	0.234	2491	2369	2440
Spk. Std. B5 (HQC)	3.3283	Stock B	3.6655	0.092	996	951	973
Spk. Std. A5	3.0134	Spk. Std. A6	3.7526	0.197	491	467	481
Spk. Std. B4	2.9495	Spk. Std. B5	3.9377	0.251	250	239	244
Spk. Std. A4	3.0215	Spk. Std. A5	3.7741	0.199	97.8	93.1	95.8
Spk. Std. B3 (MQC)	2.9944	Spk. Std. B4	3.7386	0.199	49.8	47.5	48.6
Spk. Std. A3	3.0228	Spk. Std. A4	4.0098	0.246	24.1	22.9	23.6
Spk. Std. B2	3.0124	Spk. Std. B3	3.7474	0.196	9.76	9.32	9.54
Spk. Std. A2 (LQC)	3.0059	Spk. Std. A3	3.7510	0.199	4.78	4.55	4.69
Spk. Std. B1	3.0104	Spk. Std. B2	3.9983	0.247	2.41	2.30	2.36
Spk. Std. A1 (LLQC)	3.0118	Spk. Std. A2	3.7579	0.199	0.950	0.903	0.930

**6.2 Calibration Standards**

Calibration standards were prepared in headspace vials at eleven different concentrations per analyte (DIPE, isopropanol, and acetone) using the calibration spiking solutions, blank Fischer 344 rat

blood, and the internal standard solution. Three replicates were prepared at each concentration level for generation of the standard curves. In addition to the eleven DIPE standard concentrations, three blank blood samples (no internal standard), and six blank blood samples with internal standard were prepared. Table 3 details the amount of each component in all blanks and standard concentrations.

**Table 5.**  
**Calibration Standard and QC Sample Components**

Standard or QC Sample ID	Nominal DIPE Conc. (µg/ml)	Nominal Acetone Conc. (µg/ml)	Nominal Isopropanol Conc. (µg/ml)	Blank Blood (µl)	Spiking Solution ID	Spiking Solution Aliquot or water Volume (µl)	Volume Internal Standard Solution (µl)
Blood Blank	n/a	n/a	n/a	90	n/a	20	0
ISTD Blank	n/a	n/a	n/a	90	n/a	10	10
Std A1 (LLQC)	0.0887	0.0862	0.0880	90	A1	10	10
Std B1	0.237	0.226	0.225	90	B1	10	10
Std A2 (LQC)	0.451	0.438	0.448	90	A2	10	10
Std B2	0.962	0.917	0.916	90	B2	10	10
Std A3	2.30	2.23	2.28	90	A3	10	10
Std B3 (MQC)	4.88	4.66	4.65	90	B3	10	10
Std A4	9.41	9.14	9.33	90	A4	10	10
Std B4	24.9	23.7	23.7	90	B4	10	10
Std A5	47.4	46.1	47.1	90	A5	10	10
Std B5 (HQC)	102	96.9	96.7	90	B5	10	10
Std A6	244	237	242	90	A6	10	10

**Table 5 (Continued).**  
**Calibration Standard and QC Sample Components**  
**(Repeat of blood stability at 16 h and 24 h at 4 °C).**

Standard or QC Sample ID	Nominal DIPE Conc. (µg/ml)	Nominal Acetone Conc. (µg/ml)	Nominal Isopropanol Conc. (µg/ml)	Blank Blood (µl)	Spiking Solution ID	Spiking Solution Aliquot or water Volume (µl)	Volume Internal Standard Solution (µl)
Blood Blank	n/a	n/a	n/a	90	n/a	20	0
ISTD Blank	n/a	n/a	n/a	90	n/a	10	10
Std A1 (LLQC)	0.0903	0.0871	0.0864	90	A1	10	10
Std B1	0.227	0.218	0.220	90	B1	10	10
Std A2 (LQC)	0.461	0.445	0.441	90	A2	10	10
Std B2	0.921	0.882	0.890	90	B2	10	10
Std A3	2.34	2.25	2.24	90	A3	10	10
Std B3 (MQC)	4.68	4.48	4.52	90	B3	10	10
Std A4	9.53	9.19	9.12	90	A4	10	10
Std B4	23.9	22.9	23.1	90	B4	10	10
Std A5	48.6	46.8	46.5	90	A5	10	10
Std B5 (HQC)	97.6	93.6	94.3	90	B5	10	10
Std A6	251	242	240	90	A6	10	10

### 6.3 LOQ, Accuracy, and Precision

Three additional replicate samples were prepared at each of the 0.451 µg/ml (LQC), 4.88 µg/ml (MQC) and 102 µg/ml (HQC) concentrations for use in accuracy and precision determinations in addition to the three samples prepared for use as calibration standards. Three additional replicate samples were also prepared at the 0.0887 µg/ml (LLQC) concentration for use in establishing the Lower Limit of Quantitation (LLOQ) also referred to as the LOQ.

## 7.0 STABILITY STUDIES

### 7.1 Stability in Solution

Stability of DIPE in spiking solutions was investigated over a seven-day period following storage at -20 °C and after six hours of storage on wet ice (ca. 4 °C). The two spiking solutions used to prepare the MQC and LQC calibration standards were prepared and stored at approximately these conditions. At the analysis timepoint, three aliquots of the two spiking solutions were transferred to separate headspace autosampler vials. Aliquots (10 µl) of the multi internal standard solution were then added to each vial prior to analysis.

### 7.2 Stability in Blood

The stability of DIPE in blood samples was tested under sample storage conditions expected to be utilized during the animal studies. During the studies, samples were expected to be analyzed within 24 h following collection. Note: The expectation was changed to 16 h due to stability results. Four sets of blank blood samples were prepared. Each set consisted of three samples at the LQC and HQC concentrations. One set was analyzed immediately following preparation. The second set was stored for 8 hours at room temperature prior to analysis. The third and fourth sets were stored in refrigerator temperatures at approximately 4 °C for 16 h and 24 h, respectively. Aliquots (10 µl) of the 50 µg/ml internal standard solution were then added to each vial prior to analysis.

### 7.3 Internal Standard Solution Stability

The stability of MTBE and 1-propanol internal standard spiking solutions was investigated after storage at ca. -20 °C for seven days, and on wet ice (ca. 4 °C) for six hours. At each timepoint, three sets (triplicate samples per set) of the HQC concentration before and after storage of the internal standard solution were transferred to individual headspace autosampler vials. At each timepoint, 10 µl aliquots of the internal standard solution were injected into each vial. At the zero timepoint, freshly prepared internal standard solution was added to the first set of spiking solutions samples followed by analysis of this set. An aliquot of freshly prepared internal standard solution was stored for seven days at -20 °C for seven days. At the seven day timepoint, the second set of samples was created using the stored internal standard solution and stored spiking (HQC) solution. A third set of samples was stored on wet ice for six hours. The internal standard ratios for isopropanol/MTBE and isopropanol/1-propanol was calculated for each sample. Ratios at the 6 h and seven day timepoints were compared to the ratios at the respective zero timepoint.

## 8.0 STANDARD/SAMPLE LIST

The following tables (Table 6 through Table 9) detail the list of samples/standards prepared and analyzed during conduct of the validation. Standards and samples listed in Tables 6 through Table 8 contained all three analytes, but these standards are listed separately by analyte to detail the multiple uses (i.e., calibration using a different analyte) for each standard.

**Table 6.**  
**DIPE Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	DIPE Concentration (µg/ml)	Purpose <sup>2</sup>
Blank Blood	1	-	Blank
Blank Blood	2	-	Blank
Blank Blood	3	-	Blank
Blank Blood	1	-	Blank
Blank Blood	2	-	Blank
Blank Blood	3	-	Blank
Blank Blood + IS	1	-	Blank
Blank Blood + IS	2	-	Blank
Blank Blood + IS	3	-	Blank
Blank Blood + IS	4	-	Blank
Blank Blood + IS	5	-	Blank
Blank Blood + IS	6	-	Blank
Blank Blood + IS	7	-	Blank
Blank Blood + IS	1	-	Blank
Blank Blood + IS	2	-	Blank
Blank Blood + IS	3	-	Blank
Std A1	1	0.0887	Calib Std, LLOQ
Std A1	2	0.0887	Calib Std, LLOQ
Std A1	3	0.0887	Calib Std, LLOQ
Std A1	4	0.0887	Calib Std, LLOQ
Std A1	5	0.0887	Calib Std, LLOQ
Std A1	6	0.0887	Calib Std, LLOQ
Std A1	1	0.100	Calib Std
Std A1	2	0.100	Calib Std
Std A1	3	0.100	Calib Std
Std B1	1	0.237	Calib Std
Std B1	2	0.237	Calib Std
Std B1	3	0.237	Calib Std
Std B1	1	0.250	Calib Std
Std B1	2	0.250	Calib Std
Std B1	3	0.250	Calib Std
Std A2	1	0.451	Calib Std, P + A
Std A2	2	0.451	Calib Std, P + A
Std A2	3	0.451	Calib Std, P + A
Std A2	4	0.451	Calib Std, P + A
Std A2	5	0.451	Calib Std, P + A
Std A2	6	0.451	Calib Std, P + A

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 6 (Continued).**  
**DIPE Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	DIPE Concentration (µg/ml)	Purpose <sup>2</sup>
Std A1	1	0.0887	Calib Std
Std A1	2	0.0887	Calib Std
Std A1	3	0.0887	Calib Std
Std A1	4	0.0887	Calib Std
Std A1	5	0.0887	Calib Std
Std A1	6	0.0887	Calib Std
Std B1	1	0.237	Calib Std
Std B1	2	0.237	Calib Std
Std B1	3	0.237	Calib Std
Std A2	1	0.451	Calib Std, P + A
Std A2	2	0.451	Calib Std, P + A
Std A2	3	0.451	Calib Std, P + A
Std A2	1	0.451	Calib Std, P + A
Std A2	2	0.451	Calib Std, P + A
Std A2	3	0.451	Calib Std, P + A
Std B2	1	0.962	Calib Std
Std B2	2	0.962	Calib Std
Std B2	3	0.962	Calib Std
Std B2	1	0.995	Calib Std
Std B2	2	0.995	Calib Std I
Std B2	3	0.995	Calib Std
Std A3	1	2.30	Calib Std
Std A3	2	2.30	Calib Std
Std A3	3	2.30	Calib Std
Std A3	1	2.47	Calib Std
Std A3	2	2.47	Calib Std
Std A3	3	2.47	Calib Std
Std B3	1	4.88	Calib Std, P + A
Std B3	2	4.88	Calib Std, P + A
Std B3	3	4.88	Calib Std, P + A
Std B3	4	4.88	Calib Std, P + A
Std B3	5	4.88	Calib Std, P + A
Std B3	6	4.88	Calib Std, P + A.
Std B3	1	5.02	Calib Std
Std B3	2	5.02	Calib Std
Std B3	3	5.02	Calib Std
Std A4	1	9.41	Calib Std

Std A4	2	9.41	Calib Std
Std A4	3	9.41	Calib Std
Std A4	1	9.98	Calib Std
Std A4	2	9.98	Calib Std
Std A4	3	9.98	Calib Std
Std B4	1	24.9	Calib Std
Std B4	2	24.9	Calib Std
Std B4	3	24.9	Calib Std
Std B4	1	25.6	Calib Std
Std B4	2	25.6	Calib Std
Std B4	3	25.6	Calib Std

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 6 (Continued).**  
**DIPE Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	DIPE Concentration (µg/ml)	Purpose <sup>2</sup>
Std A5	1	47.4	Calib Std
Std A5	2	47.4	Calib Std
Std A5	3	47.4	Calib Std
Std A5	1	49.1	Calib Std
Std A5	2	49.1	Calib Std
Std A5	3	49.1	Calib Std
Std B5	1	102	Calib Std, P + A
Std B5	2	102	Calib Std, P + A
Std B5	3	102	Calib Std, P + A
Std B5	4	102	Calib Std, P + A
Std B5	5	102	Calib Std, P + A
Std B5	6	102	Calib Std, P + A
Std B5	1	96.7	Calib Std
Std B5	2	96.7	Calib Std
Std B5	3	96.7	Calib Std
Std A6	1	244	Calib Std
Std A6	2	244	Calib Std
Std A6	3	244	Calib Std
Std A6	1	250	Calib Std
Std A6	2	250	Calib Std
Std A6	3	250	Calib Std
LQC RT 0 h	1	0.451	Stab in Blood
LQC RT 0 h	2	0.451	Stab in Blood
LQC RT 0 h	3	0.451	Stab in Blood
HQC RT 0 h	1	102	Stab in Blood
HQC RT 0 h	2	102	Stab in Blood
HQC RT 0 h	3	102	Stab in Blood
LQC RT 8 h	1	0.451	Stab in Blood
LQC RT 8 h	2	0.451	Stab in Blood
LQC RT 8 h	3	0.451	Stab in Blood
HQC RT 8 h	1	102	Stab in Blood
HQC RT 8 h	2	102	Stab in Blood
HQC RT 8 h	3	102	Stab in Blood
LQC RT 0 h	1	0.495	Stab in Blood (0 h for 4°C storage)

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 6 (Continued).  
DIPE Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	DIPE Concentration (µg/ml)	Purpose <sup>2</sup>
LQC RT 0 h	2	0.451	Stab in Blood (0 h for 4°C storage)
LQC RT 0 h	3	0.451	Stab in Blood (0 h for 4°C storage)
HQC RT 0 h	1	102	Stab in Blood (0 h for 4°C storage)
HQC RT 0 h	2	102	Stab in Blood (0 h for 4°C storage)
HQC RT 0 h	3	102	Stab in Blood (0 h for 4°C storage)
LQC 4 °C 16 h	1	0.451	Stab in Blood
LQC 4 °C 16 h	2	0.451	Stab in Blood
LQC 4 °C 16 h	3	0.451	Stab in Blood
HQC 4 °C 16 h	1	102	Stab in Blood
HQC 4 °C 16 h	2	102	Stab in Blood
HQC 4 °C 16 h	3	102	Stab in Blood
LQC 4 °C 24 h	1	0.451	Stab in Blood
LQC 4 °C 24 h	2	0.451	Stab in Blood
LQC 4 °C 24 h	3	0.451	Stab in Blood
HQC 4 °C 24 h	1	102	Stab in Blood
HQC 4 °C 24 h	2	102	Stab in Blood
HQC 4 °C 24 h	3	102	Stab in Blood
Spk LQC (0 days)	1	4.51	Spike Sol Stab
Spk LQC (0 days)	2	4.51	Spike Sol Stab
Spk LQC (0 days)	3	4.51	Spike Sol Stab
Spk MQC (0 days)	1	46.8	Spike Sol Stab
Spk MQC (0 days)	2	46.8	Spike Sol Stab
Spk MQC (0 days)	3	46.8	Spike Sol Stab
Spk LQC (7 days)	1	4.51	Spike Sol Stab
Spk LQC (7 days)	2	4.51	Spike Sol Stab
Spk LQC (7 days)	3	4.51	Spike Sol Stab
Spk MQC (7 days)	1	46.8	Spike Sol Stab
Spk MQC (7 days)	2	46.8	Spike Sol Stab
Spk MQC (7 days)	3	46.8	Spike Sol Stab
Spk LQC (zero time)	1	4.95	Spike Sol Stab
Spk LQC (zero time)	2	4.95	Spike Sol Stab
Spk LQC (zero time)	3	4.95	Spike Sol Stab
Spk MQC (zero time)	1	50.2	Spike Sol Stab

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 6 (Continued).**  
**DIPE Standard and Sample Analysis List<sup>1</sup>**

<b>Standard/Sample</b>	<b>Replicate Number</b>	<b>DIPE Concentration (µg/ml)</b>	<b>Purpose<sup>2</sup></b>
Spk MQC (zero time)	2	50.2	Spike Sol Stab
Spk MQC (zero time)	3	50.2	Spike Sol Stab
Spk LQC (6 hours on ice)	1	4.95	Spike Sol Stab
Spk LQC (6 hours on ice)	2	4.95	Spike Sol Stab
Spk LQC (6 hours on ice)	3	4.95	Spike Sol Stab
Spk MQC (6 hours on ice)	1	50.2	Spike Sol Stab
Spk MQC (6 hours on ice)	2	50.2	Spike Sol Stab
Spk MQC (6 hours on ice)	3	50.2	Spike Sol Stab

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 7.**  
**Isopropanol Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Isopropanol Concentration (µg/ml)	Purpose <sup>2</sup>
Blank Blood	1	-	Blank
Blank Blood	2	-	Blank
Blank Blood	3	-	Blank
Blank Blood	1	-	Blank
Blank Blood	2	-	Blank
Blank Blood	3	-	Blank
Blank Blood + IS	1	-	Blank
Blank Blood + IS	2	-	Blank
Blank Blood + IS	3	-	Blank
Blank Blood + IS	4	-	Blank
Blank Blood + IS	5	-	Blank
Blank Blood + IS	6	-	Blank
Blank Blood + IS	7	-	Blank
Blank Blood + IS	1	-	Blank
Blank Blood + IS	2	-	Blank
Blank Blood + IS	3	-	Blank
Std A3	1	2.28	Calib Std
Std A3	2	2.28	Calib Std
Std A3	3	2.28	Calib Std
Std A3	1	2.43	Calib Std
Std A3	2	2.43	Calib Std
Std A3	3	2.43	Calib Std

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 7 (Continued).**  
**Isopropanol Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Isopropanol Concentration (µg/ml)	Purpose <sup>2</sup>
Std B3	1	4.65	Calib Std, P + A
Std B3	2	4.65	Calib Std, P + A
Std B3	3	4.65	Calib Std, P + A
Std B3	4	4.65	Calib Std, P + A
Std B3	5	4.65	Calib Std, P + A
Std B3	6	4.65	Calib Std, P + A.
Std B3	1	4.94	Calib Std
Std B3	2	4.94	Calib Std
Std B3	3	4.94	Calib Std
Std A4	1	9.33	Calib Std
Std A4	2	9.33	Calib Std
Std A4	3	9.33	Calib Std
Std A4	1	9.86	Calib Std
Std A4	2	9.86	Calib Std
Std A4	3	9.86	Calib Std
Std B4	1	23.7	Calib Std
Std B4	2	23.7	Calib Std
Std B4	3	23.7	Calib Std
Std B4	1	25.1	Calib Std
Std B4	2	25.1	Calib Std
Std B4	3	25.1	Calib Std
Std A5	1	47.1	Calib Std
Std A5	2	47.1	Calib Std
Std A5	3	47.1	Calib Std
Std A5	1	48.5	Calib Std, Reval
Std A5	2	48.5	Calib Std, Reval
Std A5	3	48.5	Calib Std, Reval
Std B5	1	96.7	Calib Std, P + A
Std B5	2	96.7	Calib Std, P + A
Std B5	3	96.7	Calib Std, P + A
Std B5	4	96.7	Calib Std, P + A
Std B5	5	96.7	Calib Std, P + A
Std B5	6	96.7	Calib Std, P + A
Std B5	1	100	Calib Std
Std B5	2	100	Calib Std
Std B5	3	100	Calib Std

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 7 (Continued).  
Isopropanol Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Isopropanol Concentration (µg/ml)	Purpose <sup>2</sup>
Std A6	1	242	Calib Std
Std A6	2	242	Calib Std
Std A6	3	242	Calib Std
Std A6	1	247	Calib Std
Std A6	2	247	Calib Std
Std A6	2	247	Calib Std
HQC RT 0 h	1	96.7	Stab in Blood
HQC RT 0 h	2	96.7	Stab in Blood
HQC RT 0 h	3	96.7	Stab in Blood
HQC RT 8 h	1	96.7	Stab in Blood
HQC RT 8 h	2	96.7	Stab in Blood
HQC RT 8 h	3	96.7	Stab in Blood
HQC RT 0 h	1	100	Stab in Blood (0 h for 4 °C storage)
HQC RT 0 h	2	100	Stab in Blood (0 h for 4 °C storage)
HQC RT 0 h	3	100	Stab in Blood (0 h for 4 °C storage)
HQC 4 °C 16 h	1	100	Stab in Blood
HQC 4 °C 16 h	2	100	Stab in Blood
HQC 4 °C 16 h	3	100	Stab in Blood
HQC 4 °C 24 h	1	100	Stab in Blood
HQC 4 °C 24 h	2	100	Stab in Blood
HQC 4 °C 24 h	3	100	Stab in Blood
Spk LQC (0 days)	1	4.48	Spike Sol Stab
Spk LQC (0 days)	2	4.48	Spike Sol Stab
Spk LQC (0 days)	3	4.48	Spike Sol Stab
Spk MQC (0 days)	1	45.2	Spike Sol Stab
Spk MQC (0 days)	2	45.2	Spike Sol Stab
Spk MQC (0 days)	3	45.2	Spike Sol Stab
Spk LQC (7 days)	1	4.48	Spike Sol Stab
Spk LQC (7 days)	2	4.48	Spike Sol Stab
Spk LQC (7 days)	3	4.48	Spike Sol Stab
Spk MQC (7 days)	1	45.2	Spike Sol Stab
Spk MQC (7 days)	2	45.2	Spike Sol Stab
Spk MQC (7 days)	3	45.2	Spike Sol Stab
Spk LQC (zero time)	1	4.89	Spike Sol Stab
Spk LQC (zero time)	2	4.89	Spike Sol Stab
Spk LQC (zero time)	3	4.89	Spike Sol Stab

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 7 (Continued).**  
**Isopropanol Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Isopropanol Concentration (µg/ml)	Purpose <sup>2</sup>
Spk MQC (zero time)	1	49.4	Spike Sol Stab
Spk MQC (zero time)	2	49.4	Spike Sol Stab
Spk MQC (zero time)	3	49.4	Spike Sol Stab
Spk LQC (6 hours on ice)	1	4.89	Spike Sol Stab
Spk LQC (6 hours on ice)	2	4.89	Spike Sol Stab
Spk LQC (6 hours on ice)	3	4.89	Spike Sol Stab
Spk MQC (6 hours on ice)	1	49.4	Spike Sol Stab
Spk MQC (6 hours on ice)	2	49.4	Spike Sol Stab
Spk MQC (6 hours on ice)	3	49.4	Spike Sol Stab

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 8.**  
**Acetone Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Acetone Concentration (µg/ml)	Purpose <sup>2</sup>
Blank Blood	1	-	Blank
Blank Blood	2	-	Blank
Blank Blood	3	-	Blank
Blank Blood	1	-	Blank
Blank Blood	2	-	Blank
Blank Blood	3	-	Blank
Blank Blood + IS	1	-	Blank
Blank Blood + IS	2	-	Blank
Blank Blood + IS	3	-	Blank
Blank Blood + IS	4	-	Blank
Blank Blood + IS	5	-	Blank
Blank Blood + IS	6	-	Blank
Blank Blood + IS	7	-	Blank
Blank Blood + IS	1	-	Blank
Blank Blood + IS	2	-	Blank
Blank Blood + IS	3	-	Blank

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 8 (Continued).**  
**Acetone Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Acetone Concentration (µg/ml)	Purpose <sup>2</sup>
Std B2	1	0.974	Calib Std
Std B2	2	0.974	Calib Std
Std B2	3	0.974	Calib Std
Std A3	1	2.42	Calib Std
Std A3	2	2.42	Calib Std
Std A3	3	2.42	Calib Std
Std B3	1	4.66	Calib Std, P + A
Std B3	2	4.66	Calib Std, P + A
Std B3	3	4.66	Calib Std, P + A
Std B3	4	4.66	Calib Std, P + A
Std B3	5	4.66	Calib Std, P + A
Std B3	6	4.66	Calib Std, P + A.
Std B3	1	4.92	Calib Std
Std B3	2	4.92	Calib Std
Std B3	3	4.92	Calib Std
Std A4	1	9.14	Calib Std
Std A4	2	9.14	Calib Std
Std A4	3	9.14	Calib Std
Std A4	1	9.82	Calib Std
Std A4	2	9.82	Calib Std
Std A4	3	9.82	Calib Std
Std B4	1	23.7	Calib Std
Std B4	2	23.7	Calib Std
Std B4	3	23.7	Calib Std
Std B4	1	25.0	Calib Std
Std B4	2	25.0	Calib Std
Std B4	3	25.0	Calib Std
Std A5	1	46.1	Calib Std
Std A5	2	46.1	Calib Std
Std A5	3	46.1	Calib Std
Std A5	1	48.3	Calib Std
Std A5	2	48.3	Calib Std
Std A5	3	48.3	Calib Std

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 8 (Continued).**  
**Acetone Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Acetone Concentration (µg/ml)	Purpose <sup>2</sup>
Std B5	1	96.9	Calib Std, P + A
Std B5	2	96.9	Calib Std, P + A
Std B5	3	96.9	Calib Std, P + A
Std B5	4	96.9	Calib Std, P + A
Std B5	5	96.9	Calib Std, P + A
Std B5	6	96.9	Calib Std, P + A
Std B5	1	99.7	Calib Std
Std B5	2	99.7	Calib Std
Std B5	3	99.7	Calib Std
Std A6	1	237	Calib Std
Std A6	2	237	Calib Std
Std A6	3	237	Calib Std
Std A6	1	246	Calib Std
Std A6	2	246	Calib Std
Std A6	3	246	Calib Std
HQC RT 0 h	1	96.9	Stab in Blood
HQC RT 0 h	2	96.9	Stab in Blood
HQC RT 0 h	3	96.9	Stab in Blood
HQC RT 8 h	1	96.9	Stab in Blood
HQC RT 8 h	2	96.9	Stab in Blood
HQC RT 8 h	3	96.9	Stab in Blood
HQC RT 0 h	1	99.7	Stab in Blood (0 h for 4 °C storage)
HQC RT 0 h	2	99.7	Stab in Blood (0 h for 4 °C storage)
HQC RT 0 h	3	99.7	Stab in Blood (0 h for 4 °C storage)
HQC 4 °C 16 h	1	99.7	Stab in Blood
HQC 4 °C 16 h	2	99.7	Stab in Blood
HQC 4 °C 16 h	3	99.7	Stab in Blood
HQC 4 °C 24 h	1	99.7	Stab in Blood
HQC 4 °C 24 h	2	99.7	Stab in Blood
HQC 4 °C 24 h	3	99.7	Stab in Blood
Spk LQC (0 days)	1	4.38	Spike Sol Stab
Spk LQC (0 days)	2	4.38	Spike Sol Stab
Spk LQC (0 days)	3	4.38	Spike Sol Stab

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 8 (Continued).**  
**Acetone Standard and Sample Analysis List<sup>1</sup>**

Standard/Sample	Replicate Number	Acetone Concentration (µg/ml)	Purpose <sup>2</sup>
Spk MQC (0 days)	1	44.8	Spike Sol Stab
Spk MQC (0 days)	2	44.8	Spike Sol Stab
Spk MQC (0 days)	3	44.8	Spike Sol Stab
Spk LQC (7 days)	1	4.38	Spike Sol Stab
Spk LQC (7 days)	2	4.38	Spike Sol Stab
Spk LQC (7 days)	3	4.38	Spike Sol Stab
Spk MQC (7 days)	1	8	Spike Sol Stab
Spk MQC (7 days)	2	8	Spike Sol Stab
Spk MQC (7 days)	3	8	Spike Sol Stab
Spk LQC (zero time)	1	4.87	Spike Sol Stab
Spk LQC (zero time)	2	4.87	Spike Sol Stab
Spk LQC (zero time)	3	4.87	Spike Sol Stab
Spk MQC (zero time)	1	48.2	Spike Sol Stab
Spk MQC (zero time)	2	48.2	Spike Sol Stab
Spk MQC (zero time)	3	48.2	Spike Sol Stab
Spk LQC (6 hours on ice)	1	4.87	Spike Sol Stab
Spk LQC (6 hours on ice)	2	4.87	Spike Sol Stab
Spk LQC (6 hours on ice)	3	4.87	Spike Sol Stab
Spk MQC (6 hours on ice)	1	48.2	Spike Sol Stab
Spk MQC (6 hours on ice)	2	48.2	Spike Sol Stab
Spk MQC (6 hours on ice)	3	48.2	Spike Sol Stab

<sup>1</sup> Note: Order in the table does not indicate order of analysis in the sample set.

<sup>2</sup> P + A = Precision and Accuracy, Calib Std = Calibration Standard, Stab in Blood = Stability in Blood

**Table 9.**  
**Internal Standard<sup>1</sup> Sample Analysis List<sup>2</sup>**

Standard/Sample	Replicate Number	Isopropanol Concentration (µg/ml)	Purpose <sup>2</sup>
Spk HQC (0day)	1	967	ISTD Sol Stab
Spk HQC (0day)	2	967	ISTD Sol Stab
Spk HQC (0day)	3	967	ISTD Sol Stab
Spk HQC (7day)	1	967	ISTD Sol Stab
Spk HQC (7day)	2	967	ISTD Sol Stab
Spk HQC (7day)	3	967	ISTD Sol Stab
Spk HQC (zero time)	1	1019	ISTD Sol Stab
Spk HQC (zero time)	2	1002	ISTD Sol Stab
Spk HQC (zero time)	3	1002	ISTD Sol Stab
Spk HQC (6 hours on ice)	1	1002	ISTD Sol Stab
Spk HQC (6 hours on ice)	2	1002	ISTD Sol Stab
Spk HQC (6 hours on ice)	3	1002	ISTD Sol Stab

<sup>1</sup> Internal standard concentration: MTBE: 50 (µg/ml) and 1-Propanol: 500 (µg/ml)

<sup>2</sup> Note: Order in the table does not indicate order of analysis in the sample set.  
ISTD Sol Stab = Internal Solution Stability.

## 9.0 GC/MS ANALYSIS OF SAMPLES AND STANDARDS

Samples and standards were assayed using an Agilent Model 6890 gas chromatograph fitted with an Agilent Model 5973 Mass Selective Detector. Samples of air from the headspace of sample vials were introduced into the gas chromatograph using an Agilent Model G1888A headspace autosampler. Data acquisition and instrument control was performed by Agilent MSD Security Chemstation Software Version A.01.02 (Agilent technologies, Wilmington, DE). Instrumental operating parameters and other analysis parameters are detailed in Table 10. A representative chromatogram, shown in Figure 1, contains selected ion chromatograms for DIPE (m/z 87); isopropanol (m/z 45); acetone (m/z 58); MTBE (m/z 73), and 1-propanol (31 m/z).

**Table 10.**  
**Instrument Parameters**

**6890 GC**

Injection type	split/splitless
Injector temperature	150 °C
Split ratio	15:1 (calculated by adding flow rate of HSS)
Carrier gas	Helium
Flow rate	2.5 ml/min
Column	DB-624 30 m x 0.32 mm i.d. 1.8 µm film thickness (J&W, Agilent technologies, Wilmington, DE)

**GC oven program**

Initial temperature	30 °C
Initial time	2.5 min
Temperature rate	5 °C/min
Final temperature	35 °C
Final time	1.25 min
Temperature rate A	90 °C/min
Final temperature A	150 °C
Run time	6.03 min

**G1888A headspace sampler**

Loop size	<u>1 ml</u>
Vial Pressure	15 psig
Carrier pressure	6.0 psig
Headspace oven	65 °C
loop temperature	90 °C
Transfer line temperature	110 °C
Equilibration time	10 min
GC cycle time	10 min
Pressurization	0.2 min
Loop Fill	0.2 min
Loop Equilibration	0.05 min
Inject	0.5 min
Shake	low

**5973 MSD**

SIM	DIPE (m/z 87); Isopropanol(m/z 45); Acetone (m/z 58); MTBE (m/z 73), 1-Propanol (31 m/z)
Quad temperature	150 °C
Source temperature	230 °C
Transfer line	230 °C
Tune	Atune.u
Solvent delay	2.5 min

## 10.0 CALCULATIONS

Peak areas for each analyte and the respective internal standard were used to calculate the internal standard ratio. For each chromatographic run, the Peak Area ratio was computed using the equation shown below:

$$\text{Peak Area Ratio} = \frac{\text{Analyte Peak Area}}{\text{Internal Standard Peak Area}}$$

MTBE peak areas were used in the above equation as the internal standard for quantitation of DIPE. The 1-propanol peak area was substituted into the above equation for quantitation of isopropanol and acetone analytes. Resulting concentration and internal standard ratios for all calibration standards were input into the curve fitting software TableCurve 2D for Windows (Version 2.04, Jandel Scientific) for calculation of the linear regression coefficients. Regression coefficients of slope and intercept were then used to quantitate the three analyte standards and samples.

## 11.0 VALIDATION RESULTS AND DISCUSSION

### 11.1 DIPE Calibration Curves

Two calibration curves were constructed to encompass the entire standard concentration range. The low concentration curve included standards from 0.0887 µg/ml to 4.88 µg/ml DIPE (Table 6 and Figure 2). The high concentration curve encompassed standard concentrations from 4.88 µg/ml to 244 µg/ml DIPE (Figure 2). Both curves were weighted by a factor of 1/x in order to provide accuracy at the lower end of each calibration range. Calculation of linear (least squares) regression generated a slope of 0.05121 and intercept value of -0.00001467 for the low curve. The linear correlation coefficient (r) for the low concentration curve was 0.995. Slope and intercept values were 0.0564 and -0.0307, respectively for the high concentration curve. The linear correlation coefficient (r) was 0.997 for the high curve. Correlation coefficients resulting from both curves exceeded the minimum acceptance criterion of 0.990.

Concentrations of the individual calibration standards were then calculated using the respective regression equations. Resulting concentrations (Table 11) were within ±15% of the nominal values for more than 75% of the calibration standards assayed per curve. The method validation plan included this limit for acceptance of the calibration curves. Therefore, the standard curves constructed by the analytical method met both criteria stated for acceptance in the validation plan for DIPE.

### 11.2 Acetone and Isopropanol Calibration Curves

A single calibration curve was constructed to encompass the standard concentration range of 2.28 µg/ml to 242 µg/ml for isopropanol. For acetone, a single calibration curve was constructed to encompass the range of 4.66 µg/ml to 237 µg/ml. Both curves were weighted by a factor of 1/x in order to provide accuracy at the lower end of each calibration range. Calculation of linear (least squares) regression generated a slope of 0.04305 and intercept value of -0.0397 for the isopropanol curve

(Figure 3). The linear correlation coefficient ( $r$ ) for the isopropanol curve was 0.999. Slope and intercept values were 0.02336 and -0.004077, respectively for the acetone curve (Figure 3). The linear correlation coefficient ( $r$ ) was 0.999 for the acetone curve. Correlation coefficients resulting from both curves exceeded the minimum acceptance criterion of 0.990. Calibration ranges were chosen to ensure that both the correlation coefficient criterion was obtained and that precision and accuracy values were below  $\pm 20\%$  for the lowest calibration point when standards were quantitated using the regression equations. Calculated concentrations of the individual calibration standards are shown in Table 12 and Table 13. Slight variance in endogenous levels of isopropanol and acetone in the blank blood may allow selection of slightly higher or lower calibration points in future calibrations according to these selection criteria.

### 11.3 Limit of Quantitation

Results of six analyses of standards at the 0.0887  $\mu\text{g/ml}$  DIPE concentration yielded a mean concentration of 0.0899  $\mu\text{g/ml}$  DIPE (Table 14). The mean value was within  $\pm 20\%$  of nominal concentration stated in the validation plan for acceptance. The individual concentration determinations for each replicate varied around the mean by 11.0% (%CV). The precision around the mean was also within the  $\pm 20\%$  CV criterion specified in the validation plan. Therefore, this standard concentration met the criteria in the validation plan for establishing it as the limit of quantitation.

### 11.4 Accuracy and Precision

Determination of precision and accuracy utilized results obtained from analyses of six standards at each of three DIPE standard concentrations: 0.451  $\mu\text{g/ml}$ , 4.88  $\mu\text{g/ml}$ , and 102  $\mu\text{g/ml}$ . Table 15 details assay results and precision and accuracy calculation results. The accuracy of the analytical method was defined as the closeness of test results obtained by the method to the nominal values of each standard. The acceptance criterion for accuracy of the method was a mean concentration determination within  $\pm 15\%$  of the nominal concentration at each of the three standard concentrations in addition to three replicates (LQC, MQC and HQC). Precision was measured by calculating the Percent Coefficient of Variation (%CV) around the mean for each set of determinations per concentration. The acceptance limit for precision was  $\pm 15\%$  for each set of determinations per concentration.

Mean calculated concentrations for the six replicates of DIPE samples analysis were 0.430  $\mu\text{g/ml}$ , 4.86  $\mu\text{g/ml}$ , and 103  $\mu\text{g/ml}$ , respectively. These mean concentration results were well within the acceptance criterion of  $\pm 15\%$  of nominal concentration, and correspond to accuracy values of 95.4%, 99.7%, and 102% of the nominal concentrations. Therefore, accuracy of the method was established within the calibration range. The precision (CV%) around three concentration determinations were 9.91%, 10.8% and 7.92%, which were within 15% of the required criteria. An additional measure of accuracy in this validation was provided by linearity of the standard curve. Adjacent calibration concentrations in the curve were prepared from different stock solutions. Therefore, attainment of acceptable calibration curves provided this additional measure.

### 11.5 Storage Stability of DIPE, Isopropanol, and Acetone in Blood

Mean values resulting from analyses of blood samples of 0.451 µg/ml and 102 µg/ml DIPE concentrations stored at room temperature for 8 h were determined to be 89.6% and 91.1% of the initial concentration prior to storage (Table 16). Analysis of blood samples at 0.451 µg/ml and 102 µg/ml DIPE following storage at 4 °C for 16 h yielded mean concentrations that were 86.2% and 87.9% of the initial concentration respectively (Table 17). Following storage for 24 h at 4 °C, DIPE concentration was determined to be 87.0% and 81.8% for the two concentrations, respectively. Measured concentrations were within the ± 15% of initial concentration acceptance criterion following storage for 8 h at room temperature. Measured concentrations of these standards stored at 4 °C for 16 h were also within the acceptance criterion. At 24 h following storage at 4 °C for 24 h, the lower concentration standard met the acceptance criterion, but the high concentration was outside the acceptance criterion (Table 17). Therefore, blood samples containing DIPE cannot be stored for periods exceeding 16 h at 4 °C.

Analysis of blood samples stored at room temperature for 8 h containing isopropanol (96.7 µg/ml) and acetone (96.9 µg/ml) yielded mean concentrations that were 97.8% and 95.0% of the initial concentration measured at preparation (Table 18 and Table 20). Storage of blood samples containing isopropanol (100 µg/ml) and acetone (99.7 µg/ml) for 16 h and 24 h at 4 °C prior to analysis yielded concentrations that were 98.8 – 103% and 94.1% - 102% of initial concentrations measured at preparation, respectively (Table 19 and Table 21). These results indicate that isopropanol and acetone concentrations are stable at room temperature for 8 h, and up to 24 h at ca. 4 °C.

### 11.6 Storage Stability of DIPE in Spiking Solutions

Mean concentrations obtained from analyses of two DIPE spiking solutions of 4.51 µg/ml and 46.8 µg/ml stored at -20 °C for seven days were within the acceptance criterion of ± 15% of original measured concentrations at preparation. Analysis results are detailed in Table 22. Mean analysis results for each of the two solutions at preparation were 4.39 µg/ml and 46.8 µg/ml DIPE, respectively. Following storage for seven days, mean analysis results were 3.77 µg/ml and 45.3 µg/ml, respectively. These concentrations were 85.9% and 96.7% of the initial concentrations prior to storage for the 4.39 µg/ml and 46.8 µg/ml solutions, respectively.

Concentration of DIPE in spiking solutions stored on wet ice for six hours following preparation were also determined to be within ± 15% of the initial measured concentration at preparation. Analysis results are shown in Table 23. The concentration of the two spiking solutions at 4.95 µg/ml and 50.2 µg/ml were measured to be 5.05 µg/ml and 48.8 µg/ml at the six-hour timepoint corresponding to 103% and 98.8% of the initial measured concentration, respectively.

### 11.7 Storage Stability of MTBE and 1-Propanol in Internal Standard Solution

Results obtained from assays determining concentration stability of the internal standards MTBE and 1-propanol in solution stored at room temperature for 6 h on wet ice (ca. 4 °C) and at -20 °C for

seven days were within the  $\pm 10\%$  of the initial analysis results. Following storage of the internal standard solution at  $-20\text{ }^{\circ}\text{C}$  for seven days, analyses yielded Isopropanol/MTBE and Isopropanol/1-Propanol peak area ratios that were 95.9% and 92.1% of the initial ratios measured at initial preparation of the solution, respectively (Table 24). Analyses using the internal standard solution stored in wet ice for six hours following preparation yielded peak area ratios that were 98.0% and 98.8% for the Isopropanol/MTBE and Isopropanol/1-propanol internal standards, respectively (Table 25).

## 12.0 REFERENCES

Metabolism and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats: Pilot, Study Protocol, RTI Protocol RTI-934, February 23, 2007.

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US Food and Drug Administration (2001). Guidance for Industry: Bioanalytical Method Validation.

**Table 11.**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A1	0.00450	0.0887	0.0882	99.4
Std A1	0.00521	0.0887	0.102	115
Std A1	0.00419	0.0887	0.0821	92.6
Std A1	0.00496	0.100	0.107	107
Std A1	0.00505	0.100	0.109	109
Std A1	0.00522	0.100	0.112	112
Std B1	0.0113	0.237	0.221	93.1
Std B1	0.0138	0.237	0.270	114
Std B1	0.0117	0.237	0.229	96.5
Std B1	0.0119	0.250	0.243	97.2
Std B1	0.0121	0.250	0.247	98.7
Std B1	0.0127	0.250	0.259	104

**Table 11 (Continued).****Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Calculated Conc. (µg/ml)</b>	<b>Accuracy (%)</b>
Std A2	0.0201	0.451	0.392	86.9
Std A2	0.0247	0.451	0.483	107
Std A2	0.0214	0.451	0.419	92.8
Std A2	0.0225	0.495	0.451	91.1
Std A2	0.0215	0.495	0.431	87.7
Std A2	0.0250	0.495	0.500	101
Std B2	0.0478	0.962	0.933	97.0
Std B2	0.0545	0.962	1.06	111
Std B2	0.0458	0.962	0.895	93.1
Std B2	0.0495	0.995	0.980	98.5
Std B2	0.0449	0.995	0.889	89.4
Std B2	0.0514	0.995	1.02	102

**Table 11 (Continued).**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A3	0.115	2.30	2.24	97.4
Std A3	0.128	2.30	2.49	108
Std A3	0.113	2.30	2.20	95.7
Std A3	0.124	2.47	2.44	98.8
Std A3	0.123	2.47	2.41	97.6
Std A3	0.132	2.47	2.59	105
Std B3 (low curve)	0.230	4.88	4.50	92.2
Std B3 (low curve)	0.286	4.88	5.59	114
Std B3 (low curve)	0.233	4.88	4.56	93.3

**Table 11 (Continued).**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std B3 (low curve)	0.259	5.02	5.09	101
Std B3 (low curve)	0.245	5.02	4.82	96.0
Std B3 (low curve)	0.270	5.02	5.30	106
Std B3 (high curve)	0.230	4.88	4.63	94.9
Std B3 (high curve)	0.286	4.88	5.62	115
Std B3 (high curve)	0.233	4.88	4.68	95.9

**Table 11 (Continued).**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std B3 (high curve)	0.259	5.02	5.24	104
Std B3 (high curve)	0.245	5.02	5.00	100
Std B3 (high curve)	0.270	5.02	5.42	108
Std A4	0.485	9.41	9.14	97.1
Std A4	0.548	9.41	10.3	109
Std A4	0.482	9.41	9.10	96.7
Std A4	0.527	9.98	9.88	99.0
Std A4	0.501	9.98	9.44	94.6
Std A4	0.553	9.98	10.3	104

**Table 11 (Continued).**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Calculated Conc. (µg/ml)</b>	<b>Accuracy (%)</b>
Std B4	1.34	24.9	24.3	97.5
Std B4	1.52	24.9	27.5	110
Std B4	1.10	24.9	20.0	80.4
Std B4	1.44	25.6	25.7	100
Std B4	1.40	25.6	25.1	98.2
Std B4	1.48	25.6	26.4	103
Std A5	2.39	47.4	43.0	90.6
Std A5	2.85	47.4	51.1	108
Std A5	2.58	47.4	46.2	97.5

**Table 11 (Continued).**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Calculated Conc. (µg/ml)</b>	<b>Accuracy (%)</b>
Std A5	2.72	49.1	48.0	97.8
Std A5	2.62	49.1	46.3	94.3
Std A5	2.83	49.1	50.0	102
Std B5	5.30	102	94.5	93.0
Std B5	6.54	102	116	115
Std B5	5.86	102	104	103
Std B5	5.73	102	100	98.5
Std B5	5.27	102	92.3	90.5
Std B5	5.93	102	104	102

**Table 11 (Continued).**  
**Concentration of DIPE in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A6	13.5	244	240	98.4
Std A6	14.5	244	258	106
Std A6	12.9	244	230	94
Std A6	14.6	250	255	102
Std A6	14.2	250	247	98.8
Std A6	14.9	250	260	104

**Table 12.**  
**Concentration of Isopropanol in Calibration Standards**

Standard	Internal Standard Ratio	Nominal Conc. ( $\mu\text{g/ml}$ )	Calculated Conc. ( $\mu\text{g/ml}$ )	Accuracy (%)
Std A3	0.0754	2.28	2.67	117
Std A3	0.0774	2.28	2.72	119
Std A3	0.0777	2.28	2.73	120
Std A3	0.116	2.43	3.38	139
Std A3	0.0806	2.43	2.57	106
Std A3	0.0868	2.43	2.71	112
Std B3	0.159	4.65	4.62	99.4
Std B3	0.166	4.65	4.78	103
Std B3	0.165	4.65	4.74	102

**Table 12 (Continued).**  
**Concentration of Isopropanol in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std B3	0.201	4.94	5.33	108
Std B3	0.170	4.94	4.63	93.6
Std B3	0.179	4.94	4.82	97.5
Std A4	0.328	9.33	8.54	91.6
Std A4	0.339	9.33	8.79	94.2
Std A4	0.338	9.33	8.76	93.9
Std A4	0.390	9.86	9.65	97.9
Std A4	0.389	9.86	9.63	97.6
Std A4	0.364	9.86	9.06	91.9

**Table 12 (Continued).**  
**Concentration of Isopropanol in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std B4	0.879	23.7	21.4	90.1
Std B4	0.917	23.7	22.2	93.8
Std B4	0.915	23.7	22.2	93.6
Std B4	0.968	25.1	22.9	91.1
Std B4	0.960	25.1	22.7	90.5
Std B4	1.00	25.1	23.6	94.1
Std A5	1.82	47.1	43.1	91.6
Std A5	1.88	47.1	44.6	94.6
Std A5	1.88	47.1	44.6	94.8

**Table 12 (Continued).**  
**Concentration of Isopropanol in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A5	1.94	48.5	45.1	93.1
Std A5	1.89	48.5	44.0	90.8
Std A5	1.99	48.5	46.2	95.2
Std B5	3.84	96.7	90.2	93.3
Std B5	4.12	96.7	96.7	100
Std B5	4.08	96.7	95.8	99.0
Std B5	4.15	100	95.8	95.6
Std B5	4.18	100	96.5	96.3
Std B5	4.35	100	100	100

**Table 12 (Continued).**  
**Concentration of Isopropanol in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A6	10.3	242	240	99
Std A6	10.8	242	252	104
Std A6	11.0	242	256	106
Std A6	11.0	247	253	102
Std A6	10.8	247	247	100
Std A6	11.5	247	263	107

**Table 13.**  
**Concentration of Acetone in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std B2	0.0294	0.974	1.08	111
Std B2	0.0291	0.974	1.07	110
Std B2	0.0281	0.974	1.03	106
Std A3	0.0603	2.42	2.41	100
Std A3	0.0603	2.42	2.41	100
Std A3	0.0642	2.42	2.58	106
Std B3	0.103	4.66	4.58	98.3
Std B3	0.115	4.66	5.10	109
Std B3	0.113	4.66	5.02	108

**Table 13 (Continued).**  
**Concentration of Acetone in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Calculated Conc. (µg/ml)</b>	<b>Accuracy (%)</b>
Std B3	0.113	4.92	4.67	94.9
Std B3	0.114	4.92	4.72	96.0
Std B3	0.120	4.92	4.95	101
Std A4	0.203	9.14	8.86	96.9
Std A4	0.214	9.14	9.31	102
Std A4	0.214	9.14	9.32	102
Std A4	0.221	9.82	9.32	94.9
Std A4	0.232	9.82	9.76	99.4
Std A4	0.228	9.82	9.59	97.7

**Table 13 (Continued).**  
**Concentration of Acetone in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Calculated Conc. (µg/ml)</b>	<b>Accuracy (%)</b>
Std B4	0.522	23.7	22.5	95.1
Std B4	0.553	23.7	23.9	101
Std B4	0.531	23.7	22.9	96.7
Std B4	0.549	25.0	23.3	93.4
Std B4	0.571	25.0	24.3	97.1
Std B4	0.588	25.0	25.0	100
Std A5	0.980	46.1	42.1	91.4
Std A5	1.09	46.1	46.9	102
Std A5	1.05	46.1	45.0	97.6

**Table 13 (Continued).**  
**Concentration of Acetone in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A5	1.08	48.3	46.0	95.3
Std A5	1.09	48.3	46.4	96.0
Std A5	1.12	48.3	47.7	98.8
Std B5	2.10	96.9	90.0	92.9
Std B5	2.35	96.9	101	104
Std B5	2.26	96.9	96.8	99.9
Std B5	2.27	99.7	97.1	97.4
Std B5	2.31	99.7	98.8	99.1
Std B5	2.37	99.7	101	102

**Table 13 (Continued).**  
**Concentration of Acetone in Calibration Standards**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Calculated Conc. (<math>\mu\text{g/ml}</math>)</b>	<b>Accuracy (%)</b>
Std A6	5.34	237	229	96.5
Std A6	5.74	237	246	104
Std A6	5.71	237	245	103
Std A6	5.72	246	245	100
Std A6	5.69	246	244	99.0
Std A6	6.03	246	258	105

**Table 14.**  
**Limit of Quantitation Determination Results for DIPE in Blood**

<b>Standard</b>	<b>Internal Standard Ratio</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Calculated Conc. (µg/ml)</b>	<b>S.D.</b>	<b>% RSD</b>	<b>Mean Calc. Conc. (µg/ml)</b>	<b>Mean Accuracy (%)</b>
Std A1	0.00450	0.0887	0.0882	0.00990	11.0	0.0899	101
Std A1	0.00521	0.0887	0.102				
Std A1	0.00419	0.0887	0.0821				
LLQC	0.00417	0.0887	0.0817				
LLQC	0.00525	0.0887	0.103				
LLQC	0.00423	0.0887	0.0829				

**Table 15.**  
**Precision and Accuracy Determination Results for DIPE in Blood**

Standard	Internal Standard Ratio	Nominal Conc. (µg/ml)	Calculated Conc. (µg/ml)	S.D.	% RSD	Mean Calc. Conc. (µg/ml)	Mean Accuracy (%)
Std A2	0.0201	0.451	0.392	0.0426	9.91	0.430	95.4
Std A2	0.0247	0.451	0.483				
Std A2	0.0214	0.451	0.419				
LQC	0.0201	0.451	0.393				
LQC	0.0248	0.451	0.484				
LQC	0.0210	0.451	0.410				
Std B3	0.230	4.88	4.50	0.524	10.8	4.86	99.7
Std B3	0.286	4.88	5.59				
Std B3	0.233	4.88	4.56				
MQC	0.230	4.88	4.49				
MQC	0.281	4.88	5.49				
MQC	0.234	4.88	4.57				
Std B5	5.30	102	94.5	8.17	7.92	103	102
Std B5	6.54	102	116				
Std B5	5.86	102	104				
HQC	5.37	102	95.8				
HQC	6.03	102	107				
HQC	5.64	102	100.6				

**Table 16.**  
**Stability of DIPE Concentration in Blood Samples Stored at Room Temperature**

<b>Analysis Timepoint</b>	<b>Nominal Conc. (µg/ml)</b>	<b>Measured Conc. (µg/ml)</b>	<b>Mean Measured Conc. (µg/ml)</b>	<b>SD</b>	<b>%RSD</b>	<b>Percent of Time 0 Conc. (%)</b>
Time 0	0.451	0.428 0.420 0.413	0.421	0.00737	1.75	n/a
	102	101.2 99.9 93.5	98.2	4.10	4.17	n/a
8 h	0.451	0.384 0.372 0.374	0.377	0.00610	1.62	89.6
	102	88.8 90.1 89.6	89.5	0.692	0.773	91.1

**Table 17.**  
**Stability of DIPE Concentration in Blood Samples Stored at Approximately 4 °C**

Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%RSD	Percent of Time 0 Conc. (%)
Time 0	0.495	0.447 0.428 0.445	0.440	0.0106	2.40	n/a
	102	93.6 94.7 94.4	94.2	0.575	0.610	n/a
16 h	0.495	0.393 0.366 0.379	0.379	0.0137	3.62	86.2
	102	79.7 81.4 87.4	82.9	4.05	4.89	87.9
24 h	0.495	0.378 0.382 0.388	0.383	0.00514	1.34	87.0
	102	79.6 79.4 72.2	77.1	4.21	5.46	81.8

**Table 18.****Stability of Isopropanol Concentration in Blood Samples Stored at Room Temperature**

Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%RSD	Percent of Time 0 Conc. (%)
Time 0	96.7	96.1	95.1	1.08	1.13	n/a
		95.3				
		94.0				
8 h	96.7	94.2	93.1	1.00	1.07	97.8
		92.3				
		92.7				

**Table 19.****Stability of Isopropanol Concentration in Blood Samples Stored at Approximately 4 °C**

Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%RSD	Percent of Time 0 Conc. (%)
Time 0	100	96.1	97.0	0.392	0.404	n/a
		97.4				
		96.9				
16 h	100	95.1	99.8	7.99	8.01	103
		95.2				
		109				
24 h	100	95.8	95.8	0.368	0.384	98.8
		96.2				
		95.5				

**Table 20.****Stability of Acetone Concentration in Blood Samples Stored at Room Temperature**

Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%RSD	Percent of Time 0 Conc. (%)
Time 0	96.9	96.1	94.8	1.93	2.03	n/a
		95.7				
		92.5				
8 h	96.9	91.1	90.0	0.999	1.11	95.0
		89.2				
		89.7				

**Table 21.****Stability of Acetone Concentration in Blood Samples Stored at Approximately 4 °C**

Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%RSD	Percent of Time 0 Conc. (%)
Time 0	99.7	96.1	96.5	0.392	0.406	n/a
		96.8				
		96.7				
16 h	99.7	94.7	98.5	5.43	5.51	102
		96.2				
		105				
24 h	99.7	92.8	90.8	1.79	1.97	94.1
		90.4				
		89.3				

**Table 22.**  
**Concentration of DIPE Calibration Spiking Solutions Stored at Approximately -20 °C**

Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%RSD	Percent of Day 0 Conc. (%)
Day 0	4.51	4.40	4.39	0.00501	1.14	n/a
		4.44				
		4.34				
Day 0	46.8	46.0	46.8	0.702	1.50	n/a
		47.4				
		46.9				
7 Days	4.51	3.86	3.77	0.0827	2.19	85.9
		3.75				
		3.70				
7 Days	46.8	45.5	45.3	0.204	0.451	96.7
		45.3				
		45.1				

**Table 23.**  
**Concentration of DIPE Calibration Spiking Solutions Stored on Wet Ice**

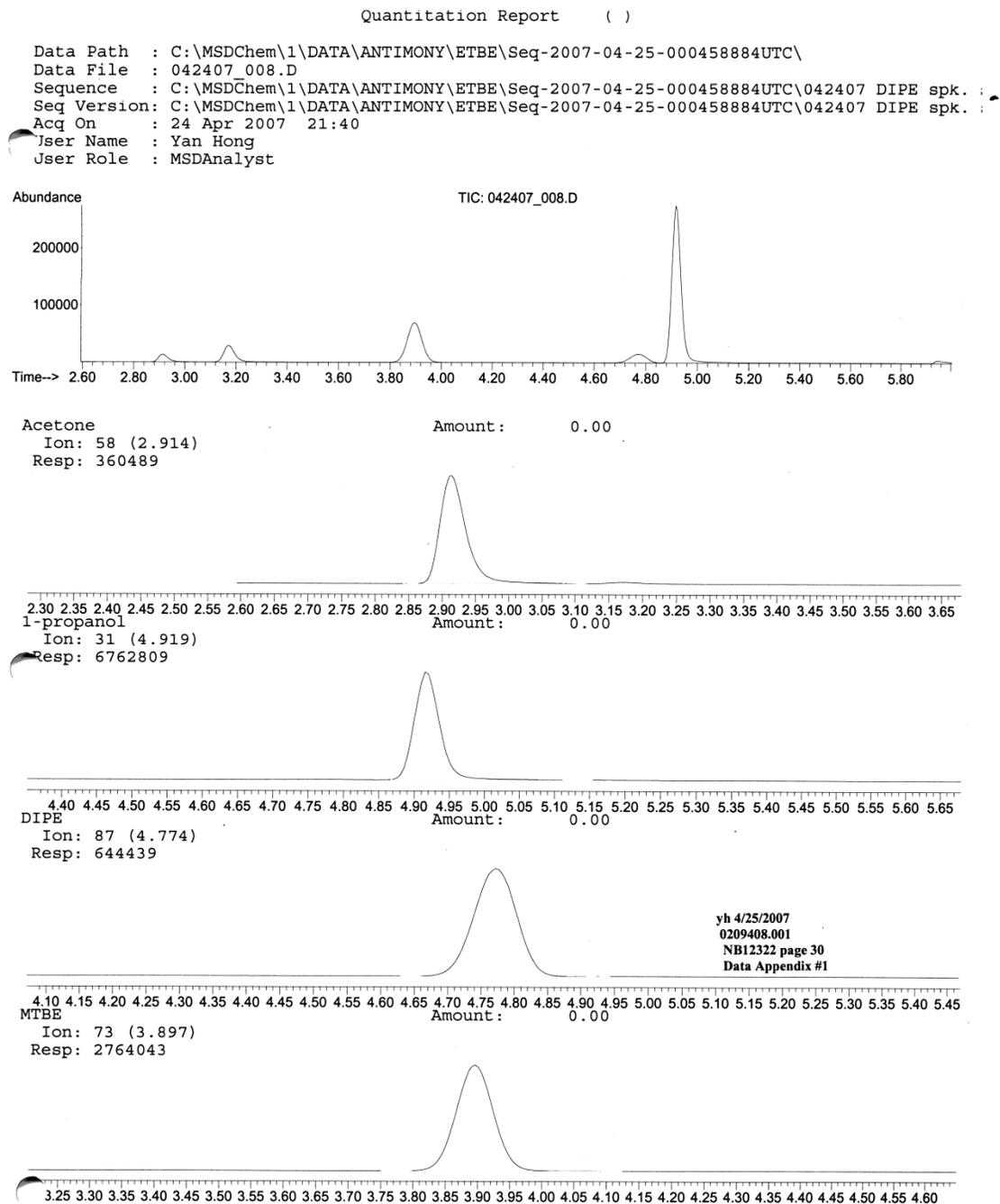
Analysis Timepoint	Nominal Conc. (µg/ml)	Measured Conc. (µg/ml)	Mean Measured Conc. (µg/ml)	SD	%CV	Percent of Day 0 Conc. (%)
Time 0	4.95	5.00	4.89	0.103	2.10	n/a
		4.86				
		4.80				
Time 0	50.2	50.3	49.4	0.742	1.50	n/a
		49.0				
		49.0				
6 h	4.95	5.04	5.05	0.0145	0.287	103
		5.07				
		5.05				
6 h	50.2	49.1	48.8	1.73	3.55	98.8
		50.3				
		46.9				

**Table 24.**  
**Concentration of Internal Standard Solution Stored at Approximately -20 °C**

Analysis Timepoint	Internal Standard	Isopropanol Conc. (µg/ml)	Peak Area Ratio (vs Isopropanol)	Mean Peak Area Ratio	SD	%RSD	Percent of Day 0 Peak Area Ratio (%)
Day 0	MTBE	967	0.122	0.113	0.00749	6.63	n/a
			0.110				
			0.108				
Day 0	1-Propanol	967	0.293	0.289	0.00327	1.13	n/a
			0.286				
			0.288				
7 Days	MTBE	967	0.106	0.108	0.00252	2.32	95.9
			0.108				
			0.111				
7 Days	1-Propanol	967	0.260	0.267	0.00569	2.13	92.1
			0.270				
			0.269				

**Table 25.**  
**Concentration of Internal Standard Solution Stored on Wet Ice**

Analysis Timepoint	Internal Standard	Isopropanol Conc. (µg/ml)	Peak Area Ratio (vs Isopropanol)	Mean Peak Area Ratio	SD	%RSD	Percent of Day 0 Peak Area Ratio (%)
Time 0	MTBE	1002	0.104	0.106	0.00161	1.52	n/a
			0.107				
			0.106				
Time 0	1-Propanol	1002	0.272	0.271	0.00133	0.491	n/a
			0.269				
			0.271				
6 h	MTBE	1002	0.103	0.104	0.000833	0.805	98.0
			0.104				
			0.104				
6 h	1-Propanol	1002	0.269	0.267	0.00357	1.34	98.6
			0.263				
			0.269				

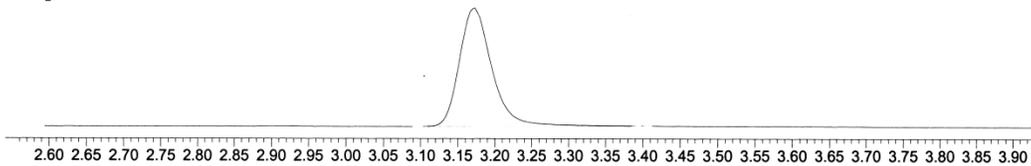
**Figure 1. Representative Total Ion Chromatogram and Single Ion Chromatograms Obtained During Analysis of a DIPE Standard**

**Figure 1 (Continued). Representative Total Ion Chromatogram and Single-Ion Chromatograms  
Obtained During Analysis of a DIPE Standard**

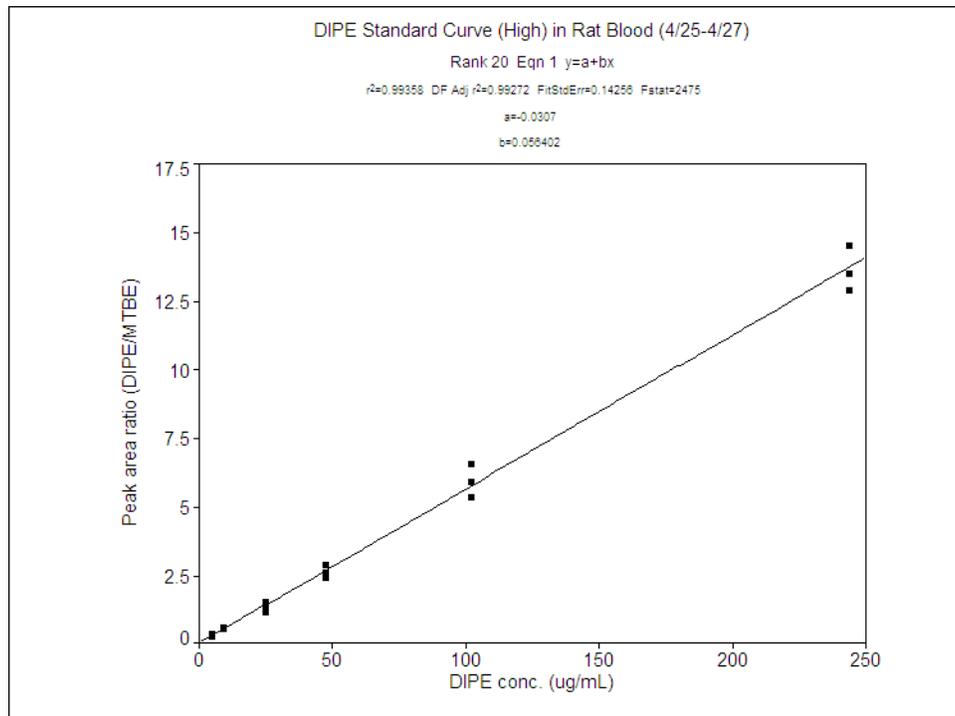
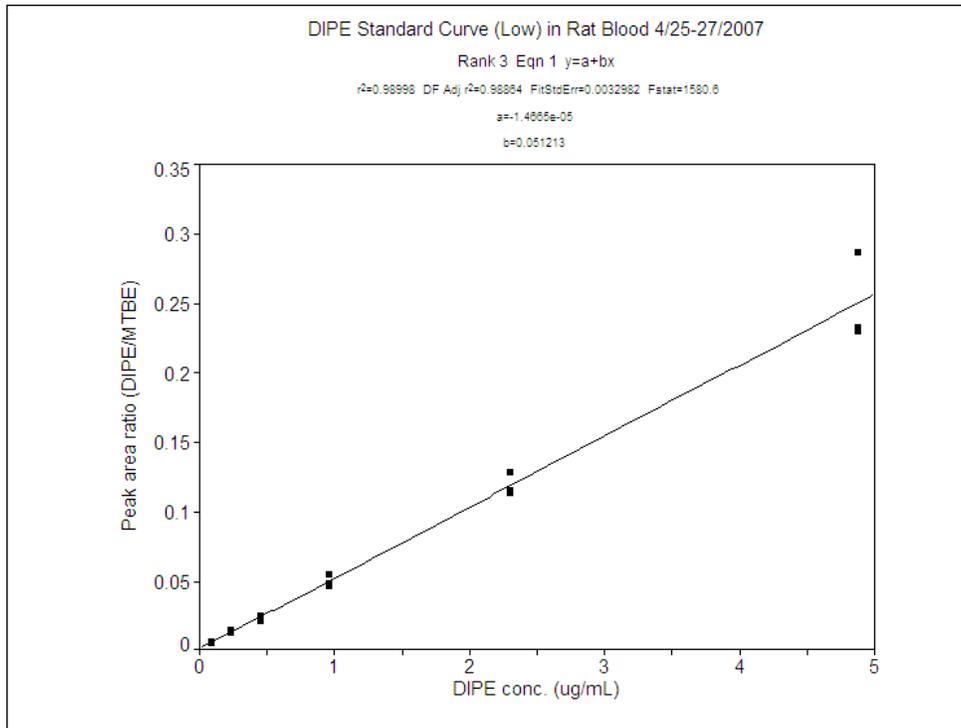
## Quantitation Report ( )

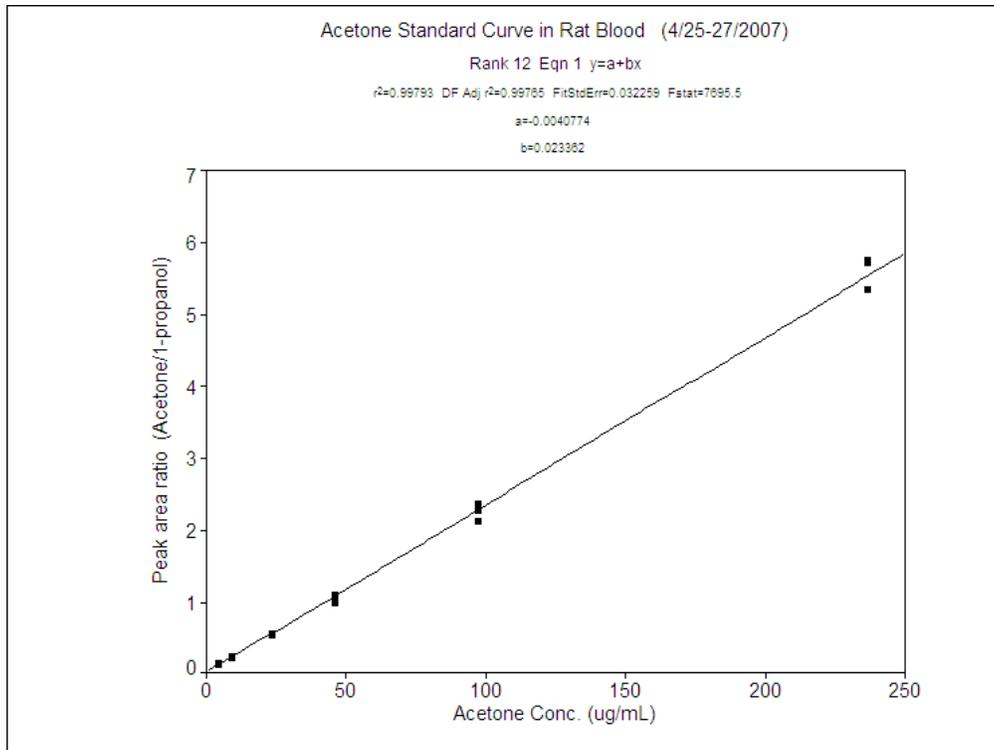
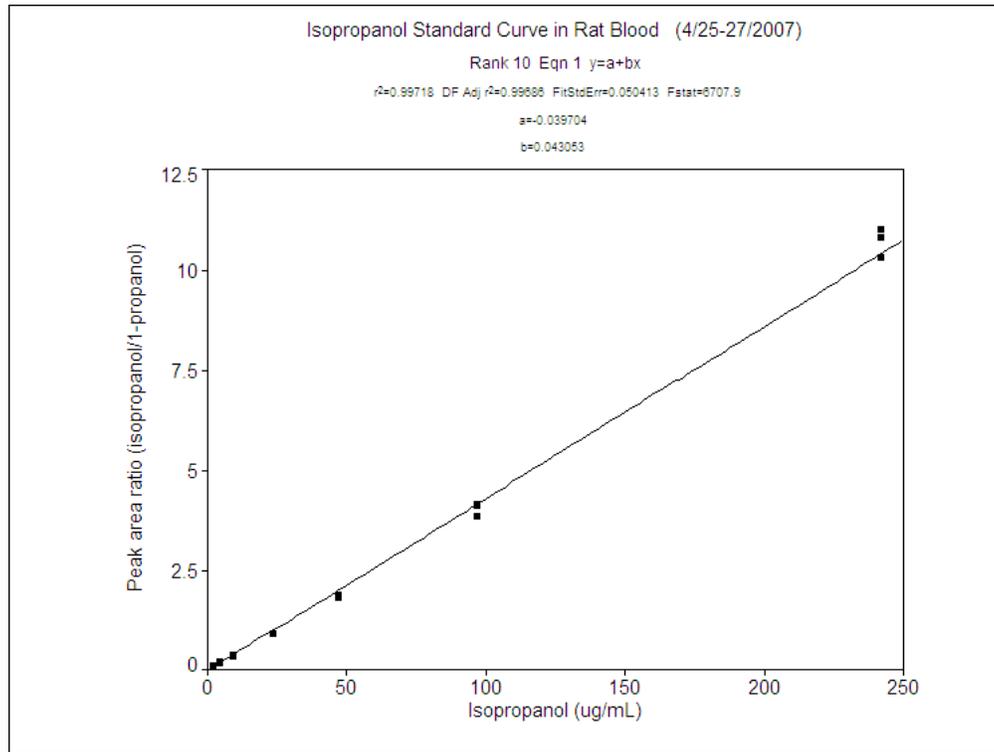
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Data File : 042407\_008.D  
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Seq Version: C:\MSDCHEM\1\DATA\ANTIMONY\ETBE\Seq-2007-04-25-000458884UTC\042407 DIPE spk. :  
Acq On : 24 Apr 2007 21:40  
User Name : Yan Hong  
User Role : MSDAnalyst

Isopropanol Amount: 0.00  
Ion: 45 (3.173)  
Resp: 877366



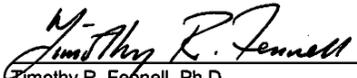
yh 4/25/2007  
0209408.001  
NB12322 page 30  
Data Appendix #1

**Figure 2. DIPE Standard Calibration Curves**

**Figure 3. Isopropanol and Acetone Standard Calibration Curves**

# Appendix 1

## Method Validation Plan

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<b>TITLE: DIISOPROPYL ETHER BIOANALYTICAL METHOD VALIDATION</b>		
<b>SPONSOR:</b>	Section 211(b) Research Group American Petroleum Institute 1220 L Street NW Washington, DC 20005	
<b>TESTING FACILITY:</b>	Science and Engineering RTI International* Post Office Box 12194 Research Triangle Park, NC 27709	
<b>RTI PROJECT NO.:</b>	0209408.001	
<b>RTI STUDY DIRECTOR:</b>	Timothy R. Fennell	
<b>APPROVAL SIGNATURES:</b>		
	 Timothy R. Fennell, Ph.D. Study Director, RTI	<u>04-24-07</u> Date
<small>*RTI International is a tradename of Research Triangle Institute.</small>		

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<p><b>1.0 INTRODUCTION</b></p> <p>This document details components and acceptance criteria for validating a method designed to determine the concentration of Diisopropyl Ether (DIPE) in rat blood. Analysis of all samples, standards, and quality control samples will be performed using gas chromatography-mass spectrometry (GC/MS) coupled with headspace sample introduction. The primary focus of this validation is the quantitation of DIPE in the blood matrix. However, the analytical method incorporates acetone and isopropanol in the calibration standards in order to measure amounts of these compounds in blood samples. These two analytes are both potential metabolites of DIPE, but may arise from other endogenous sources. Acetone is known to be present as an endogenous metabolite in blood with the potential for a high and variable background. The methodology for quantitation of acetone and isopropanol in the blood samples will be validated to the extent possible. However, it is expected that that background levels of these compounds will vary significantly, and will constrain the limit of quantitation, accuracy, and precision of the method for acetone and isopropanol. In the event that the validation criteria are met for DIPE, but not for acetone and isopropanol, the method will be considered suitable for analysis of DIPE in blood.</p> <p><b>2.0 PROJECT SPECIFIC ANALYTICAL METHOD (PSAM)</b></p> <ul style="list-style-type: none"> <li>• A detailed project specific analytical method (PSAM) describing the analysis method will be written prior to validating the method. The analytical method will contain procedures for quantitating DIPE, acetone, and isopropanol in blood samples.</li> <li>• Included in the PSAM will be a statement of the objective of the analytical method, a listing of necessary reagents, test solutions and mixtures with directions for their preparation, specified storage conditions and usable shelf life. It will also contain a listing of the required instrumentation, instrumental parameters and a step-by-step description of the entire analytical procedure.</li> </ul> <p><b>3.0 LOQ-</b></p> <ul style="list-style-type: none"> <li>• <b>Definition:</b> The limit of quantitation (LOQ) is the lowest concentration of analyte in a sample that can be determined with acceptable accuracy and precision under the stated experimental conditions. The response at the LOQ is at least 5 times the response compared to blank response.</li> <li>• <b>Raw data required:</b> The LOQ will be established for DIPE by analyzing at least five replicate blood samples (LLQC samples) at the lowest DIPE standard concentration. Lower limit of quantitation for acetone and isopropanol will be estimated per assay with the three replicate standard curve points per assay. Background levels of these compounds are expected to</li> </ul>		

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<p>change from animal to animal preventing establishment of a single LOQ that can be used over numerous assays.</p> <ul style="list-style-type: none"> <li>• <b>Acceptance specifications:</b> For DIPE, the mean value must be within <math>\pm 20\%</math> of the actual value. The precision around the mean value must not exceed 20% CV. For isopropanol and acetone, the lowest standard concentration meeting these criteria per assay will be utilized as the LOQ.</li> </ul> <p><b>4.0 QUALITY CONTROL STANDARDS</b></p> <ul style="list-style-type: none"> <li>• <b>Definition:</b> A spiked sample used to monitor the performance of a bioanalytical method to assess the integrity and validity of the results of the unknown samples analyzed in an individual batch.</li> <li>• <b>Raw data required:</b> Quality control samples (QCSs), containing DIPE, will be prepared in blood at a minimum of three concentrations (LQC, MQC and HQC). For DIPE, LQC will be near the LOQ, MQC will be near the center, and HQC will be near the upper limit of the standard curves. The QC samples will be prepared in duplicate at each concentration, and they will be analyzed within each sample batch. Acetone and isopropanol will be present in the QC samples only to provide an estimate of the performance of the method in regard to these analytes.</li> <li>• <b>Acceptance specifications:</b> Measured DIPE concentrations for at least 4 out of 6 of QCSs must fall within 15% of the nominal value of DIPE. The two QC samples determined to exceed 15% of nominal concentration must not be at the same concentration. No acceptance criteria are set for analytes of acetone and isopropanol.</li> </ul> <p><b>5.0 ACCURACY AND PRECISION</b></p> <ul style="list-style-type: none"> <li>• <b>Definition:</b> The accuracy of an analytical method is the closeness of test results obtained by that method to the nominal or true value. The Precision is a measure of the degree of reproducibility of the analytical method under normal operating circumstances.</li> <li>• <b>Raw data required:</b> Determination of accuracy and precision for DIPE will be accomplished by analyzing replicate sets of known DIPE concentration in the blood matrix. At a minimum, accuracy and precision of DIPE will be determined using three replicate samples at the three concentrations (LQC, MQC and HQC). Accuracy and precision per QC concentrations will then be calculated using these three replicates in addition to the three replicate standards</li> </ul>		

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<p>used to construct the calibration curve. Accuracy and precision for the lowest point, the LLQC, will be determined as described in section 1.2.</p> <ul style="list-style-type: none"> <li>• <b>Acceptance specifications:</b> For DIPE, the mean value for the MQC and HQC sets must be within <math>\pm 15\%</math> of the nominal value. The precision around the mean value must not exceed 15% coefficient of variation (CV). No acceptance criteria are set for analytes of acetone and isopropanol.</li> </ul> <p><b>6.0 STANDARD CURVE</b></p> <ul style="list-style-type: none"> <li>• <b>Definition:</b> A standard curve is the relationship between instrument response and known concentration of the analyte.</li> <li>• <b>Raw data required:</b> <ol style="list-style-type: none"> <li>1. A minimum number of six standards, consisting of DIPE, acetone and isopropanol, will define the relationship between concentration and response, respectively.</li> <li>2. A standard curve will be prepared by spiking rat blood with known concentration of DIPE, acetone and isopropanol and a consistent amount of the internal standard mixture of MTBE and 1-propanol.</li> <li>3. Standard concentration and curve range will be determined based on the requirement of the study.</li> <li>4. A standard curve must consist of a blank sample (matrix sample processed without internal standard), a zero sample (matrix sample processed with internal standard), and minimum of six non-zero samples covering the expected range, including LOQ. Standard curve ranges for acetone and isopropanol will be determined per assay during and after validation. For these analytes, standard points will be included in the curves starting from the most concentrated standard to the least concentrated standard until the acceptance criteria detailed below for linear correlation coefficient and accuracy fall within 20% for the lowest point.</li> <li>5. Regression equations, describing the concentration and response relationship of DIPE, acetone and isopropanol, will be determined.</li> <li>6. <b>Acceptance specifications:</b> The linear regression correlation coefficient ( <math>r</math> ) must be greater than or equal to 0.990. For DIPE, calculated concentrations of individual standards must be within <math>\pm 15\%</math> of the nominal value for at least 75% of the calibration standards, except the lowest concentration point (LOQ) which must be <math>\pm 20\%</math>.</li> </ol> </li> </ul>		

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<p><b>7.0 STABILITY</b></p> <ul style="list-style-type: none"> <li>• <b>Definition:</b> The chemical stability of an analyte in a given matrix under specific conditions for specified time intervals. Stability procedures will evaluate the stability of the analyte during sample handling and short-term storage.</li> <li>• <b>Raw data required for stability:</b> To demonstrate storage stability for DIPE, a minimum of three determinations at two concentrations (LQC and HQC) will be examined. If possible, acetone and isopropanol will also be examined.</li> <li>• <b>Expected sample collection and sample storage intervals during studies:</b> During animal studies, rat blood samples will be drawn from the jugular vein cannula into a heparinized 1 ml syringe, and the sample will be immediately placed in a preweighed headspace vial that will be immediately crimped. If cannulas fail between the 8 and 24 hr blood samples, blood will be collected by cardiac puncture under CO<sub>2</sub> anesthesia at sacrifice at 24 hr. The blood samples will be maintained at room temperature until analyzed. It is anticipated that blood samples will be analyzed by GC within 24 hours of collection.</li> <li>• <b>DIPE, acetone and isopropanol in blood:</b> Analyte stability will be determined by preparing four sample sets (a sample set will consist of three replicates at two concentrations). One set of samples will be analyzed immediately. The second set will be stored at room temperature for at least 8 hours before analysis. The third set will be stored at ca. 4° C for 16 hours before analysis. The fourth set will be stored at ca. 4° C for 24 hours before analysis. Stability sample analyses will include calibration standards, stability samples, and QC samples.</li> <li>• <b>Spiking solution stability (DIPE, acetone and isopropanol):</b> The stability of DIPE, acetone, and isopropanol concentrations in spiking solutions stored at on ice for 6 hours, and at ca -20° C for 7 days will be determined through analysis by headspace GC/MS. A minimum of two spiking solutions at the low (LQC) and medium (MQC) concentrations will be analyzed in three replicates per concentration with a set of calibration standards for quantitation. The 18-point calibration curve will consist of triplicate standard analyses per standard concentration. Each standard will be created from freshly prepared spiking solution. The curve will be constructed by plotting the DIPE to MTBE internal standard ratio versus spiking solution concentration, or acetone or isopropanol to 1-propanol internal standard ratio versus spiking solution concentration. For each sample or standard, 10 µl of spiking solution will be added into a headspace vial and the vial will be crimp-sealed; then 10 µl of the multi internal standard solution will be injected into the vial through septum using a 10-µl syringe.</li> </ul>		

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<p>In addition, the stability of the internal standard solution will be evaluated by comparing the relative ratio of internal standards (MTBE and 1-propanol) with storage.</p> <ul style="list-style-type: none"> <li>• <b>Internal standard solution stability</b> The stability of MTBE and 1-propanol concentrations in internal standard spiking solution stored in ice for 6 h and at ca -20° C for 7 days will be determined through analysis by headspace GC/MS. The stability of internal standard solutions stored at -20 °C will be determined by analyzing three sets (triplicate samples per set) of spiking solution at the HQC concentration before and after storage of the internal standard solution. At each timepoint, three 10 µL aliquots of the HQC spiking solution will be sealed in individual headspace vials. Then, 10 µL of the internal standard solution will be injected into each vial using a 10-µL syringe. At the zero timepoint, freshly prepared internal standard solution will be added to the first set of spiking solution samples, and this first set will be analyzed immediately. An aliquot of the freshly prepared internal standard solution will then be stored for 7 days at -20 °C. At the 7 day timepoint, the second set of samples will be created using the stored internal standard solution and stored spiking solution, and the second set will be analyzed. A third set of samples will be stored in ice for 6 hours and analyzed to show stability of the internal standard solutions during use. The internal standard ratios for Isopropanol/MTBE and Isopropanol/1-propanol at 7 days will be calculated for each sample, and the ratios calculated for the 6 h and 7 day timepoints will be compared to the ratios at the respective zero timepoint.</li> <li>• <b>Acceptance specifications:</b> The mean value determined for each concentration level of DIPE must be within ±15% of its initial analyzed concentration. The precision around the mean value must not exceed 15% CV. Similar calculations will be performed for acetone and isopropanol. For internal standard stability, the internal standard ratios for isopropanol/MTBE and Isopropanol/1-Propanol must be within ±10% of the zero timepoint values.</li> </ul> <p><b>8.0 PREPARATION AND ANALYSIS ORDER</b></p> <ul style="list-style-type: none"> <li>• <b>Sample and standard preparation</b> <ol style="list-style-type: none"> <li>1 Prepare three sets of calibration standards. Along with each set of calibration standards, prepare one set of QC samples including the LLQC, LQC, MQC and HQC concentrations. Store the calibration standards and QC samples in the refrigerator at approximately 4° C between preparation and analysis.</li> <li>2 Prepare four sets of the LQC and HQC concentrations in triplicate which will be analyzed as follows for determination of stability:</li> </ol> </li> </ul>		

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<p>a) Immediately following preparation,</p> <p>b) After storage at room temperature for 8 hours</p> <p>c) After storage at approximately 4° C for 16 hours</p> <p>d) After storage at approximately 4° C for 24 hours</p> <ul style="list-style-type: none"><li>• <b>Analysis order:</b> The prepared calibration curves will be stored at approximately -4 °C before analysis.<ol style="list-style-type: none"><li>1) Calibration standard set 1, including one set of QC samples.</li><li>2) Stability samples</li><li>3) Calibration standard set 2, including one set of QC samples.</li><li>4) Stability samples.</li><li>5) Calibration standard set 3, including one set of QC samples.</li></ol></li></ul> <p><b>9.0 INSTRUMENT VALIDATION</b></p> <ul style="list-style-type: none"><li>• Analysis of samples, standards and quality control samples will be performed using GC-MS instrumentation coupled with a headspace autosampler. The instrumentation has been previously validated with respect to the accuracy and reliability of generated data and computed results. Complete documentation of the system validation procedure will be available for inspection.</li></ul>		

## Appendix 2

### Analytical Method

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<b>TITLE:</b>	Project Specific Analytical Method for Analysis of Diisopropyl Ether in Blood	
<b>SOURCE:</b>	Science and Engineering	
<b>AUTHOR:</b>	Signed <u><i>Norman F. Gaudette Jr.</i></u> Norman F. Gaudette Jr.	
	Date <u>4-24-07</u>	
<b>APPROVED BY:</b>	Signed <u><i>Timothy R. Fennell</i></u> Timothy R. Fennell	
	Date <u>04-24-07</u>	
<b>EFFECTIVE DATE:</b>	<u>04-24-07</u> (This version)	

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<p><b>1.0 INTRODUCTION</b></p> <p>The methodology described in this document details procedures necessary for the quantitation of Diisopropyl Ether (DIPE), acetone and isopropanol in rat blood. This method is intended to be utilized for quantitation of DIPE in rat blood during the study "Metabolism, and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats: Pilot Study" under the RTI study protocol number RTI-934, and in subsequent studies on the pharmacokinetics of DIPE. In this method, blood samples are analyzed using Gas Chromatography coupled with Mass Spectrometry detection (GC/MS). A headspace autosampler is utilized for introduction of samples into the instrumentation. Methyl tertiary butyl ether (MTBE) and 1-propanol serve as internal standards. Procedures and other content of this method include preparation of calibration standards, analysis of standards and samples, regression analyses, acceptance criteria, and sample concentration calculation. The method is designed with standards ranging from 0.1 µg/ml to 250 µg/ml in blood for each of the analytes.</p> <p><b>2.0 REAGENTS AND CHEMICALS</b></p> <p><u>Diisopropyl ether</u>: 99% Sigma-Aldrich, St. Louis, MO. This test article lot was approved by the study sponsor, and it will be used for preparation of all standards and QC samples.</p> <p><u>Acetone</u>: Chromasolv for HPLC, Sigma-Aldrich, St. Louis, MO.</p> <p><u>Isopropanol</u>: Chromasolv for HPLC 99.8%, Sigma-Aldrich, St. Louis, MO.</p> <p><u>Methyl Tertiary Butyl Ether</u>: 99.9%, HPLC, Sigma-Aldrich, St. Louis, MO. This compound serves as the internal standard for diisopropyl ether.</p> <p><u>1-Propanol</u>: Chromasolv for HPLC, 99.9% Aldrich, Sigma-Aldrich, St. Louis, MO. This compound serves as the internal standard for acetone and isopropanol.</p> <p><u>N, N-dimethylformamide</u>: 99.8% A.C.S. Reagent, Aldrich, Sigma-Aldrich, St. Louis, MO.</p> <p><u>Distilled/deionized water</u>: Obtain this reagent from the Corning Still in Hermann 210.</p> <p><u>Helium</u>: 99.996% purity, used as the carrier gas in GC/MS instrumentation.</p> <p><b>3.0 EQUIPMENT</b></p> <p>Equipment necessary for completion of procedures in this method are detailed in the Table 1 below.</p>		

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Table 1 Equipment	
Item	Description
Electronic Balance	Four-place electronic balance, Mettler AG 204, or equivalent
Gas Chromatograph/Mass Spectrometer (GC/MS)	Agilent Model 6890 gas chromatograph equipped with an Agilent Model 5973 Mass Selective Detector (MSD), or equivalent
Headspace Autosampler interfaced to the GC/MS	Agilent G1888A headspace autosampler, or equivalent
Electronic Pipet (0.5—5mL)	Rainin Model LTS-0.5-5mL, or equivalent
Electronic Pipet (100 $\mu$ L)	Rainin Model LTS-100, or equivalent
Electronic Pipet (1000 $\mu$ L)	Rainin Model LTS-1000, or equivalent
Electronic Pipet (10 $\mu$ L)	Rainin Model LTS-10, or equivalent
Microsyringe (10 $\mu$ L)	Hamilton, Gastight #1801, or equivalent
Syringe (1 mL)	Hamilton, Gastight #1001, or equivalent
Headspace Vials	Agilent Technologies, 10 ml Flat bottom, or equivalent, silylated with hexamethyldisilazane using a vacuum oven at 225 °C, or equivalent silylation method.
Headspace Crimp Cap	Agilent Technologies, PTFE/Si sep, 20mm, or equivalent
Electronic Crimper	Agilent Technologies, 20mm, or equivalent

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<p><b>4.0 SOLUTION PREPARATION (STOCK, INTERNAL STANDARD, AND SPIKING)</b></p> <p><b>4.1 Analyte Stock Solutions Preparation</b></p> <p>Prepare two stock solutions containing each analyte (DIPE, Isopropanol, and acetone – six solutions in all) at a target concentration of 30000 µg/ml. Calculate the concentration of each stock solution in units of micrograms of analyte per milliliter (mL) of solution. Prepare each stock solution according to the following procedure:</p> <ol style="list-style-type: none"> <li>1) Fill a 10-ml volumetric flask with distilled/deionized water (for acetone and isopropanol) or dimethylformamide (for DIPE) to the volume mark. Consult Table 2 for the correct solvent. Using a pipet, remove the volume listed in Table 2 from the flask.</li> <li>2) With the flask on the balance pan, tare/zero the balance.</li> <li>3) Add the amount of analyte listed in Table 2 to the flask completing the 10-mL solution volume. Use an electronic pipet for transfer of isopropanol and acetone. Use the 1-mL syringe for transfer of DIPE.</li> <li>4) Record the mass of analyte solution added to the flask.</li> <li>5) Immediately place the volumetric flask on ice. Cool and mix the stock solution by hand.</li> <li>6) Calculate the actual concentration for two stock solutions per analyte.</li> <li>7) Prepare two multi-analyte stock solutions (Stock A and Stock B) containing all three analytes at a target concentration of 10000 µg/ml per analyte. Combine 1 mL of each analyte stock solution (1:1:1) in a tared vial to make each multi-analyte stock. Record the weight of each stock solution added. Cool and mix the stock solutions by hand. The dilution factor for each analyte is calculated by the equation:  (The weight of added stock/density)/(the total weight of multi-analyte stock)  The density for Isopropanol and acetone stock = 1 mg/mL;  The density for DIPE stock = 0.944 mg/mL.</li> </ol>		

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**Table 2.**  
**Preparation of DIPE, Isopropanol and Acetone Stock Solutions**

Analyte	Solvent	Removal Amount of Solvent ( $\mu\text{L}$ )	Addition amount of Analyte ( $\mu\text{L}$ )
DIPE	N, N-dimethylformamide	408	430
Isopropanol	Distilled/Deionized water	366	388
Acetone	Distilled/Deionized water	356	386

4.2 MTBE and 1-Propanol Internal Standard Solution

Two internal standards are utilized in this method. MTBE is used as the internal standard for quantitating DIPE, and the other internal standard 1-propanol is used for quantitation of acetone and isopropanol. Target concentrations of the internal standard stock solutions are 1 mg/mL and 10 mg/mL for MTBE and 1-propanol, respectively, in a single internal standard solution. Prepare two stock solutions as described below.

- 1) Prepare the MTBE stock
  - a. Fill a 10 mL volumetric flask with distilled/deionized water.
  - b. With the flask on the analytical balance, remove 12  $\mu\text{L}$  of water.
  - c. Tare/zero the balance and add 16  $\mu\text{L}$  of MTBE.
  - d. Record the weight of MTBE added and mix the flask contents.
  - e. Calculate the actual concentration of MTBE
- 2) Prepare the 1-Propanol stock.
  - a. Fill a 10 mL volumetric flask with distilled/deionized water.
  - b. With the flask on the analytical balance, remove 120  $\mu\text{L}$  of water.
  - c. Tare/zero the balance and add 130 $\mu\text{L}$  of 1-propanol.
  - d. Record the weight of 1-propanol added.

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<p>e. Mix the flask contents calculate the actual concentration of 1-propanol.</p> <p>3) Calculate the amount of each stock solution required for preparation of a 50 µg/ml of MTBE and 500 µg/ml of 1-Propanol in a 10-mL solution. Then, transfer the required amount of each MTBE and 1-propanol stock solution into a single 10-mL volumetric flask. Complete the dilution with distilled/deionized water. The resulting internal standard working solution should consist of 50 µg/ml of MTBE and 500 µg/ml of 1-Propanol.</p> <p>4) Aliquot the internal standard working solution into multiple vials and store aliquots at approximately -20 °C. This ensures an unopened solution is used for preparation of each set of calibration standards and QC samples.</p> <p>4.3 Preparation of DIPE, Isopropanol and Acetone Calibration Spiking Standards Prepare a set of eleven calibration spiking solutions encompassing the concentration range between 1 and 2500 µg/mL for DIPE, isopropanol and acetone. Adjacent spiking standard concentrations are prepared using different stock solutions. Prepare the spiking solutions in wet ice. Ensure water used for solution preparation is cooled in wet ice prior to use. Prepare the spiking calibration standards as described below in the step below. The amount of stock solution or standard solution, distilled/deionized water, and the target final volume required for preparation of calibration spiking solution for DIPE are shown in Table 3. Collect weights of added distilled/deionized water, stock or spiking standard solution, and total solution volumes where noted.</p> <p>1) Add cold distilled/deionized water into a tared vial, and record the weight of water added.</p> <p>2) Transfer the appropriate amount of stock or spiking standard solution into the vial. Record the weight after stock or spiking standard solution added. Total amount of solution = stock or spiking standard solution wt + water wt.</p> <p>3) Mix the spiking standard solution by hand.</p> <p>4) Calculate dilution factors for each spiking standard solution of three analytes by dividing the weight of stock or spiking standard solution by the total weight of solution.</p>		

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<p>5) Aliquot the spiking solutions into multiple vials and store the aliquots at approximately -20 °C when not in use. This ensures an unopened solution is used for preparation of each set of calibration standards and QC samples,</p>		

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<b>Table 3.</b> <b>Preparation of DIPE Spiking Standard and QC Solution</b>					
Spiking Standard Solution	Water Required (mL)	Stock or Spiking Solution required	Stock or Spiking Standard Aliquot Volume (mL)	Target Dilution Factor	Target Spiking Solution Conc. (µg/mL)
Spk. Std. A6	X	Stock A	0.900	1:4	2500
Spk. Std. B5 HQC	Y	Stock B	0.350	1:10	1000
Spk. Std. A5	3	Spk. Std. A6	0.75	1:5	500
Spk. Std. B4	3	Spk. Std. B5	1	1:4	250
Spk. Std. A4	3	Spk. Std. A5	0.75	1:5	100
Spk. Std. B3 (MQC)	3	Spk. Std. B4	0.75	1:5	50
Spk. Std. A3	3	Spk. Std. A4	1	1:4	25
Spk. Std. B2	3	Spk. Std. B3	0.75	1:5	10
Spk. Std. A2 (LQC)	3	Spk. Std. A3	0.75	1:5	5.0
Spk. Std. B1	3	Spk. Std. B2	1	1:4	2.5
Spk. Std. A1 (LLQC)	3	Spk. Std. A2	0.75	1:5	1.0
$X = [(Conc. Stock A \times 0.900 \text{ mL}) / (2500 \text{ } \mu\text{g/mL})] - 0.900 \text{ mL}$ $Y = [(Conc. Stock B \times 0.350 \text{ mL}) / 1000 \text{ } \mu\text{g/mL}] - 0.350 \text{ mL}$ <p>Note: Dilution factors shown in the table are targets for using pipets to perform the dilution. Weights of added water and total dilution weights must be collected for calculation of dilution factors by weight.</p>					

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<p><b>5.0 PREPARATION OF CALIBRATION STANDARDS AND QUALITY CONTROL SAMPLES</b></p> <p>A set of calibration standards consists of a blank sample (without internal standard), a zero sample (with internal standard), analyte standards, and QC Samples. Consult Table 4 for components and amounts when preparing each calibration standard. Use a new unopened set of calibration spiking solutions (stored in the freezer at -20 °C) and internal standard solution when preparing each set of calibration standards and QC samples.</p> <ol style="list-style-type: none"><li>1) Transfer 90 µl of blank blood into a 10-ml headspace vial.</li><li>2) Add 10 µl of the appropriate spiking standard solution to the vial.</li><li>3) Immediately crimp-seal the vial.</li><li>4) For all vials, except for the blank sample, add 10 µl of internal standard working solution to the vial through septum using a 10-µl microsyringe.</li></ol>		

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<p><b>Table 4.</b></p> <p><b>Calibration Standard Preparation</b></p>						
Standard ID	Spiking Solution ID	Volume of Rat Blood (µl)	Volume of Spiking Solution or water (µl)	Final Volume (µl)	Target Final concentration (µg/ml)	Volume of ISTD added (µl)
Blood Blank	Water	90	20 <sup>1</sup>	100	0	0
ISTD Blank	Water	90	10	100	0	10
Std A1 (LLQC)	A1	90	10	100	0.1	10
Std B1	B1	90	10	100	0.25	10
Std A2 (LQC)	A2	90	10	100	0.50	10
Std B2	B2	90	10	100	1.00	10
Std A3	A3	90	10	100	2.50	10
Std B3 (MQC)	B3	90	10	100	5.00	10
Std A4	A4	90	10	100	10.0	10
Std B4	B4	90	10	100	25.0	10
Std A5	A5	90	10	100	50.0	10
Std B5 (HQC)	B5	90	10	100	100	10
Std A6	A6	90	10	100	250	10
<p><sup>1</sup> Note: 20 µL distilled/deionized water is substituted for both volumes (10 µL each) of spiking solution and internal standard solution for this sample</p>						

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<p><b>6.0 PREPARATION OF STUDY SAMPLES FOR ANALYSIS</b></p> <p>Procedures for collection of blood samples from study animals are detailed in the appropriate study protocol. Therefore, the sample analysis procedure will not include sample collection procedures. It is expected that blood collection will occur in heparinized syringes. The procedure detailed below begins following removal of the blood sample from each study subject</p> <ol style="list-style-type: none"><li>1) Upon collection, immediately place the 100 <math>\mu</math>L blood sample in a pre-weighed 10-ml headspace vial.</li><li>2) Crimp-seal and weigh the filled vial to determine the weight of the blood collection.</li><li>3) Add 10 <math>\mu</math>l of the internal standard solution containing MTBE (50 <math>\mu</math>g/ml) and 1-propanol (500 <math>\mu</math>g/ml) by injection through the septum with a 10-<math>\mu</math>l microsyringe.</li><li>4) Analyze the samples according to Section 6.0. Storage of samples between preparation and analysis should not exceed the following limits:<ol style="list-style-type: none"><li>a. Room temperature for no longer than 8 hours,</li><li>b. Refrigerated (approximately 4 <math>^{\circ}</math>C) for no longer than 24 hours prior to loading in the headspace autosampler.</li></ol></li></ol> <p><b>7.0 ANALYSIS OF SAMPLES AND STANDARDS</b></p> <p>All samples and standards will be analyzed by GC/MS with injection of headspace from the sample and standard vials.</p> <p>7.1 Analysis Sequence</p> <ol style="list-style-type: none"><li>1) Daily Tune of the MS spectrometer (prior to sample analysis)</li><li>2) Chromatography system verification sample ( A spiking solution used for checking peak shape of analytes)</li><li>3) Calibration standard (Set 1)</li><li>4) Rat blood samples</li><li>5) Calibration standards (Set 2)</li></ol>		

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<p>6) Rat blood samples</p> <p>7) Calibration standards (Set 3)</p> <p>7.2 Analysis Parameters</p> <p>Instrumental and other analysis parameters are listed below in Table 5.</p>		

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<b>Table 5 Instrument Conditions</b>		
<b><u>6890 GC</u></b>		
Injection port	split/splitless	
Temperature	150 °C	
Split ratio	15:1 (calculated by adding flow rate of HSS )	
Carrier gas	Helium	
Flow rate	2.5 ml/min	
Column	DB-624 30m x 0.32 mm i.d. 1.8 um film thickness (J&W, Agilent technologies, Wilmington, DE)	
<b><u>GC oven program</u></b>		
Initial temperature	30 °C	
Initial time	2.5 min	
Temperature rate	5 °C/min	
Final temperature	35°C	
Final time	1.25 min	
Temperature rate A	90 °C/min	
Final temperature A	150 °C	
Run time	6.03 min	
<b><u>G1888A headspace sampler</u></b>		
Loop size	1 ml	
Vial Pressure	15 psig	
Carrier pressure	6.0 psig	
Headspace oven	65 °C	
loop temperature	90 °C	
Transfer line temperature	110 °C	
Equilibration time	10 min	
GC cycle time	10 min	
Pressurization	0.2 min	
Loop Fill	0.2 min	
Loop Equilibration	0.05 min	
Inject	0.5 min	
Shake	low	
<b><u>5973 MSD</u></b>		
SIM	DIPE(m/z 87); Isopropanol(m/z 45); Acetone (m/z 58); MTBE (m/z 73), 1-Propanol (31 m/z)	
Quad temperature	150 °C	
Source temperature	230°C	
Transfer line	230 °C	
Tune	Atune.u	
Solvent delay	2.5 min	

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<p><b>8.0 DATA PROCESSING AND CALCULATIONS</b></p> <p><b>8.1 Peak Integration</b></p> <p>Integrate all analyte peaks (DIPE, isopropanol, and acetone) and internal standard peaks (MTBE and 1-propanol) in the result chromatograms. Perform the following calculations using calibration standard assay results. When possible, use Microsoft Excel spreadsheets for the calculation.</p> <p>Note: The calculations performed within these spreadsheets must be verified before the results can be reported.</p> <p><b>8.2 Peak Area Ratios</b></p> <p>Compute the peak area ratio for each analyte using chromatographic results. Perform the calculation by dividing peak area for each analyte (DIPE, isopropanol, acetone) by the corresponding internal standard. The analytes and their corresponding internal standards are as follows: DIPE/MTBE, isopropanol/1-propanol, and acetone/1-propanol.</p> $\frac{\text{Analyte Peak Area}}{\text{Internal Standard Peak Area}}$ <p><b>8.3 Standard Curves</b></p> <p>Two standard curves are necessary for quantitation of DIPE over the entire concentration range detailed by this method. A single calibration curve is necessary for quantitation of isopropanol, and another single calibration curve is necessary for quantitating acetone.</p> <ol style="list-style-type: none"><li>1) For each analyte/internal standard pair (DIPE/MTBE, isopropanol/1-propanol, and acetone/1-propanol), generate separate plots of peak area ratio versus nominal standard concentration as described below. Use the program TableCurve (Systat Software Inc., Richmond Ca) to calculate the weighted linear regression equation (<math>Y=mx+b</math>) for each set of plotted calibration standards. Use a weighting of <math>1/x</math>. Note: During validation, six replicates are generated for analysis at specific standard concentrations of DIPE. In this case, use the first three replicates in each set of six for computing the regression equation. Concentration ranges for the plots are as follows:</li></ol>		

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<p>a. <u>DIPE/MTBE</u>: Generate two calibration curves. Use results from Standards A1, B1, A2, B2, A3, and B3 for the first curve ( ca. 0.1 µg/mL to ca. 5.0 µg/mL); Use results from Standards B3, A4, B4, A5, B5, and A6 for the second curve (ca. 5.0 µg/mL to ca. 250 µg/mL).</p> <p>b. <u>Isopropanol/1-propanol and acetone/1-propanol</u>: Generate a single calibration curve containing the six most concentrated standards. Use the regression equation and coefficients of slope and intercept to calculate the concentration and accuracy of each standard (as described in later steps. Determine if the linear correlation coefficient ( r ) and accuracy meet acceptance criteria in Section 9.1. If necessary, remove calibration points from the lowest portion of the curve and repeat the calculations in order to obtain an acceptable curve for acetone and isopropanol analytes.</p> <p>2) Compute the concentration of all calibration standards using the corresponding regression equations.</p> <p>3) Compute the accuracy (%) for each standard by dividing the calculated concentration by the nominal standard concentration for each analyte. Multiply the result by 100 to complete the calculation.</p> <p>4) Using the calculated concentrations for individual calibration standards at each level, determine the mean, standard deviation, and relative standard deviation (%RSD) for the triplicate standard concentration points.</p> <p>5) Compute the accuracy (%) of the method by dividing the mean calculated concentration for each standard concentration by the nominal standard concentration. Multiply the result by 100 to complete the calculation.</p> <p>6) For the method validation results, compute concentration, mean concentration, standard deviation, and relative standard deviation for each of the six replicates prepared at multiple concentrations (three calibration standards plus the three additional standards prepared per concentration) for DIPE. Also, calculate the mean accuracy value for each of the four QC concentrations (LLQC, LQC, MQC, and HQC). These results are used to establish accuracy and precision of the method during validation.</p>		

<b>Project Specific Analytical Method</b>	<b>RESEARCH TRIANGLE INSTITUTE POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709</b>	<b>AM-0209408.001.0</b>
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<p>8.4 Quality Control (QC) Samples</p> <ol style="list-style-type: none"> <li>1) Use the appropriate linear regression equation to calculate concentration DIPE in the QC samples.</li> <li>2) Divide the calculated concentration by the nominal concentration and multiply the result by 100 to obtain the Accuracy % value for DIPE.</li> </ol> <p>8.5 Blood Samples</p> <p>If calibration curves are acceptable according to criteria stated in Section 9.0, use the corresponding linear regression equation to calculate sample concentration. Use Microsoft Excel spreadsheets to perform these calculations. Use the weight of sample collected to correct for differences between the actual sample weight and the volume of blood used for generation of standards.</p> <p><b>9.0 ACCEPTANCE CRITERIA</b></p> <p>9.1 Linear Regression and Calibration Curve</p> <p>Acceptance criteria for the calibration curves are as follows:</p> <ol style="list-style-type: none"> <li>1) Correlation coefficient ( <math>r</math> ) must be greater than or equal to 0.990 for all four analytes.</li> <li>2) For DIPE, calculated concentrations of individual standards must be within <math>\pm 15\%</math> of the nominal value, except the lowest concentration point (LLQC) which must be <math>\pm 20\%</math>.</li> </ol> <p>9.2 QC Samples</p> <p>If QC samples are distributed throughout the sample set, measured concentrations of DIPE at least 4 out of 6 of QC samples must fall within 15% (20% for LLOQ) of the nominal value for acceptance of results for samples bracketed in between them.</p>		

<p><b>Project Specific Analytical Method</b></p>	<p><b>RESEARCH TRIANGLE INSTITUTE POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709</b></p>	<p><b>AM-0209408.001.0</b></p>						
		<p><b>Page 18 of 18</b></p>						
<p><b>REVIEW/ REVISION LOG</b></p> <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th data-bbox="397 548 456 569"><u>Rev. #</u></th> <th data-bbox="532 548 618 569"><u>Rev. Date</u></th> <th data-bbox="667 548 764 569"><u>Description</u></th> </tr> </thead> <tbody> <tr> <td data-bbox="418 590 435 611" style="text-align: center;">0</td> <td data-bbox="558 590 591 611" style="text-align: center;">NA</td> <td data-bbox="667 590 805 611">Original version.</td> </tr> </tbody> </table>			<u>Rev. #</u>	<u>Rev. Date</u>	<u>Description</u>	0	NA	Original version.
<u>Rev. #</u>	<u>Rev. Date</u>	<u>Description</u>						
0	NA	Original version.						

## **Appendix E**

### **Pre-Study Inhalation Report**

Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether

**Protocol Title**

Metabolism and Pharmacokinetics of Diisopropyl Ether (DIPE) in Male and Female Rats:  
Pilot Study

**Appendix Title**

Inhalation Summary Report for Setup and Evaluation of the Nose Only Inhalation Exposure  
System at RTI International <sup>[1]</sup>.

**Study Protocol**

RTI-934

**Author**

Kay C. Roberts, CIIT Centers for Health Research  
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Research Triangle Park, NC 27709

**Study Sponsor**

American Petroleum Institute  
1220 L Street NW  
Washington, DC 20005

<sup>[1]</sup> RTI International is Research Triangle Institute or RTI  
<sup>[2]</sup> CIIT Centers for Health Research is CIIT-CHR or CIIT

Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether

Report Prepared by:

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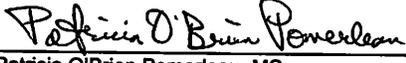
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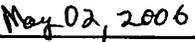
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#### Statement of Quality Assurance

This setup and evaluation of the nose only inhalation exposure system for diisopropyl ether was conducted at Research Triangle Institute (RTI) under CIIT Research Quality Standards. These standards are designed to help assure the quality and integrity of the studies. Data generated from this study is well documented and will be retained in the archive at CIIT for up to 10 years. The study was subjected to Quality Assessments conducted by CIIT's independent Quality Assurance personnel. Quality Assurance reviewed the data and inhalation summary report in April 2006.

  
Patricia O'Brien Pomerleau, MS  
Quality Assurance Director, CIIT

  
Date

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### Introduction

The purpose of this study was to develop a nose only inhalation exposure system for exposure of male and female rats to diisopropyl ether (DIPE). This report describes the inhalation exposure system as designed, tested, and evaluated at RTI.

### Summary

A nose only exposure system including the generation and exposure system was set up at RTI and the system performance verified. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified for system performance verification by the draft protocol. The system verification at RTI was completed between October of 2005 and December of 2005. The system was evaluated and found to be acceptable.

### Materials and Methods

#### Chemical

All liquid diisopropyl ether (CAS No. 108-20-3) used during system performance verification was obtained in aliquots as needed from RTI personnel.

Information regarding source, identity, purity, storage conditions and stability of the test chemical are the responsibility of RTI.

#### Generation and Exposure System

Exposure atmospheres were generated by metering liquid DIPE from a gas tight syringe using a syringe pump (Harvard Apparatus, Model 956, Holliston, MA) into a stainless steel tee where the liquid mixed with approximately 50 mL/min of nitrogen. The DIPE vapor was further diluted with a mixture of HEPA filtered and humidified dilution air. The dilution air was set to deliver approximately 0.25 L/min at each of the open ports on the nose only exposure system. The number of open ports on the nose only exposure system determined the size of syringe needed to generate exposure atmospheres. The system air supply was set, and the total exhaust was adjusted to attain a slightly negative ( $\geq -0.2$  in of H<sub>2</sub>O) static pressure.

Exposure trials were conducted using a Cannon-style nose only exposure system (Lab Products, Seaford, DE) without animals. The nose only exposure system is a dynamic, nonbreathing system. The components of the generation system and delivery line were composed of glass, stainless steel, or teflon. At RTI, the exposure system was contained within a fume hood as an additional safety measure.

Figure 1 is a diagrammatic representation of the exposure system setup.

#### Analytical System

DIPE exposure atmospheres were measured with two calibrated infrared spectrophotometers (MIRAN 1A, The Foxboro Co., Foxboro, MA) with one infrared spectrophotometer (MIRAN) sampling from the inlet of the nose only tower and the other MIRAN sampling the exhaust of the nose only tower.

Concentrations of DIPE in the room were also monitored as an added safety precaution using one additional MIRAN. This MIRAN also served as a backup analytical instrument.

#### Nose only Exposure System Distribution

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The nose only exposure system was checked twice during pre-study trials for uniformity of distribution of test compound using MIRANs, by measuring the concentration at various positions on the nose only exposure system. The number of locations sampled during the distribution varied, dependent upon the total number of open ports. The nose-only exposure system distributions were completed at CIIT-CHR and were not repeated at RTI.

#### Nose Only Concentration Analysis

DIPE exposure atmospheres were analyzed continuously during the exposure period using MIRANs, sampling at the inlet of the nose only tower and sampling the exhaust of the nose only tower. The criteria used to determine acceptable system verification of the nose-only inhalation exposure system was agreement ( $\leq 10\%$ ) between the target and actual concentration based on the average of all concentration readings during the exposure period.

The operating conditions for each MIRAN are listed in Table 1. The inlet MIRAN sampled at a flow rate of approximately 200 mL/min. The total exhaust flow of the nose only tower, which varied, depending upon the total number of open ports, went through the exhaust MIRAN. Voltages from the MIRAN corresponding to the exposure concentrations were recorded by a chart recorder.

#### Analytical Instrument Calibration

Each MIRAN was calibrated using liquid injections of DIPE into a closed loop, using a metal bellows pump to circulate the test chemical vapor. The data from the calibration curves was plotted using the Concentration (ppm) on the X-axis and the Mean Chart Divisions on the Y-axis. The calibration procedures were completed during the system verifications completed at RTI. Each MIRAN will be recalibrated prior to any animal exposures.

#### Estimated Limit of Detection

The accuracy of concentration values depends on how accurately the numbers can be determined from the chart recorder. The best accuracy was determined to be 0.1 chart divisions. Table 2 contains the Estimated Limit of Detection (ELOD) for each MIRAN used, based on the lowest calibration point and number of chart divisions of the lowest calibration point.

#### **Exposure Day**

An exposure day for these system verifications was defined as a 6.5 hour exposure. When using animals, exposure start times for each animal will be stagger-started to facilitate sample collection with each animal being exposed for approximately 6 hours.

#### **Environmental Parameters**

##### Nose-Only Exposure System

The temperature and relative humidity in the nose only exposure system was monitored at an open exposure port by a temperature/relative humidity transmitter, (Rotronic Hygromer Series 200, Rotronic Instrument Corp., Huntington, NY) which was connected to an LCD display. Calibration of the temperature transmitter was checked by comparing the temperature reported by the probe to a certified mercury thermometer. The relative humidity was calibrated by immersing the transmitter in an atmosphere of known humidity generated from saturated salt solutions. To the extent possible, the temperature was maintained between 64° and 79° F and the relative humidity was maintained between 30 and 70%.

##### Domiciliary Area

There were no animals used.

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Statistical Procedures

At the end of each exposure, the reported summary data (grand mean and standard deviation) for temperature, humidity, static pressure, and MIRAN concentrations at each location sampled were determined.

Nominal Concentration Calculation

The nominal chamber concentration (NCC or Nominal) for each exposure can be calculated by using the following formula:

Nominal Chamber Concentration (NCC) Working Equation<sup>(a)</sup>:

$$NCC = \frac{V_{liq}}{V_{AF}} * \frac{\rho * MV}{MW} * 10^6$$

Where:

NCC : Nominal Chamber Concentration, (ppm)  
V liq : Syringe Pump nominal flow rate for the day, (mL/min)  
V AF: Air flow rate through nose only tower, (L/min)  
Mass Density (rho): 0.724 g/ml  
Molecular Volume (MV): 24.5 L/mole  
Molecular Weight (MW): 102.18 g/mole

<sup>(a)</sup> Reference: Moss, OR: Calibration of gas and vapor samplers, in Sampling Instruments, 8<sup>th</sup> ed., edited by S. Hering, Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1994.

**Project Personnel**

CIIT-CHR Study Personnel

Principal Investigator	R. Arden James, B. A.
Inhalation Manager	R. Arden James, B. A.
Research Associate	Kay C. Roberts, A. S.

**Results**

Chemical

The purity of the diisopropyl ether was evaluated and will be reported by RTI.

Nose-Only Exposure System Distribution

Results of the port-to-port variability measurements are shown in Table 3. The relative standard deviation was less than 2% for all of the sampling sites for both distributions.

Generation and Chamber Concentration

Table 4 shows the summary data for DIPE generation and characterization for each of the system verifications. The mean and standard deviation of the values for analytical concentration (ACC) at the inlet and outlet, exposure temperature, exposure relative humidity and exposure static pressure are shown during the entire exposure. The inlet and outlet concentrations during the last 2.5 or 3.5 hours are also shown.

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The individual analytical chamber concentration readings during system verification are shown in Tables 5a (17 open ports) and 5b (7 open ports) on the nose only tower, respectively. The mean (with standard deviation) represents the average of the daily mean.

**Deviations**

Exposure

There were no deviations.

SOP

There were no deviations.

**Discussion**

The daily mean for both system verifications fall slightly outside the draft protocol criteria of the target concentration of 3600 ppm +/- 10% (3240 to 3960 ppm). This occurred because the initial concentrations were purposely set lower than the anticipated target settings, then the settings were slowly adjusted during the system verification tests to hit the target concentrations. The average concentrations during the final 2.5 – 3.5 hours were within the draft protocol criteria and considered acceptable.

**Conclusion**

The target concentration of DIPE was generated and maintained for the required exposure time of 6.5 hours. The temperature and humidity were maintained within the limits specified by the draft protocol. Acceptable system verification testing at RTI was completed from October 2005 thru December 2005.

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Table 1. MIRAN <sup>(1)</sup> Operating Conditions at RTI.

Instrument	MIRAN	MIRAN	MIRAN
Serial No.	4211	4121	4159
Sampling Location:	Inlet	Exhaust	Room Air
Pathlength:	0.75 meters	2.25 meters	0.75 meters
Wavelength:	9.0 microns	9.8 microns	10.0 microns
Slit:	1 mm	1 mm	1 mm
Coarse Zero:	X 10	X 10	X 10
Range:	1A	1A	1A
Meter Response:	1	1	10
Calibration Range: Low	0 – 918 ppm	0 – 918 ppm	0 – 918 ppm
Calibration Range: Mid	918 – 2754 ppm	918 – 2754 ppm	918 – 2754 ppm
Calibration Range: High	2754 – 4284 ppm	2754 – 4284 ppm	2754 – 4284 ppm
Curve Fit	Piecewise	Piecewise	Piecewise

<sup>(1)</sup>MIRAN is an Infrared Spectrophotometer

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Table 2. Estimated Limit of Detection for each MIRAN.

The estimated limit of detection (ELOD) was determined based on the lowest readable chart division. It was determined that the lowest readable chart division was 0.1. Conversion to a concentration in parts per million (ppm) was accomplished by using the calibration curve for each MIRAN where the concentrations is the X variable and the chart divisions are the Y variable.

	MIRAN 4211	MIRAN 4121	MIRAN 4159
Lowest Readable Chart Division	0.1	0.1	0.1
Calibration Curve Slope <sup>[1]</sup>	$y = 0.03x$	$y = 0.0458x$	$y = 0.0196x$
Calibration Curve Y Intercept <sup>[1]</sup>	0.0	0.0	0.0
ELOD (ppm)	3	2	5

<sup>[1]</sup>The calibration curve for each MIRAN was broken into 3 segments and a linear regression was calculated for each segment. The linear regression for the lowest segment was used to determine the ELOD.

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Table 3. DIPE Port-to-Port Variability within the Nose Only Exposure System at CIIT-CHR.

Exposure Target Concentration	3600 ppm	3600 ppm
Sample Position	(17 Open Ports)	(7 Open Ports)
Home <sup>(1)</sup>	1720	1779
1	1736	1801
2	1723	1814
3	1723	1814
4	1736	1736
5	1736	1757
6	1736	
7	1692	
8	1692	
TPCV (%) <sup>(2)</sup>	1.05	1.80
WPCV (%) <sup>(3)</sup>	1.22	0.0
BPCV (%) <sup>(4)</sup>	0 <sup>(5)</sup>	1.8

<sup>(1)</sup> Average of sample position 9 MIRAN readings. Sample position 9 was the home port.

<sup>(2)</sup> TPCV = Total Port Measurements Coefficient of Variation: (St Dev TP/Average TP) \* 100

<sup>(3)</sup> WPCV = Within Port Measurements Coefficient of Variation: (St Dev WP/Average WP) \* 100

<sup>(4)</sup> BPCV = Between Port Measurements Coefficient of Variation:  $\text{Sqrt}[(\text{TPCV})^2 - (\text{WPCV})^2]$

<sup>(5)</sup> Could not be calculated (value less than 0)

NOTE: When completing the Port to Port Variability, the Room Air MIRAN was used to sample at each port selected. Using a rotameter, 200 mL/min of test atmosphere was pulled from each sampling location. This sample was further diluted with 200 mL/min of house air to decrease the time needed to fill the MIRAN cell and reach a stable concentration reading.

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Table 4. Summary of Data for DIPE System Verification Exposures at RTI.

		Target (ppm)	3600	3600	3600	3600
		Number of Open Ports	17	7	17	7
					Last 2.5 Hours	Last 3.5 Hours
ACC - Inlet (ppm)	Daily mean	3058	3208	3409	3557	
	Std Dev	677	507	30	224	
	No. of Data Points	12	14	6	8	
ACC - Exhaust (ppm)	Daily mean	3257	3394	3541	3707	
	Std Dev	479	452	0	235	
	No. of Data Points	12	14	6	8	
Exposure Temperature (°F)	Daily mean	72.7	72.6			
	Std Dev	0.2	0.2			
	No. of Data Points	12	14			
Exposure Relative Humidity (%)	Daily mean	44.3	47.6			
	Std Dev	0.5	0.2			
	No. of Data Points	12	14			
Exposure Static Pressure (in H <sub>2</sub> O)	Daily mean	-0.11	-0.10			
	Std Dev	0.05	0.2			
	No. of Data Points	12	14			

ACC: Analytical Chamber Concentration

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Table 5a. Nose only System Verification Data with 17 Open Ports.

These data represent the individual concentration readings during system verification.

Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)
35	1017	1860
80	2733	2967
105	3048	3254
130	3123	3254
170	3160	3254
205	3160	3254
235	3347	3541
265	3421	3541
295	3421	3541
325	3421	3541
355	3421	3541
385	3421	3541
<hr/>		
<b>Mean</b>	<b>3058</b>	<b>3257</b>
St Dev	677	479
No of Data Points	12	12
<hr/>		
<b>Mean (Last 2.5 Hours)</b>	<b>3409</b>	<b>3541</b>
St Dev	30	0
No of Data Points	6	6

ACC: Analytical Chamber Concentration

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**Table 5b. Nose only System Verification Data with 7 Open Ports.**

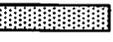
These data represent the individual concentration readings during system verification.

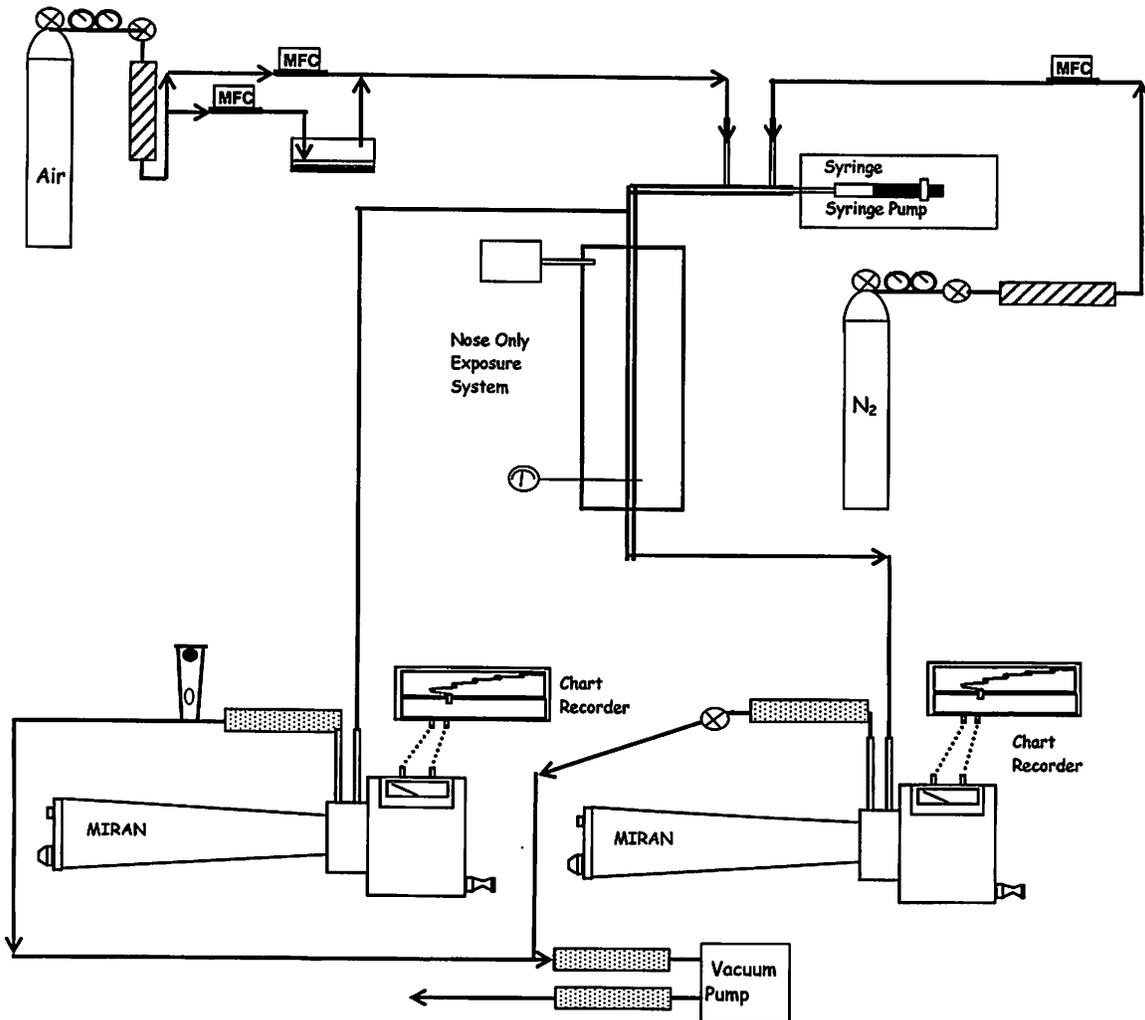
Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)
25	2105	2737
60	2554	2737
90	2733	2909
125	2899	3024
155	3197	3541
185	2973	2909
215	3645	3944
245	3757	3886
275	3869	4001
305	3720	3714
335	3347	3312
365	3235	3484
395	3384	3656
425	3496	3656
<b>Mean</b>	<b>3208</b>	<b>3394</b>
St Dev	507	452
No of Data Points	14	14
<b>Mean (Last 2.5 Hours)</b>	<b>3557</b>	<b>3707</b>
St Dev	224	235
No of Data Points	8	8

ACC: Analytical Chamber Concentration

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Figure 1: Example of Diagram of Exposure System.

	Mass Flow Controller		Temperature/ RH Probe with Output Receiver		Charcoal Filter
	Pressure Vessel Containing water (Humidifier)		Pressure Regulator		Rotameter
	Needle Valve		Magnehelic		HEPA Filter



## **Appendix F**

### **Inhalation Reports**

RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study A

**Protocol Title**

Metabolism and Pharmacokinetics of Diisopropyl Ether (DIPE) in Male and Female Rats: Pilot Study

**Appendix Title**

Inhalation Summary Report: DIPE Nose Only Inhalation Exposure at RTI International <sup>[1]</sup>.

**Study Protocol**

RTI-934

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RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study A

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The Hamner Institutes for Health Research

**QUALITY ASSURANCE STATEMENT**

RTI Study Number: RTI-934      The Hamner Principal Investigator: Kay C. Roberts

RTI Protocol Title: Metabolism and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats: Pilot Study

Inhalation Portion: DIPE Nose Only Inhalation Exposure at RTI International

Protocol No.: RTI-934 (RTI International)      Testing Facility's Study Director: Timothy R. Fennell, Ph.D. RTI International

Sponsor: American Petroleum Institute      Testing Facility: RTI International

The following statement pertains to the work performed by The Hamner Institutes for Health Sciences (The Hamner) staff. Phase inspections, data and inhalation summary report reviews were performed by The Hamner Quality Assurance Unit in accordance with the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards for Inhalation Exposure Health Effects Testing (40 CFR Part 79.60). The dates of The Hamner Quality Assurance Unit inspections and the dates the results were reported to The Hamner Principal Investigator, The Hamner Management, the Testing Facility's Study Director and Testing Facility's Management are noted below.

Phase(s)	Inspection	Date Reported to	Date Reported to
	Date(s)	The Hamner Principal	Testing Facility's Study
	(MM/DD/YY)	Investigator/Management	Director/Management
	(MM/DD/YY)	(MM/DD/YY)	(MM/DD/YY)
Protocol	09/25/2007	09/25/2007	01/08/2009
Inhalation Phase	10/03/2007	10/31/2007	01/08/2009
Draft Inhalation Summary Report and Data	12/12-13/2007	12/18/2007	01/08/2009
Protocol Amendment	03/13/2008	03/13/2008	01/08/2008
Protocol Amendment	12/10/2008	12/11/2008	01/08/2009
Final Inhalation Summary Report	12/19/2008	12/19/2008	01/08/2009

  
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01/09/2009  
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**Inhalation Summary Report GLP Compliance Statement for Protocol No. RTI-934**

This study was performed in compliance with the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards for Inhalation Exposure Health Effects Testing (40 CFR Part 79.60), with the following exceptions.

There were no exceptions.

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09-Jan-09  
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Timothy R. Fennell  
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05-29-2017  
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RTI Inhalation Summary Report  
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### Introduction

The purpose of this study was to conduct a single Nose Only inhalation exposure of male and female F-344 rats to diisopropyl ether (DIPE) and evaluate the effects. This report describes the inhalation portion of the exposure which was completed at RTI.

### Summary

Male and female rats were exposed nose only to 3643 ( $\pm 177$ ) ppm DIPE for 6 hours. The concentration of DIPE delivered to the nose only exposure system was monitored using a calibrated infrared spectrophotometer (MIRAN 1A, The Foxboro Co., Foxboro, MA). One additional MIRAN was used to monitor the exhaust of the nose only exposure system during the exposure period. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified by the protocol. The exposure occurred on 27-Sep-07 at RTI.

### Materials and Methods

#### Chemical

All liquid diisopropyl ether (CAS No. 108-20-3) used during the exposure were obtained from RTI personnel.

Information regarding source, identity, purity, storage conditions and stability of the test chemical was the responsibility of RTI.

The stability of the test atmosphere was checked twice during the exposure by RTI. The stability was also monitored continuously by two infrared spectrophotometers for the duration of the exposures.

#### Generation and Exposure System

The target exposure atmosphere concentration was 3600 ppm DIPE. Exposure atmospheres were generated by metering liquid DIPE from a gas tight syringe. Using a syringe pump (Harvard Apparatus, Model 956, Holliston, MA) liquid DIPE was metered into a stainless steel tee where the liquid mixed with approximately 50 mL/min of nitrogen supplied by RTI (National Welders Supply Co, Inc. Durham, NC). The DIPE vapor was further diluted with a mixture of HEPA filtered and humidified dilution air. The humidified dilution air was created by bubbling a portion of the dilution air flow into a pressure vessel (manufactured by Amicon Corp., Lexington, MA for Alloy Products Corp., Waukesha, WI) containing approximately 0.5 liter of distilled water. The dilution air, supplied by RTI (National Welders Supply Co, Inc. Durham, NC) was set to deliver approximately 0.25 L/min at each of the open ports on the nose only exposure system. The number of open ports on the nose only exposure system determined the size of syringe needed to generate exposure atmospheres. The system air supply was set, and the total exhaust was adjusted to attain a slightly negative ( $\leq -0.25$  in H<sub>2</sub>O) static pressure.

The exposure was conducted using a Cannon-style nose only exposure system (Lab Products, Seaford, DE) with 16 animals received from RTI. Open nose only tubes were used to hold the animals during the inhalation exposure. The nose only exposure system is a dynamic, nonbreathing system. The components of the generation system and delivery line were composed of glass, stainless steel, or teflon. These materials are chemically compatible with DIPE to minimize chemical loss. At RTI, the exposure system was contained within a fume hood as an additional safety measure.

Figure 1 is a diagrammatic representation of the exposure system setup.

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#### Analytical System

The DIPE exposure atmosphere was measured with two calibrated MIRANs with one MIRAN sampling from the inlet of the nose only tower and the other MIRAN sampling the exhaust of the nose only tower.

Concentrations of DIPE in the room were also monitored as an added safety precaution using one additional MIRAN. This MIRAN also served as a backup analytical instrument.

#### Nose Only Exposure System Distribution

The nose only exposure system was checked once during pre-study trials for uniformity of distribution of test compound using a MIRAN, by measuring the concentration at various positions on the nose only exposure system. The number of locations sampled during the distribution varied, dependent upon the total number of open ports. The nose only exposure system distribution was completed at CIIT-CHR and was not repeated at RTI.

#### Nose Only Concentration Analysis

The DIPE exposure atmosphere was analyzed continuously during the exposure period using calibrated MIRANs, sampling at the inlet of the nose only tower and sampling the exhaust of the nose only tower.

The operating conditions for each MIRAN are listed in Table 1. The inlet MIRAN sampled at a flow rate of approximately 200 mL/min. The exhaust MIRAN sampled the total exhaust flow of the nose only tower. Voltages from the MIRAN corresponding to the exposure concentration were recorded by a chart recorder.

#### Analytical Instrument Calibration

Each MIRAN was calibrated using liquid injections of unlabeled DIPE into a closed loop, using a metal bellows pump to circulate the test chemical (RTI-934 – Method\_1 Rev. 1). The data from the calibration curves were plotted using the Concentration (ppm) on the X-axis and the Mean Chart Divisions on the Y-axis. The calibration procedures were completed prior to the exposure.

#### Estimated Limit of Detection

The accuracy of concentration values depends on how accurately the numbers can be determined from the chart recorder. The best accuracy was determined to be 0.1 chart divisions. Table 2 contains the Estimated Limit of Detection (ELOD) for each MIRAN used, based on the lowest calibration point and number of chart divisions of the lowest calibration point. The estimated ELOD for each of the three MIRANs was 2.2, 2.1, and 1.9 ppm, respectively, for the MIRANs listed in Table 1.

#### **Exposure Day**

An exposure day was defined as a 6.5 hour exposure. Exposure start times for each animal were stagger-started to facilitate sample collection with each animal being exposed for approximately 6 hours.

#### **Environmental Parameters**

##### Nose Only Exposure System

The temperature and relative humidity in the nose only exposure system was monitored at an open exposure port by a temperature/ relative humidity transmitter, (Rotronic Hygromer Series 200

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Humidity-Temperature Transmitter, Rotronic Instrument Corp., Huntington, NY) which was connected to an LCD display. Calibration of the temperature transmitter was checked by comparing the temperature reported by the probe to a certified mercury thermometer. The relative humidity calibration was checked by immersing the transmitter in an atmosphere of known humidity generated from saturated salt solutions. To the extent possible, the temperature was maintained between 64° and 79° F and the relative humidity was maintained between 30 and 70%.

#### Domiciliary Area

Information regarding the domiciliary area for the exposure animals was the responsibility of RTI personnel.

#### Statistical Procedures

At the end of the exposure, the reported summary data (grand mean and standard deviation) for temperature, humidity, static pressure, and MIRAN exposure concentrations at each location sampled were determined.

#### Nominal Concentration Calculation

The nominal chamber concentration (NCC or Nominal) for each exposure can be calculated by using the following formula:

Nominal Chamber Concentration (NCC) Working Equation<sup>[6]</sup>:

$$\text{NCC} = \frac{V_{\text{liq}}}{V_{\text{AF}}} * \frac{\rho * \text{MV}}{\text{MW}} * 10^6$$

Where:

NCC : Nominal Chamber Concentration, (ppm)  
V liq : Syringe Pump nominal flow rate for the day, (mL/min)  
V AF: Air flow rate through nose only tower, (L/min)  
Mass Density (rho): 0.724 g/ml  
Molecular Volume (MV): 24.5 L/mole  
Molecular Weight (MW): 102.18 g/mole

<sup>[6]</sup> Reference: Moss, OR: Calibration of gas and vapor samplers, in Sampling Instruments, 8<sup>th</sup> ed., edited by S. Hering, Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1994.

#### **Project Personnel**

##### The Hamner Study Personnel

Principal Investigator	Kay C. Roberts, A.S. R. Arden James, B.A.	Effective: 04-Sep-08 Prior to: 31-Dec-07
Manager, Inhalation Exposure and Aerosol Sciences Facility	Dr. Brian Wong, Ph.D.	Effective: 01-Jan-08
Manager, Exposure Facility Operations	R. Arden James	Prior to: 31-Dec-07

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## Results

### Chemical

The purity of the diisopropyl ether was evaluated and will be reported by RTI. The stability of DIPE concentrations during exposure will be reported by RTI.

### Nose Only Exposure System Distribution

Results of the port-to-port variability measurements were documented in Inhalation Summary Report for Setup and Evaluation of the Nose Only Inhalation Exposure System at RTI International (Issued 25-Apr-06). The relative standard deviation was less than 2% for all of the sampling sites.

### Generation and Chamber Concentration

Table 3 shows the summary data for the DIPE exposure. The mean and standard deviation of the values for analytical concentration (ACC) for inlet and exhaust, exposure temperature, exposure relative humidity and exposure static pressure are shown. The mean (with standard deviation) were ACC-Inlet 3643 ( $\pm 177$ ) ppm, ACC-Outlet 3470 ( $\pm 48$ ) ppm; temperature, 76.3 ( $\pm 2.2$ ) deg F; relative humidity, 37 ( $\pm 3$ ) %, and static pressure, 0.0 ( $\pm 0.0$ ) inches of H<sub>2</sub>O. The mean concentration of 3643 ( $\pm 177$ ) ppm DIPE at the inlet was within the 10% difference requirement between the target and actual concentration on the nose only tower.

The system air supply was set at 4.58 L/min and remained unchanged through out the exposure. Approximately 46.5 mL of DIPE was loaded into the syringe. The syringe pump was set to deliver a nominal flow rate of 0.105 mL/min and remained unchanged through out the exposure. Animals were loaded onto the nose only tower at 4 to 6 minute intervals beginning at 9:45 am with the last animal being loaded at 10:40 am. Each animal was exposed for a total of 6 hours. The first animal was removed from the nose only tower at 3:50 pm and the last animal removed at 4:48 pm. The total exposure time, from the first animal being loaded onto the nose only tower to the last animal being removed from the nose only tower was 7 hours and 3 minutes.

The individual analytical chamber concentration readings, along with the individual temperature, relative humidity, and static pressure readings during the exposure are shown in Table 4. The mean (with standard deviation) represents the average of the exposure readings.

### Nominal Concentration

The mean calculated nominal concentration was 3980 ppm DIPE. The ratio of analytical concentration to nominal concentration was 92%.

## Deviations

### Protocol

There were no deviations.

### Exposure Chambers

There were no deviations.

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SOP

There were no deviations.

**Archive**

Documentation and raw data generated by The Hamner personnel while conducting the study will be retained within The Hamner Archive for ten years following issuance of the final report.

**Conclusion**

Male and female rats were exposed nose only to 3643 ( $\pm 177$ ) ppm DIPE for 6 hours. The concentration of DIPE delivered to the nose only exposure system was monitored using a MIRAN. One additional MIRAN was used to monitor the exhaust of the nose only exposure system during the exposure period. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified by the protocol. The exposure occurred on 27-Sep-07 at RTI.

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Table 1. MIRAN <sup>[1]</sup> Operating Conditions at RTI.

Instrument	MIRAN	MIRAN	MIRAN
Serial No.	4121	4159	3064
Sampling Location:	Inlet	Exhaust	Room Air
Pathlength:	2.25 meters	2.25 meters	2.25 meters
Wavelength:	9.8 microns	9.9 microns	9.9 microns
Slit:	1 mm	1 mm	1 mm
Coarse Zero:	X 10	X 1	X 10
Range:	1A	1A	1A
Meter Response:	1	1	4
Calibration Range: Low	0 – 918 ppm	0 – 918 ppm	0 – 918 ppm
Linear Regression	$y = 0.0464x + 0.0$	$y = 0.0480x + 0.0$	$y = 0.0534x + 0.0$
Calibration Range: Mid-High	918 – 4284 ppm	918 – 4284 ppm	918 – 4284 ppm
Linear Regression	$y = 0.0130x + 35.284$	$y = 0.0143x + 37.491$	$y = 0.0137x + 44.69$
Curve Fit <sup>[2]</sup>	Linear	Linear	Linear

<sup>[1]</sup> MIRAN is an Infrared Spectrophotometer

<sup>[2]</sup> The calibration curve for each MIRAN was broken into 2 segments and a linear regression was calculated for each segment.

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Table 2. Estimated Limit of Detection for each MIRAN <sup>[1]</sup>.

The estimated limit of detection (ELOD) was determined based on the lowest readable chart division. It was determined that the lowest readable chart division was 0.1. Conversion to a concentration in parts per million (ppm) was accomplished by using the calibration curve for each MIRAN where the concentrations were the X variable and the chart divisions were the Y variable.

	MIRAN 4121 Inlet	MIRAN 4159 Exhaust	MIRAN 3064 Room Air
Lowest Readable Chart Division	0.1	0.1	0.1
Calibration Curve Slope <sup>[2]</sup>	$y = 0.0464x$	$y = 0.048x$	$y = 0.0534x$
Calibration Curve Y Intercept <sup>[2]</sup>	0.0	0.0	0.0
ELOD (ppm)	2.2	2.1	1.9

<sup>[1]</sup> MIRAN is an infrared spectrophotometer

<sup>[2]</sup> The calibration curve for each MIRAN was broken into 2 segments and a linear regression was calculated for each segment. The linear regression for the lowest segment was used to determine the ELOD.

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Table 3. Summary of Data for DIPE Exposure at RTI.

	Target (ppm)	3600
	Number of Open Ports	17
ACC - Inlet (ppm)	<b>Daily mean</b>	<b>3643</b>
	Std Dev	177
	No. of Data Points	14
ACC - Exhaust (ppm)	<b>Daily mean</b>	<b>3470</b>
	Std Dev	48
	No. of Data Points	14
Exposure Temperature (°F)	<b>Daily mean</b>	<b>76.3</b>
	Std Dev	2.2
	No. of Data Points	14
Exposure Relative Humidity (%)	<b>Daily mean</b>	<b>37</b>
	Std Dev	3
	No. of Data Points	14
Exposure Static Pressure (in H <sub>2</sub> O)	<b>Daily mean</b>	<b>0.0</b>
	Std Dev	0.0
	No. of Data Points	14

ACC: Analytical Chamber Concentration  
DIPE: Diisopropyl Ether

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Table 4. DIPE Nose Only Exposure Data.

These data represent the individual readings during exposure.

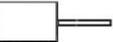
Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)	Temperature (deg F)	Relative Humidity (%)	Static Pressure (in of H <sub>2</sub> O)
15	3055 <sup>[1]</sup>	3462	76.9	38	0.00
45	3517	3427	77.6	37	0.00
75	3670	3392	78.3	35	0.00
105	3709	3427	78.4	35	0.00
135	3709	3392	78.5	34	0.00
165	3709	3462	78.8	34	0.00
195	3709	3497	77.5	35	0.00
225	3709	3462	76.8	35	0.00
255	3709	3532	76.0	37	0.00
285	3709	3532	75.8	37	0.00
315	3670	3497	74.7	39	0.00
345	3709	3462	73.3	41	0.00
375	3709	3497	72.8	41	0.00
405	3709	3532	72.5	41	0.00
<b>Mean</b>	<b>3643</b>	<b>3470</b>	<b>76.3</b>	<b>37</b>	<b>0.0</b>
<b>St Dev</b>	<b>177</b>	<b>48</b>	<b>2.2</b>	<b>3</b>	<b>0.0</b>
<b>No of Data Points</b>	<b>14</b>	<b>14</b>	<b>14</b>	<b>14</b>	<b>14</b>

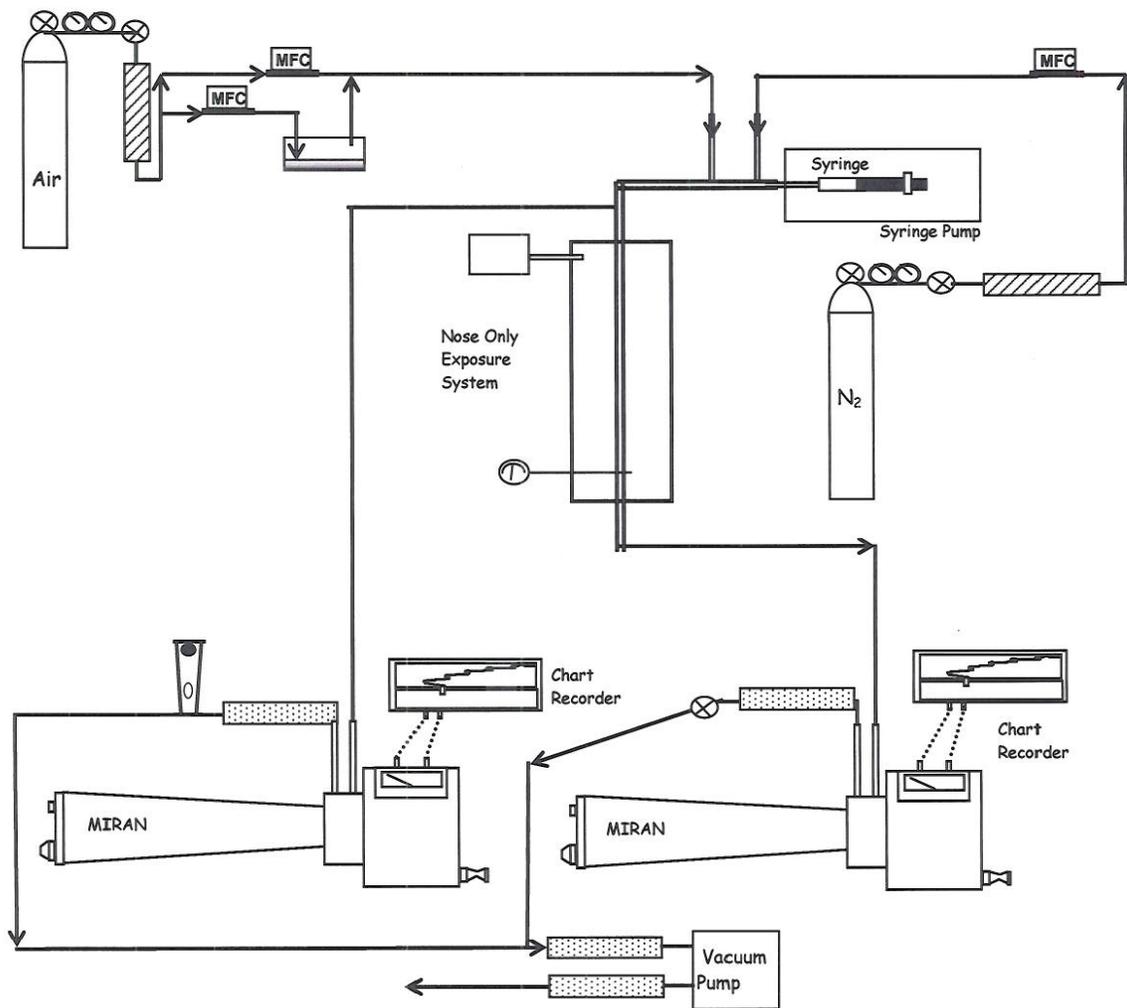
ACC: Analytical Chamber Concentration  
DIPE: Diisopropyl Ether

<sup>[1]</sup> The flow rate of approximately 200 mL/min into the Inlet MIRAN (5.6 L volume) gives a  $t_{90}$  time of 64 minutes. The MIRAN had not reached 90% of equilibration value at this time, however the concentration value is included in the data.

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Figure 1: Example of Diagram of Exposure System.

	Mass Flow Controller		Temperature/ RH Probe with Output Receiver		Charcoal Filter
	Pressure Vessel Containing water (Humidifier)		Pressure Regulator		Rotameter
	Needle Valve		Magnehelic		HEPA Filter



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**Protocol Title**

Metabolism and Pharmacokinetics of Diisopropyl Ether (DIPE) in Male and Female Rats: Pilot Study

**Appendix Title**

Inhalation Summary Report: <sup>14</sup>C-DIPE/DIPE Nose Only Inhalation Exposure at RTI International <sup>[1]</sup>.

**Study Protocol**

RTI-934

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<sup>[3]</sup> The Hamner Institutes for Health Sciences was previously CIIT Centers for Health Research or CIIT-CHR or CIIT

RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study BReport Prepared by:

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The Hamner Institutes for Health Research

**QUALITY ASSURANCE STATEMENT**

RTI Study Number: RTI-934      The Hamner Principal Investigator: Kay C. Roberts

RTI Protocol Title: Metabolism and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats: Pilot Study

Inhalation Portion: <sup>14</sup>C-DIPE/DIPE Nose Only Inhalation Exposure at RTI International

Protocol No.: RTI-934 (RTI International)      Testing Facility's Study Director: Timothy R. Fennell, Ph.D. RTI International

Sponsor: American Petroleum Institute      Testing Facility: RTI International

The following statement pertains to the work performed by The Hamner Institutes for Health Sciences (The Hamner) staff. Phase inspections, data and inhalation summary report reviews were performed by The Hamner Quality Assurance Unit in accordance with the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards for Inhalation Exposure Health Effects Testing (40 CFR Part 79.60). The dates of The Hamner Quality Assurance Unit inspections and the dates the results were reported to The Hamner Principal Investigator, The Hamner Management, the Testing Facility's Study Director and Testing Facility's Management are noted below.

Phase(s)	Inspection Date(s)	Date Reported to The Hamner Principal Investigator/Management	Date Reported to Testing Facility's Study Director/Management
	(MM/DD/YY)	(MM/DD/YY)	(MM/DD/YY)
Protocol	09/25/2007	09/25/2007	01/08/2009
Inhalation Phase	10/03/2007	10/31/2007	01/08/2009
Draft Inhalation Summary Report and Data	12/12-13/2007	12/18/2007	01/08/2009
Protocol Amendment	03/13/2008	03/13/2008	01/08/2009
Protocol Amendment	12/10/2008	12/11/2008	01/08/2009
Final Inhalation Summary Report	12/19/2008	12/19/2008	01/08/2009

 01/09/2009  
Quality Assurance Director      Date  
The Hamner Institutes for Health Sciences

RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study B

**Inhalation Summary Report GLP Compliance Statement for Protocol No. RTI-934**

This study was performed in compliance with the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards for Inhalation Exposure Health Effects Testing (40 CFR Part 79.60), with the following exceptions.

There were no exceptions.

K Roberts  
Kay C. Roberts, A.S.  
Principal Investigator (The Hamner Institutes for Health Sciences)

09-Jan-09  
Date

Timothy R Fennell  
Timothy R. Fennell, Ph.D.  
Study Director (RTI International)

05-29-2017  
Date

RTI Inhalation Summary Report  
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Diisopropyl Ether – Study B

### Introduction

The purpose of this study was to conduct a single nose only inhalation exposure of male F-344 rats to  $^{14}\text{C}$  diisopropyl ether (DIPE) and evaluate the effects. This report describes the inhalation portion of the exposure which was completed at RTI.

### Summary

Male rats were exposed nose only to 3529 ( $\pm 40$ ) ppm  $^{14}\text{C}$ -DIPE/DIPE for 6 hours. The concentration of  $^{14}\text{C}$ -DIPE/DIPE delivered to the nose only exposure system was monitored using a calibrated infrared spectrophotometer. One additional infrared spectrophotometer was used to monitor the exhaust of the nose only exposure system during the exposure period. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified by the protocol. The exposure occurred on 03-Oct-07 at RTI.

### Materials and Methods

#### Chemical

All liquid  $^{14}\text{C}$  diisopropyl ether (CAS No. 108-20-3) and unlabeled DIPE ( $^{14}\text{C}$ -DIPE/DIPE) used during the exposure were obtained from RTI personnel.

Information regarding source, identity, purity, storage conditions and stability of the test chemical mixture was the responsibility of RTI.

The stability of the test atmosphere was checked twice during the exposure by RTI by sampling from the inlet to the nose only unit. The stability was also monitored continuously by two infrared spectrophotometers for the duration of the exposures.

#### Generation and Exposure System

The target exposure atmosphere concentration was 3600 ppm  $^{14}\text{C}$ -DIPE/DIPE. Exposure atmospheres were generated by metering liquid  $^{14}\text{C}$ -DIPE/DIPE from a gas tight syringe. Using a syringe pump (Harvard Apparatus, Model 956, Holliston, MA) liquid  $^{14}\text{C}$ -DIPE/DIPE was metered into a stainless steel tee where the liquid mixed with approximately 55 mL/min of nitrogen supplied by RTI (National Welders Supply Co, Inc. Durham, NC). The DIPE vapor was further diluted with a mixture of HEPA filtered and humidified dilution air. The humidified dilution air was created by bubbling a portion of the dilution air flow into a pressure vessel (manufactured by Amicon Corp., Lexington, MA for Alloy Products Corp., Waukesha, WI) containing approximately 0.5 liter of distilled water. The dilution air, supplied by RTI (National Welders Supply Co, Inc. Durham, NC) was set to deliver approximately 0.25 L/min at each of the open ports on the nose only exposure system. The number of open ports on the nose only exposure system determined the size of syringe needed to generate exposure atmospheres. The system air supply was set, and the total exhaust was adjusted to attain a slightly negative ( $\leq -0.25$  in  $\text{H}_2\text{O}$ ) static pressure.

The exposure was conducted using a Cannon-style nose only exposure system (Lab Products, Seaford, DE) with 4 animals received from RTI. Closed nose only tubes were used to hold the animals during the inhalation exposure. The nose only exposure system is a dynamic, nonbreathing system. The components of the generation system and delivery line were composed of glass, stainless steel, or teflon. These materials are chemically compatible with  $^{14}\text{C}$ -DIPE/DIPE to minimize chemical loss. At RTI, the exposure system was contained within a fume hood as an additional safety measure.

Figure 1 is a diagrammatic representation of the exposure system setup.

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#### Analytical System

The <sup>14</sup>C-DIPE/DIPE exposure atmosphere was measured with two calibrated MIRANs, (MIRAN 1A, The Foxboro Co., Foxboro, MA) with one MIRAN sampling from the inlet of the nose only tower and the other MIRAN sampling the exhaust of the nose only exposure system.

Concentrations of <sup>14</sup>C-DIPE/DIPE in the room were also monitored as an added safety precaution using one additional MIRAN. This MIRAN also served as a backup analytical instrument.

#### Nose Only Exposure System Distribution

The nose only exposure system was checked once during pre-study trials for uniformity of distribution of test compound using a MIRAN, by measuring the concentration at various positions on the nose only exposure system. The number of locations sampled during the distribution varied, dependent upon the total number of open ports. The nose only exposure system distribution was completed at CIIT-CHR and was not repeated at RTI.

#### Nose Only Concentration Analysis

The <sup>14</sup>C-DIPE/DIPE exposure atmosphere was analyzed continuously during the exposure period using calibrated MIRANs, sampling at the inlet of the nose only exposure system and sampling the exhaust of the nose only exposure system.

The operating conditions for each MIRAN are listed in Table 1. The inlet MIRAN sampled at a flow rate of approximately 200 mL/min. The exhaust MIRAN sampled the total exhaust flow of the nose only exposure system. Voltages from the MIRAN corresponding to the exposure concentration were recorded by a chart recorder.

#### Analytical Instrument Calibration

Each MIRAN was calibrated using liquid injections of unlabeled DIPE into a closed loop, using a metal bellows pump to circulate the test chemical (RTI-934 – Method\_1 Rev. 1). The data from the calibration curves were plotted using the Concentration (ppm) on the X-axis and the Mean Chart Divisions on the Y-axis. The calibration procedures were completed prior to the exposure.

#### Estimated Limit of Detection

The accuracy of concentration values depends on how accurately the numbers can be determined from the chart recorder. The best accuracy was determined to be 0.1 chart divisions. Table 2 contains the Estimated Limit of Detection (ELOD) for each MIRAN used, based on the lowest calibration point and number of chart divisions of the lowest calibration point.

#### **Exposure Day**

An exposure day was defined as a 6.5 hour exposure. Exposure start times for each animal were stagger-started to facilitate sample collection with each animal being exposed for approximately 6 hours.

#### **Environmental Parameters**

##### Nose Only Exposure System

The temperature and relative humidity in the nose only exposure system was monitored at an open exposure port by a temperature/ relative humidity transmitter, (Rotronic Hygromer Series 200 Humidity-Temperature Transmitter, Rotronic Instrument Corp., Huntington, NY) which was

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connected to an LCD display. Calibration of the temperature transmitter was checked by comparing the temperature reported by the probe to a certified mercury thermometer. The relative humidity calibration was checked by immersing the transmitter in an atmosphere of known humidity generated from saturated salt solutions. To the extent possible, the temperature was maintained between 64° and 79° F and the relative humidity was maintained between 30 and 70%.

#### Domiciliary Area

Information regarding the domiciliary area for the exposure animals was the responsibility of RTI personnel.

#### Statistical Procedures

At the end of the exposure, the reported summary data (grand mean and standard deviation) for temperature, humidity, static pressure, and MIRAN exposure concentrations at each location sampled were determined.

#### Nominal Concentration Calculation

The nominal chamber concentration (NCC or Nominal) for each exposure can be calculated by using the following formula:

Nominal Chamber Concentration (NCC) Working Equation<sup>[a]</sup>:

$$NCC = \frac{V_{liq}}{V_{AF}} * \frac{\rho * MV}{MW} * 10^6$$

Where:

NCC : Nominal Chamber Concentration, (ppm)  
V liq : Syringe Pump nominal flow rate for the day, (mL/min)  
V AF: Air flow rate through nose only exposure system, (L/min)  
Mass Density (rho): 0.724 g/ml  
Molecular Volume (MV): 24.5 L/mole  
Molecular Weight (MW): 102.18 g/mole

<sup>[a]</sup> Reference: Moss, OR: Calibration of gas and vapor samplers, in Sampling Instruments, 8<sup>th</sup> ed., edited by S. Hering, Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1994.

#### **Project Personnel**

##### The Hamner Study Personnel

Principal Investigator	Kay C. Roberts, A.S. R. Arden James, B.A.	Effective: 04-Sep-08 Prior to: 31-Dec-07
Manager, Inhalation Exposure and Aerosol Sciences Facility	Dr. Brian Wong, Ph.D.	Effective: 01-Jan-08
Manager, Exposure Facility Operations	R. Arden James	Prior to: 31-Dec-07

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## Results

### Chemical

The purity of the <sup>14</sup>C diisopropyl ether was evaluated and will be reported by RTI. The stability of <sup>14</sup>C-DIPE/DIPE concentrations as determined from samples taken from the nose only exposure system inlet during exposure will be reported by RTI.

### Nose Only Exposure System Distribution

Results of the port-to-port variability measurements were documented in Inhalation Summary Report for Setup and Evaluation of the Nose Only Inhalation Exposure System at RTI International (Issued 25-Apr-06). The relative standard deviation was less than 2% for all of the sampling sites.

### Estimated Limit of Detection

The estimated ELOD for each of the three MIRANs determined from 0.1 chart divisions was 2.2, 2.1, and 1.9 ppm, for the inlet, exhaust, and room air MIRANs.

### Generation and Chamber Concentration

Table 3 shows the summary data for the <sup>14</sup>C-DIPE/DIPE exposure. The mean and standard deviation of the values for analytical concentration (ACC) for inlet and exhaust, exposure temperature, exposure relative humidity and exposure static pressure are shown. The mean (with standard deviation) were ACC-Inlet 3529 (+40) ppm, ACC-Outlet 3357 (±130) ppm; temperature, 72.3 (±0.2) deg F; relative humidity, 42 (±0) %, and static pressure, -0.2 (±0.0) inches of H<sub>2</sub>O. The mean concentration of 3529 (+40) ppm DIPE at the inlet was within the 10% difference requirement between the target and actual concentration on the nose only exposure system.

The system air supply was set at 1.98 L/min and remained unchanged through out the exposure. Approximately 21 mL of DIPE was loaded into the syringe. The syringe pump was set to deliver a nominal flow rate of 0.048 mL/min and remained unchanged through out the exposure. Animals were loaded onto the nose only tower beginning at 10:05 am with the last animal being loaded at 10:07 am. Each animal was exposed for a total of 6 hours. The first animal was removed from the nose only tower at 4:07 pm and the last animal removed at 4:15 pm. The total exposure time, from the first animal being loaded onto the nose only tower to the last animal being removed from the nose only tower was 6 hours and 10 minutes.

The individual analytical chamber concentration readings, along with the individual temperature, relative humidity, and static pressure readings during the exposure are shown in Table 4. The mean (with standard deviation) represents the average of the exposure readings.

### Nominal Concentration

The mean calculated nominal concentration was 4208 ppm DIPE. The ratio of analytical concentration to nominal concentration was 84%.

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**Deviations**Protocol

There were no deviations.

Exposure Chambers

There were no deviations.

SOP

There were no deviations.

**Archive**

Documentation and raw data generated by The Hamner personnel while conducting the study will be retained within The Hamner Archive for ten years following issuance of the final report.

**Conclusion**

Male rats were exposed nose only to 3529 ( $\pm 40$ ) ppm  $^{14}\text{C}$ -DIPE/DIPE for 6 hours. The concentration of  $^{14}\text{C}$ -DIPE/DIPE delivered to the nose only exposure system was monitored using a MIRAN. One additional MIRAN was used to monitor the exhaust of the nose only exposure system during the exposure period. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified by the protocol. The exposure occurred on 03-Oct-07 at RTI.

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Table 1. MIRAN <sup>[1]</sup> Operating Conditions at RTI.

Instrument	MIRAN	MIRAN	MIRAN
Serial No.	4121	4159	3064
Sampling Location:	Inlet	Exhaust	Room Air
Pathlength:	2.25 meters	2.25 meters	2.25 meters
Wavelength:	9.8 microns	9.9 microns	9.9 microns
Slit:	1 mm	1 mm	1 mm
Coarse Zero:	X 10	X 1	X 10
Range:	1A	1A	1A
Meter Response:	1	1	4
Calibration Range: Low	0 – 918 ppm	0 – 918 ppm	0 – 918 ppm
Linear Regression	$y = 0.0464x + 0.0$	$y = 0.0480x + 0.0$	$y = 0.0534x + 0.0$
Calibration Range: Mid-High	918 – 4284 ppm	918 – 4284 ppm	918 – 4284 ppm
Linear Regression	$y = 0.0130x + 35.284$	$y = 0.0143x + 37.491$	$y = 0.0137x + 44.69$
Curve Fit <sup>[2]</sup>	Linear	Linear	Linear

<sup>[1]</sup> MIRAN is an Infrared Spectrophotometer

<sup>[2]</sup> The calibration curve for each MIRAN was broken into 2 segments and a linear regression was calculated for each segment.

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Table 2. Estimated Limit of Detection for each MIRAN <sup>[1]</sup>.

The estimated limit of detection (ELOD) was determined based on the lowest readable chart division. It was determined that the lowest readable chart division was 0.1. Conversion to a concentration in parts per million (ppm) was accomplished by using the calibration curve for each MIRAN where the concentrations were the X variable and the chart divisions were the Y variable.

	MIRAN 4121 Inlet	MIRAN 4159 Exhaust	MIRAN 3064 Room Air
Lowest Readable Chart Division	0.1	0.1	0.1
Calibration Curve Slope <sup>[2]</sup>	$y = 0.0464x$	$y = 0.048x$	$y = 0.0534x$
Calibration Curve Y Intercept <sup>[2]</sup>	0.0	0.0	0.0
ELOD (ppm)	2.2	2.1	1.9

<sup>[1]</sup> MIRAN is an infrared spectrophotometer

<sup>[2]</sup> The calibration curve for each MIRAN was broken into 2 segments and a linear regression was calculated for each segment. The linear regression for the lowest segment was used to determine the ELOD.

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Table 3. Summary of Data for <sup>14</sup>C-DIPE/DIPE Exposure at RTI.

	Target (ppm)	3600
	Number of Open Ports	5
ACC - Inlet (ppm)	<b>Daily mean</b>	<b>3529</b>
	Std Dev	40
	No. of Data Points	13
ACC - Exhaust (ppm)	<b>Daily mean</b>	<b>3357</b>
	Std Dev	130
	No. of Data Points	13
Exposure Temperature (°F)	<b>Daily mean</b>	<b>72.3</b>
	Std Dev	0.2
	No. of Data Points	13
Exposure Relative Humidity (%)	<b>Daily mean</b>	<b>42</b>
	Std Dev	0
	No. of Data Points	13
Exposure Static Pressure (in H <sub>2</sub> O)	<b>Daily mean</b>	<b>-0.15</b>
	Std Dev	0.0
	No. of Data Points	13

ACC: Analytical Chamber Concentration  
DIPE: Diisopropyl Ether

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Table 4. <sup>14</sup>C-DIPE/DIPE Nose Only Exposure Data.

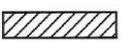
These data represent the individual readings during exposure.

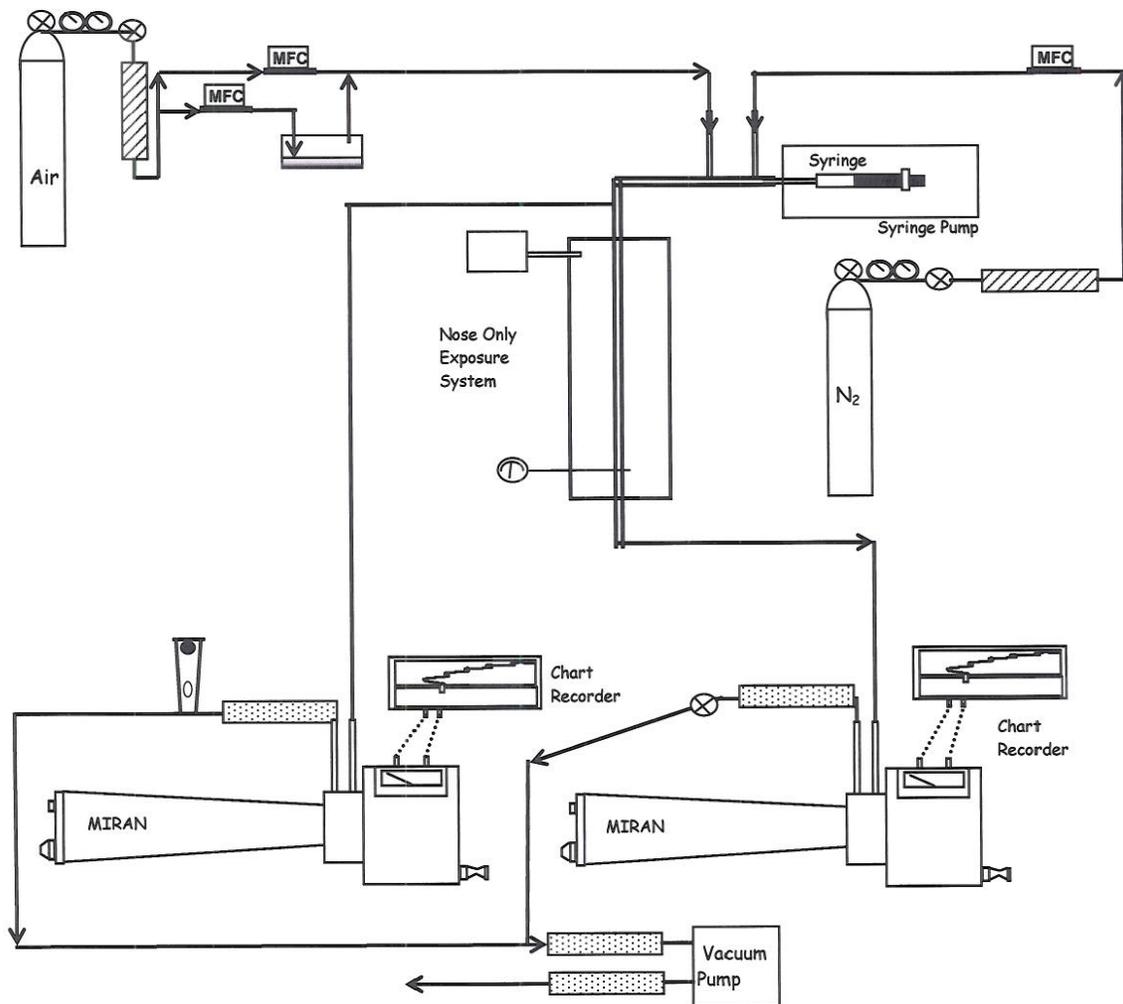
Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)	Temperature (deg F)	Relative Humidity (%)	Static Pressure (in of H <sub>2</sub> O)
10	3594	3462	72.4	42	-0.15
40	3517	2973	72.5	42	-0.17
70	3594	3427	72.3	42	-0.10
100	3517	3322	72.6	42	-0.20
130	3594	3462	72.1	42	-0.10
160	3517	3322	72.3	42	-0.20
190	3517	3322	72.4	42	-0.18
220	3517	3462	72.3	42	-0.15
250	3517	3357	72.3	42	-0.18
280	3517	3462	72.1	42	-0.15
310	3478	3322	72.1	42	-0.15
340	3478	3357	72.2	42	-0.14
355	3517	3392	72.2	42	-0.03
<hr/>					
<b>Mean</b>	<b>3529</b>	<b>3357</b>	<b>72.3</b>	<b>42</b>	<b>-0.15</b>
St Dev	40	130	0.2	0	0.05
No of Data Points	13	13	13	13	13

ACC: Analytical Chamber Concentration  
DIPE: Diisopropyl Ether

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Figure 1: Example of Diagram of Exposure System.

	Mass Flow Controller		Temperature/ RH Probe with Output Receiver		Charcoal Filter
	Pressure Vessel Containing water (Humidifier)		Pressure Regulator		Rotameter
	Needle Valve		Magnehelic		HEPA Filter



Version 1.1

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10-Dec-08

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Diisopropyl Ether – Study C

**Protocol Title**

Metabolism and Pharmacokinetics of Diisopropyl Ether (DIPE) in Male and Female Rats: Pilot Study

**Appendix Title**

Inhalation Summary Report: <sup>14</sup>C-DIPE/<sup>13</sup>C-DIPE Nose Only Inhalation Exposure at RTI International <sup>[1]</sup>.

**Study Protocol**

RTI-934

**Author**

Kay C. Roberts, The Hamner Institutes for Health Sciences

**Performing Laboratory**

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**Study Sponsor**

American Petroleum Institute  
1220 L Street NW  
Washington, DC 20005

<sup>[1]</sup> RTI International is Research Triangle Institute or RTI

<sup>[2]</sup> The Hamner Institutes for Health Sciences is The Hamner

<sup>[3]</sup> The Hamner Institutes for Health Sciences was previously CIIT Centers for Health Research or CIIT-CHR or CIIT

RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study CReport Prepared by:

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The Hamner Institutes for Health Research

**QUALITY ASSURANCE STATEMENT**

RTI Study Number: RTI-934      The Hamner Principal Investigator: Kay C. Roberts

RTI Protocol Title: Metabolism and Pharmacokinetics of Diisopropyl Ether in Male and Female Rats: Pilot Study

Inhalation Portion: <sup>14</sup>C-DIPE/<sup>13</sup>C-DIPE Nose Only Inhalation Exposure at RTI International

Protocol No.: RTI-934 (RTI International)      Testing Facility's Study Director: Timothy R. Fennell, Ph.D. RTI International

Sponsor: American Petroleum Institute      Testing Facility: RTI International

The following statement pertains to the work performed by The Hamner Institutes for Health Sciences (The Hamner) staff. Phase inspections, data and inhalation summary report reviews were performed by The Hamner Quality Assurance Unit in accordance with the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards for Inhalation Exposure Health Effects Testing (40 CFR Part 79.60). The dates of The Hamner Quality Assurance Unit inspections and the dates the results were reported to The Hamner Principal Investigator, The Hamner Management, the Testing Facility's Study Director and Testing Facility's Management are noted below.

Phase(s)	Inspection Date(s) (MM/DD/YY)	Date Reported to The Hamner Principal Investigator/Management (MM/DD/YY)	Date Reported to Testing Facility's Study Director/Management (MM/DD/YY)
Protocol	09/25/2007	09/25/2007	01/08/2009
Inhalation Phase	10/03/2007	10/13/2007	01/08/2009
Protocol Amendment	03/13/2008	03/13/2008	01/08/2009
Draft Inhalation Summary Report and Data	08/05/2008	08/07/2008	01/08/2009
Protocol Amendment	12/10/2008	12/11/2008	01/08/2009
Final Inhalation Summary Report	12/19/2008	12/19/2008	01/08/2009

  
 Quality Assurance Director      Date  
 The Hamner Institutes for Health Sciences

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**Inhalation Summary Report GLP Compliance Statement for Protocol No. RTI-934**

This study was performed in compliance with the U.S. Environmental Protection Agency's Good Laboratory Practice (GLP) Standards for Inhalation Exposure Health Effects Testing (40 CFR Part 79.60), with the following exceptions.

Animals received 3600 ppm nose only inhalation exposures for ca. five hours and forty minutes instead of six hours as stated in the protocol, because of insufficient volume of  $^{14}\text{C}$  DIPE/ $^{13}\text{C}$  DIPE formulation. This deviation should not affect the integrity of the study. The protocol deviation was written and distributed on October 04, 2007 by Study Director, Timothy R. Fennell.

*K. Roberts*

\_\_\_\_\_  
Kay C. Roberts, A.S.  
Principal Investigator (The Hamner Institutes for Health Sciences)

*09 Jan 09*

\_\_\_\_\_  
Date

*Timothy R Fennell*

\_\_\_\_\_  
Timothy R. Fennell, Ph.D.  
Study Director (RTI International)

*05-29-2017*

\_\_\_\_\_  
Date

RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study C

### Introduction

The purpose of this study was to conduct a single nose only inhalation exposure of male F-344 rats to  $^{14}\text{C}$ -diisopropyl ether /  $^{13}\text{C}$ -diisopropyl ether ( $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE) and evaluate the effects. This report describes the inhalation portion of the exposure which was completed at RTI.

### Summary

Male rats were exposed nose only to 3334 (+99) ppm  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE for 5 hours and 40 minutes. The concentration of  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE delivered to the nose only exposure system was monitored using a calibrated infrared spectrophotometer. One additional infrared spectrophotometer was used to monitor the exhaust of the nose only exposure system during the exposure period. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified by the protocol. The exposure occurred on 04-Oct-07 at RTI.

### Materials and Methods

#### Chemical

All liquid  $^{14}\text{C}$ -diisopropyl ether /  $^{13}\text{C}$ -diisopropyl ether (CAS No. 108-20-3) ( $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE) used during the exposure was obtained from RTI personnel.

Information regarding source, identity, purity, storage conditions and stability of the test chemical mixture was the responsibility of RTI.

The stability of the test atmosphere was checked twice by sampling at the inlet to the nose only exposure system during the exposure by RTI. The stability was also monitored continuously by two infrared spectrophotometers for the duration of the exposures.

#### Generation and Exposure System

The target exposure atmosphere concentration was 3600 ppm  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE. Exposure atmospheres were generated by metering liquid  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE from a gas tight syringe. Using a syringe pump (Harvard Apparatus, Model 956, Holliston, MA) liquid  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE was metered into a stainless steel tee where the liquid mixed with approximately 70 mL/min of nitrogen supplied by RTI (National Welders Supply Co, Inc. Durham, NC). The DIPE vapor was further diluted with a mixture of HEPA filtered and humidified dilution air. The humidified dilution air was created by bubbling a portion of the dilution air flow into a pressure vessel (manufactured by Amicon Corp., Lexington, MA for Alloy Products Corp., Waukesha, WI) containing approximately 0.5 liter of distilled water. The dilution air, supplied by RTI (National Welders Supply Co, Inc. Durham, NC) was set to deliver approximately 0.25 L/min at each of the open ports on the nose only exposure system. The number of open ports on the nose only exposure system determined the size of syringe needed to generate exposure atmospheres. The system air supply was set, and the total exhaust was adjusted to attain a slightly negative ( $\leq -0.25$  in  $\text{H}_2\text{O}$ ) static pressure.

The exposure was conducted using a Cannon-style nose only exposure system (Lab Products, Seaford, DE) with 4 animals received from RTI. Closed nose only tubes were used to hold the animals during the inhalation exposure. The nose only exposure system is a dynamic, nonbreathing system. The components of the generation system and delivery line were composed of glass, stainless steel, or teflon. These materials are chemically compatible with  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE to minimize chemical loss. At RTI, the exposure system was contained within a fume hood as an additional safety measure.

Figure 1 is a diagrammatic representation of the exposure system setup.

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#### Analytical System

The  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE exposure atmosphere was measured with two calibrated MIRANs, (MIRAN 1A, The Foxboro Co., Foxboro, MA) with one MIRAN sampling from the inlet of the nose only exposure system and the other MIRAN sampling the exhaust of the nose only exposure system.

Concentrations of  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE in the room were also monitored as an added safety precaution using one additional MIRAN. This MIRAN also served as a backup analytical instrument.

#### Nose Only Exposure System Distribution

The nose only exposure system was checked once during pre-study trials for uniformity of distribution of test compound using a MIRAN, by measuring the concentration at various positions on the nose only exposure system. The number of locations sampled during the distribution varied, dependent upon the total number of open ports. The nose only exposure system distribution was completed at CIIT-CHR and was not repeated at RTI.

#### Nose Only Concentration Analysis

The  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE exposure atmosphere was analyzed continuously during the exposure period using calibrated MIRANs, sampling at the inlet of the nose only tower and sampling the exhaust of the nose only exposure system.

The operating conditions for each MIRAN are listed in Table 1. The inlet MIRAN sampled at a flow rate of approximately 200 mL/min. The exhaust MIRAN sampled the total exhaust flow of the nose only exposure system. Voltages from each MIRAN corresponding to the exposure concentration were recorded by chart recorders.

#### Analytical Instrument Calibration

Each MIRAN was calibrated using liquid injections of unlabeled DIPE into a closed loop, using a metal bellows pump to circulate the test chemical (RTI-934 – Method\_1 Rev. 1). The data from the calibration curves were plotted using the Concentration (ppm) on the X-axis and the Mean Chart Divisions on the Y-axis. The calibration procedures were completed prior to the exposure.

#### Estimated Limit of Detection

The accuracy of concentration values depends on how accurately the numbers can be determined from the chart recorder. The best accuracy was determined to be 0.1 chart divisions. Table 2 contains the Estimated Limit of Detection (ELOD) for each MIRAN used, based on the lowest calibration point and number of chart divisions of the lowest calibration point

#### **Exposure Day**

An exposure day was defined as a 6.5 hour exposure. Exposure start times for each animal were stagger-started to facilitate sample collection with each animal being exposed for approximately 6 hours.

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### Environmental Parameters

#### Nose Only Exposure System

The temperature and relative humidity in the nose only exposure system was monitored at an open exposure port by a temperature/relative humidity transmitter, (Rotronic Hygromer Series 200 Humidity-Temperature Transmitter, Rotronic Instrument Corp., Huntington, NY) which was connected to an LCD display. Calibration of the temperature transmitter was checked by comparing the temperature reported by the probe to a certified mercury thermometer. The relative humidity calibration was checked by immersing the transmitter in an atmosphere of known humidity generated from saturated salt solutions. To the extent possible, the temperature was maintained between 64° and 79° F and the relative humidity was maintained between 30 and 70%.

#### Domiciliary Area

Information regarding the domiciliary area for the exposure animals was the responsibility of RTI personnel.

#### Statistical Procedures

At the end of the exposure, the reported summary data (grand mean and standard deviation) for temperature, humidity, static pressure, and MIRAN exposure concentrations at each location sampled were determined.

#### Nominal Concentration Calculation

The nominal chamber concentration (NCC or Nominal) for each exposure can be calculated by using the following formula:

Nominal Chamber Concentration (NCC) Working Equation<sup>[a]</sup>:

$$NCC = \frac{V_{liq}}{V_{AF}} * \frac{\rho * MV}{MW} * 10^6$$

Where:

NCC : Nominal Chamber Concentration, (ppm)  
V liq : Syringe Pump nominal flow rate for the day, (mL/min)  
V AF: Air flow rate through nose only exposure system, (L/min)  
Mass Density (rho): 0.724 g/ml  
Molecular Volume (MV): 24.5 L/mole  
Molecular Weight (MW): 102.18 g/mole

<sup>[a]</sup> Reference: Moss, OR: Calibration of gas and vapor samplers, in Sampling Instruments, 8<sup>th</sup> ed., edited by S. Hering, Cincinnati, OH: American Conference of Governmental Industrial Hygienists, 1994.

### Project Personnel

#### The Hamner Study Personnel

Principal Investigator	Kay C. Roberts, A.S. R. Arden James, B.A.	Effective: 04-Sep-08 Prior to: 31-Dec-07
Manager, Inhalation Exposure and Aerosol Sciences Facility	Dr. Brian Wong, Ph.D.	Effective: 01-Jan-08

RTI Inhalation Summary Report  
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Manager, Exposure Facility Operations

R. Arden James

Prior to: 31-Dec-07

**Results**Chemical

The purity of the  $^{14}\text{C}$ -diisopropyl ether /  $^{13}\text{C}$ -diisopropyl ether was evaluated and will be reported by RTI. The stability of  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE concentrations during exposure will be reported by RTI.

Nose Only Exposure System Distribution

Results of the port-to-port variability measurements were documented in Inhalation Summary Report for Setup and Evaluation of the Nose Only Inhalation Exposure System at RTI International (Issued 25-Apr-06). The relative standard deviation was less than 2% for all of the sampling sites.

Estimated Limit of Detection

The estimated ELOD for each of the three MIRANs was 2.2, 2.1, and 1.9 ppm, for the inlet, exhaust, and room air MIRANs.

Generation and Chamber Concentration

Table 3 shows the summary data for the  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE exposure. The mean and standard deviation of the values for analytical concentration (ACC) for inlet and exhaust, exposure temperature, exposure relative humidity and exposure static pressure are shown. The mean (with standard deviation) were ACC-Inlet 3334 ( $\pm 99$ ) ppm, ACC-Outlet 1650 ( $\pm 113$ ) ppm; temperature, 72.2 ( $\pm 0.2$ ) deg F; relative humidity, 42 ( $\pm 0$ ) %, and static pressure, -0.2 ( $\pm 0.0$ ) inches of  $\text{H}_2\text{O}$ . The mean concentration of 3334 ( $\pm 99$ ) ppm DIPE at the inlet was within the 10% difference requirement between the target and actual concentration on the nose only tower.

The system air supply was set at 2.0 L/min and remained unchanged through out the exposure. Approximately 19 mL of DIPE was loaded into the syringe. The syringe pump was set to deliver a nominal flow rate of 0.040 mL/min. After approximately 80 exposure minutes the syringe pump setting was increased to deliver a nominal flow rate of 0.044 mL/min and remained unchanged through out the remainder of the exposure. Animals were loaded onto the nose only tower beginning at 9:40 am with the last animal being loaded at 9:45 am. Each animal was exposed for a total of 5 hours and 40 minutes. The first animal was removed from the nose only tower at 3:23 pm and the last animal removed at 3:25 pm. The total exposure time, from the first animal being loaded onto the Nose only tower to the last animal being removed from the nose only tower was 5 hours and 40 minutes.

The individual analytical chamber concentration readings, along with the individual temperature, relative humidity, and static pressure readings during the exposure are shown in Table 4. The mean (with standard deviation) represents the average of the exposure readings.

Nominal Concentration

The mean calculated nominal concentration was 3732 ppm DIPE. The ratio of analytical concentration to nominal concentration was 90%.

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### **Deviations**

#### Protocol

Due to the limited amount of test chemical mixture, the total number of exposure minutes was 5 hours and 40 minutes, instead of 6 hours as stated in the protocol. The cause for the deviation and its effect on the outcome of the study were documented by the Study Director.

#### Exposure Chambers

There were no deviations.

#### SOP

There were no deviations.

### **Archive**

Documentation and raw data generated by The Hamner personnel while conducting the study will be retained within The Hamner Archive for ten years following issuance of the final report.

### **Conclusion**

Male rats were exposed Nose only to 3334 ( $\pm 99$ ) ppm  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE for 5 hours and 40 minutes. The concentration of  $^{14}\text{C}$ -DIPE/ $^{13}\text{C}$ -DIPE delivered to the nose only exposure system was monitored using a MIRAN. One additional MIRAN was used to monitor the exhaust of the nose only exposure system during the exposure period. The environmental parameters (temperature, relative humidity and airflow) were maintained within the range specified by the protocol. The exposure occurred on 04-Oct-07 at RTI.

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Table 1. MIRAN <sup>[1]</sup> Operating Conditions at RTI.

Instrument	MIRAN	MIRAN	MIRAN
Serial No.	4121	4159	3064
Sampling Location:	Inlet	Exhaust	Room Air
Pathlength:	2.25 meters	2.25 meters	2.25 meters
Wavelength:	9.8 microns	9.9 microns	9.9 microns
Slit:	1 mm	1 mm	1 mm
Coarse Zero:	X 10	X 1	X 10
Range:	1A	1A	1A
Meter Response:	1	1	4
Calibration Range: Low	0 – 918 ppm	0 – 918 ppm	0 – 918 ppm
Linear Regression	$y = 0.0464x + 0.0$	$y = 0.0480x + 0.0$	$y = 0.0534x + 0.0$
Calibration Range: Mid-High	918 – 4284 ppm	918 – 4284 ppm	918 – 4284 ppm
Linear Regression	$y = 0.0130x + 35.284$	$y = 0.0143x + 37.491$	$y = 0.0137x + 44.69$
Curve Fit <sup>[2]</sup>	Linear	Linear	Linear

<sup>[1]</sup> MIRAN is an Infrared Spectrophotometer

<sup>[2]</sup> The calibration curve for each MIRAN was broken into 2 segments and a linear regression was calculated for each segment.

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Table 2. Estimated Limit of Detection for each MIRAN <sup>[1]</sup>.

The estimated limit of detection (ELOD) was determined based on the lowest readable chart division. It was determined that the lowest readable chart division was 0.1. Conversion to a concentration in parts per million (ppm) was accomplished by using the calibration curve for each MIRAN where the concentrations were the X variable and the chart divisions were the Y variable.

	MIRAN 4121 Inlet	MIRAN 4159 Exhaust	MIRAN 3064 Room Air
Lowest Readable Chart Division	0.1	0.1	0.1
Calibration Curve Slope <sup>[2]</sup>	$y = 0.0464x$	$y = 0.048x$	$y = 0.0534x$
Calibration Curve Y Intercept <sup>[2]</sup>	0.0	0.0	0.0
ELOD (ppm)	2.2	2.1	1.9

<sup>[1]</sup> MIRAN is an infrared spectrophotometer

<sup>[2]</sup> The calibration curve for each MIRAN was broken into 2 segments and a linear regression was calculated for each segment. The linear regression for the lowest segment was used to determine the ELOD.

RTI Inhalation Summary Report  
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Diisopropyl Ether – Study C

Table 3. Summary of Data for <sup>14</sup>C-DIPE/<sup>13</sup>C-DIPE Exposure at RTI.

	Target (ppm)	3600
	Number of Open Ports	5
ACC - Inlet (ppm)	<b>Daily mean</b>	<b>3334</b>
	Std Dev	99
	No. of Data Points	12
ACC - Exhaust (ppm)	<b>Daily mean</b>	<b>1650</b>
	Std Dev	113
	No. of Data Points	12
Exposure Temperature (°F)	<b>Daily mean</b>	<b>72.2</b>
	Std Dev	0.2
	No. of Data Points	12
Exposure Relative Humidity (%)	<b>Daily mean</b>	<b>42</b>
	Std Dev	0
	No. of Data Points	12
Exposure Static Pressure (in H <sub>2</sub> O)	<b>Daily mean</b>	<b>-0.15</b>
	Std Dev	0.03
	No. of Data Points	12

ACC: Analytical Chamber Concentration  
DIPE: Diisopropyl Ether

RTI Inhalation Summary Report  
Protocol RTI-934  
Diisopropyl Ether – Study C

Table 4. <sup>14</sup>C-DIPE/<sup>13</sup>C-DIPE Nose Only Exposure Data.

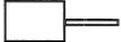
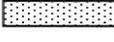
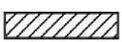
These data represent the individual readings during exposure.

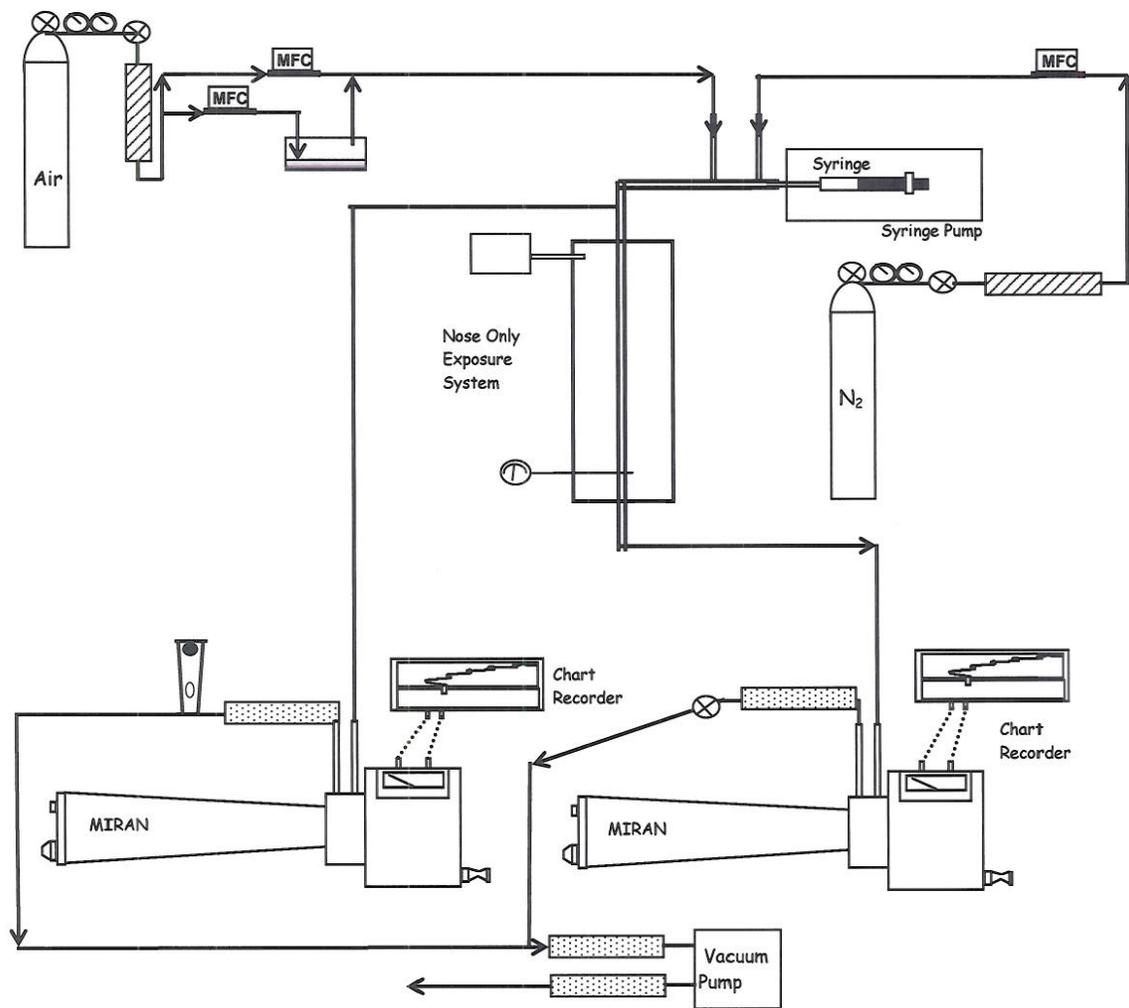
Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)	Temperature (deg F)	Relative Humidity (%)	Static Pressure (in of H <sub>2</sub> O)
20	3594	1854	72.3	43	-0.15
50	3363	1434	72.4	42	-0.08
80	3247	1504	72.2	42	-0.16
110	3209	1539	72.2	42	-0.19
140	3363	1749	72.4	42	-0.19
170	3363	1714	72.3	42	-0.14
200	3401	1714	72.5	42	-0.15
230	3286	1644	72.1	43	-0.13
260	3286	1644	72.2	42	-0.16
290	3286	1644	71.9	43	-0.14
320	3286	1679	72.1	43	-0.10
335	3324	1679	71.9	43	-0.15
<hr/>					
<b>Mean</b>	<b>3334</b>	<b>1650</b>	<b>72.2</b>	<b>42</b>	<b>-0.15</b>
St Dev	99	113	0.2	0	0.03
No of Data Points	12	12	12	12	12

ACC: Analytical Chamber Concentration  
DIPE: Diisopropyl Ether

RTI Inhalation Summary Report  
 Protocol RTI-934  
 Diisopropyl Ether – Study C

Figure 1: Example of Diagram of Exposure System.

	Mass Flow Controller		Temperature/ RH Probe with Output Receiver		Charcoal Filter
	Pressure Vessel Containing water (Humidifier)		Pressure Regulator		Rotameter
	Needle Valve		Magnehelic		HEPA Filter



Version 1.0

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10-Dec-08

## Analysis of Exposure Atmospheres

### Diisopropyl Ether

**SUBMITTED TO:**

Section 211(b) Research Group  
American Petroleum Institute  
1220 L Street NW  
Washington, DC 20005

**TESTING FACILITY:**

RTI International\*  
3040 Cornwallis Road  
P.O. Box 12194  
Research Triangle Park, NC 27709-2194

  
\_\_\_\_\_  
Timothy R. Fennell, Ph.D.

05-29-2017  
\_\_\_\_\_  
Date

**Exposure Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04**

Three inhalation studies were conducted: Study A, exposure to unlabeled DIPE, 3600 ppm; Study B, exposure to <sup>14</sup>C-DIPE/DIPE; Study C, exposure to <sup>14</sup>C-DIPE/<sup>13</sup>C<sub>6</sub>-DIPE. During the course of the studies, samples of the exposure atmosphere were drawn for analysis to evaluate the stability of the DIPE in the inhalation system. A qualitative analysis was conducted using GC-MS analysis (Studies A, B and C) and HPLC coupled with detection of radioactivity (Studies B and C).

**Sampling**

For analysis by GC-MS, samples were removed with a glass syringe from a sampling port at the inlet of the nose only inhalation system. Exposure atmosphere samples (1 mL) were collected and placed in a 20 mL headspace vial for analysis by GC-MS. Exposure atmosphere samples (1 mL) were collected and bubbled through 300 µL of 50 % acetonitrile:water.

**GC-MS analysis**

Equipment: Agilent 6890 gas chromatograph equipped with a split-splitless injector and a flame ionization detector.  
Agilent 5973 Mass Selective Detector.

Column DB-624 30m x 0.32 mm i.d., 1.8 µm film thickness  
(J&W, Agilent technologies, Wilmington, DE)

Injection port	split/splitless
Temperature	150 °C
Split ratio	5:1
Carrier gas	Helium
Flow rate	1.7 ml/min
Injection volume	1 µl

Initial temperature	30 °C
Initial time	3 min
Temperature rate	5 °C/min
Final temperature	80 °C
Final time	0
Ramp	100 °C/min
Final temperature	200 °C
Final time	1 min

**Exposure Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04****5973 MSD**

Mode	El mode
Scan	10-150 amu
Source temperature	230 °C
Quad temperature	150 °C
Transfer line	250 °C
Tune	Atune.u
Solvent delay	2.75 min

**HPLC Analysis**

HPLC Analysis of <sup>14</sup>C-DIPE was conducted on a Waters Atlantis dC18 column, 4.6 mm i.d. x 25 cm, 5 µm particle size. The mobile phase consisted of 75 % water and 25% acetonitrile, with a 20-minute linear gradient to 5% water, 95% acetonitrile. Chromatography was conducted using a system that consisted of 2X Waters 515 Pumps, a Waters 717 Plus Autoinjector, or a Rheodyne manual injector, with a ABI 759A UV detector, and a β-RAM Model 3 radioactivity detector. The column flow rate was 1.0 ml/min, and 100% of the flow went to the radioactivity detector. A 500 µl solid phase cell was used for detection. UV absorbance was monitored at 195 nm. After injection of a 10-µl aliquot of the 2-<sup>14</sup>C DIPE solution, the HPLC effluent was collected in scintillation vials, and after addition of Ultima Gold scintillant, radioactivity in each of the fractions was determined by scintillation counting using a Packard 1900 CA Tricarb scintillation counter. For determination of recovery of radioactivity from the column, triplicate aliquots of the 2-<sup>14</sup>C DIPE solution were prepared for scintillation counting and were counted directly to determine the total amount of radioactivity injected on the column.

**Results****GC-MS Analysis**

Identity was verified by GC-MS analysis. A sample of DIPE atmosphere was sampled from a headspace vial.

**Study A**

For the first sample of the exposure atmosphere obtained at 1.33 h following the start of the exposure, the total ion chromatogram showed a single peak at approximately 4.7 min (Figure 1, upper panel). The mass spectrum of this peak (Figure 1, lower panel) showed a molecular ion at *m/z* 102, and major fragment ions at 87 (consistent with *M*-CH<sub>3</sub>), 59, and 45 (consistent with CH<sub>3</sub>-CH=OH<sup>+</sup>). A library search indicated a match with the spectrum of diisopropyl ether (Figure 2).

**Exposure Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04**

A second sample of the exposure atmosphere obtained at 7.1 h (after the last animal was removed from the tower) provided a similar chromatogram (Figure 3). The major peak was at 4.7 min, and gave the same mass spectrum as that obtained at 1.2 h. The mass spectrum matched that of the DIPE obtained at 1.33 h.

**Study B**

For the first sample of the exposure atmosphere obtained at 1.5 h following the start of the exposure, the total ion chromatogram showed a single peak at approximately 4.7 min (Figure 4, upper panel). The mass spectrum of this peak (Figure 4, lower panel) showed a molecular ion at  $m/z$  102, and major fragment ions at 87 (consistent with  $M-CH_3$ ), 59, and 45 (consistent with  $CH_3-CH=OH^+$ ). A library search indicated a match with the spectrum of diisopropyl ether (not shown). For the second sample obtained at 6.17 h, the main peak at 4.7 min was observed again (Figure 5). The mass spectrum was consistent with that of DIPE. An additional peak was observed in this last sample that eluted earlier than that of DIPE. A library match indicated that this peak was from acetonitrile (Figure 6). It is thought that contamination of this sample occurred during the collection of a sample for radioactivity analysis where the sample was bubbled through a mixture of acetonitrile and water.

**Study C**

For the first sample of the exposure atmosphere obtained at 1.1 h following the start of the exposure, the total ion chromatogram showed a single peak at approximately 4.7 min (Figure 7, upper panel). The mass spectrum of this peak (Figure 7, lower panel) showed a molecular ion at  $m/z$  108, and major fragment ions at 92 (consistent with  $M-CH_3$ ), 74, 62, and 47 (consistent with  $CH_3-CH=OH^+$ ). The spectrum was consistent with that expected for  $^{13}C_6$ - diisopropyl ether (not shown). For the second sample obtained at 5.53 h, the main peak at 4.7 min was observed again (Figure 8). The mass spectrum was consistent with that of DIPE.

**HPLC Analysis**

For the HPLC analysis of  $^{14}C$ -DIPE, a qualitative analysis was attempted using collection of exposure atmosphere from Studies B and C, with sampling by bubbling 1 or 2 mL of atmosphere from the exposure tower through 50% acetonitrile in water. The results were variable, with the concentration of radioactivity being low, and the assessment of purity not possible, because of insufficient radioactivity obtained in the sample injected on the HPLC.

**Exposure Analysis Report****Diisopropyl Ether  
RTI Reference 12322-04****Conclusion**

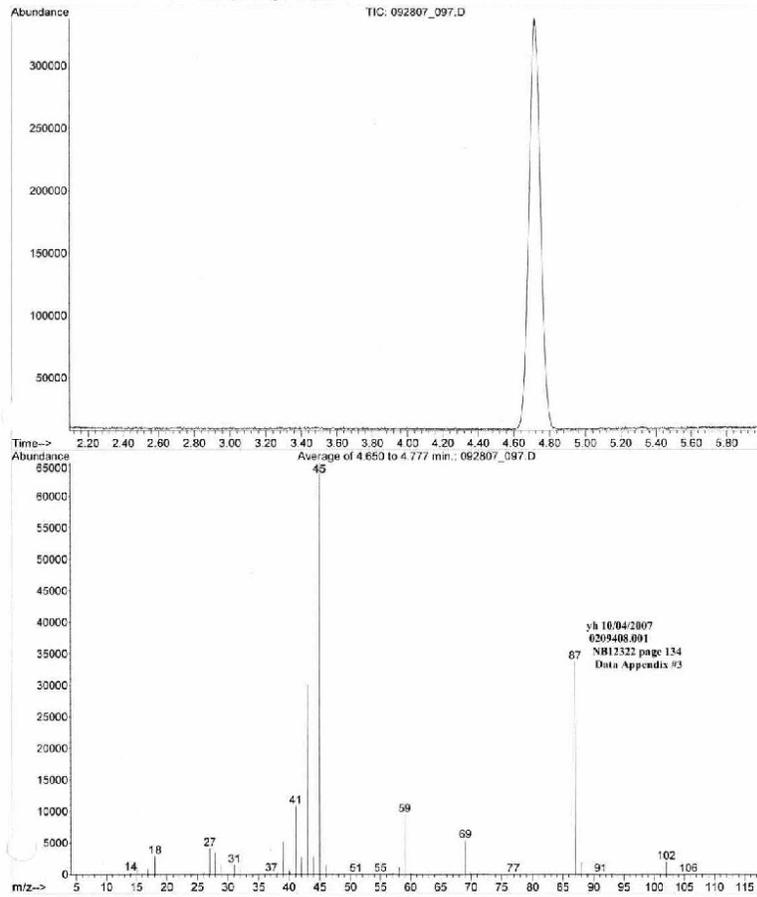
The GC-MS analysis of the atmosphere confirmed the presence of DIPE in studies A and B. No other significant peaks were detected, with the exception of a peak assigned to acetonitrile, thought to arise from contamination during sample collection. In study C, the GC-MS spectrum was consistent with  $^{13}\text{C}_6$  DIPE. The absence of additional peaks in the chromatograms indicates the stability of DIPE under the conditions of exposure.

Insufficient radioactivity was obtained in the samples collected to verify the stability of the radiolabeled material under the conditions of exposure.

Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

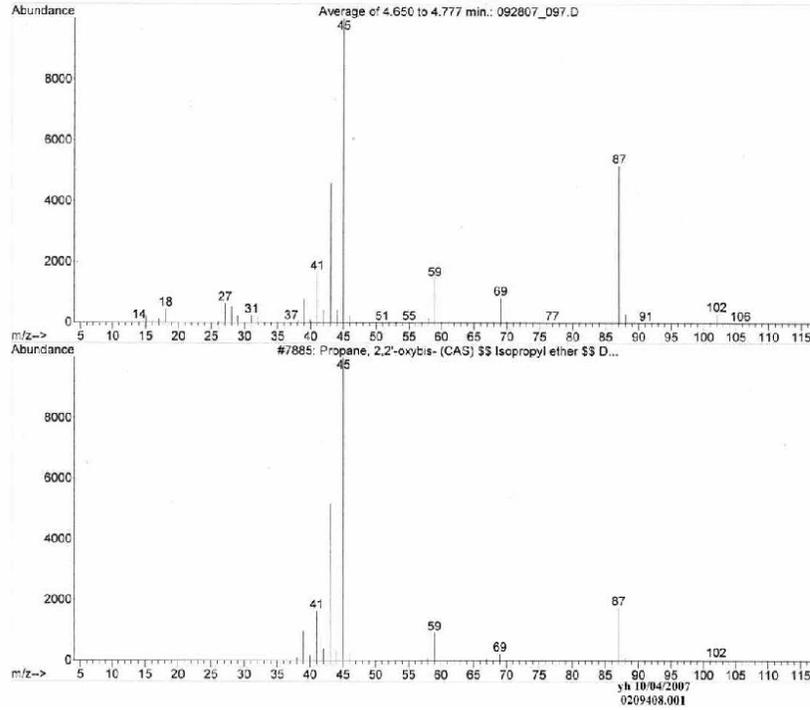
Figure 1. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study A at 1.2 h.



## Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

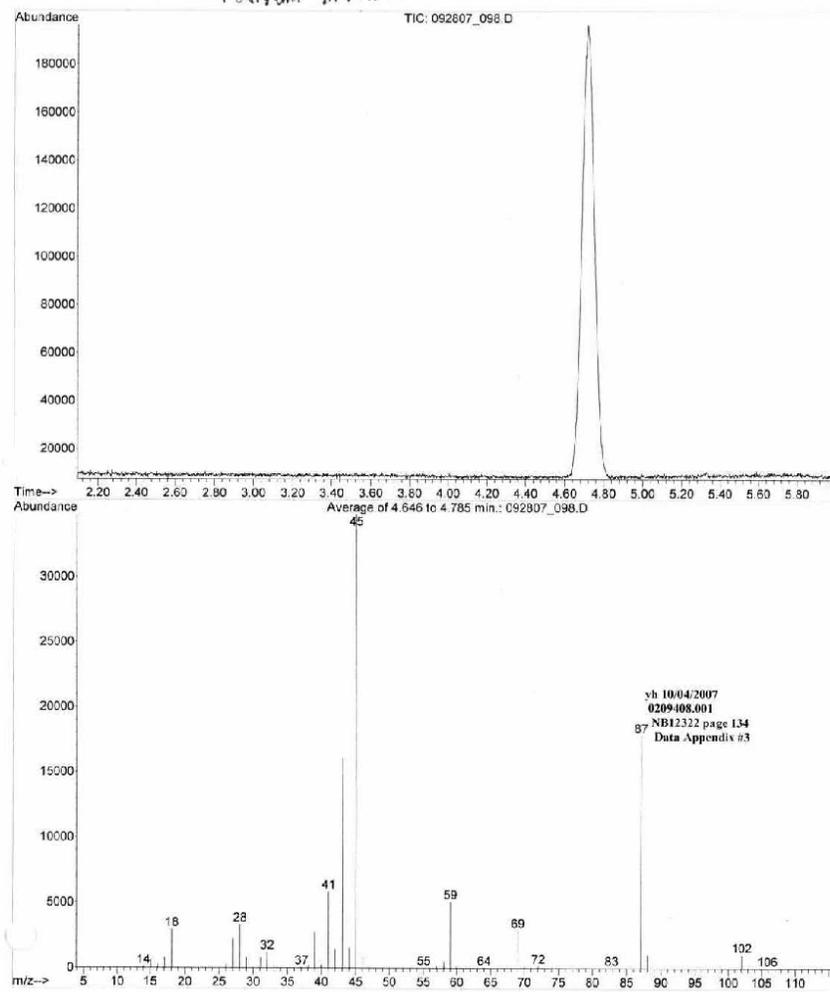
Figure 2. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study A at 1.2 h. Spectral Library Match



## Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

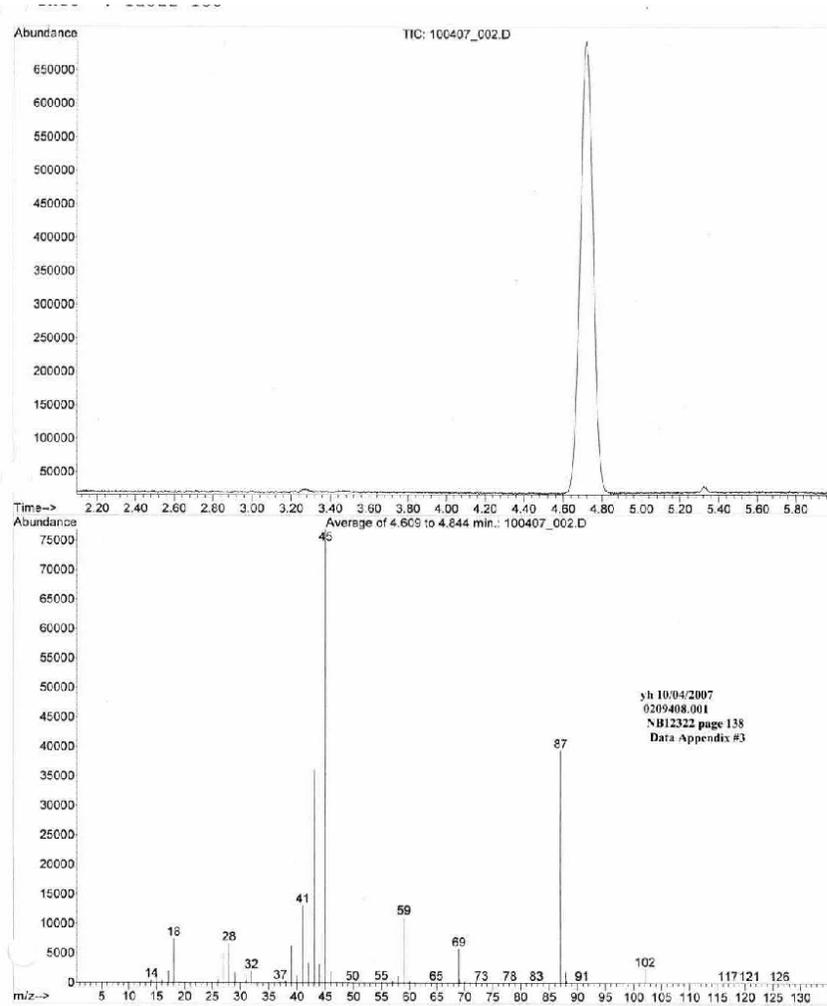
Figure 3. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study A at 7.1 h.



## Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

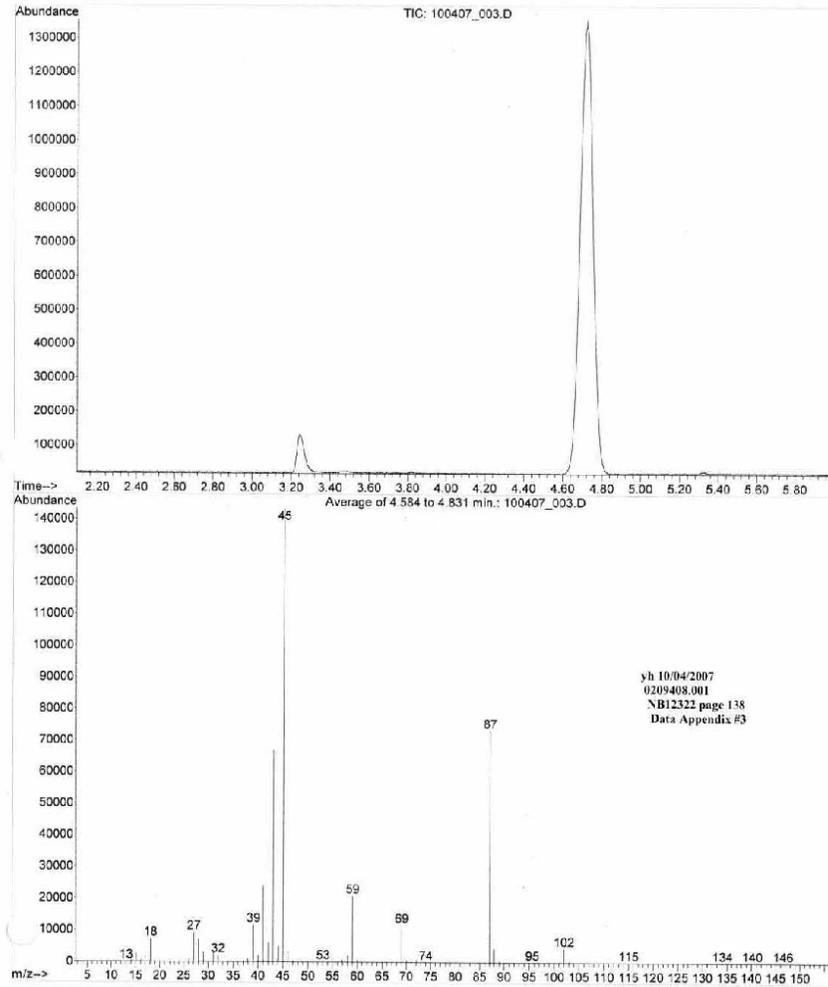
Figure 4. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study B at 1.5 h.



## Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

Figure 5. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study B at 6.17 h.

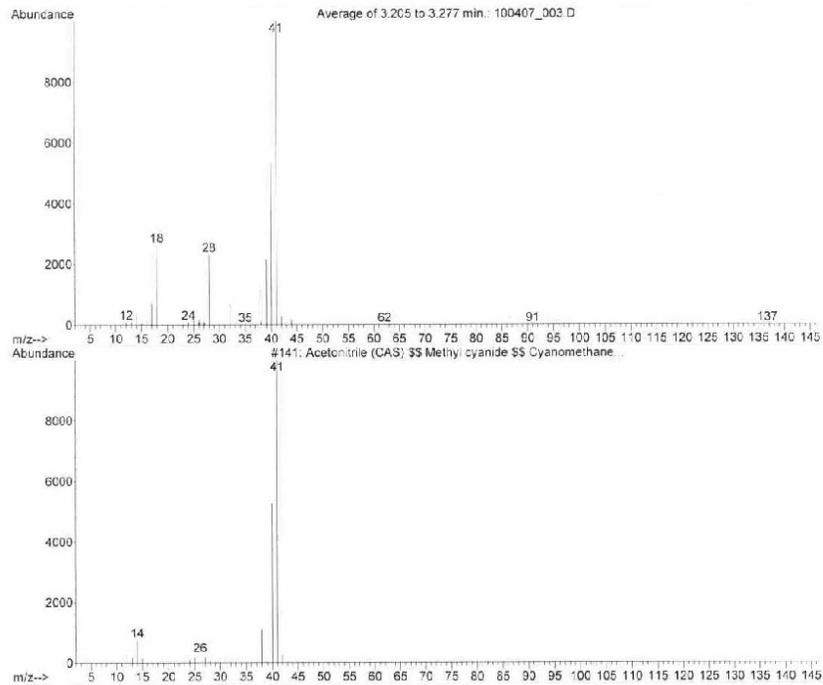


## Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

Figure 6. Library match of the Peak at 3.25 min with Acetonitrile

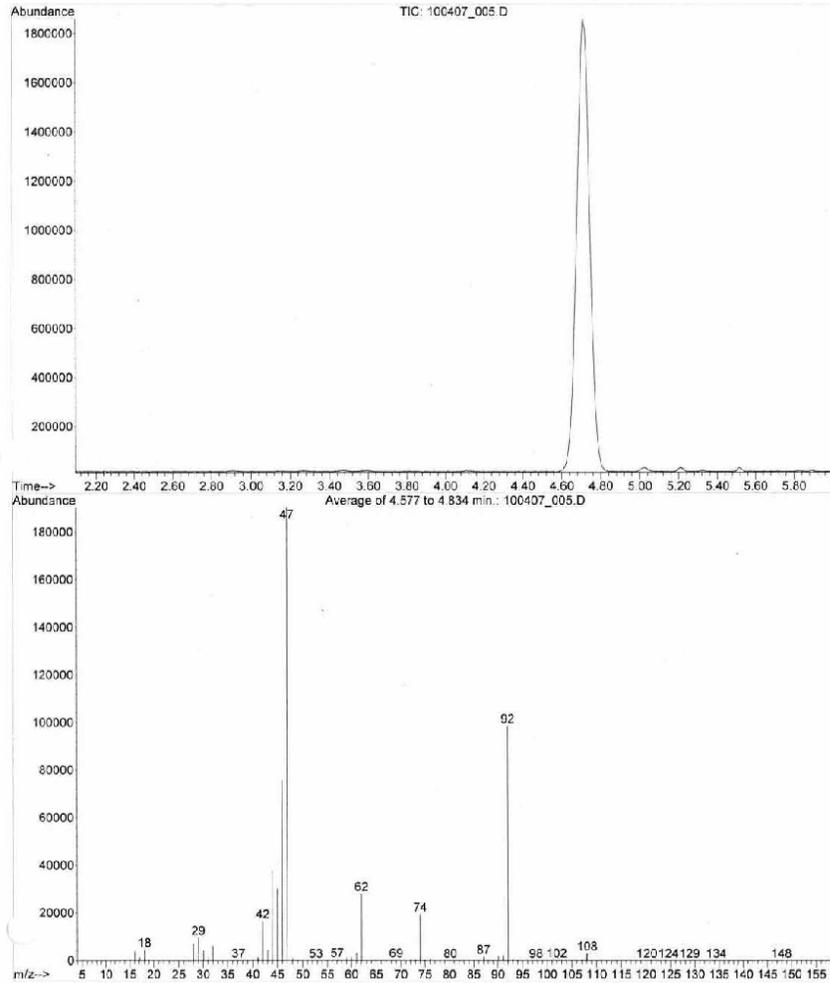
Library Searched : C:\Database\Wiley275.L  
Quality : 64  
ID : Acetonitrile (CAS) \$\$ Methyl cyanide \$\$ Cyanomethane \$\$  
Ethanenitrile \$\$ Ethyl nitrile \$\$ Methane, cyano- \$\$ M  
ethanecarbonitrile \$\$ CH3CN \$\$ Acetonitril \$\$ Cyanure d  
e methyl \$\$ USAF ek-489 \$\$ Methylcyanid \$\$ NA 1648 \$\$ N  
Cl-C60822 \$\$ Rera waste number 0003



Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

Figure 7. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study C at 1.1 h.

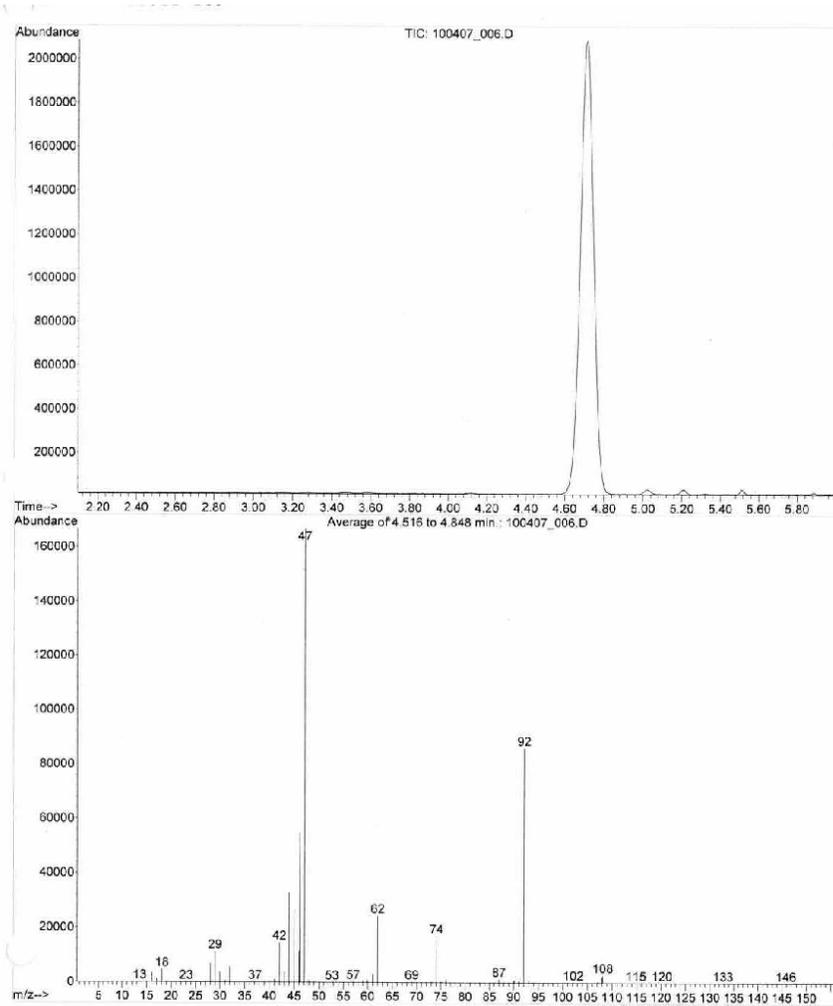


MS 12322-04  
4h 10/5/2007

Exposure Analysis Report

Diisopropyl Ether  
RTI Reference 12322-04

Figure 8. GC-MS Analysis of DIPE in an Exposure Atmosphere Sample from Study C at 5.53 h.



*MS 12322-1174  
on 10/5/2007*

## **Appendix G**

### **Example Calculations**

### Formulae for Calculations

Formulae used in the Debra system for calculations are shown below:

Aliquot dpm/g is calculated by:

$$\text{dpm/g} = \text{aliquot corrected count (dpm)} / \text{aliquot weight (g)}$$

The mean of these aliquot figures for the sample is calculated by:

$$\text{Mean dpm/g} = \text{Sum of all aliquot dpm/g} / \text{number of aliquots}$$

The weight of the sample used for calculations is calculated from the pot weight (before sample collection) and sample weight (weight of pot + sample):

$$\text{Weight of the sample} = \text{sample weight} - \text{pot weight}$$

Depending on the nature of the sample processing, **Effective weight** may be one of a number of values:

If processing type does not use homogenate (e.g. for urine, exhaled VOC trap, CO<sub>2</sub> trap, Cage Wash) then:

$$\text{Effective weight} = \text{weight of the sample}$$

If processing type uses homogenate (e.g. for feces and carcass) then:

$$\text{Effective weight} = \text{Homogenate weight}$$

To calculate the total dpm in the sample, Debra uses the previously calculated figures;

$$\text{Total Dpm} = \text{Mean dpm/g} * \text{Effective weight}$$

For inhalation exposure, where the dpm administered is not precisely known, the **Dose Dpm** is calculated as the sum of all dpm recovered for each animal.

Recovery is calculated by:

$$\text{Recovery} = 100 * (\text{Total Dpm} / \text{Dose Dpm})$$

Concentration is calculated by:

$$\text{Concentration} = (\text{Total dpm} / \text{effective weight}) / (\text{Specific activity})$$

Results are expressed in terms of the arithmetic mean  $\pm$  standard deviation. Mean values were calculated using:

$$Mean = \frac{\sum_1^n Sample}{n}$$

Standard deviation was calculated using:

$$SD = \sqrt{\frac{\left( \left( n \sum_1^n sample^2 \right) - \left( \sum_1^n sample \right)^2 \right)}{n(n-1)}}$$

## Example Calculations

Examples of the calculations for urine, feces, exhaled VOC trap, CO<sub>2</sub> trap, and cage rinse made in the Debra™ system are shown below:

**Subject** = CM-02  
**Tissue** = Urine  
**Timepoint** = 24 h

Aliquot 1 dpm/g = 9129.865/0.0532  
 = 171614.003759398 dpm/g  
 Aliquot 2 dpm/g = 9167.455/0.053  
 = 172970.849056604 dpm/g  
 Sum of dpm/g = 344584.852816002 dpm/g  
 Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots  
 = 344584.852816002 / 2  
 = 172292.426408001 dpm/g  
 = 0.393762316049851 %

Recovery Effective Wt  
 = Sample weight  
 = 2.9801g

Concentration Effective Weight  
 = Sample weight  
 = 2.9801g

Total dpm = Dpm/g x Recovery effective weight  
 = 172292.426408001 x 2.9801  
 = 513448.659938484 dpm

Dose dpm (using Exposure Dose)  
 = sum of all dpm recovered  
 = 57600304.4347919 dpm

Recovery = 100 x (Total dpm / Dose dpm)  
 = 100 x (513448.659938484 / 57600304.4347919)  
 = 100 x (0.00891399212168659)  
 = 0.891399212168659%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)  
 = (513448.659938484 / 2.9801) / (0.4241438147 μCi/mg)  
 = (172292.426408001 dpm/g) / (941599.268634 dpm/mg)  
 = 182.978504919561 μg/g

**Subject** = CM-02  
**Tissue** = Feces  
**Timepoint** = 24 h

Aliquot 1 dpm/g = 1953.942/0.082  
 = 23828.5609756098 dpm/g  
 Aliquot 2 dpm/g = 2254.492/0.0927  
 = 24320.3020496224 dpm/g  
 Sum of dpm/g = 48148.8630252322 dpm/g  
 Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots  
 = 48148.8630252322 / 2  
 = 24074.4315126161 dpm/g

Recovery Effective Weight  
 = Homogenate weight  
 = 8.8412g

Concentration Effective Weight  
 = Sample weight  
 = 4.247699999999999g

Total dpm = Dpm/g x Recovery effective weight  
 = 24074.4315126161 x 8.8412  
 = 212846.863889341 dpm

Dose dpm (using Exposure Dose)  
 = sum of all dpm recovered  
 = 57600304.4347919 dpm

Recovery = 100 x (Total dpm / Dose dpm)  
 = 100 x (212846.863889341 / 57600304.4347919)  
 = 100 x (0.00369523852309324)  
 = 0.369523852309324%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)  
 = (212846.863889341 / 4.247699999999999) / (0.4241438147  $\mu$ Ci/mg)  
 = (50108.7326998945 dpm/g) / (941599.268634 dpm/mg)  
 = 53.2166223669527  $\mu$ g/g

**Subject** = CM-02  
**Tissue** = Exhaled VOC Trap 1  
**Timepoint** = 1 h

Aliquot 1 dpm/g = 121058.01375/0.0482  
 = 2511577.04875519 dpm/g

Aliquot 2 dpm/g = 121584.00375/0.0469  
 = 2592409.46162047 dpm/g

Sum of dpm/g = 5103986.51037566 dpm/g

Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots  
 = 5103986.51037566 / 2  
 = 2551993.25518783 dpm/g

Recovery Effective Wt  
 = Sample weight  
 = 7.7495g

Concentration Effective Weight  
 = Sample weight  
 = 7.7495g

Total dpm = Dpm/g x Recovery effective weight  
 = 2551993.25518783 x 7.7495  
 = 19776671.7310781 dpm

Dose dpm (using Exposure Dose)  
 = sum of all dpm recovered  
 = 57600304.4347919 dpm

Recovery = 100 x (Total dpm / Dose dpm)  
 = 100 x (19776671.7310781 / 57600304.4347919)  
 = 100 x (0.343343180650492)  
 = 34.3343180650492%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)  
 = (19776671.7310781 / 7.7495) / (0.4241438147  $\mu$ Ci/mg)

$$= (2551993.25518783 \text{ dpm/g}) / (941599.268634 \text{ dpm/mg})$$

$$= 2710.27531583586 \text{ } \mu\text{g/g}$$

**Subject** = **CM-02**

**Tissue** = **CO2 Trap 1**

**Timepoint** = **1 h**

Aliquot 1 dpm/g =  $489.773333333333/1.064$

$$= 460.31328320802 \text{ dpm/g}$$

Aliquot 2 dpm/g =  $470.083333333333/1.031$

$$= 455.94891690915 \text{ dpm/g}$$

Sum of dpm/g =  $916.26220011717 \text{ dpm/g}$

Number of aliquots = 2

Mean dpm/g =  $\text{Sum of dpm/g} / \text{Number of aliquots}$

$$= 916.26220011717 / 2$$

$$= 458.131100058585 \text{ dpm/g}$$

Recovery Effective Wt

$$= \text{Sample weight}$$

$$= 523.6\text{g}$$

Concentration Effective Weight

$$= \text{Sample weight}$$

$$= 523.6\text{g}$$

Total dpm =  $\text{Dpm/g} \times \text{Recovery effective weight}$

$$= 458.131100058585 \times 523.6$$

$$= 239877.443990675 \text{ dpm}$$

Dose dpm (using Exposure Dose)

$$= \text{sum of all dpm recovered}$$

$$= 57600304.4347919 \text{ dpm}$$

Recovery

$$= 100 \times (\text{Total dpm} / \text{Dose dpm})$$

$$= 100 \times (239877.443990675 / 57600304.4347919)$$

$$= 100 \times (0.00416451694734071)$$

$$= 0.416451694734071\%$$

Concentration =  $(\text{Total dpm} / \text{Concentration effective weight}) / (\text{Specific activity})$

$$= (239877.443990675 / 523.6) / (0.4241438147 \text{ } \mu\text{Ci/mg})$$

$$= (458.131100058585 \text{ dpm/g}) / (941599.268634 \text{ dpm/mg})$$

$$= 0.486545726318592 \text{ } \mu\text{g/g}$$

**Subject** = **CM-02**

**Tissue** = **Exhaled VOC Trap 1**

**Timepoint** = **168 h**

Aliquot 1 dpm/g =  $42.3866666666667/0.049$

$$= 865.034013605443 \text{ dpm/g}$$

Aliquot 2 dpm/g =  $40.9566666666667/0.0464$

$$= 882.686781609196 \text{ dpm/g}$$

Sum of dpm/g =  $1747.72079521464 \text{ dpm/g}$

Number of aliquots = 2

Mean dpm/g =  $\text{Sum of dpm/g} / \text{Number of aliquots}$

$$= 1747.72079521464 / 2$$

$$= 873.860397607319 \text{ dpm/g}$$

Sum of (dpm/g)<sup>2</sup> =  $(\text{Aliquot 1 dpm/g})^2 + \dots + (\text{Aliquot n dpm/g})^2$

$$= 1527419.79912194$$

SD of dpm/g =  $\text{Sqrt}((n \times \text{Sum of (dpm/g)}^2 - (\text{Sum of dpm/g})^2) / n(n-1))$

$$= \text{Sqrt}((\text{Sum of (dpm/g)}^2 - ((\text{Sum of dpm/g})^2 / n) / (n-1))$$

$$= \text{Sqrt}(1527419.79912194 - (3054527.97802569 / 2) / (2-1))$$

$$= 12.4823919621806$$

Dpm/g %Variance (2 aliquot rule)  
 =  $100 \times ((\text{Mean dpm/g} - \text{aliquot 1 dpm/g}) / \text{Mean dpm/g})$   
 =  $100 \times ((873.860397607319 - 865.034013605443) / 873.860397607319)$   
 =  $100 \times 0.0101004508569603$   
 = 1.01004508569603 %

Recovery Effective Wt  
 = Sample weight  
 = 3.894g

Concentration Effective Weight  
 = Sample weight  
 = 3.894g

Total dpm = Dpm/g x Recovery effective weight  
 =  $873.860397607319 \times 3.894$   
 = 3402.8123882829 dpm

Dose dpm (using Exposure Dose)  
 = sum of all dpm recovered  
 = 57600304.4347919 dpm

Recovery =  $100 \times (\text{Total dpm} / \text{Dose dpm})$   
 =  $100 \times (3402.8123882829 / 57600304.4347919)$   
 =  $100 \times (5.90762917257695\text{E}-5)$   
 = 0.00590762917257695%

Recovery SD = Recovery x (dpm/g SD / dpm/g)  
 =  $0.00590762917257695 \times (12.4823919621806 / 873.860397607319)$   
 =  $0.00590762917257695 / 0.0142841945880121$   
 = 8.43857246549062E-5

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)  
 =  $(3402.8123882829 / 3.894) / (0.4241438147 \mu\text{Ci/mg})$   
 =  $(873.860397607319 \text{ dpm/g}) / (941599.268634 \text{ dpm/mg})$   
 = 0.928059766736065  $\mu\text{g/g}$

Concentration SD = Concentration x (dpm/g SD / dpm/g)  
 =  $0.000928059766736065 \text{ mg/g} \times (12.4823919621806 / 873.860397607319)$   
 =  $0.000928059766736065 / 0.0142841945880121$   
 = 0.0132565862973631

**Subject = CM-02**  
**Tissue = Cage Rinse**  
**Timepoint = 168 h**

Aliquot 1 dpm/g = 60.16/0.5662  
 = 106.252207700459 dpm/g

Aliquot 2 dpm/g = 67.4/0.5789  
 = 116.427707721541 dpm/g

Sum of dpm/g = 222.679915422 dpm/g

Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots  
 =  $222.679915422 / 2$   
 = 111.339957711 dpm/g

Recovery Effective Wt  
 = Sample weight  
 = 202.8g

Concentration Effective Weight  
 = Sample weight  
 = 202.8g

Total dpm = Dpm/g x Recovery effective weight  
 =  $111.339957711 \times 202.8$   
 = 22579.7434237908 dpm

Dose dpm (using Exposure Dose)  
= sum of all dpm recovered  
= 57600304.4347919 dpm

Recovery = 100 x (Total dpm / Dose dpm)  
= 100 x (22579.7434237908 / 57600304.4347919)  
= 100 x (0.00039200736255401)  
= 0.039200736255401%

Recovery SD = Recovery x (dpm/g SD / dpm/g)  
= 0.039200736255401 x (7.1951650668709 / 111.339957711)  
= 0.039200736255401 / 0.0646233860223573  
= 0.0025332843113934

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)  
= (22579.7434237908 / 202.8) / (0.4241438147  $\mu$ Ci/mg)  
= (111.339957711 dpm/g) / (941599.268634 dpm/mg)  
= 0.118245586439891  $\mu$ g/g

## **Appendix H**

### **Raw Data Tables for Radioactivity**

### Sample Naming and Abbreviations

In the tables of raw data values in this appendix, the table headings are derived from the conventions of sample naming used in the Debra™ system. These are explained below. Example calculations and formulae are provided in Appendix G.

Column Heading	Source	Explanation
Subject		Animal number
Sample		The type of sample, e.g. urine, feces
Time		The time of sample collection
Pot wt	Balance	The weight of the empty sample container
Samp wt	Balance	Sample weight, the weight of sample + empty sample container
Corr Samp	Calculated	The corrected sample weight, calculated from Samp wt – Pot wt
Homog wt	Balance	For samples requiring homogenization, the weight of pot + sample + medium for homogenization.
Corr Homog	Calculated	Corrected Homogenate Weight, calculated from Homog Wt – Samp Wt.
Alq wt	Balance	Aliquot weight, weight of aliquot removed for scintillation counting
Orig DPM	Scintillation Counter	Original DPM, the DPM measured in each aliquot.
Bkg DPM	Scintillation Counter	The background DPM determined for the batch of samples
Calc DPM	Calculated	The calculated DPM for the aliquot, = Orig DPM – Calc DPM
LOD	Calculated	Limit of Detection. If Orig DMP value is less than Bkg DMP, then the sample is flagged with an *

<b>Column Heading</b>	<b>Source</b>	<b>Explanation</b>
LOQ	Calculated	Limit of Quantitation. If Orig DMP value is less than 3 x Bkg DMP, then the sample is flagged with an *
DPM/g	Calculated	Calculated value of DPM/g sample.
Sample DPM	Calculated	Total DPM in the sample.
Reco (%)	Calculated	Percentage of the total recovered radioactivity present in the sample.
Sample DPM Sum	Calculated	The sum of radioactivity in all samples for a subject.

**Table 1. Preparation of <sup>14</sup>C DIPE Formulation (Feedstock) for Inhalation Exposure****<sup>14</sup>C DIPE for Study B****[<sup>14</sup>C] DIPE Test Material**

Specific activity 2 mCi/mmol (A)

**Nonradiolabeled DIPE Test Material**

Molecular weight 102.18 g/mol or mg/mmol (B)

Density 0.724 mg/ $\mu$ L (C)**Formulation (Feedstock)**

Nonradiolabeled DIPE 15.8176 g (D)

[<sup>14</sup>C] DIPE 0.2975 g (E)

Net Feedstock Weight 16.1151 g (F)=(D+E)

Total DIPE present in the formulation (by weight) 16.1151 g (F)=(D+E)

Total DIPE present in the formulation (by weight) 16115.1 mg (G)=(D+E)\*100

**Nominal Specific Activity**

Activity present 6.114210217 mCi (H)=((E)\*1000/(B))\*(A)

[<sup>14</sup>C]DIPE and nonradiolabeled DIPE 16115.1 mg (G)

Nominal Specific Activity 0.000379409 mCi/mg (I)=(H)/(G)

Nominal Specific Activity 0.379408767  $\mu$ Ci/mg (J)=(I)\*1000Nominal Specific Activity 38.76798779  $\mu$ Ci/mmol (K)=(((H)\*1000)/(G))\*(B)**Formulation Dilution**

Weight of Feedstock 0.0679 g (L)

Weight of DMF 20.4687 g (M)

Total weight of solution 20.5366 g (N)=(L)+(M)

**Dose Formulation Dilution Aliquots**

Aliq # Aliquot Wt (g) DPM DPM/g

1 0.0876 301093 3437135

2 0.0862 291199 3378179

3 0.0955 295976 3099225

Mean dilution DPM/g (O) 3304846

**Total Radioactivity in the Dilution** 67870303.71 DPM (P)=(M)\*(O)**Radiochemical Concentration of the Feedstock** 999562646.6 DPM/g (Q)=(P)/(L)**Radiochemical Concentration of the Feedstock** 450.2534444  $\mu$ Ci/g (R)=(Q)/2220000**Specific Activity ( $\mu$ Ci/mg DIPE)** 0.450253444  $\mu$ Ci/mg (S)=(R)\*(F)/(G)**Specific Activity ( $\mu$ Ci/mmol DIPE)** 46.00689695  $\mu$ Ci/mmol (T)=(R)\*(B)/1000

**Table 1 (contd). Preparation of <sup>14</sup>C DIPE Formulation (Feedstock) for Inhalation Exposure****<sup>13</sup>C/<sup>14</sup>C DIPE For Study C****[<sup>14</sup>C] DIPE Test Material**

Specific activity 2.1 mCi/mmol (A)

**Nonradiolabeled DIPE Test Material**

Molecular weight 108.18 g/mol or mg/mmol (B)

Density 0.724 mg/ $\mu$ L (C)**Formulation (Feedstock)**

Nonradiolabeled DIPE 14.4207 g (D)

[<sup>14</sup>C] DIPE 0.2707 g (E)

Net Feedstock Weight 14.6914 g (F)=(D+E)

Total DIPE present in the formulation (by weight) 14.6914 g (F)=(D+E)

Total DIPE present in the formulation (by weight) 14691.4 mg (G)=(D+E)\*100

**Nominal Specific Activity**

Activity present 5.254853023 mCi (H)=((E)\*1000/(B))\*(A)

[<sup>14</sup>C]DIPE and nonradiolabeled DIPE 14691.4 mg (G)

Nominal Specific Activity 0.000357682 mCi/mg (I)=(H)/(G)

Nominal Specific Activity 0.357682251  $\mu$ Ci/mg (J)=(I)\*1000Nominal Specific Activity 38.69406592  $\mu$ Ci/mmol (K)=(((H)\*1000)/(G))\*(B)**Formulation Dilution**

Weight of Feedstock 0.0673 g (L)

Weight of DMF 18.9894 g (M)

Total weight of solution 19.0567 g (N)=(L)+(M)

**Dose Formulation Dilution Aliquots**

Aliq #	Aliquot Wt (g)	DPM	DPM/g
1	0.0911	304041	3337442
2	0.0856	288454	3369790
3	0.0881	287975	3268729

Mean dilution DPM/g 3325320 (O)

**Total Radioactivity in the Dilution** 63369630.78 DPM (P)=(M)\*(O)**Radiochemical Concentration of the Feedstock** 941599268.6 DPM/g (Q)=(P)/(L)**Radiochemical Concentration of the Feedstock** 424.1438147  $\mu$ Ci/g (R)=(Q)/2220000**Specific Activity ( $\mu$ Ci/mg DIPE)** 0.424143815  $\mu$ Ci/mg (S)=(R)\*(F)/(G)**Specific Activity ( $\mu$ Ci/mmol DIPE)** 45.88387787  $\mu$ Ci/mmol (T)=(R)\*(B)/1000

**Table 2. Recovery of Radioactivity in Samples from Group B.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Homog wt	Corr Homog	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	Mean DPM/g	Sample DPM	Recovery (%)
BM-01	Carcass Digest	0 h	109.00	299.11	190.11	740.66	631.66	0.1074 0.1048	12678.60 12836.32	26.41 26.41	12652.190 12809.910			120018.171	75810677.8347	98.200
BM-01	Exhaled VOC	0 h	17.0963	24.8570	7.7607	N.A.	N.A.	0.0447 0.0460	2854.75 2879.17	25.67 25.67	2829.084 2853.504			62661.577	486297.7023	0.630
BM-01	Exposure Urine	0 h	16.3491	Missing	Missing	N.A.	N.A.	N.C. N.C.	N.C. N.C.	N.C. N.C.	N.A. N.A.			N.A.	N.C.	N.A.
BM-01	Exposure Feces	0 h	16.5629	22.1174	5.5545	23.7509	7.1880	0.0636 0.0668	6646.67 6672.22	9.66 9.66	6637.007 6662.557			102047.161	733514.9968	0.950
BM-01	Nose Only Tube Rinse	0 h	107.09	301.24	194.15	N.A.	N.A.	0.4110 0.4885 0.4619	357.85 429.17 416.26	4.86 4.86 4.86	352.990 424.310 411.400			872.708	169436.2046	0.219
BM-02	Carcass Digest	0 h	108.94	303.89	194.95	751.53	642.59	0.1053 0.1150	11916.17 13281.38	26.41 26.41	11889.760 13254.970			114086.905	73311103.9869	97.751
BM-02	Exhaled VOC	0 h	17.3547	25.0557	7.7010	N.A.	N.A.	0.0456 0.0468	5250.95 5340.78	25.67 25.67	5225.284 5315.114			114080.181	878531.4760	1.171
BM-02	Exposure Urine	0 h	16.5909	Missing	Missing	N.A.	N.A.	N.C. N.C.	N.C. N.C.	N.C. N.C.	N.A. N.A.			N.A.	N.C.	N.A.
BM-02	Exposure Feces	0 h	16.3308	20.9757	4.6449	22.5426	6.2118	0.1079 0.0662	12210.99 7020.68	9.66 9.66	12201.327 7011.017			109493.273	680150.3155	0.907
BM-02	Nose Only Tube Rinse	0 h	108.03	271.25	163.22	N.A.	N.A.	0.5193 0.4742 0.4911	404.39 367.04 406.95	4.86 4.86 4.86	399.530 362.180 402.090			783.962	127958.3311	0.171
BM-03	Carcass Digest	0 h	108.55	304.29	195.74	753.38	644.83	0.1089 0.1076	12673.66 12160.60	26.41 26.41	12647.250 12134.190			114453.823	73803258.7380	99.529
BM-03	Exhaled VOC	0 h	17.2584	25.0178	7.7594	N.A.	N.A.	0.0474 0.0476	21.73 23.28	28.57 28.57	0.000 0.000	*	*	0.000	0.0000	0.000
BM-03	Exposure Urine	0 h	16.7684	Missing	Missing	N.A.	N.A.	N.C. N.C.	N.C. N.C.	N.C. N.C.	N.A. N.A.			N.A.	N.C.	N.A.
BM-03	Exposure Feces	0 h	16.1935	18.1833	1.9898	19.7737	3.5802	0.0723 0.0856	3400.14 4134.72	9.66 9.66	3390.477 4125.057			47542.237	170210.7170	0.230
BM-03	Nose Only Tube Rinse	0 h	107.42	261.91	154.49	N.A.	N.A.	0.6756 0.4754 0.5117	800.72 554.35 586.39	4.86 4.86 4.86	795.860 549.490 581.530			1156.773	178709.8671	0.241
BM-04	Carcass Digest	0 h	109.11	296.36	187.25	719.52	610.41	0.0922 0.0927	11057.51 10924.22	26.41 26.41	11031.100 10897.810			118601.573	72395586.0081	98.726
BM-04	Exhaled VOC	0 h	17.2089	25.0106	7.8017	N.A.	N.A.	0.0458 0.0448	2202.97 2249.47	25.67 25.67	2177.304 2223.804			48588.930	379076.2542	0.517
BM-04	Exposure Urine	0 h	16.6977	Missing	Missing	N.A.	N.A.	N.C. N.C.	N.C. N.C.	N.C. N.C.	N.A. N.A.			N.A.	N.C.	N.A.
BM-04	Exposure Feces	0 h	16.7245	20.0300	3.3055	23.1542	6.4297	0.0788 0.0934	3576.95 3938.10	9.66 9.66	3567.287 3928.437			43665.243	280754.4101	0.383
BM-04	Exp. Urine Sample 2	0 h	107.82	121.65	13.83	625.14	517.32	0.9189 0.9323 0.6153	277.92 278.43 188.89	4.88 4.88 4.88	273.040 273.550 184.010			296.536	153404.2432	0.209
BM-04	Nose Only Tube Rinse		106.88	184.10	77.22	N.A.	N.A.	0.4604 0.5673 0.6552	738.94 911.27 987.73	4.86 4.86 4.86	734.080 906.410 982.870			1564.103	120780.0023	0.165

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 3. Total Recovery of Radioactivity in Samples from Group B.**

Subject	Sample	Time	Sample dpm	Sample dpm Sum	Reco (%)
BM-01	Carcass Digest	0 h	75810677.83 dpm	77199926.730 dpm	98.200
BM-01	Exhaled VOC	0 h	486297.70 dpm		0.630
BM-01	Exposure Feces	0 h	733515.00 dpm		0.950
BM-01	Nose Only Tube Rinse	0 h	169436.20 dpm		0.219
BM-02	Carcass Digest	0 h	73311103.99 dpm	74997744.120 dpm	97.751
BM-02	Exhaled VOC	0 h	878531.48 dpm		1.171
BM-02	Exposure Feces	0 h	680150.32 dpm		0.907
BM-02	Nose Only Tube Rinse	0 h	127958.33 dpm		0.171
BM-03	Carcass Digest	0 h	73803258.74 dpm	74152179.330 dpm	99.529
BM-03	Exhaled VOC	0 h	0.00 dpm		0.000
BM-03	Exposure Feces	0 h	170210.72 dpm		0.230
BM-03	Nose Only Tube Rinse	0 h	178709.87 dpm		0.241
BM-04	Carcass Digest	0 h	72395586.01 dpm	73329600.910 dpm	98.726
BM-04	Exhaled VOC	0 h	379076.25 dpm		0.517
BM-04	Exposure Feces	0 h	280754.41 dpm		0.383
BM-04	Exp. Urine Sample 2	0 h	153404.24 dpm		0.209
BM-04	Nose Only Tube Rinse	0 h	120780.00 dpm		0.165

**Table 4. Recovery of Radioactivity in Urine from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Urine	8 h	16.3833 g	20.0443 g	3.6610 g	0.0538 g 0.0535 g	25207.96 24753.04	25.83 25.83	25182.130 24727.210			468069.331 462190.841	1702841.2448	3.083
CM-01	Urine	24 h	49.6715 g	54.2445 g	4.5730 g	0.0546 g 0.0539 g	13386.21 13071.36	31.37 31.37	13354.845 13039.995			244594.231 241929.406	1112436.2962	2.014
CM-01	Urine	48 h	49.8979 g	54.5841 g	4.6862 g	0.1980 g 0.2073 g	6452.76 6718.38	4.60 4.60	6448.165 6713.785			32566.490 32386.807	152192.0689	0.276
CM-01	Urine	72 h	50.0245 g	57.9905 g	7.9660 g	0.2080 g 0.2110 g	1112.64 1144.74	3.90 3.90	1108.740 1140.840			5330.481 5406.825	42766.6875	0.077
CM-01	Urine	96 h	49.4195 g	58.9340 g	9.5145 g	0.2084 g 0.2055 g	448.37 457.20	3.90 3.90	444.470 453.300			2132.774 2205.839	20639.8664	0.037
CM-01	Urine	120 h	49.3183 g	60.9000 g	11.5817 g	0.2033 g 0.2057 g	246.10 235.53	3.90 3.90	242.200 231.630			1191.343 1126.057	13419.7170	0.024
CM-01	Urine	144 h	49.4390 g	58.4684 g	9.0294 g	0.2040 g 0.2054 g	163.49 181.32	0.00 0.00	163.490 181.320			801.422 882.765	7603.5986	0.014
CM-01	Urine	168 h	48.4712 g	58.6314 g	10.1602 g	0.2080 g 0.2077 g	138.30 139.64	3.10 3.10	135.205 136.545			650.024 657.415	6641.9187	0.012
CM-02	Urine	8 h	16.4290 g	20.4321 g	4.0031 g	0.0542 g 0.0527 g	23333.85 22766.29	25.83 25.83	23308.020 22740.460			430037.269 431507.780	1724425.4933	2.994
CM-02	Urine	24 h	49.6840 g	52.6641 g	2.9801 g	0.0532 g 0.0530 g	9161.23 9198.82	31.37 31.37	9129.865 9167.455			171614.004 172970.849	513448.6599	0.891
CM-02	Urine	48 h	49.5056 g	52.9815 g	3.4759 g	0.1035 g 0.1029 g	2965.31 2965.80	4.60 4.60	2960.715 2961.205			28605.942 28777.502	99729.5573	0.173
CM-02	Urine	72 h	49.4267 g	61.2787 g	11.8520 g	0.2068 g 0.2109 g	794.63 830.46	3.90 3.90	790.730 826.560			3823.646 3919.203	45884.1258	0.080
CM-02	Urine	96 h	50.2654 g	69.1980 g	18.9326 g	0.2001 g 0.2011 g	277.13 278.05	3.90 3.90	273.230 274.150			1365.467 1363.252	25830.8763	0.045
CM-02	Urine	120 h	49.7676 g	58.2694 g	8.5018 g	0.2089 g 0.2121 g	336.87 329.49	3.90 3.90	332.970 325.590			1593.921 1535.078	13301.0590	0.023

CM-02	Urine	144 h	47.7846 g	56.7403 g	8.9557 g	0.2044 g 0.2075 g	181.95 192.33	0.00 0.00	181.950 192.330			890.166 926.892	8136.5127	0.014
CM-02	Urine	168 h	50.0855 g	60.5776 g	10.4921 g	0.2077 g 0.2101 g	161.69 174.21	3.10 3.10	158.595 171.115			763.577 814.446	8278.3864	0.014

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 4 (contd). Recovery of Radioactivity in Urine from Group 2.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-03	Urine	8 h	16.5370 g	21.0053 g	4.4683 g	0.0512 g 0.0517 g	20958.37 21257.53	25.83 25.83	20932.540 21231.700			408838.672 410671.180	1830907.9353	2.954
CM-03	Urine	24 h	49.3539 g	55.0547 g	5.7008 g	0.0518 g 0.0540 g	9358.26 9916.82	31.37 31.37	9326.895 9885.455			180055.888 183063.981	1035036.8761	1.670
CM-03	Urine	48 h	50.0055 g	59.6864 g	9.6809 g	0.1978 g 0.2022 g	1933.32 1965.51	4.60 4.60	1928.725 1960.915			9750.885 9697.898	94140.8610	0.152
CM-03	Urine	72 h	49.5513 g	62.8301 g	13.2788 g	0.2049 g 0.2116 g	578.91 570.88	3.90 3.90	575.010 566.980			2806.296 2679.490	36422.3233	0.059
CM-03	Urine	96 h	49.3539 g	64.2525 g	14.8986 g	0.2054 g 0.2046 g	296.89 301.67	3.90 3.90	292.990 297.770			1426.436 1455.376	21467.4863	0.035
CM-03	Urine	120 h	49.2192 g	59.1509 g	9.9317 g	0.2087 g 0.2067 g	247.53 264.03	3.90 3.90	243.630 260.130			1167.369 1258.491	12046.4569	0.019
CM-03	Urine	144 h	50.0996 g	62.7657 g	12.6661 g	0.2021 g 0.2055 g	181.35 176.72	0.00 0.00	181.350 176.720			897.328 859.951	11128.9383	0.018
CM-03	Urine	168 h	50.2428 g	59.7059 g	9.4631 g	0.2064 g 0.2097 g	143.11 144.77	3.10 3.10	140.015 141.675			678.367 675.608	6406.4016	0.010
CM-04	Urine	8 h	16.6114 g	18.5065 g	1.8951 g	0.0529 g 0.0539 g	27836.60 27971.21	25.83 25.83	27810.770 27945.380			525723.440 518467.161	989422.8048	6.461
CM-04	Urine	24 h	50.0264 g	57.2613 g	7.2349 g	0.0542 g 0.0524 g	10002.06 9761.64	31.37 31.37	9970.695 9730.275			183961.162 185692.271	1337202.8125	8.732
CM-04	Urine	48 h	49.1938 g	65.1926 g	15.9988 g	0.2017 g 0.2051 g	2284.97 2395.29	4.60 4.60	2280.375 2390.695			11305.776 11656.241	183682.3569	1.199
CM-04	Urine	72 h	49.9395 g	61.4514 g	11.5119 g	0.2071 g 0.2098 g	684.50 742.74	3.90 3.90	680.600 738.840			3286.335 3521.640	39186.3623	0.256
CM-04	Urine	96 h	48.7565 g	56.3028 g	7.5463 g	0.2121 g 0.2096 g	393.63 372.46	3.90 3.90	389.730 368.560			1837.482 1758.397	13567.7919	0.089
CM-04	Urine	120 h	49.9636 g	60.1770 g	10.2134 g	0.2100 g 0.2111 g	251.37 243.74	3.90 3.90	247.470 239.840			1178.429 1136.144	11819.8278	0.077
CM-04	Urine	144 h	49.5018 g	58.9107 g	9.4089 g	0.2049 g 0.2084 g	194.47 210.64	0.00 0.00	194.470 210.640			949.097 1010.749	9219.9960	0.060
CM-04	Urine	168 h	50.9518 g	60.1408 g	9.1890 g	0.2091 g 0.2125 g	166.56 163.50	3.10 3.10	163.465 160.405			781.755 754.847	7059.9188	0.046

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 5. Recovery of Radioactivity in Feces from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Homog wt	Corr Homog	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Feces	24 h	50.1379	55.4465	5.3086	60.6817	10.5438	0.0775 0.0906	2308.16 2566.50	16.42 16.42	2291.742 2550.082			29570.865 28146.600	304280.7035	0.551
CM-01	Feces	48 h	50.3414	54.0328	3.6914	57.8248	7.4834	0.0859 0.0947	962.37 1042.05	16.42 16.42	945.952 1025.632			11012.247 10830.327	81728.3597	0.148
CM-01	Feces	72 h	50.1662	58.1751	8.0089	65.3328	15.1666	0.0899 0.0995	669.00 609.90	16.42 16.42	652.582 593.482			7258.977 5964.643	100278.6765	0.182
CM-01	Feces	96 h	49.1753	55.0041	5.8288	61.1627	11.9874	0.0599 0.0621	176.87 182.11	9.08 9.08	167.788 173.028			2801.127 2786.272	33489.1935	0.061
CM-01	Feces	120 h	50.2002	55.1973	4.9971	60.9793	10.7791	0.0731 0.0547	145.04 112.66	9.08 9.08	135.958 103.578			1859.884 1893.556	20229.3497	0.037
CM-01	Feces	144 h	49.7822	60.6093	10.8271	72.6359	22.8537	0.0538 0.0536	65.59 64.40	9.08 9.08	56.508 55.318			1050.325 1032.043	23794.9089	0.043
CM-01	Feces	168 h	50.0089	56.5155	6.5066	65.8127	15.8038	0.0567 0.0546	63.24 65.20	9.08 9.08	54.158 56.118			955.159 1027.793	15669.0866	0.028
CM-02	Feces	24 h	50.5312	54.7789	4.2477	59.3724	8.8412	0.0820 0.0927	1970.36 2270.91	16.42 16.42	1953.942 2254.492			23828.561 24320.302	212846.8639	0.370
CM-02	Feces	48 h	49.8609	53.3509	3.4900	57.3311	7.4702	0.0631 0.0781	901.67 1143.17	16.42 16.42	885.252 1126.752			14029.350 14427.042	106287.4716	0.185
CM-02	Feces	72 h	49.6047	61.2648	11.6601	75.0969	25.4922	0.0862 0.0725	255.68 228.62	16.42 16.42	239.262 212.202			2775.661 2926.924	72685.7237	0.126
CM-02	Feces	96 h	50.2877	56.0242	5.7365	66.1906	15.9029	0.0932 0.0802	169.07 155.96	9.08 9.08	159.988 146.878			1716.604 1831.390	28211.6997	0.049
CM-02	Feces	120 h	49.6526	56.7563	7.1037	64.4761	14.8235	0.0433 0.0472	93.49 108.15	9.08 9.08	84.408 99.068			1949.365 2098.888	30004.6363	0.052
CM-02	Feces	144 h	49.9225	62.9517	13.0292	73.9735	24.0510	0.0588 0.0563	60.96 51.89	9.08 9.08	51.878 42.808			882.270 760.346	19753.2879	0.034
CM-02	Feces	168 h	49.9487	61.8468	11.8981	72.1372	22.1885	0.0562 0.0623	60.23 63.87	9.08 9.08	51.148 54.788			910.098 879.414	19853.2934	0.034

**Table 5 (contd). Recovery of Radioactivity in Feces from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Homog wt	Corr Homog	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-03	Feces	24 h	50.0987	57.2064	7.1077	61.1449	11.0462	0.0755 0.0688	3729.77 3194.62	16.42 16.42	3713.352 3178.202			49183.470 46194.797	526783.7049	0.850
CM-03	Feces	48 h	50.7997	58.1472	7.3475	66.0725	15.2728	0.0828 0.0773	964.40 681.73	16.42 16.42	947.982 665.312			11449.058 8606.882	153155.1821	0.247
CM-03	Feces	72 h	49.8413	56.3469	6.5056	64.7264	14.8851	0.0633 0.0572	231.90 231.98	16.42 16.42	215.482 215.562			3404.139 3768.566	53383.2190	0.086
CM-03	Feces	96 h	50.2371	60.9189	10.6818	70.5728	20.3357	0.0462 0.0735	232.63 237.32	9.08 9.08	223.548 228.238			4838.690 3105.272	80773.0200	0.130
CM-03	Feces	120 h	50.1252	56.3624	6.2372	64.0774	13.9522	0.0568 0.0510	133.91 120.79	9.08 9.08	124.828 111.708			2197.667 2190.343	30611.1993	0.049
CM-03	Feces	144 h	50.7243	52.3823	1.6580	57.2336	6.5093	0.0715 0.0905	98.58 113.16	9.08 9.08	89.498 104.078			1251.713 1150.028	7816.8261	0.013
CM-03	Feces	168 h	49.9673	71.7209	21.7536	84.8118	34.8445	0.0780 0.0563	98.24 68.84	9.08 9.08	89.158 59.758			1143.045 1061.412	38406.6001	0.062
CM-04	Feces	24 h	48.8431	53.9673	5.1242	58.0589	9.2158	0.0677 0.0766	2107.54 2386.85	16.42 16.42	2091.122 2370.432			30888.065 30945.587	284923.2872	1.861
CM-04	Feces	48 h	49.6202	55.5099	5.8897	61.3325	11.7123	0.0709 0.0863	749.79 722.30	16.42 16.42	733.372 705.882			10343.752 8179.397	108474.3403	0.708
CM-04	Feces	72 h	49.2083	56.6738	7.4655	65.2357	16.0274	0.0846 0.0584	230.38 187.77	16.42 16.42	213.962 171.352			2529.102 2934.110	43780.5359	0.286
CM-04	Feces	96 h	49.8824	64.1021	14.2197	75.6956	25.8132	0.0669 0.0458	181.88 108.94	9.08 9.08	172.798 99.858			2582.922 2180.295	61476.9369	0.401
CM-04	Feces	120 h	49.3234	56.7203	7.3969	66.3387	17.0153	0.0470 0.0558	74.43 87.23	9.08 9.08	65.348 78.148			1390.372 1400.493	23743.7041	0.155
CM-04	Feces	144 h	48.8169	59.0513	10.2344	68.8185	20.0016	0.0608 0.0514	59.60 63.18	9.08 9.08	50.518 54.098			830.880 1052.481	18835.1115	0.123
CM-04	Feces	168 h	50.5895	60.6736	10.0841	71.0874	20.4979	0.0501 0.0520	50.21 51.19	9.08 9.08	41.128 42.108			820.908 809.760	16712.6327	0.109

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 6. Recovery of Radioactivity in CO<sub>2</sub> Trap 1 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	CO2 Trap 1	1 h	0.00	490.30	490.30	1.0077 1.0011	570.60 478.29	18.43 18.43	552.173 459.863			547.954 459.358	246942.5678	0.447
CM-01	CO2 Trap 1	3 h	0.00	523.66	523.66	1.0389 1.0392	46.46 50.37	29.40 29.40	17.065 20.975		*	16.426 20.184	9585.5499	0.017
CM-01	CO2 Trap 1	6 h	0.00	491.96	491.96	1.2911 1.0336	4759.31 3348.04	15.38 15.38	4743.929 3332.659			3674.331 3224.322	1696930.6986	3.073
CM-01	CO2 Trap 1	24 h	0.00	500.33	500.33	1.0572 1.0435	14427.83 14047.58	15.38 15.38	14412.449 14032.199			13632.661 13447.244	6774444.4008	12.267
CM-01	CO2 Trap 1	48 h	0.00	452.14	452.14	1.0312 1.0924	1007.16 1207.98	15.38 15.38	991.779 1192.599			961.772 1091.724	464233.7551	0.841
CM-01	CO2 Trap 1	72 h	0.00	497.20	497.20	1.0415 1.0380	500.58 490.91	15.38 15.38	485.199 475.529			465.866 458.121	229702.9637	0.416
CM-01	CO2 Trap 1	96 h	0.00	499.65	499.65	1.0526 1.0451	241.98 220.18	15.38 15.38	226.599 204.799			215.276 195.961	102737.2420	0.186
CM-01	CO2 Trap 1	120 h	0.01	511.23	511.22	1.0510 1.0423	180.24 188.74	15.38 15.38	164.859 173.359			156.859 166.324	82608.7736	0.150
CM-01	CO2 Trap 1	144 h	0.00	495.81	495.81	1.0236 1.0339	159.56 151.52	40.80 40.80	118.760 110.720			116.022 107.090	55310.4673	0.100
CM-01	CO2 Trap 1	168 h	0.00	432.25	432.25	1.0576 1.0552	146.06 133.70	48.32 48.32	97.737 85.377		*	92.414 80.910	37459.6609	0.068
CM-02	CO2 Trap 1	1 h	0.00	523.60	523.60	1.0640 1.0310	508.20 488.51	18.43 18.43	489.773 470.083			460.313 455.949	239877.4440	0.416
CM-02	CO2 Trap 1	3 h	0.00	504.82	504.82	1.0022 1.0333	1348.34 1431.13	18.43 18.43	1329.913 1412.703			1326.994 1367.176	680035.5272	1.181
CM-02	CO2 Trap 1	6 h	0.00	503.45	503.45	1.1323 1.0111	4729.43 4098.53	15.38 15.38	4714.049 4083.149			4163.251 4038.324	2064541.3872	3.584
CM-02	CO2 Trap 1	24 h	0.00	520.27	520.27	1.0500 1.0381	12921.11 13081.04	15.38 15.38	12905.729 13065.659			12291.171 12586.128	6471455.9649	11.235
CM-02	CO2 Trap 1	48 h	0.00	503.79	503.79	1.2546 1.0504	870.87 775.99	15.38 15.38	855.489 760.609			681.882 724.114	354163.2880	0.615
CM-02	CO2 Trap 1	72 h	0.00	540.28	540.28	1.0407 1.0324	292.56 275.18	15.38 15.38	277.179 259.799			266.339 251.646	139928.4295	0.243
CM-02	CO2 Trap 1	96 h	0.00	503.82	503.82	1.0489 1.0422	195.67 188.68	15.38 15.38	180.289 173.299			171.884 166.282	85187.3860	0.148
CM-02	CO2 Trap 1	120 h	0.00	383.39	383.39	1.0480 1.0314	188.00 169.96	15.38 15.38	172.619 154.579			164.713 149.873	60304.5536	0.105
CM-02	CO2 Trap 1	144 h	0.00	481.47	481.47	1.0445 1.0342	161.63 170.29	40.80 40.80	120.830 129.490			115.682 125.208	57990.6625	0.101
CM-02	CO2 Trap 1	168 h	0.00	441.91	441.91	1.0512 1.0547	137.23 140.04	48.32 48.32	88.907 91.717		*	84.576 86.960	37901.8063	0.066

**Table 6 (contd). Recovery of Radioactivity in CO<sub>2</sub> Trap 1 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-03	CO2 Trap 1	1 h	0.00	526.18	526.18	1.0554 1.0172	491.89 475.81	18.43 18.43	473.463 457.383			448.610 449.649	236323.1407	0.381
CM-03	CO2 Trap 1	3 h	0.00	468.45	468.45	1.0701 1.0076	1592.14 1404.32	18.43 18.43	1573.713 1385.893			1470.623 1375.440	666619.0295	1.076
CM-03	CO2 Trap 1	6 h	0.00	491.87	491.87	1.3109 1.3951	5863.37 6424.76	15.38 15.38	5847.989 6409.379			4461.049 4594.208	2227004.5358	3.593
CM-03	CO2 Trap 1	24 h	0.00	480.16	480.16	1.0451 1.0455	15663.79 17512.06	15.38 15.38	15648.409 17496.679			14973.121 16735.226	7612540.0951	12.283
CM-03	CO2 Trap 1	48 h	0.00	537.25	537.25	1.1550 0.9654	1619.51 1332.57	15.38 15.38	1604.129 1317.189			1388.856 1364.397	739592.7459	1.193
CM-03	CO2 Trap 1	72 h	0.00	475.62	475.62	1.0470 1.0434	572.84 546.39	15.38 15.38	557.459 531.009			532.435 508.922	247644.9995	0.400
CM-03	CO2 Trap 1	96 h	0.00	499.88	499.88	1.0463 1.0202	296.67 262.08	15.38 15.38	281.289 246.699			268.842 241.814	127633.4003	0.206
CM-03	CO2 Trap 1	120 h	0.00	497.56	497.56	1.0396 1.0529	249.39 256.31	15.38 15.38	234.009 240.929			225.095 228.824	112926.1187	0.182
CM-03	CO2 Trap 1	144 h	0.00	457.74	457.74	1.0297 1.0297	242.00 246.60	40.80 40.80	201.200 205.800			195.397 199.864	90463.3291	0.146
CM-03	CO2 Trap 1	168 h	0.00	426.92	426.92	1.0598 1.0556	183.58 171.41	48.32 48.32	135.257 123.087			127.625 116.604	52132.9559	0.084
CM-04	CO2 Trap 1	1 h	0.00	522.07	522.07	1.0378 1.0418	508.33 504.27	18.43 18.43	489.903 485.843			472.059 466.350	244957.6956	1.600
CM-04	CO2 Trap 1	3 h	0.06	479.56	479.50	1.1495 1.2601	1653.86 2024.88	18.43 18.43	1635.433 2006.453			1422.735 1592.297	722853.7853	4.720
CM-04	CO2 Trap 1	6 h	0.00	530.40	530.40	1.0388 1.0498	3363.69 3498.30	15.38 15.38	3348.309 3482.919			3223.247 3317.698	1734658.5733	11.328
CM-04	CO2 Trap 1	24 h	0.00	484.93	484.93	1.0492 1.0588	12420.51 13959.27	15.38 15.38	12405.129 13943.889			11823.417 13169.521	6059912.7649	39.572
CM-04	CO2 Trap 1	48 h	0.00	495.21	495.21	1.0911 1.0175	944.90 762.90	15.38 15.38	929.519 747.519			851.910 734.662	392843.3036	2.565
CM-04	CO2 Trap 1	72 h	0.00	492.82	492.82	1.0427 1.0333	421.32 396.78	15.38 15.38	405.939 381.399			389.315 369.108	186883.0429	1.220
CM-04	CO2 Trap 1	96 h	0.00	534.71	534.71	1.0459 1.0354	240.52 242.19	15.38 15.38	225.139 226.809			215.259 219.055	116115.8257	0.758
CM-04	CO2 Trap 1	120 h	0.00	514.48	514.48	1.0429 1.0431	189.01 177.93	15.38 15.38	173.629 162.549			166.487 155.833	82913.4703	0.541
CM-04	CO2 Trap 1	144 h	0.00	481.13	481.13	1.0371 1.0425	170.62 180.41	40.80 40.80	129.820 139.610			125.176 133.918	62329.0532	0.407
CM-04	CO2 Trap 1	168 h	0.00	447.61	447.61	1.0445 1.0402	150.64 134.50	48.32 48.32	102.317 86.177		*	97.958 82.846	40464.7951	0.264

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 7. Recovery of Radioactivity in CO<sub>2</sub> Trap 2 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	CO2 Trap 2	1 h	0.00	467.41	467.41	1.0360 1.0249	19.37 18.92	18.43 18.43	0.943 0.493		*	0.911 0.481	325.2943	0.001
CM-01	CO2 Trap 2	3 h	0.00	494.91	494.91	1.0448 1.0472	1523.93 1487.19	29.40 29.40	1494.535 1457.795			1430.451 1392.088	698451.4452	1.265
CM-01	CO2 Trap 2	6 h	0.00	500.60	500.60	0.8320 1.0826	24.58 35.51	15.38 15.38	9.199 20.129		*	11.057 18.593	7421.3663	0.013
CM-01	CO2 Trap 2	24 h	0.00	480.62	480.62	1.0296 1.0314	1111.84 1102.61	15.38 15.38	1096.459 1087.229			1064.937 1054.129	509232.8427	0.922
CM-01	CO2 Trap 2	48 h	0.00	485.06	485.06	1.0431 1.0131	262.34 252.68	15.38 15.38	246.959 237.299			236.755 234.231	114228.1428	0.207
CM-01	CO2 Trap 2	72 h	0.00	500.26	500.26	1.0470 1.0260	137.03 144.06	15.38 15.38	121.649 128.679			116.188 125.418	60433.0240	0.109
CM-01	CO2 Trap 2	96 h	0.01	550.29	550.28	1.0437 1.0423	78.87 74.13	15.38 15.38	63.489 58.749			60.831 56.365	32245.2092	0.058
CM-01	CO2 Trap 2	120 h	0.00	529.51	529.51	1.0278 1.0170	59.03 53.50	15.38 15.38	43.649 38.119			42.468 37.482	21167.2592	0.038
CM-01	CO2 Trap 2	144 h	0.00	496.85	496.85	1.0352 1.0031	94.44 85.95	40.80 48.32	53.640 37.627		*	51.816 37.510	22190.9255	0.040
CM-01	CO2 Trap 2	168 h	0.00	423.49	423.49	1.0497 1.0511	95.33 92.32	48.32 48.32	47.007 43.997		*	44.781 41.858	18345.3295	0.033
CM-02	CO2 Trap 2	1 h	0.00	571.59	571.59	1.0299 1.0281	22.36 18.92	18.43 18.43	3.933 0.493		*	3.819 0.480	1228.6300	0.002
CM-02	CO2 Trap 2	3 h	0.00	507.47	507.47	1.1457 1.0445	32.47 32.25	18.43 18.43	14.043 13.823		*	12.257 13.234	6468.1692	0.011
CM-02	CO2 Trap 2	6 h	0.01	548.70	548.69	1.0659 1.0745	40.36 38.96	15.38 15.38	24.979 23.579		*	23.435 21.944	12449.4979	0.022
CM-02	CO2 Trap 2	24 h	0.00	480.57	480.57	1.0380 1.0382	710.86 719.07	15.38 15.38	695.479 703.689			670.018 677.797	323859.8783	0.562
CM-02	CO2 Trap 2	48 h	0.00	596.52	596.52	0.9346 1.0249	217.10 245.07	15.38 15.38	201.719 229.689			215.835 224.109	131217.5367	0.228
CM-02	CO2 Trap 2	72 h	0.00	526.52	526.52	1.0378 1.0208	175.16 163.67	15.38 15.38	159.779 148.289			153.959 145.268	78774.4871	0.137
CM-02	CO2 Trap 2	96 h	0.00	519.50	519.50	1.0404 1.0380	107.02 101.02	15.38 15.38	91.639 85.639			88.081 82.504	44309.3432	0.077
CM-02	CO2 Trap 2	120 h	0.00	539.26	539.26	1.0359 1.0388	90.59 89.39	15.38 15.38	75.209 74.009			72.603 71.245	38785.5875	0.067
CM-02	CO2 Trap 2	144 h	0.05	466.00	465.95	1.0233 1.0246	86.46 89.29	40.80 40.80	45.660 48.490		*	44.620 47.326	21421.1500	0.037
CM-02	CO2 Trap 2	168 h	0.00	446.26	446.26	1.0401 1.0393	93.68 80.83	48.32 48.32	45.357 32.507		*	43.608 31.277	16709.1902	0.029

**Table 7 (contd). Recovery of Radioactivity in CO2 Trap 2 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-03	CO2 Trap 2	1 h	0.00	521.17	521.17	1.0485 1.0273	18.69 22.82	18.43 18.43	0.263 4.393		*	0.251 4.277	1179.8598	0.002
CM-03	CO2 Trap 2	3 h	0.00	493.38	493.38	1.1244 1.0920	31.80 29.95	18.43 18.43	13.373 11.523		*	11.894 10.553	5537.2663	0.009
CM-03	CO2 Trap 2	6 h	0.00	490.60	490.60	1.0724 1.1990	52.79 56.31	15.38 15.38	37.409 40.929			34.884 34.136	16930.4947	0.027
CM-03	CO2 Trap 2	24 h	0.00	483.12	483.12	1.0375 1.0375	1691.43 1674.66	15.38 15.38	1676.049 1659.279			1615.469 1599.305	776560.8440	1.253
CM-03	CO2 Trap 2	48 h	0.00	525.06	525.06	1.0157 1.0333	500.41 537.45	15.38 15.38	485.029 522.069			477.532 505.244	258008.2599	0.416
CM-03	CO2 Trap 2	72 h	0.00	533.66	533.66	1.0400 1.0266	186.17 166.03	15.38 15.38	170.789 150.649			164.220 146.746	82975.0407	0.134
CM-03	CO2 Trap 2	96 h	0.00	534.21	534.21	1.0271 1.0309	96.23 99.43	15.38 15.38	80.849 84.049			78.716 81.530	42802.4303	0.069
CM-03	CO2 Trap 2	120 h	0.00	499.09	499.09	1.0322 1.0397	60.01 65.28	15.38 15.38	44.629 49.899			43.237 47.994	22766.1409	0.037
CM-03	CO2 Trap 2	144 h	0.00	434.99	434.99	1.0310 1.0316	100.62 109.29	40.80 40.80	59.820 68.490		*	58.021 66.392	27059.2818	0.044
CM-03	CO2 Trap 2	168 h	0.00	463.41	463.41	1.0418 1.0509	97.90 98.78	48.32 48.32	49.577 50.457		*	47.588 48.013	22151.0729	0.036
CM-04	CO2 Trap 2	1 h	0.00	506.47	506.47	1.0202 1.1229	18.23 19.42	18.43 18.43	0.000 0.993	*		0.000 0.885	224.0153	0.001
CM-04	CO2 Trap 2	3 h	0.00	470.22	470.22	1.0450 1.1401	25.34 22.15	18.43 18.43	6.913 3.723		*	6.616 3.266	2323.2219	0.015
CM-04	CO2 Trap 2	6 h	0.00	507.89	507.89	1.0146 1.0599	41.43 36.22	15.38 15.38	26.049 20.839		*	25.674 19.661	11512.7544	0.075
CM-04	CO2 Trap 2	24 h	0.00	498.50	498.50	1.0329 1.0390	1334.77 1321.62	15.38 15.38	1319.389 1306.239			1277.364 1257.208	631742.0214	4.125
CM-04	CO2 Trap 2	48 h	0.00	503.19	503.19	1.0357 1.0142	306.46 313.37	15.38 15.38	291.079 297.989			281.046 293.817	144632.5680	0.944
CM-04	CO2 Trap 2	72 h	0.00	497.60	497.60	1.0417 1.0332	164.92 149.96	15.38 15.38	149.539 134.579			143.553 130.255	68123.3236	0.445
CM-04	CO2 Trap 2	96 h	0.00	500.89	500.89	1.0382 1.0325	84.11 92.55	15.38 15.38	68.729 77.169			66.200 74.740	35297.7887	0.231
CM-04	CO2 Trap 2	120 h	0.00	503.19	503.19	1.0374 1.0343	57.23 75.47	15.38 15.38	41.849 60.089			40.340 58.096	24766.1932	0.162
CM-04	CO2 Trap 2	144 h	0.00	460.40	460.40	1.0349 1.0484	85.61 78.99	40.80 40.80	44.810 38.190		*	43.299 36.427	18352.8805	0.120
CM-04	CO2 Trap 2	168 h	0.00	431.17	431.17	1.0396 1.0489	85.88 84.29	48.32 48.32	37.557 35.967		*	36.126 34.290	15180.6258	0.099

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 8. Recovery of Radioactivity in Exhaled VOC Trap 1 from Group C.**

Subject <sup>a</sup>	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Exhaled VOC Trap 1	1 h	17.5558	25.3023	7.7465	0.0474 0.0483	99943.74 100749.81	25.67 25.67	99918.074 100724.144			2107976.239 2085385.999	16241940.2899	29.410
CM-01	Exhaled VOC Trap 1	3 h	17.5676	25.3483	7.7807	0.0460 0.0474	59579.64 61273.30	25.67 25.67	59553.974 61247.634			1294651.603 1292144.172	10063540.9441	18.222
CM-01	Exhaled VOC Trap 1	6 h	17.5313	25.3044	7.7731	0.0476 0.0482	44284.21 44039.05	25.67 25.67	44258.544 44013.384			929801.339 913140.742	7162686.5449	12.970
CM-01	Exhaled VOC Trap 1	24 h	17.6593	25.4422	7.7829	0.0469 0.0480	13796.80 13896.70	25.67 25.67	13771.134 13871.034			293627.585 288979.870	2267187.7811	4.105
CM-01	Exhaled VOC Trap 1	48 h	17.6284	21.4364	3.8080	0.0497 0.0485	378.90 367.05	25.59 25.59	353.308 341.458			7108.803 7040.361	26940.0076	0.049
CM-01	Exhaled VOC Trap 1	72 h	17.6258	21.4358	3.8100	0.0484 0.0494	156.32 165.32	25.59 25.59	130.728 139.728			2700.981 2828.492	10533.6467	0.019
CM-01	Exhaled VOC Trap 1	96 h	17.6580	21.5898	3.9318	0.0458 0.0480	90.47 81.26	26.58 26.58	63.886 54.676			1394.891 1139.083	4981.5398	0.009
CM-01	Exhaled VOC Trap 1	120 h	17.7819	21.7156	3.9337	0.0477 0.0508	70.95 67.63	26.58 26.58	44.366 41.046		*	930.105 807.992	3418.5760	0.006
CM-01	Exhaled VOC Trap 1	144 h	17.4693	21.3647	3.8954	0.0492 0.0482	57.05 59.98	26.58 26.58	30.466 33.396		*	619.228 692.863	2555.5591	0.005
CM-01	Exhaled VOC Trap 1	168 h	17.7322	21.6433	3.9111	0.0459 0.0485	51.39 50.39	25.67 25.67	25.717 24.717		*	560.276 509.622	2092.2389	0.004
CM-02	Exhaled VOC Trap 1	1 h	17.5459	25.2954	7.7495	0.0482 0.0469	121083.68 121609.67	25.67 25.67	121058.014 121584.004			2511577.049 2592409.462	19776671.7311	34.334
CM-02	Exhaled VOC Trap 1	3 h	17.5840	25.3323	7.7483	0.0472 0.0491	73991.88 76960.98	25.67 25.67	73966.214 76935.314			1567080.800 1566910.667	12141553.0411	21.079
CM-02	Exhaled VOC Trap 1	6 h	17.4309	25.1783	7.7474	0.0474 0.0493	45609.96 45949.57	25.67 25.67	45584.294 45923.904			961693.961 931519.346	7333740.3866	12.732
CM-02	Exhaled VOC Trap 1	24 h	17.5774	25.3569	7.7795	0.0475 0.0472	4309.84 4259.25	25.67 25.67	4284.174 4233.584			90193.132 89694.571	699718.1910	1.215
CM-02	Exhaled VOC Trap 1	48 h	17.5024	21.3221	3.8197	0.0488 0.0492	303.20 285.14	25.59 25.59	277.608 259.548			5688.678 5275.356	20939.6603	0.036
CM-02	Exhaled VOC Trap 1	72 h	17.7206	21.5519	3.8313	0.0485 0.0478	214.15 210.72	25.59 25.59	188.558 185.128			3887.784 3872.960	14866.8688	0.026
CM-02	Exhaled VOC Trap 1	96 h	17.6396	21.5424	3.9028	0.0479 0.0464	151.99 165.19	26.58 26.58	125.406 138.606			2618.079 2987.198	10938.1387	0.019
CM-02	Exhaled VOC Trap 1	120 h	17.8302	21.7374	3.9072	0.0472 0.0477	99.39 98.53	26.58 26.58	72.806 71.946			1542.500 1508.302	5960.0466	0.010
CM-02	Exhaled VOC Trap 1	144 h	17.6197	21.5368	3.9171	0.0470 0.0471	76.54 89.14	26.58 26.58	49.956 62.556		*	1062.894 1328.153	4682.9841	0.008
CM-02	Exhaled VOC Trap 1	168 h	17.9646	21.8586	3.8940	0.0490 0.0464	68.06 66.63	25.67 25.67	42.387 40.957		*	865.034 882.687	3402.8124	0.006

<sup>a</sup> Samples from CM-04 were not analyzed.

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 8 (contd). Recovery of Radioactivity in Exhaled VOC Trap 1 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-03	Exhaled VOC Trap 1	1 h	17.4816	25.5681	8.0865	0.0471 0.0462	103897.22 100125.02	25.67 25.67	103871.554 100099.354			2205340.844 2166652.679	17677062.8099	28.523
CM-03	Exhaled VOC Trap 1	3 h	17.5195	25.2592	7.7397	0.0457 0.0492	74391.22 76154.17	25.67 25.67	74365.554 76128.504			1627255.005 1547327.312	12285157.3812	19.823
CM-03	Exhaled VOC Trap 1	6 h	17.6374	25.3807	7.7433	0.0479 0.0469	53799.01 52072.05	25.67 25.67	53773.344 52046.384			1122616.780 1109730.997	8642869.2691	13.946
CM-03	Exhaled VOC Trap 1	24 h	17.9299	25.7339	7.8040	0.0486 0.0497	22022.07 22080.24	25.67 25.67	21996.404 22054.574			452600.900 443753.999	3497576.8167	5.644
CM-03	Exhaled VOC Trap 1	48 h	17.6796	21.4889	3.8093	0.0478 0.0484	545.00 551.15	25.59 25.59	519.408 525.558			10866.266 10858.626	41378.3150	0.067
CM-03	Exhaled VOC Trap 1	72 h	17.6713	21.4801	3.8088	0.0490 0.0484	167.96 173.29	25.59 25.59	142.368 147.698			2905.459 3051.601	11344.6259	0.018
CM-03	Exhaled VOC Trap 1	96 h	17.7283	21.6241	3.8958	0.0486 0.0483	102.20 101.46	26.58 26.58	75.616 74.876			1555.885 1550.228	6050.3966	0.010
CM-03	Exhaled VOC Trap 1	120 h	17.5743	21.4510	3.8767	0.0453 0.0449	71.37 72.05	26.58 26.58	44.786 45.466		*	988.653 1012.606	3879.1408	0.006
CM-03	Exhaled VOC Trap 1	144 h	17.5994	21.5116	3.9122	0.0457 0.0481	62.68 59.05	26.58 26.58	36.096 32.466		*	789.847 674.969	2865.3259	0.005
CM-03	Exhaled VOC Trap 1	168 h	17.7335	21.6304	3.8969	0.0458 0.0480	48.47 61.56	25.67 25.67	22.797 35.887		*	497.744 747.639	2426.5659	0.004

<sup>a</sup> Samples from CM-04 were not analyzed.

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 9. Recovery of Radioactivity in VOC Trap 2 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Exhaled VOC Trap 2	1 h	17.2870	25.0071	7.7201	0.0473 0.0459	31.00 31.97	25.67 25.67	5.334 6.304		*	112.764 137.337	965.4019	0.002
CM-01	Exhaled VOC Trap 2	3 h	17.3408	25.1146	7.7738	0.0472 0.0474	39.11 34.60	25.67 25.67	13.444 8.934		*	284.825 188.476	1839.6735	0.003
CM-01	Exhaled VOC Trap 2	6 h	17.4090	25.1304	7.7214	0.0476 0.0453	34.18 25.69	25.67 25.67	8.514 0.024		*	178.860 0.524	692.5500	0.001
CM-01	Exhaled VOC Trap 2	24 h	17.6242	25.4035	7.7793	0.0456 0.0483	18230.11 17956.97	25.67 25.67	18204.444 17931.304			399220.258 371248.525	2996853.8999	5.426
CM-01	Exhaled VOC Trap 2	48 h	17.7448	21.5501	3.8053	0.0477 0.0479	51.12 44.42	25.59 25.59	25.528 18.828		*	535.168 393.058	1766.0895	0.003
CM-01	Exhaled VOC Trap 2	72 h	17.7050	21.5138	3.8088	0.0483 0.0489	30.73 30.07	25.59 25.59	5.138 4.478		*	106.366 91.564	376.9396	0.001
CM-01	Exhaled VOC Trap 2	96 h	17.4716	21.3689	3.8973	0.0486 0.0461	30.75 31.00	26.58 26.58	4.166 4.416		*	85.720 95.792	353.7032	0.001
CM-01	Exhaled VOC Trap 2	120 h	17.6334	21.5254	3.8920	0.0470 0.0473	31.23 28.06	26.58 26.58	4.646 1.476		*	98.851 31.205	253.0892	0.000
CM-01	Exhaled VOC Trap 2	144 h	17.6692	21.5887	3.9195	0.0478 0.0472	27.41 25.84	26.58 26.58	0.826 0.000	*	*	17.280 0.000	33.8651	0.000
CM-01	Exhaled VOC Trap 2	168 h	17.7720	21.6849	3.9129	0.0477 0.0484	25.14 27.80	25.67 25.67	0.000 2.127	*		0.000 43.939	85.9652	0.000
CM-02	Exhaled VOC Trap 2	1 h	17.3715	25.1200	7.7485	0.0469 0.0454	35.90 36.32	25.67 25.67	10.234 10.654		*	218.204 234.664	1754.5228	0.003
CM-02	Exhaled VOC Trap 2	3 h	17.4032	25.1312	7.7280	0.0471 0.0491	39.92 40.17	25.67 25.67	14.254 14.504		*	302.627 295.392	2310.7471	0.004
CM-02	Exhaled VOC Trap 2	6 h	17.5781	25.3211	7.7430	0.0484 0.0467	48.46 43.98	25.67 25.67	22.794 18.314		*	470.945 392.157	3341.5019	0.006
CM-02	Exhaled VOC Trap 2	24 h	17.7019	25.4820	7.7801	0.0490 0.0488	14165.28 13924.75	25.67 25.67	14139.614 13899.084			288563.546 284817.290	2230480.1206	3.872
CM-02	Exhaled VOC Trap 2	48 h	17.6271	21.4378	3.8107	0.0475 0.0490	56.54 67.04	25.59 25.59	30.948 41.448		*	651.526 845.867	2853.0590	0.005
CM-02	Exhaled VOC Trap 2	72 h	17.6103	21.4186	3.8083	0.0478 0.0488	31.18 30.71	25.59 25.59	5.588 5.118		*	116.893 104.867	422.2645	0.001
CM-02	Exhaled VOC Trap 2	96 h	17.6750	21.5940	3.9190	0.0454 0.0428	32.99 27.39	26.58 26.58	6.406 0.806		*	141.101 18.832	313.3889	0.001
CM-02	Exhaled VOC Trap 2	120 h	17.6740	21.5859	3.9119	0.0476 0.0483	25.36 30.32	26.58 26.58	0.000 3.736	*	*	0.000 77.350	151.2925	0.000
CM-02	Exhaled VOC Trap 2	144 h	17.6951	21.6186	3.9235	0.0459 0.0487	33.24 28.52	26.58 26.58	6.656 1.936		*	145.011 39.754	362.4617	0.001
CM-02	Exhaled VOC Trap 2	168 h	17.6282	21.6312	4.0030	0.0458 0.0488	26.96 26.75	25.67 25.67	1.287 1.077		*	28.093 22.063	100.3872	0.000

**Table 9 (contd). Recovery of Radioactivity in VOC Trap 2 from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-03	Exhaled VOC Trap 2	1 h	17.3866	25.1682	7.7816	0.0465 0.0443	35.47 36.14	25.67 25.67	9.804 10.474		*	210.833 236.428	1740.2035	0.003
CM-03	Exhaled VOC Trap 2	3 h	17.3954	25.1348	7.7394	0.0486 0.0448	41.02 39.29	25.67 25.67	15.354 13.624		*	315.921 304.102	2399.3005	0.004
CM-03	Exhaled VOC Trap 2	6 h	17.5554	25.3039	7.7485	0.0479 0.0481	41.73 46.66	25.67 25.67	16.064 20.994		*	335.360 436.460	2990.2261	0.005
CM-03	Exhaled VOC Trap 2	24 h	17.2213	24.9328	7.7115	0.0475 0.0477	27.57 25.34	28.57 28.57	0.000 0.000	*		0.000 0.000	0.0000	0.000
CM-03	Exhaled VOC Trap 2	48 h	17.6937	21.5069	3.8132	0.0477 0.0476	4014.48 3934.03	25.59 25.59	3988.888 3908.438			83624.476 82110.032	315989.4118	0.510
CM-03	Exhaled VOC Trap 2	72 h	17.7882	21.6101	3.8219	0.0475 0.0495	26.22 29.61	25.59 25.59	0.627 4.018		*	13.211 81.162	180.3404	0.000
CM-03	Exhaled VOC Trap 2	96 h	17.5522	21.4615	3.9093	0.0467 0.0467	25.79 26.04	26.58 26.58	0.000 0.000	*		0.000 0.000	0.0000	0.000
CM-03	Exhaled VOC Trap 2	120 h	17.7909	21.6904	3.8995	0.0463 0.0475	26.48 25.16	26.58 26.58	0.000 0.000	*		0.000 0.000	0.0000	0.000
CM-03	Exhaled VOC Trap 2	144 h	17.6494	21.5537	3.9043	0.0477 0.0493	26.48 24.69	26.58 26.58	0.000 0.000	*		0.000 0.000	0.0000	0.000
CM-03	Exhaled VOC Trap 2	168 h	17.5559	21.4776	3.9217	0.0485 0.0474	22.91 30.93	25.67 25.67	0.000 5.257	*		0.000 110.900	217.4585	0.000

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 10. Recovery of Radioactivity in Nose Only Tube Rinse from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Nose Only Tube Rinse	0 h	227.64	296.44	68.80	0.5421	951.59	0.92	950.670			1753.680	119843.93	0.217
						0.8379	1458.96	0.92	1458.040			1740.112		
						0.5657	980.69	0.92	979.770			1731.960		
CM-02	Nose Only Tube Rinse	0 h	229.08	334.99	105.91	0.5700	565.56	0.92	564.640			990.596	107893.96	0.187
						0.5653	582.71	0.92	581.790			1029.170		
						0.5704	592.10	0.92	591.180			1036.431		
CM-03	Nose Only Tube Rinse	0 h	228.50	347.05	118.55	0.5328	969.56	0.92	968.640			1818.018	216547.99	0.349
						0.5842	1072.25	0.92	1071.330			1833.841		
						0.5047	923.54	0.92	922.620			1828.056		
CM-04	Nose Only Tube Rinse	0 h	228.72	303.75	75.03	0.5872	714.90	0.92	713.980			1215.906	91240.85	0.596
						0.5935	701.06	0.92	700.140			1179.680		
						0.5891	738.82	0.92	737.900			1252.589		

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 11. Recovery of Radioactivity in Cage Rinse from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Cage Rinse	168 h	291.92	563.41	271.49	0.5431	76.81	33.22	43.590		*	80.261	21756.9550	0.039
						0.4799	71.62	33.22	38.400		*	80.017		
CM-02	Cage Rinse	168 h	290.04	492.84	202.80	0.5662	93.38	33.22	60.160		*	106.252	22579.7434	0.039
						0.5789	100.62	33.22	67.400		*	116.428		
CM-03	Cage Rinse	168 h	290.11	525.72	235.61	0.6135	95.84	33.22	62.620		*	102.070	22706.1049	0.037
						0.5854	86.30	33.22	53.080		*	90.673		
CM-04	Cage Rinse	168 h	290.30	588.24	297.94	0.5235	69.56	33.22	36.340		*	69.417	21354.7493	0.139
						0.6671	82.54	33.22	49.320		*	73.932		

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 14. Recovery of Radioactivity in Carcass Digest from Group C.**

Subject	Sample	Time	Pot wt	Samp wt	Corr Samp	Homog wt	Corr Homog	Alq wt	Orig DPM	Bkg DPM	Calc DPM	LOD	LOQ	DPM/g	Sample DPM	Reco (%)
CM-01	Carcass Digest	168 h	108.43	298.32	189.89	743.25	634.82	0.9660 0.7788	2243.64 1783.77	2.02 2.02	2241.623 1781.753			2320.521 2287.819	1462733.1726 dpm	2.649
CM-02	Carcass Digest	168 h	108.49	264.06	155.57	713.15	604.66	0.7983 0.7838	1639.65 1587.32	2.02 2.02	1637.633 1585.303			2051.401 2022.587	1231688.6221 dpm	2.138
CM-03	Carcass Digest	168 h	108.50	321.55	213.05	764.55	656.05	0.8878 0.7609	2545.03 2140.18	2.02 2.02	2543.013 2138.163			2864.399 2810.045	1861359.4966 dpm	3.003
CM-04	Carcass Digest	168 h	108.96	308.51	199.55	750.02	641.06	0.7264 0.7493	1645.23 1678.70	2.02 2.02	1643.213 1676.683			2262.133 2237.666	1442320.6245 dpm	9.419

\* in LOD column indicates that sample DPM were less than background DPM, and in LOQ column indicates that sample DPM was less than 3 x background.

**Table 15. Total Recovery of Radioactivity in Samples from Group C.**

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Reco (%)
CM-01	Urine	8 h	1702841.2448 dpm	55226372.663 dpm	3.083
CM-01	Urine	24 h	1112436.2962 dpm		2.014
CM-01	Urine	48 h	152192.0689 dpm		0.276
CM-01	Urine	72 h	42766.6875 dpm		0.077
CM-01	Urine	96 h	20639.8664 dpm		0.037
CM-01	Urine	120 h	13419.7170 dpm		0.024
CM-01	Urine	144 h	7603.5986 dpm		0.014
CM-01	Urine	168 h	6641.9187 dpm		0.012
CM-01	Feces	24 h	304280.7035 dpm		0.551
CM-01	Feces	48 h	81728.3597 dpm		0.148
CM-01	Feces	72 h	100278.6765 dpm		0.182
CM-01	Feces	96 h	33489.1935 dpm		0.061
CM-01	Feces	120 h	20229.3497 dpm		0.037
CM-01	Feces	144 h	23794.9089 dpm		0.043
CM-01	Feces	168 h	15669.0866 dpm		0.028
CM-01	Carcass Digest	168 h	1462733.1726 dpm		2.649
CM-01	CO2 Trap 1	1 h	246942.5678 dpm		0.447
CM-01	CO2 Trap 1	3 h	9585.5499 dpm		0.017
CM-01	CO2 Trap 1	6 h	1696930.6986 dpm		3.073
CM-01	CO2 Trap 1	24 h	6774444.4008 dpm		12.267
CM-01	CO2 Trap 1	48 h	464233.7551 dpm		0.841
CM-01	CO2 Trap 1	72 h	229702.9637 dpm		0.416
CM-01	CO2 Trap 1	96 h	102737.2420 dpm		0.186
CM-01	CO2 Trap 1	120 h	82608.7736 dpm		0.150
CM-01	CO2 Trap 1	144 h	55310.4673 dpm		0.100
CM-01	CO2 Trap 1	168 h	37459.6609 dpm		0.068
CM-01	CO2 Trap 2	1 h	325.2943 dpm		0.001
CM-01	CO2 Trap 2	3 h	698451.4452 dpm		1.265
CM-01	CO2 Trap 2	6 h	7421.3663 dpm		0.013
CM-01	CO2 Trap 2	24 h	509232.8427 dpm		0.922
CM-01	CO2 Trap 2	48 h	114228.1428 dpm		0.207
CM-01	CO2 Trap 2	72 h	60433.0240 dpm		0.109
CM-01	CO2 Trap 2	96 h	32245.2092 dpm		0.058
CM-01	CO2 Trap 2	120 h	21167.2592 dpm		0.038
CM-01	CO2 Trap 2	144 h	22190.9255 dpm		0.040
CM-01	CO2 Trap 2	168 h	18345.3295 dpm		0.033
CM-01	Exhaled VOC Trap 1	1 h	16241940.2899 dpm		29.410
CM-01	Exhaled VOC Trap 1	3 h	10063540.9441 dpm		18.222
CM-01	Exhaled VOC Trap 1	6 h	7162686.5449 dpm		12.970
CM-01	Exhaled VOC Trap 1	24 h	2267187.7811 dpm		4.105
CM-01	Exhaled VOC Trap 1	48 h	26940.0076 dpm		0.049
CM-01	Exhaled VOC Trap 1	72 h	10533.6467 dpm		0.019
CM-01	Exhaled VOC Trap 1	96 h	4981.5398 dpm		0.009
CM-01	Exhaled VOC Trap 1	120 h	3418.5760 dpm		0.006
CM-01	Exhaled VOC Trap 1	144 h	2555.5591 dpm		0.005
CM-01	Exhaled VOC Trap 1	168 h	2092.2389 dpm		0.004
CM-01	Exhaled VOC Trap 2	1 h	965.4019 dpm		0.002
CM-01	Exhaled VOC Trap 2	3 h	1839.6735 dpm		0.003
CM-01	Exhaled VOC Trap 2	6 h	692.5500 dpm		0.001
CM-01	Exhaled VOC Trap 2	24 h	2996853.8999 dpm		5.426
CM-01	Exhaled VOC Trap 2	48 h	1766.0895 dpm		0.003
CM-01	Exhaled VOC Trap 2	72 h	376.9396 dpm		0.001
CM-01	Exhaled VOC Trap 2	96 h	353.7032 dpm		0.001
CM-01	Exhaled VOC Trap 2	120 h	253.0892 dpm		0.000
CM-01	Exhaled VOC Trap 2	144 h	33.8651 dpm		0.000
CM-01	Exhaled VOC Trap 2	168 h	85.9652 dpm		0.000
CM-01	Cage Rinse	168 h	21756.9550 dpm		0.039
CM-01	Exposure Urine	0 h	N.C.		N.A.
CM-01	Exposure Feces	0 h	10931.7067 dpm		0.020
CM-01	Nose Only Tube Rinse	0 h	119843.9291 dpm		0.217

**Table 15 (contd). Total Recovery of Radioactivity in Samples from Group C.**

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Reco (%)
CM-02	Urine	8 h	1724425.4933 dpm	57600304.435 dpm	2.994
CM-02	Urine	24 h	513448.6599 dpm		0.891
CM-02	Urine	48 h	99729.5573 dpm		0.173
CM-02	Urine	72 h	45884.1258 dpm		0.080
CM-02	Urine	96 h	25830.8763 dpm		0.045
CM-02	Urine	120 h	13301.0590 dpm		0.023
CM-02	Urine	144 h	8136.5127 dpm		0.014
CM-02	Urine	168 h	8278.3864 dpm		0.014
CM-02	Feces	24 h	212846.8639 dpm		0.370
CM-02	Feces	48 h	106287.4716 dpm		0.185
CM-02	Feces	72 h	72685.7237 dpm		0.126
CM-02	Feces	96 h	28211.6997 dpm		0.049
CM-02	Feces	120 h	30004.6363 dpm		0.052
CM-02	Feces	144 h	19753.2879 dpm		0.034
CM-02	Feces	168 h	19853.2934 dpm		0.034
CM-02	Carcass Digest	168 h	1231688.6221 dpm		2.138
CM-02	CO2 Trap 1	1 h	239877.4440 dpm		0.416
CM-02	CO2 Trap 1	3 h	680035.5272 dpm		1.181
CM-02	CO2 Trap 1	6 h	2064541.3872 dpm		3.584
CM-02	CO2 Trap 1	24 h	6471455.9649 dpm		11.235
CM-02	CO2 Trap 1	48 h	354163.2880 dpm		0.615
CM-02	CO2 Trap 1	72 h	139928.4295 dpm		0.243
CM-02	CO2 Trap 1	96 h	85187.3860 dpm		0.148
CM-02	CO2 Trap 1	120 h	60304.5536 dpm		0.105
CM-02	CO2 Trap 1	144 h	57990.6625 dpm		0.101
CM-02	CO2 Trap 1	168 h	37901.8063 dpm		0.066
CM-02	CO2 Trap 2	1 h	1228.6300 dpm		0.002
CM-02	CO2 Trap 2	3 h	6468.1692 dpm		0.011
CM-02	CO2 Trap 2	6 h	12449.4979 dpm		0.022
CM-02	CO2 Trap 2	24 h	323859.8783 dpm		0.562
CM-02	CO2 Trap 2	48 h	131217.5367 dpm		0.228
CM-02	CO2 Trap 2	72 h	78774.4871 dpm		0.137
CM-02	CO2 Trap 2	96 h	44309.3432 dpm		0.077
CM-02	CO2 Trap 2	120 h	38785.5875 dpm		0.067
CM-02	CO2 Trap 2	144 h	21421.1500 dpm		0.037
CM-02	CO2 Trap 2	168 h	16709.1902 dpm		0.029
CM-02	Exhaled VOC Trap 1	1 h	19776671.7311 dpm		34.334
CM-02	Exhaled VOC Trap 1	3 h	12141553.0411 dpm		21.079
CM-02	Exhaled VOC Trap 1	6 h	7333740.3866 dpm		12.732
CM-02	Exhaled VOC Trap 1	24 h	699718.1910 dpm		1.215
CM-02	Exhaled VOC Trap 1	48 h	20939.6603 dpm		0.036
CM-02	Exhaled VOC Trap 1	72 h	14866.8688 dpm		0.026
CM-02	Exhaled VOC Trap 1	96 h	10938.1387 dpm		0.019
CM-02	Exhaled VOC Trap 1	120 h	5960.0466 dpm		0.010
CM-02	Exhaled VOC Trap 1	144 h	4682.9841 dpm		0.008
CM-02	Exhaled VOC Trap 1	168 h	3402.8124 dpm		0.006
CM-02	Exhaled VOC Trap 2	1 h	1754.5228 dpm		0.003
CM-02	Exhaled VOC Trap 2	3 h	2310.7471 dpm		0.004
CM-02	Exhaled VOC Trap 2	6 h	3341.5019 dpm		0.006
CM-02	Exhaled VOC Trap 2	24 h	2230480.1206 dpm		3.872
CM-02	Exhaled VOC Trap 2	48 h	2853.0590 dpm		0.005
CM-02	Exhaled VOC Trap 2	72 h	422.2645 dpm		0.001
CM-02	Exhaled VOC Trap 2	96 h	313.3889 dpm		0.001
CM-02	Exhaled VOC Trap 2	120 h	151.2925 dpm		0.000
CM-02	Exhaled VOC Trap 2	144 h	362.4617 dpm		0.001
CM-02	Exhaled VOC Trap 2	168 h	100.3872 dpm		0.000
CM-02	Blood	168 h	772.7276 dpm		0.001
CM-02	Spleen	168 h	2713.8934 dpm		0.005

**Table 15 (contd). Total Recovery of Radioactivity in Samples from Group C.**

CM-02	Liver	168 h	68605.0539 dpm	0.119
CM-02	Kidney	168 h	14078.4226 dpm	0.024
CM-02	Brain	168 h	8225.5161 dpm	0.014
CM-02	Heart	168 h	3921.6704 dpm	0.007
CM-02	Lung	168 h	7171.9886 dpm	0.012
CM-02	Stomach	168 h	6507.6618 dpm	0.011
CM-02	Small Intestine	168 h	15092.2206 dpm	0.026
CM-02	Femur	168 h	3312.0956 dpm	0.006
CM-02	Testes	168 h	14999.9728 dpm	0.026
CM-02	Cecum+Lg Int+Rect	168 h	10720.4814 dpm	0.019
CM-02	Adip (Abdominal)	168 h	3212.4929 dpm	0.006
CM-02	Cage Rinse	168 h	22579.7434 dpm	0.039
CM-02	Skin (Neck)	168 h	2992.3947 dpm	0.005
CM-02	Adipose (subcut)	168 h	3668.5436 dpm	0.006
CM-02	Stomach Contents	168 h	604.4376 dpm	0.001
CM-02	Muscle Gastrocnemius	168 h	2454.9885 dpm	0.004
CM-02	Small Intestine Cont	168 h	10854.0784 dpm	0.019
CM-02	Lg Int + Cecum Cont	168 h	8382.2992 dpm	0.015
CM-02	Exposure Urine	0 h	N.C.	N.A.
CM-02	Exposure Feces	0 h	N.C.	N.A.
CM-02	Nose Only Tube Rinse	0 h	107893.9562 dpm	0.187

**Table 15 (contd). Total Recovery of Radioactivity in Samples from Group C.**

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Reco (%)
CM-03	Urine	8 h	1830907.9353 dpm	61974739.573 dpm	2.954
CM-03	Urine	24 h	1035036.8761 dpm		1.670
CM-03	Urine	48 h	94140.8610 dpm		0.152
CM-03	Urine	72 h	36422.3233 dpm		0.059
CM-03	Urine	96 h	21467.4863 dpm		0.035
CM-03	Urine	120 h	12046.4569 dpm		0.019
CM-03	Urine	144 h	11128.9383 dpm		0.018
CM-03	Urine	168 h	6406.4016 dpm		0.010
CM-03	Feces	24 h	526783.7049 dpm		0.850
CM-03	Feces	48 h	153155.1821 dpm		0.247
CM-03	Feces	72 h	53383.2190 dpm		0.086
CM-03	Feces	96 h	80773.0200 dpm		0.130
CM-03	Feces	120 h	30611.1993 dpm		0.049
CM-03	Feces	144 h	7816.8261 dpm		0.013
CM-03	Feces	168 h	38406.6001 dpm		0.062
CM-03	Carcass Digest	168 h	1861359.4966 dpm		3.003
CM-03	CO2 Trap 1	1 h	236323.1407 dpm		0.381
CM-03	CO2 Trap 1	3 h	666619.0295 dpm		1.076
CM-03	CO2 Trap 1	6 h	2227004.5358 dpm		3.593
CM-03	CO2 Trap 1	24 h	7612540.0951 dpm		12.283
CM-03	CO2 Trap 1	48 h	739592.7459 dpm		1.193
CM-03	CO2 Trap 1	72 h	247644.9995 dpm		0.400
CM-03	CO2 Trap 1	96 h	127633.4003 dpm		0.206
CM-03	CO2 Trap 1	120 h	112926.1187 dpm		0.182
CM-03	CO2 Trap 1	144 h	90463.3291 dpm		0.146
CM-03	CO2 Trap 1	168 h	52132.9559 dpm		0.084
CM-03	CO2 Trap 2	1 h	1179.8598 dpm		0.002
CM-03	CO2 Trap 2	3 h	5537.2663 dpm		0.009
CM-03	CO2 Trap 2	6 h	16930.4947 dpm		0.027
CM-03	CO2 Trap 2	24 h	776560.8440 dpm		1.253
CM-03	CO2 Trap 2	48 h	258008.2599 dpm		0.416
CM-03	CO2 Trap 2	72 h	82975.0407 dpm		0.134
CM-03	CO2 Trap 2	96 h	42802.4303 dpm		0.069
CM-03	CO2 Trap 2	120 h	22766.1409 dpm		0.037
CM-03	CO2 Trap 2	144 h	27059.2818 dpm		0.044
CM-03	CO2 Trap 2	168 h	22151.0729 dpm		0.036
CM-03	Exhaled VOC Trap 1	1 h	17677062.8099 dpm		28.523
CM-03	Exhaled VOC Trap 1	3 h	12285157.3812 dpm		19.823
CM-03	Exhaled VOC Trap 1	6 h	8642869.2691 dpm		13.946
CM-03	Exhaled VOC Trap 1	24 h	3497576.8167 dpm		5.644
CM-03	Exhaled VOC Trap 1	48 h	41378.3150 dpm		0.067
CM-03	Exhaled VOC Trap 1	72 h	11344.6259 dpm		0.018
CM-03	Exhaled VOC Trap 1	96 h	6050.3966 dpm		0.010
CM-03	Exhaled VOC Trap 1	120 h	3879.1408 dpm		0.006
CM-03	Exhaled VOC Trap 1	144 h	2865.3259 dpm		0.005
CM-03	Exhaled VOC Trap 1	168 h	2426.5659 dpm		0.004
CM-03	Exhaled VOC Trap 2	1 h	1740.2035 dpm		0.003
CM-03	Exhaled VOC Trap 2	3 h	2399.3005 dpm		0.004
CM-03	Exhaled VOC Trap 2	6 h	2990.2261 dpm		0.005
CM-03	Exhaled VOC Trap 2	24 h	0.0000 dpm		0.000
CM-03	Exhaled VOC Trap 2	48 h	315989.4118 dpm		0.510
CM-03	Exhaled VOC Trap 2	72 h	180.3404 dpm		0.000
CM-03	Exhaled VOC Trap 2	96 h	0.0000 dpm		0.000
CM-03	Exhaled VOC Trap 2	120 h	0.0000 dpm		0.000
CM-03	Exhaled VOC Trap 2	144 h	0.0000 dpm		0.000
CM-03	Exhaled VOC Trap 2	168 h	217.4585 dpm		0.000
CM-03	Cage Rinse	168 h	22706.1049 dpm		0.037
CM-03	Exposure Urine	0 h	N.C.		N.A.
CM-03	Exposure Feces	0 h	72660.3202 dpm		0.117
CM-03	Nose Only Tube Rinse	0 h	216547.9918 dpm		0.349

**Table 15 (contd). Total Recovery of Radioactivity in Samples from Group C.**

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Reco (%)
CM-04	Urine	8 h	989422.8048 dpm	15313501.108 dpm	6.461
CM-04	Urine	24 h	1337202.8125 dpm		8.732
CM-04	Urine	48 h	183682.3569 dpm		1.199
CM-04	Urine	72 h	39186.3623 dpm		0.256
CM-04	Urine	96 h	13567.7919 dpm		0.089
CM-04	Urine	120 h	11819.8278 dpm		0.077
CM-04	Urine	144 h	9219.9960 dpm		0.060
CM-04	Urine	168 h	7059.9188 dpm		0.046
CM-04	Feces	24 h	284923.2872 dpm		1.861
CM-04	Feces	48 h	108474.3403 dpm		0.708
CM-04	Feces	72 h	43780.5359 dpm		0.286
CM-04	Feces	96 h	61476.9369 dpm		0.401
CM-04	Feces	120 h	23743.7041 dpm		0.155
CM-04	Feces	144 h	18835.1115 dpm		0.123
CM-04	Feces	168 h	16712.6327 dpm		0.109
CM-04	Carcass Digest	168 h	1442320.6245 dpm		9.419
CM-04	CO2 Trap 1	1 h	244957.6956 dpm		1.600
CM-04	CO2 Trap 1	3 h	722853.7853 dpm		4.720
CM-04	CO2 Trap 1	6 h	1734658.5733 dpm		11.328
CM-04	CO2 Trap 1	24 h	6059912.7649 dpm		39.572
CM-04	CO2 Trap 1	48 h	392843.3036 dpm		2.565
CM-04	CO2 Trap 1	72 h	186883.0429 dpm		1.220
CM-04	CO2 Trap 1	96 h	116115.8257 dpm		0.758
CM-04	CO2 Trap 1	120 h	82913.4703 dpm		0.541
CM-04	CO2 Trap 1	144 h	62329.0532 dpm		0.407
CM-04	CO2 Trap 1	168 h	40464.7951 dpm		0.264
CM-04	CO2 Trap 2	1 h	224.0153 dpm		0.001
CM-04	CO2 Trap 2	3 h	2323.2219 dpm		0.015
CM-04	CO2 Trap 2	6 h	11512.7544 dpm		0.075
CM-04	CO2 Trap 2	24 h	631742.0214 dpm		4.125
CM-04	CO2 Trap 2	48 h	144632.5680 dpm		0.944
CM-04	CO2 Trap 2	72 h	68123.3236 dpm		0.445
CM-04	CO2 Trap 2	96 h	35297.7887 dpm		0.231
CM-04	CO2 Trap 2	120 h	24766.1932 dpm		0.162
CM-04	CO2 Trap 2	144 h	18352.8805 dpm		0.120
CM-04	CO2 Trap 2	168 h	15180.6258 dpm		0.099
CM-04	Exhaled VOC Trap 1	1 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	3 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	6 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	24 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	48 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	72 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	96 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	120 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	144 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 1	168 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	1 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	3 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	6 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	24 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	48 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	72 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	96 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	120 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	144 h	N.C.		N.A.
CM-04	Exhaled VOC Trap 2	168 h	N.C.		N.A.
CM-04	Cage Rinse	168 h	21354.7493 dpm		0.139
CM-04	Exposure Urine	0 h	N.C.		N.A.
CM-04	Exposure Feces	0 h	13388.7664 dpm		0.087
CM-04	Nose Only Tube Rinse	0 h	91240.8456 dpm		0.596

## **Appendix I**

### **Blood Sample Collection and Analysis for DIPE, Acetone and Isopropanol**

**Table 1. Blood Sample Collection Times.**

Subject	Sample	Dose Time	Nominal Time	Elapsed Time (hh:mm:ss)	Capture Time
AM-01	Blood	No Dose	Predose	NA	9/27/2007 8:42:04 AM
AM-02	Blood	No Dose	Predose	NA	9/27/2007 8:46:04 AM
AM-03	Blood	No Dose	Predose	NA	9/27/2007 8:51:53 AM
AM-04	Blood	No Dose	Predose	NA	9/27/2007 8:56:58 AM
AM-05	Blood	No Dose	Predose	NA	9/27/2007 9:01:48 AM
AM-06	Blood	No Dose	Predose	NA	9/27/2007 9:06:53 AM
AM-07	Blood	No Dose	Predose	NA	9/27/2007 9:43:54 AM
AM-08	Blood	No Dose	Predose	NA	9/27/2007 9:49:50 AM
AF-01	Blood	No Dose	Predose	NA	9/27/2007 9:10:32 AM
AF-02	Blood	No Dose	Predose	NA	9/27/2007 9:17:04 AM
AF-03	Blood	No Dose	Predose	NA	9/27/2007 9:22:33 AM
AF-04	Blood	No Dose	Predose	NA	9/27/2007 9:27:41 AM
AF-05	Blood	No Dose	Predose	NA	9/27/2007 9:32:50 AM
AF-06	Blood	No Dose	Predose	NA	9/27/2007 9:37:48 AM
AF-07	Blood	No Dose	Predose	NA	9/27/2007 9:54:48 AM
AF-08	Blood	No Dose	Predose	NA	9/27/2007 10:00:43 AM
AM-01	Blood	9/27/2007 9:45:07 AM	5 m	0 h 5 m 11 s	9/27/2007 9:50:18 AM
AM-01	Blood	9/27/2007 9:45:07 AM	15 m	0 h 15 m 26 s	9/27/2007 10:00:33 AM
AM-01	Blood	9/27/2007 9:45:07 AM	1 h	1 h 4 m 29 s	9/27/2007 10:49:36 AM
AM-02	Blood	9/27/2007 9:49:10 AM	5 m	0 h 4 m 47 s	9/27/2007 9:53:57 AM
AM-02	Blood	9/27/2007 9:49:10 AM	15 m	0 h 15 m 16 s	9/27/2007 10:04:26 AM
AM-02	Blood	9/27/2007 9:49:10 AM	1 h	1 h 0 m 29 s	9/27/2007 10:49:39 AM
AM-03	Blood	9/27/2007 9:53:01 AM	5 m	0 h 5 m 43 s	9/27/2007 9:58:44 AM
AM-03	Blood	9/27/2007 9:53:01 AM	15 m	0 h 15 m 12 s	9/27/2007 10:08:13 AM
AM-03	Blood	9/27/2007 9:53:01 AM	1 h	0 h 59 m 58 s	9/27/2007 10:52:59 AM
AM-04	Blood	9/27/2007 9:59:24 AM	10 m	0 h 10 m 25 s	9/27/2007 10:09:49 AM
AM-04	Blood	9/27/2007 9:59:24 AM	30 m	0 h 29 m 29 s	9/27/2007 10:28:53 AM
AM-04	Blood	9/27/2007 9:59:24 AM	2 h	2 h 0 m 13 s	9/27/2007 11:59:37 AM
AM-05	Blood	9/27/2007 10:05:57 AM	10 m	0 h 9 m 33 s	9/27/2007 10:15:30 AM
AM-05	Blood	9/27/2007 10:05:57 AM	30 m	0 h 29 m 1 s	9/27/2007 10:34:58 AM
AM-05	Blood	9/27/2007 10:05:57 AM	2 h	2 h 0 m 30 s	9/27/2007 12:06:27 PM
AM-06	Blood	9/27/2007 10:10:12 AM	10 m	0 h 10 m 14 s	9/27/2007 10:20:26 AM
AM-06	Blood	9/27/2007 10:10:12 AM	30 m	0 h 29 m 56 s	9/27/2007 10:40:08 AM
AM-06	Blood	9/27/2007 10:10:12 AM	2 h	2 h 0 m 2 s	9/27/2007 12:10:14 PM

Table 1 (continued). Blood Sample Collection Times.

Subject	Sample	Dose Time	Nominal Time	Elapsed Time (hh:mm:ss)	Capture Time
AF-01	Blood	9/27/2007 10:14:32 AM	5 m	0 h 4 m 40 s	9/27/2007 10:19:12 AM
AF-01	Blood	9/27/2007 10:14:32 AM	15 m	0 h 14 m 30 s	9/27/2007 10:29:02 AM
AF-01	Blood	9/27/2007 10:14:32 AM	1 h	0 h 59 m 33 s	9/27/2007 11:14:05 AM
AF-02	Blood	9/27/2007 10:19:07 AM	5 m	0 h 5 m 3 s	9/27/2007 10:24:10 AM
AF-02	Blood	9/27/2007 10:19:07 AM	15 m	0 h 15 m 11 s	9/27/2007 10:34:18 AM
AF-02	Blood	9/27/2007 10:19:07 AM	1 h	1 h 0 m 0 s	9/27/2007 11:19:07 AM
AF-03	Blood	9/27/2007 10:24:59 AM	5 m	0 h 3 m 56 s	9/27/2007 10:28:55 AM
AF-03	Blood	9/27/2007 10:24:59 AM	15 m	0 h 13 m 53 s	9/27/2007 10:38:52 AM
AF-03	Blood	9/27/2007 10:24:59 AM	1 h	1 h 0 m 11 s	9/27/2007 11:25:10 AM
AF-04	Blood	9/27/2007 10:30:31 AM	10 m	0 h 9 m 37 s	9/27/2007 10:40:08 AM
AF-04	Blood	9/27/2007 10:30:31 AM	30 m	0 h 29 m 41 s	9/27/2007 11:00:12 AM
AF-04	Blood	9/27/2007 10:30:31 AM	2 h	2 h 0 m 38 s	9/27/2007 12:31:09 PM
AF-05	Blood	9/27/2007 10:35:13 AM	10 m	0 h 10 m 11 s	9/27/2007 10:45:24 AM
AF-05	Blood	9/27/2007 10:35:13 AM	30 m	0 h 29 m 46 s	9/27/2007 11:04:59 AM
AF-05	Blood	9/27/2007 10:35:13 AM	2 h	2 h 1 m 19 s	9/27/2007 12:36:32 PM
AF-06	Blood	9/27/2007 10:40:17 AM	10 m	0 h 9 m 45 s	9/27/2007 10:50:02 AM
AF-06	Blood	9/27/2007 10:40:17 AM	30 m	0 h 30 m 17 s	9/27/2007 11:10:34 AM
AF-06	Blood	9/27/2007 10:40:17 AM	2 h	2 h 0 m 12 s	9/27/2007 12:40:29 PM
AM-01	Blood	9/27/2007 9:45:07 AM	4 h	4 h 1 m 20 s	9/27/2007 1:46:27 PM
AM-02	Blood	9/27/2007 9:49:10 AM	4 h	3 h 59 m 55 s	9/27/2007 1:49:05 PM
AM-03	Blood	9/27/2007 9:53:01 AM	4 h	4 h 1 m 22 s	9/27/2007 1:54:23 PM
AF-01	Blood	9/27/2007 10:14:32 AM	4 h	4 h 0 m 11 s	9/27/2007 2:14:43 PM
AF-02	Blood	9/27/2007 10:19:07 AM	4 h	4 h 0 m 15 s	9/27/2007 2:19:22 PM
AF-03	Blood	9/27/2007 10:24:59 AM	4 h	3 h 59 m 39 s	9/27/2007 2:24:38 PM
AM-01	Blood	9/27/2007 9:45:07 AM	6.25 h	6 h 15 m 21 s	9/27/2007 4:00:28 PM
AM-01	Blood	9/27/2007 9:45:07 AM	7 h	7 h 0 m 4 s	9/27/2007 4:45:11 PM
AM-01	Blood	9/27/2007 9:45:07 AM	8 h	8 h 0 m 48 s	9/27/2007 5:45:55 PM
AM-02	Blood	9/27/2007 9:49:10 AM	6.25 h	6 h 15 m 14 s	9/27/2007 4:04:24 PM
AM-02	Blood	9/27/2007 9:49:10 AM	7 h	6 h 59 m 54 s	9/27/2007 4:49:04 PM
AM-02	Blood	9/27/2007 9:49:10 AM	8 h	8 h 0 m 2 s	9/27/2007 5:49:12 PM
AM-03	Blood	9/27/2007 9:53:01 AM	6.25 h	6 h 14 m 53 s	9/27/2007 4:07:54 PM
AM-03	Blood	9/27/2007 9:53:01 AM	7 h	6 h 59 m 57 s	9/27/2007 4:52:58 PM
AM-03	Blood	9/27/2007 9:53:01 AM	8 h	8 h 0 m 2 s	9/27/2007 5:53:03 PM
AM-04	Blood	9/27/2007 9:59:24 AM	6 h	6 h 0 m 41 s	9/27/2007 4:00:05 PM
AM-04	Blood	9/27/2007 9:59:24 AM	6.50 h	6 h 29 m 59 s	9/27/2007 4:29:23 PM
AM-04	Blood	9/27/2007 9:59:24 AM	7.33 h	7 h 18 m 37 s	9/27/2007 5:18:01 PM
AM-05	Blood	9/27/2007 10:05:57 AM	6 h	6 h 3 m 19 s	9/27/2007 4:09:16 PM
AM-05	Blood	9/27/2007 10:05:57 AM	6.50 h	6 h 31 m 31 s	9/27/2007 4:37:28 PM
AM-05	Blood	9/27/2007 10:05:57 AM	7.33 h	7 h 18 m 8 s	9/27/2007 5:24:05 PM
AM-06	Blood	9/27/2007 10:10:12 AM	6 h	6 h 0 m 42 s	9/27/2007 4:10:54 PM
AM-06	Blood	9/27/2007 10:10:12 AM	6.50 h	6 h 30 m 40 s	9/27/2007 4:40:52 PM

**Table 1 (continued). Blood Sample Collection Times.**

Subject	Sample	Dose Time	Nominal Time	Elapsed Time (hh:mm:ss)	Capture Time
AM-06	Blood	9/27/2007 10:10:12 AM	7.33 h	7 h 18 m 33 s	9/27/2007 5:28:45 PM
AF-01	Blood	9/27/2007 10:14:32 AM	6.25 h	6 h 15 m 14 s	9/27/2007 4:29:46 PM
AF-01	Blood	9/27/2007 10:14:32 AM	7 h	7 h 0 m 24 s	9/27/2007 5:14:56 PM
AF-01	Blood	9/27/2007 10:14:32 AM	8 h	7 h 59 m 38 s	9/27/2007 6:14:10 PM
AF-02	Blood	9/27/2007 10:19:07 AM	6.25 h	6 h 14 m 44 s	9/27/2007 4:33:51 PM
AF-02	Blood	9/27/2007 10:19:07 AM	7 h	6 h 59 m 58 s	9/27/2007 5:19:05 PM
AF-02	Blood	9/27/2007 10:19:07 AM	8 h	7 h 59 m 39 s	9/27/2007 6:18:46 PM
AF-03	Blood	9/27/2007 10:24:59 AM	6.25 h	6 h 15 m 3 s	9/27/2007 4:40:02 PM
AF-03	Blood	9/27/2007 10:24:59 AM	7 h	6 h 59 m 42 s	9/27/2007 5:24:41 PM
AF-03	Blood	9/27/2007 10:24:59 AM	8 h	7 h 59 m 39 s	9/27/2007 6:24:38 PM
AF-04	Blood	9/27/2007 10:30:31 AM	6 h	6 h 0 m 38 s	9/27/2007 4:31:09 PM
AF-04	Blood	9/27/2007 10:30:31 AM	6.50 h	6 h 29 m 5 s	9/27/2007 4:59:36 PM
AF-04	Blood	9/27/2007 10:30:31 AM	7.33 h	7 h 18 m 42 s	9/27/2007 5:49:13 PM
AF-05	Blood	9/27/2007 10:35:13 AM	6 h	6 h 0 m 15 s	9/27/2007 4:35:28 PM
AF-05	Blood	9/27/2007 10:35:13 AM	6.50 h	6 h 29 m 44 s	9/27/2007 5:04:57 PM
AF-05	Blood	9/27/2007 10:35:13 AM	7.33 h	7 h 19 m 1 s	9/27/2007 5:54:14 PM
AF-06	Blood	9/27/2007 10:40:17 AM	6 h	6 h 3 m 36 s	9/27/2007 4:43:53 PM
AF-06	Blood	9/27/2007 10:40:17 AM	6.50 h	6 h 29 m 43 s	9/27/2007 5:10:00 PM
AF-06	Blood	9/27/2007 10:40:17 AM	7.33 h	7 h 19 m 6 s	9/27/2007 5:59:23 PM
AM-04	Blood	9/27/2007 9:59:24 AM	10 h	9 h 59 m 34 s	9/27/2007 7:58:58 PM
AM-05	Blood	9/27/2007 10:05:57 AM	10 h	9 h 59 m 56 s	9/27/2007 8:05:53 PM
AM-06	Blood	9/27/2007 10:10:12 AM	10 h	9 h 59 m 34 s	9/27/2007 8:09:46 PM
AF-04	Blood	9/27/2007 10:30:31 AM	10 h	10 h 0 m 8 s	9/27/2007 8:30:39 PM
AF-05	Blood	9/27/2007 10:35:13 AM	10 h	9 h 59 m 43 s	9/27/2007 8:34:56 PM
AF-06	Blood	9/27/2007 10:40:17 AM	10 h	9 h 59 m 51 s	9/27/2007 8:40:08 PM
AM-01	Blood	9/27/2007 9:45:07 AM	16 h	15 h 59 m 58 s	9/28/2007 1:45:05 AM
AM-02	Blood	9/27/2007 9:49:10 AM	16 h	16 h 0 m 17 s	9/28/2007 1:49:27 AM
AM-03	Blood	9/27/2007 9:53:01 AM	16 h	15 h 59 m 50 s	9/28/2007 1:52:51 AM
AF-01	Blood	9/27/2007 10:14:32 AM	16 h	15 h 59 m 33 s	9/28/2007 2:14:05 AM
AF-02	Blood	9/27/2007 10:19:07 AM	16 h	16 h 0 m 22 s	9/28/2007 2:19:29 AM
AF-03	Blood	9/27/2007 10:24:59 AM	16 h	16 h 0 m 38 s	9/28/2007 2:25:37 AM
AM-04	Blood	9/27/2007 9:59:24 AM	24 h	23 h 59 m 17 s	9/28/2007 9:58:41 AM
AM-05	Blood	9/27/2007 10:05:57 AM	24 h	23 h 59 m 47 s	9/28/2007 10:05:44 AM
AM-06	Blood	9/27/2007 10:10:12 AM	24 h	24 h 1 m 47 s	9/28/2007 10:11:59 AM
AF-04	Blood	9/27/2007 10:30:31 AM	24 h	23 h 59 m 25 s	9/28/2007 10:29:56 AM
AF-05	Blood	9/27/2007 10:35:13 AM	24 h	24 h 0 m 52 s	9/28/2007 10:36:05 AM
AF-06	Blood	9/27/2007 10:40:17 AM	24 h	23 h 59 m 37 s	9/28/2007 10:39:54 AM

**Table 2. Blood Sample Weights from Group A.**

Subject	Sample	Time	Pot wt	Sample wt	Corrected Sample Weight
AM-01	Blood	Predose	11.4071 g	11.5786 g	0.1715 g
AM-01	Blood	5 m	11.3700 g	11.5151 g	0.1451 g
AM-01	Blood	10 m	N.C.	N.C.	N.C.
AM-01	Blood	15 m	11.1686 g	11.3501 g	0.1815 g
AM-01	Blood	30 m	N.C.	N.C.	N.C.
AM-01	Blood	1 h	11.3947 g	11.5830 g	0.1883 g
AM-01	Blood	2 h	N.C.	N.C.	N.C.
AM-01	Blood	4 h	11.2188 g	11.4168 g	0.1980 g
AM-01	Blood	6 h	N.C.	N.C.	N.C.
AM-01	Blood	6.25 h	11.3839 g	11.5485 g	0.1646 g
AM-01	Blood	6.50 h	N.C.	N.C.	N.C.
AM-01	Blood	7 h	11.3850 g	11.5548 g	0.1698 g
AM-01	Blood	7.33 h	N.C.	N.C.	N.C.
AM-01	Blood	8 h	11.3953 g	11.5450 g	0.1497 g
AM-01	Blood	10 h	N.C.	N.C.	N.C.
AM-01	Blood	16 h	11.5298 g	11.6900 g	0.1602 g
AM-01	Blood	24 h	N.C.	N.C.	N.C.
AM-02	Blood	Predose	11.2103 g	11.3715 g	0.1612 g
AM-02	Blood	5 m	11.1642 g	11.3751 g	0.2109 g
AM-02	Blood	10 m	N.C.	N.C.	N.C.
AM-02	Blood	15 m	11.3748 g	11.5851 g	0.2103 g
AM-02	Blood	30 m	N.C.	N.C.	N.C.
AM-02	Blood	1 h	11.1905 g	11.4920 g	0.3015 g
AM-02	Blood	2 h	N.C.	N.C.	N.C.
AM-02	Blood	4 h	11.2054 g	11.3703 g	0.1649 g
AM-02	Blood	6 h	N.C.	N.C.	N.C.
AM-02	Blood	6.25 h	11.4053 g	11.5638 g	0.1585 g
AM-02	Blood	6.50 h	N.C.	N.C.	N.C.
AM-02	Blood	7 h	11.1948 g	11.3717 g	0.1769 g
AM-02	Blood	7.33 h	N.C.	N.C.	N.C.
AM-02	Blood	8 h	11.2456 g	11.3945 g	0.1489 g
AM-02	Blood	10 h	N.C.	N.C.	N.C.
AM-02	Blood	16 h	11.2549 g	11.4349 g	0.1800 g
AM-02	Blood	24 h	N.C.	N.C.	N.C.

**Table 2 (contd). Blood Sample Weights from Group A.**

Subject	Sample	Time	Pot wt	Sample wt	Corrected Sample Weight
AM-03	Blood	Predose	11.3612 g	11.5564 g	0.1952 g
AM-03	Blood	5 m	11.1794 g	11.3504 g	0.1710 g
AM-03	Blood	10 m	N.C.	N.C.	N.C.
AM-03	Blood	15 m	11.3676 g	11.5785 g	0.2109 g
AM-03	Blood	30 m	N.C.	N.C.	N.C.
AM-03	Blood	1 h	11.1694 g	11.3862 g	0.2168 g
AM-03	Blood	2 h	N.C.	N.C.	N.C.
AM-03	Blood	4 h	11.2131 g	11.4204 g	0.2073 g
AM-03	Blood	6 h	N.C.	N.C.	N.C.
AM-03	Blood	6.25 h	11.1978 g	11.3562 g	0.1584 g
AM-03	Blood	6.50 h	N.C.	N.C.	N.C.
AM-03	Blood	7 h	11.3806 g	11.5546 g	0.1740 g
AM-03	Blood	7.33 h	N.C.	N.C.	N.C.
AM-03	Blood	8 h	11.2156 g	11.3954 g	0.1798 g
AM-03	Blood	10 h	N.C.	N.C.	N.C.
AM-03	Blood	16 h	11.1806 g	11.3376 g	0.1570 g
AM-03	Blood	24 h	N.C.	N.C.	N.C.
AM-04	Blood	Predose	11.3950 g	11.5575 g	0.1625 g
AM-04	Blood	5 m	N.C.	N.C.	N.C.
AM-04	Blood	10 m	11.2204 g	11.4008 g	0.1804 g
AM-04	Blood	15 m	N.C.	N.C.	N.C.
AM-04	Blood	30 m	11.1966 g	11.3660 g	0.1694 g
AM-04	Blood	1 h	N.C.	N.C.	N.C.
AM-04	Blood	2 h	11.2190 g	11.4067 g	0.1877 g
AM-04	Blood	4 h	N.C.	N.C.	N.C.
AM-04	Blood	6 h	11.2026 g	11.4238 g	0.2212 g
AM-04	Blood	6.25 h	N.C.	N.C.	N.C.
AM-04	Blood	6.50 h	11.3147 g	11.5023 g	0.1876 g
AM-04	Blood	7 h	N.C.	N.C.	N.C.
AM-04	Blood	7.33 h	11.2080 g	11.3777 g	0.1697 g
AM-04	Blood	8 h	N.C.	N.C.	N.C.
AM-04	Blood	10 h	11.1735 g	11.3462 g	0.1727 g
AM-04	Blood	16 h	N.C.	N.C.	N.C.
AM-04	Blood	24 h	11.2187 g	11.3939 g	0.1752 g

**Table 2 (contd). Blood Sample Weights from Group A.**

Subject	Sample	Time	Pot wt	Sample wt	Corrected Sample Weight
AM-05	Blood	Predose	11.3734 g	11.5553 g	0.1819 g
AM-05	Blood	5 m	N.C.	N.C.	N.C.
AM-05	Blood	10 m	11.1943 g	11.4195 g	0.2252 g
AM-05	Blood	15 m	N.C.	N.C.	N.C.
AM-05	Blood	30 m	11.1877 g	11.4057 g	0.2180 g
AM-05	Blood	1 h	N.C.	N.C.	N.C.
AM-05	Blood	2 h	11.3557 g	11.5411 g	0.1854 g
AM-05	Blood	4 h	N.C.	N.C.	N.C.
AM-05	Blood	6 h	11.1845 g	11.2590 g	0.0745 g
AM-05	Blood	6.25 h	N.C.	N.C.	N.C.
AM-05	Blood	6.50 h	11.2168 g	11.3994 g	0.1826 g
AM-05	Blood	7 h	N.C.	N.C.	N.C.
AM-05	Blood	7.33 h	11.3649 g	11.5585 g	0.1936 g
AM-05	Blood	8 h	N.C.	N.C.	N.C.
AM-05	Blood	10 h	11.2248 g	11.4093 g	0.1845 g
AM-05	Blood	16 h	N.C.	N.C.	N.C.
AM-05	Blood	24 h	11.4001 g	11.5802 g	0.1801 g
AM-06	Blood	Predose	11.3955 g	11.5799 g	0.1844 g
AM-06	Blood	5 m	N.C.	N.C.	N.C.
AM-06	Blood	10 m	11.3726 g	11.6361 g	0.2635 g
AM-06	Blood	15 m	N.C.	N.C.	N.C.
AM-06	Blood	30 m	11.3646 g	11.5919 g	0.2273 g
AM-06	Blood	1 h	N.C.	N.C.	N.C.
AM-06	Blood	2 h	11.1996 g	11.3722 g	0.1726 g
AM-06	Blood	4 h	N.C.	N.C.	N.C.
AM-06	Blood	6 h	11.3379 g	11.5769 g	0.2390 g
AM-06	Blood	6.25 h	N.C.	N.C.	N.C.
AM-06	Blood	6.50 h	11.1831 g	11.3885 g	0.2054 g
AM-06	Blood	7 h	N.C.	N.C.	N.C.
AM-06	Blood	7.33 h	11.2140 g	11.3992 g	0.1852 g
AM-06	Blood	8 h	N.C.	N.C.	N.C.
AM-06	Blood	10 h	11.1886 g	11.3758 g	0.1872 g
AM-06	Blood	16 h	N.C.	N.C.	N.C.
AM-06	Blood	24 h	11.2312 g	11.4147 g	0.1835 g

**Table 2 (contd). Blood Sample Weights from Group A.**

Subject	Sample	Time	Pot wt	Sample wt	Corrected Sample Weight
AF-01	Blood	Predose	11.1291 g	11.3771 g	0.2480 g
AF-01	Blood	5 m	11.3310 g	11.5398 g	0.2088 g
AF-01	Blood	10 m	N.C.	N.C.	N.C.
AF-01	Blood	15 m	11.1447 g	11.3298 g	0.1851 g
AF-01	Blood	30 m	N.C.	N.C.	N.C.
AF-01	Blood	1 h	11.2324 g	11.4361 g	0.2037 g
AF-01	Blood	2 h	N.C.	N.C.	N.C.
AF-01	Blood	4 h	11.1913 g	11.3916 g	0.2003 g
AF-01	Blood	6 h	N.C.	N.C.	N.C.
AF-01	Blood	6.25 h	11.1559 g	11.3721 g	0.2162 g
AF-01	Blood	6.50 h	N.C.	N.C.	N.C.
AF-01	Blood	7 h	11.3248 g	11.5011 g	0.1763 g
AF-01	Blood	7.33 h	N.C.	N.C.	N.C.
AF-01	Blood	8 h	11.2111 g	11.3844 g	0.1733 g
AF-01	Blood	10 h	N.C.	N.C.	N.C.
AF-01	Blood	16 h	11.2184 g	11.4104 g	0.1920 g
AF-01	Blood	24 h	N.C.	N.C.	N.C.
AF-02	Blood	Predose	11.3486 g	11.5351 g	0.1865 g
AF-02	Blood	5 m	11.1955 g	11.4358 g	0.2403 g
AF-02	Blood	10 m	N.C.	N.C.	N.C.
AF-02	Blood	15 m	11.3651 g	11.5766 g	0.2115 g
AF-02	Blood	30 m	N.C.	N.C.	N.C.
AF-02	Blood	1 h	11.2009 g	11.4147 g	0.2138 g
AF-02	Blood	2 h	N.C.	N.C.	N.C.
AF-02	Blood	4 h	11.1653 g	11.3543 g	0.1890 g
AF-02	Blood	6 h	N.C.	N.C.	N.C.
AF-02	Blood	6.25 h	11.3460 g	11.5281 g	0.1821 g
AF-02	Blood	6.50 h	N.C.	N.C.	N.C.
AF-02	Blood	7 h	11.4304 g	11.6057 g	0.1753 g
AF-02	Blood	7.33 h	N.C.	N.C.	N.C.
AF-02	Blood	8 h	11.4004 g	11.5626 g	0.1622 g
AF-02	Blood	10 h	N.C.	N.C.	N.C.
AF-02	Blood	16 h	11.2027 g	11.3560 g	0.1533 g
AF-02	Blood	24 h	N.C.	N.C.	N.C.

**Table 2 (contd). Blood Sample Weights from Group A.**

Subject	Sample	Time	Pot wt	Sample wt	Corrected Sample Weight
AF-03	Blood	Predose	11.2306 g	11.3887 g	0.1581 g
AF-03	Blood	5 m	11.1761 g	11.3002 g	0.1241 g
AF-03	Blood	10 m	N.C.	N.C.	N.C.
AF-03	Blood	15 m	11.2173 g	11.3713 g	0.1540 g
AF-03	Blood	30 m	N.C.	N.C.	N.C.
AF-03	Blood	1 h	11.1616 g	11.3718 g	0.2102 g
AF-03	Blood	2 h	N.C.	N.C.	N.C.
AF-03	Blood	4 h	11.2198 g	11.4459 g	0.2261 g
AF-03	Blood	6 h	N.C.	N.C.	N.C.
AF-03	Blood	6.25 h	11.2496 g	11.4027 g	0.1531 g
AF-03	Blood	6.50 h	N.C.	N.C.	N.C.
AF-03	Blood	7 h	11.2221 g	11.3962 g	0.1741 g
AF-03	Blood	7.33 h	N.C.	N.C.	N.C.
AF-03	Blood	8 h	11.2496 g	11.4627 g	0.2131 g
AF-03	Blood	10 h	N.C.	N.C.	N.C.
AF-03	Blood	16 h	11.2962 g	11.4679 g	0.1717 g
AF-03	Blood	24 h	N.C.	N.C.	N.C.
AF-04	Blood	Predose	11.2236 g	11.4068 g	0.1832 g
AF-04	Blood	5 m	N.C.	N.C.	N.C.
AF-04	Blood	10 m	11.3813 g	11.5701 g	0.1888 g
AF-04	Blood	15 m	N.C.	N.C.	N.C.
AF-04	Blood	30 m	11.1526 g	11.3641 g	0.2115 g
AF-04	Blood	1 h	N.C.	N.C.	N.C.
AF-04	Blood	2 h	11.1605 g	11.3137 g	0.1532 g
AF-04	Blood	4 h	N.C.	N.C.	N.C.
AF-04	Blood	6 h	11.2091 g	11.3934 g	0.1843 g
AF-04	Blood	6.25 h	N.C.	N.C.	N.C.
AF-04	Blood	6.50 h	11.3755 g	11.5460 g	0.1705 g
AF-04	Blood	7 h	N.C.	N.C.	N.C.
AF-04	Blood	7.33 h	11.3771 g	11.5487 g	0.1716 g
AF-04	Blood	8 h	N.C.	N.C.	N.C.
AF-04	Blood	10 h	11.2255 g	11.3967 g	0.1712 g
AF-04	Blood	16 h	N.C.	N.C.	N.C.
AF-04	Blood	24 h	11.2811 g	11.4613 g	0.1802 g

**Table 2 (contd). Blood Sample Weights from Group A.**

Subject	Sample	Time	Pot wt	Sample wt	Corrected Sample Weight
AF-05	Blood	Predose	11.3193 g	11.4684 g	0.1491 g
AF-05	Blood	5 m	N.C.	N.C.	N.C.
AF-05	Blood	10 m	11.3289 g	11.5320 g	0.2031 g
AF-05	Blood	15 m	N.C.	N.C.	N.C.
AF-05	Blood	30 m	11.2137 g	11.3794 g	0.1657 g
AF-05	Blood	1 h	N.C.	N.C.	N.C.
AF-05	Blood	2 h	11.1775 g	11.3433 g	0.1658 g
AF-05	Blood	4 h	N.C.	N.C.	N.C.
AF-05	Blood	6 h	11.3272 g	11.5219 g	0.1947 g
AF-05	Blood	6.25 h	N.C.	N.C.	N.C.
AF-05	Blood	6.50 h	11.3682 g	11.5349 g	0.1667 g
AF-05	Blood	7 h	N.C.	N.C.	N.C.
AF-05	Blood	7.33 h	11.2059 g	11.3884 g	0.1825 g
AF-05	Blood	8 h	N.C.	N.C.	N.C.
AF-05	Blood	10 h	11.2451 g	11.4289 g	0.1838 g
AF-05	Blood	16 h	N.C.	N.C.	N.C.
AF-05	Blood	24 h	11.2257 g	11.4078 g	0.1821 g
AF-06	Blood	Predose	11.3616 g	11.5308 g	0.1692 g
AF-06	Blood	5 m	N.C.	N.C.	N.C.
AF-06	Blood	10 m	11.1749 g	11.3078 g	0.1329 g
AF-06	Blood	15 m	N.C.	N.C.	N.C.
AF-06	Blood	30 m	11.1923 g	11.4146 g	0.2223 g
AF-06	Blood	1 h	N.C.	N.C.	N.C.
AF-06	Blood	2 h	11.1812 g	11.3702 g	0.1890 g
AF-06	Blood	4 h	N.C.	N.C.	N.C.
AF-06	Blood	6 h	11.1287 g	11.3020 g	0.1733 g
AF-06	Blood	6.25 h	N.C.	N.C.	N.C.
AF-06	Blood	6.50 h	11.1532 g	11.3253 g	0.1721 g
AF-06	Blood	7 h	N.C.	N.C.	N.C.
AF-06	Blood	7.33 h	11.1741 g	11.4254 g	0.2513 g
AF-06	Blood	8 h	N.C.	N.C.	N.C.
AF-06	Blood	10 h	11.2900 g	11.4425 g	0.1525 g
AF-06	Blood	16 h	N.C.	N.C.	N.C.
AF-06	Blood	24 h	11.2421 g	11.4275 g	0.1854 g

**Table 3. Low Concentration Calibration Curve for DIPE in Blood.**

Sample I.D.	Standard/QC I.D	Conc. (ug/ml)	Peak Area		Peak Area Ratio	Cal. Conc. (ug/ml)	Mean	SD	RSD% (Precision)	Accuracy (%)
			DIPE	MTBE						
12322-127A	Blood blank	0	0	0	n/a	n/a	n/a	n/a	n/a	n/a
12322-128A	Blood blank	0	0	0	n/a	n/a	-	-	-	n/a
12322-133A	Blood blank	0	0	0	n/a	n/a	-	-	-	n/a
12322-127B	Blood ISTD blank	0	0	2214393	0	n/a	n/a	n/a	n/a	n/a
12322-128B	Blood ISTD blank	0	0	2158058	0	n/a	-	-	-	n/a
12322-133B	Blood ISTD blank	0	0	2035366	0	n/a	-	-	-	n/a
12322-127C	Std A1	0.0898	11525	2122933	0.00543	0.0911	0.0908	0.00166	1.83%	101%
12322-128C	Std A1	0.0898	11854	2153947	0.00550	0.0923	-	-	-	103%
12322-133C	Std A1	0.0898	10282	1937595	0.00531	0.0890	-	-	-	99.1%
12322-127D	Std B1	0.224	29693	2145488	0.0138	0.231	0.227	0.00412	1.82%	103%
12322-128D	Std B1	0.224	27954	2078860	0.0134	0.225	-	-	-	100%
12322-133D	Std B1	0.224	26653	1991375	0.0134	0.224	-	-	-	100%
12322-127E	Std A2	0.457	58885	2156926	0.0273	0.456	0.447	0.00909	2.03%	100%
12322-128E	Std A2	0.457	55824	2083004	0.0268	0.448	-	-	-	98.0%
12322-133E	Std A2	0.457	52944	2019835	0.0262	0.438	-	-	-	95.8%
12322-127F	Std B2	0.910	112752	2083496	0.0541	0.904	0.895	0.00974	1.09%	99.3%
12322-128F	Std B2	0.910	113998	2120200	0.0538	0.898	-	-	-	98.7%
12322-133F	Std B2	0.910	105692	1995024	0.0530	0.88	-	-	-	97.2%
12322-127G	Std A3	2.33	302874	2038370	0.149	2.48	2.38	0.0926	3.90%	106%
12322-128G	Std A3	2.33	279697	1990241	0.141	2.35	-	-	-	101%
12322-133G	Std A3	2.33	275706	1998611	0.138	2.30	-	-	-	98.8%
12322-127H	Std B3	4.61	562576	2000450	0.281	4.69	4.58	0.100	2.19%	102%
12322-128H	Std B3	4.61	581850	2135947	0.272	4.55	-	-	-	98.6%
12322-133H	Std B3	4.61	535866	1986368	0.270	4.50	-	-	-	97.7%
12322-132-13	LQC	0.457	60533	2175613	0.0278	0.465	-	-	-	102%
12322-132-14	LQC	0.457	56369	2125207	0.0265	0.443	-	-	-	97.0%
12322-130-37	MQC	4.61	584614	2141927	0.273	4.56	-	-	-	98.8%
12322-130-38	MQC	4.61	579786	2146326	0.270	4.51	-	-	-	97.8%

a INTERCEPT -0.000027814  
b SLOPE 0.059915  
r 0.9996

**Table 4. High Concentration Calibration Curve for DIPE in Blood.**

Sample I.D.	Standard/QC I.D.	Conc. (ug/ml)	Peak Area		Peak Area Ratio	Cal. Conc. (ug/ml)	Mean	SD	RSD% (Precision)	Accuracy (%)
			DIPE	MTBE						
12322-127H	Std B3	4.61	562576	2000450	0.281	5.04	4.94	0.087	1.76%	109%
12322-128H	Std B3	4.61	581850	2135947	0.272	4.91	-	-	-	107%
12322-133H	Std B3	4.61	535866	1986368	0.270	4.87	-	-	-	106%
12322-127I	Std A4	9.49	1261083	2033960	0.620	9.94	9.71	0.234	2.41%	105%
12322-128I	Std A4	9.49	1252607	2073581	0.604	9.71	-	-	-	102%
12322-133I	Std A4	9.49	1143785	1946173	0.588	9.47	-	-	-	100%
12322-127J	Std B4	23.6	3203037	2025586	1.58	23.8	22.7	1.07	4.70%	101%
12322-128J	Std B4	23.6	3127453	2093341	1.49	22.6	-	-	-	95.7%
12322-133J	Std B4	23.6	2694565	1878234	1.43	21.7	-	-	-	92.0%
12322-127K	Std A5	48.4	6861748	2011540	3.41	50.3	47.0	2.86	6.09%	104%
12322-128K	Std A5	48.4	6413305	2075118	3.09	45.7	-	-	-	94.4%
12322-133K	Std A5	48.4	6075548	1992223	3.05	45.1	-	-	-	93.1%
12322-127L	Std B5	96.8	13281090	1989035	6.68	97.5	91.5	5.27	5.76%	101%
12322-128L	Std B5	96.8	12547672	2068516	6.07	88.7	-	-	-	91.6%
12322-133L	Std B5	96.8	11245967	1865371	6.03	88.2	-	-	-	91.1%
12322-127M	Std A6	247	35964910	1925776	18.7	271	254	17.61	6.93%	110%
12322-128M	Std A6	247	36519922	2072875	17.6	256	-	-	-	103%
12322-133M	Std A6	247	29531463	1817627	16.2	236	-	-	-	95.4%
12322-129-29	HQC	96.8	16459861	2911883	5.65	82.7	-	-	-	85.5%
12322-129-30	HQC	96.8	13359906	2473443	5.40	79.1	-	-	-	81.7%

a INTERCEPT -0.067191  
b SLOPE 0.069144  
r 0.997

**Table 5. Calibration Curve for Isopropanol in Blood.**

Sample I.D.	Standard/QC I.D	Conc. (ug/ml)	Peak Area		Peak Area Ratio	Cal. Conc. (ug/ml)	Mean	SD	RSD% (Precision)	Accuracy (%)
			Isopropanol	1-Propanol						
12322-127H	Std B3	4.31	309729	2070660	0.150	5.21	5.17	0.038	0.73%	120%
12322-128H	Std B3	4.31	302155	2061227	0.147	5.15	-	-	-	-
12322-133H	Std B3	4.31	293968	2007689	0.146	5.15	-	-	-	-
12322-127I	Std A4	9.00	709288	2141030	0.331	9.08	8.98	0.091	1.01%	99.7%
12322-128I	Std A4	9.00	685643	2121227	0.323	8.91	-	-	-	-
12322-133I	Std A4	9.00	632539	1947290	0.325	8.94	-	-	-	-
12322-127J	Std B4	22.1	1808774	2147270	0.842	19.9	20.1	0.151	0.750%	91.1%
12322-128J	Std B4	22.1	1794299	2098258	0.855	20.2	-	-	-	-
12322-133J	Std B4	22.1	1617027	1893249	0.854	20.2	-	-	-	-
12322-127K	Std A5	45.9	4032278	2088233	1.93	43.1	41.6	1.35	3.23%	90.7%
12322-128K	Std A5	45.9	3673564	2032197	1.81	40.5	-	-	-	-
12322-133K	Std A5	45.9	3776924	2047611	1.84	41.3	-	-	-	-
12322-127L	Std B5	90.5	8162809	2120148	3.85	83.9	85.1	1.19	1.40%	94.0%
12322-128L	Std B5	90.5	8307486	2096739	3.96	86.3	-	-	-	-
12322-133L	Std B5	90.5	8181688	2095536	3.90	85	-	-	-	-
12322-126M	Std A6	235	23370217	2076797	11.3	241	245	4.70	1.91%	105%
12322-127M	Std A6	235	22695441	1941824	11.7	251	-	-	-	-
12322-128M	Std A6	235	21694423	1902916	11.4	245	-	-	-	-
12322-130-37	MQC	4.31	314193	2086971	0.151	5.23	-	-	-	121%
12322-130-38	MQC	4.31	333890	2221608	0.150	5.23	-	-	-	121%
12322-129-29	HQC	90.5	9881942	2686808	3.68	80.3	-	-	-	88.7%
12322-129-30	HQC	90.5	8868719	2445017	3.63	79.2	-	-	-	87.5%

a INTERCEPT -0.095588  
b SLOPE 0.047019  
r 0.997

**Table 6. Calibration Curve for Acetone in Blood.**

Sample I.D.	Standard/QC I.D	Conc. (ug/ml)	Peak Area		Peak Area Ratio	Cal. Conc. (ug/ml)	Mean		RSD% (Precision)	Accuracy (%)
			Acetone	1-Propanol			SD	SD		
12322-127G	Std A3	2.21	126646	2033231	0.0623	2.51	2.48	0.0319	1.29%	112%
12322-128G	Std A3	2.21	118493	1949997	0.0608	2.45	-	-	-	-
12322-133G	Std A3	2.21	125349	2049143	0.0612	2.47	-	-	-	-
12322-127H	Std B3	4.30	223600	2070660	0.108	4.36	4.40	0.0353	0.803%	102%
12322-128H	Std B3	4.30	224879	2061227	0.109	4.41	-	-	-	-
12322-133H	Std B3	4.30	220256	2007689	0.110	4.43	-	-	-	-
12322-127I	Std A4	9.02	454345	2141030	0.212	8.57	8.70	0.190	2.19%	96.4%
12322-128I	Std A4	9.02	451733	2121227	0.213	8.60	-	-	-	-
12322-133I	Std A4	9.02	429772	1947290	0.221	8.92	-	-	-	-
12322-127J	Std B4	22.0	1085864	2147270	0.506	20.4	20.9	0.397	1.90%	95.0%
12322-128J	Std B4	22.0	1095322	2098258	0.522	21.1	-	-	-	-
12322-133J	Std B4	22.0	990823	1893249	0.523	21.2	-	-	-	-
12322-127K	Std A5	46.0	2305559	2088233	1.10	44.6	44.0	0.524	1.19%	95.7%
12322-128K	Std A5	46.0	2203661	2032197	1.08	43.8	-	-	-	-
12322-133K	Std A5	46.0	2210602	2047611	1.08	43.6	-	-	-	-
12322-127L	Std B5	90.3	4532539	2120148	2.14	86.4	86.2	2.41	2.80%	95.5%
12322-128L	Std B5	90.3	4591627	2096739	2.19	88.5	-	-	-	-
12322-133L	Std B5	90.3	4339333	2095536	2.07	84	-	-	-	-
12322-126M	Std A6	235	12300203	2076797	5.92	239	242	8.74	3.61%	103%
12322-127M	Std A6	235	12105819	1941824	6.23	252	-	-	-	-
12322-128M	Std A6	235	11073151	1902916	5.82	235	-	-	-	-
12322-130-37	MQC	4.30	221846	2086971	0.106	4.29	-	-	-	100%
12322-130-38	MQC	4.30	241029	2221608	0.108	4.38	-	-	-	102%
12322-129-29	HQC	90.3	5361227	2686808	2.00	80.7	-	-	-	89.3%
12322-129-30	HQC	90.3	4744139	2445017	1.94	78	-	-	-	86.9%

<sup>a</sup>  
 INTERCEPT 0.00013973  
<sup>b</sup> SLOPE 0.024733  
<sup>r</sup> 0.999

**Table 7. DIPE Blood Concentration Analysis for Groups A.**

Rat.	Time point	DIPE Peak Area	MTBE Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AM 01	Predose	0	3305500	0	below LOQ	0.1715	n/a
	5 min	3942626	3094095	1.27	19.4	0.1451	13.4
	15 min	10284961	2726284	3.77	55.5	0.1815	30.6
	1 hr	11169127	2352305	4.75	69.6	0.1883	37.0
	4hr	16109055	2154766	7.48	109	0.1980	55.1
	6.25 hr	6178612	2255680	2.74	40.6	0.1646	24.7
	7 hr	1823281	2073026	0.880	14.7	0.1698	8.65
	8 hr	366955	2163484	0.170	2.83	0.1497	1.89
	16 hr	3908	1792938	0.00218	below LOQ	0.1602	n/a
AM 02	Predose	0	3220242	0	below LOQ	0.1612	n/a
	5 min	9760022	3004415	3.25	48.0	0.2109	22.7
	15 min	13281846	2657842	5.00	73.2	0.2103	34.8
	1 hr	23604618	2422594	9.74	142	0.3015	47.1
	4hr	13284866	2198086	6.04	88.4	0.1649	53.6
	6.25 hr	3309760	2183125	1.52	22.9	0.1585	14.4
	7 hr	1751996	2074241	0.845	13.2	0.1769	7.45
	8 hr	399385	1912997	0.209	3.48	0.1489	2.34
	16 hr	18213	1895367	0.00961	below LOQ	0.1800	n/a
AM 03	Predose	0	3125677	0	below LOQ	0.1952	n/a
	5 min	7370270	2937781	2.51	37.3	0.1710	21.8
	15 min	11490102	2682406	4.28	62.9	0.2109	29.8
	1 hr	16872653	2289260	7.37	108	0.2168	49.6
	4hr	17905650	2155261	8.31	121	0.2073	58.4
	6.25 hr	3369772	2182099	1.54	23.3	0.1584	14.7
	7 hr	1731385	2044830	0.847	13.2	0.1740	7.60
	8 hr	534386	2071092	0.258	4.31	0.1798	2.40
	16 hr	4515	1884535	0.00240	below LOQ	0.1570	n/a

**Table 7 (continued). DIPE Blood Concentration Analysis for Group A.**

Rat.	Time point	DIPE Peak Area	MTBE Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AM 04	Predose	0	3141737	0	below LOQ	0.1625	n/a
	10 min	6634863	2864181	2.32	34.5	0.1804	19.1
	30 min	7478194	2501719	2.99	44.2	0.1694	26.1
	2 hr	11532703	2276394	5.07	74.2	0.1877	39.6
	6 hr	16647985	2106319	7.90	115	0.2212	52.1
	6.5 hr	3001728	2155520	1.39	21.1	0.1876	11.3
	7.33 hr	1252155	2220162	0.564	9.13	0.1697	5.38
	10 hr	177616	2061463	0.0862	1.44	0.1727	0.833
	24 hr	0	2006661	0.000	below LOQ	0.1752	n/a
AM 05	Predose	0	3076076	0	below LOQ	0.1819	n/a
	10 min	11508463	2772270	4.15	61.0	0.2252	27.1
	30 min	16985066	2468288	6.88	100	0.2180	46.1
	2 hr	15454012	2326258	6.64	97.1	0.1854	52.3
	6 hr	2817350	2046662	1.38	20.9	0.0745	28.0
	6.5 hr	2061183	2113931	0.975	15.1	0.1826	8.25
	7.33 hr	729460	2040999	0.357	6.14	0.1936	3.17
	10 hr	72643	2018958	0.0360	0.601	0.1845	0.326
	24 hr	0	1776471	0.000	below LOQ	0.1801	n/a
AM 06	Predose	0	3063913	0	below LOQ	0.1844	n/a
	10 min	14799587	2794446	5.30	77.6	0.2635	29.4
	30 min	16164176	2494808	6.48	94.7	0.2273	41.7
	2 hr	14031744	2189712	6.41	93.6	0.1726	54.3
	6 hr	21078163	2053273	10.3	149	0.2390	62.5
	6.5 hr	3341880	2133667	1.57	23.6	0.2054	11.5
	7.33 hr	653874	2087767	0.313	5.50	0.1852	2.97
	10 hr	76651	1949776	0.0393	0.66	0.1872	0.351
	24 hr	0	1782700	0.000	below LOQ	0.1835	n/a

**Table 7 (continued). DIPE Blood Concentration Analysis for Group A.**

Rat.	Time point	DIPE Peak Area	MTBE Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AF 01	Predose	0	3043812	0	below LOQ	0.2480	n/a
	5 min	8818210	2820179	3.13	46.2	0.2088	22.1
	15 min	10416878	2583392	4.03	59.3	0.1851	32.0
	1 hr	14503744	2408498	6.02	88.1	0.2037	43.2
	4hr	16260076	2161490	7.52	110	0.2003	54.8
	6.25 hr	5613786	2137156	2.63	39.0	0.2162	18.0
	7 hr	1173091	2071197	0.566	9.16	0.1763	5.20
	8 hr	404239	1975565	0.205	3.42	0.1733	1.97
	16 hr	22623	1806051	0.0125	below LOQ	0.1920	n/a
AF 02	Predose	0	3083673	0	below LOQ	0.1865	n/a
	5 min	7871770	2863675	2.75	40.7	0.2403	16.9
	15 min	10413512	2643903	3.94	57.9	0.2115	27.4
	1 hr	18600188	2434213	7.64	111	0.2138	52.1
	4hr	16437024	2112136	7.78	114	0.1890	60.1
	6.25 hr	5113255	2084851	2.45	36.4	0.1821	20.0
	7 hr	1435584	2046806	0.70	11.1	0.1753	6.34
	8 hr	259829	1862016	0.140	2.33	0.1622	1.44
	16 hr	0	1806061	0.000	below LOQ	0.1533	n/a
AF 03	Predose	0	2976122	0	below LOQ	0.1581	n/a
	5 min	5179417	2901591	1.79	26.8	0.1241	21.6
	15 min	9835262	2659346	3.70	54.5	0.1540	35.4
	1 hr	15868117	2323506	6.83	99.7	0.2102	47.5
	4hr	22848033	2201343	10.4	151	0.2261	66.8
	6.25 hr	3962996	2158089	1.84	27.5	0.1531	18.0
	7 hr	1564184	2064230	0.758	11.9	0.1741	6.85
	8 hr	608280	2167471	0.281	5.03	0.2131	2.36
	16 hr	3888	1810056	0.00215	below LOQ	0.1717	n/a

**Table 7 (continued). DIPE Blood Concentration Analysis for Group A.**

Rat.	Time point	DIPE Peak Area	MTBE Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AF 04	Predose	0	3026192	0	below LOQ	0.1832	n/a
	10 min	8856549	2773431	3.19	47.2	0.1888	25.0
	30 min	15766622	2586291	6.10	89.1	0.2115	42.1
	2 hr	13960100	2236237	6.24	91.3	0.1532	59.6
	6 hr	16325203	2031561	8.04	117	0.1843	63.6
	6.5 hr	2310642	2112871	1.09	16.8	0.1705	9.85
	7.33 hr	757162	2029278	0.373	6.37	0.1716	3.71
	10 hr	82489	2028032	0.0407	0.679	0.1712	0.397
	24 hr	0	1515234	0.000	below LOQ	0.1802	n/a
AF 05	Predose	0	3050805	0	below LOQ	0.1491	n/a
	10 min	11816192	2789584	4.24	62.2	0.2031	30.6
	30 min	12151980	2539713	4.78	70.2	0.1657	42.3
	2 hr	16836252	2307750	7.30	106	0.1658	64.2
	6 hr	19612248	2042061	9.60	140	0.1947	71.8
	6.5 hr	2553966	2042423	1.25	19.1	0.1667	11.4
	7.33 hr	1033665	1961449	0.527	8.59	0.1825	4.71
	10 hr	90528	2023204	0.0447	0.747	0.1838	0.407
	24 hr	0	1451432	0.000	below LOQ	0.1821	n/a
AF 06	Predose	0	3018009	0	below LOQ	0.1692	n/a
	10 min	8351910	2776813	3.01	44.5	0.1329	33.5
	30 min	15718575	2481371	6.33	92.6	0.2223	41.6
	2 hr	16424573	2215625	7.41	108	0.1890	57.2
	6 hr	15057861	2065393	7.29	106	0.1733	61.4
	6.5 hr	3573258	2095249	1.71	25.6	0.1721	14.9
	7.33 hr	1543333	2009887	0.768	12.1	0.2513	4.81
	10 hr	65336	2000918	0.0327	0.545	0.1525	0.358
	24 hr	0	1394642	0.000	below LOQ	0.1854	n/a

**Table 8. Isopropanol Blood Concentration Analysis for Group A.**

Rat.	Time point	Isopropanol Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AM 01	Predose	6272	2153513	0.00291	below LOQ	0.1715	n/a
	5 min	109777	2380795	0.0461	below LOQ	0.1451	n/a
	15 min	351929	1766360	0.199	6.27	0.1815	3.45
	1 hr	1445382	1573428	0.919	21.6	0.1883	11.5
	4hr	5681189	1352656	4.20	91.4	0.1980	46.1
	6.25 hr	8561604	1647935	5.20	113	0.1646	68.4
	7 hr	6432824	1461713	4.40	95.6	0.1698	56.3
	8 hr	3721832	1621117	2.30	50.9	0.1497	34.0
	16 hr	115326	1437329	0.0802	below LOQ	0.1602	n/a
AM 02	Predose	4018	2146112	0.00187	below LOQ	0.1612	n/a
	5 min	151958	1654781	0.0918	below LOQ	0.2109	n/a
	15 min	310509	1543333	0.201	6.31	0.2103	3.00
	1 hr	1526965	1028884	1.48	33.6	0.3015	11.1
	4hr	4547418	1579939	2.88	63.2	0.1649	38.4
	6.25 hr	7007494	1712280	4.09	89.1	0.1585	56.2
	7 hr	5108536	1450585	3.52	76.9	0.1769	43.5
	8 hr	2980650	1617537	1.84	41.2	0.1489	27.7
	16 hr	90571	1301098	0.0696	below LOQ	0.1800	n/a
AM 03	Predose	5380	1854237	0.00290	below LOQ	0.1952	n/a
	5 min	175658	1978677	0.0888	below LOQ	0.1710	n/a
	15 min	385625	1495362	0.258	7.52	0.2109	3.56
	1 hr	1541485	1319785	1.17	26.9	0.2168	12.4
	4hr	5138002	1272284	4.04	87.9	0.2073	42.4
	6.25 hr	8226552	1717442	4.79	104	0.1584	65.6
	7 hr	6370190	1501241	4.24	92.3	0.1740	53.0
	8 hr	4429311	1594458	2.78	61.1	0.1798	34.0
	16 hr	77984	1468074	0.0531	below LOQ	0.1570	n/a

**Table 8 (continued). Isopropanol Blood Concentration Analysis for Group A.**

Rat.	Time point	Isopropanol Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AM 04	Predose	6838	2106071	0.00325	below LOQ	0.1625	n/a
	10 min	215829	1980354	0.109	below LOQ	0.1804	n/a
	30 min	488328	1750458	0.279	7.97	0.1694	4.70
	2 hr	2157660	1492442	1.45	32.8	0.1877	17.5
	6 hr	7972351	1210042	6.59	142	0.2212	64.3
	6.5 hr	6772101	1428055	4.74	103	0.1876	54.8
	7.33 hr	4637067	1579500	2.94	64.5	0.1697	38.0
	10 hr	940207	1529728	0.615	15.1	0.1727	8.75
	24 hr	3843	1413218	0.00272	below LOQ	0.1752	n/a
AM 05	Predose	3518	1933430	0.00182	below LOQ	0.1819	n/a
	10 min	244913	1511261	0.162	5.48	0.2252	2.43
	30 min	795402	1478297	0.538	13.5	0.2180	6.18
	2 hr	3048080	1479577	2.06	45.8	0.1854	24.7
	6 hr	5527552	2805396	1.97	43.9	0.0745	59.0
	6.5 hr	8254324	1433356	5.76	125	0.1826	68.2
	7.33 hr	5463418	1321156	4.14	90.0	0.1936	46.5
	10 hr	954680	1449282	0.659	16.0	0.1845	8.70
	24 hr	3161	1314654	0.00240	below LOQ	0.1801	n/a
AM 06	Predose	5676	1923945	0.00295	below LOQ	0.1844	n/a
	10 min	344130	1319405	0.261	7.58	0.2635	2.88
	30 min	836825	1338377	0.625	15.3	0.2273	6.74
	2 hr	2956336	1535211	1.93	43.0	0.1726	24.9
	6 hr	9668514	1155596	8.37	180	0.2390	75.3
	6.5 hr	8433691	1319920	6.39	138	0.2054	67.1
	7.33 hr	5640666	1401146	4.03	87.7	0.1852	47.3
	10 hr	1089471	1404476	0.776	18.5	0.1872	9.90
	24 hr	3276	1290696	0.00254	below LOQ	0.1835	n/a

**Table 8 (continued). Isopropanol Blood Concentration Analysis for Group A.**

Rat.	Time point	Isopropanol Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AF 01	Predose	4258	1870330	0.00228	below LOQ	0.2480	n/a
	5 min	80094	1646387	0.0486	below LOQ	0.2088	n/a
	15 min	175601	1669197	0.105	below LOQ	0.1851	n/a
	1 hr	537971	1418766	0.379	10.1	0.2037	4.96
	4hr	2139912	1323597	1.62	36.4	0.2003	18.2
	6.25 hr	4187519	1254062	3.34	73.1	0.2162	33.8
	7 hr	2459573	1447194	1.70	38.2	0.1763	21.7
	8 hr	1019824	1430873	0.713	17.2	0.1733	9.92
	16 hr	7248	1258028	0.00576	below LOQ	0.1920	n/a
AF 02	Predose	4955	1867624	0.00265	below LOQ	0.1865	n/a
	5 min	87060	1462557	0.0595	below LOQ	0.2403	n/a
	15 min	164656	1488895	0.111	below LOQ	0.2115	n/a
	1 hr	508099	1405743	0.361	9.72	0.2138	4.55
	4hr	2124810	1340890	1.58	35.7	0.1890	18.9
	6.25 hr	3773344	1421113	2.66	58.5	0.1821	32.1
	7 hr	2740403	1442899	1.90	42.4	0.1753	24.2
	8 hr	1104185	1535827	0.719	17.3	0.1622	10.7
	16 hr	0	1518048	0.000	below LOQ	0.1533	n/a
AF 03	Predose	4303	2114181	0.00204	below LOQ	0.1581	n/a
	5 min	60845	2478779	0.0245	below LOQ	0.1241	n/a
	15 min	178917	1929727	0.0927	below LOQ	0.1540	n/a
	1 hr	657330	1339129	0.491	12.5	0.2102	5.93
	4hr	3357579	1194444	2.81	61.8	0.2261	27.3
	6.25 hr	4856392	1691576	2.87	63.1	0.1531	41.2
	7 hr	3566768	1498467	2.38	52.7	0.1741	30.2
	8 hr	1732491	1245271	1.39	31.6	0.2131	14.8
	16 hr	4547	1376268	0.00330	below LOQ	0.1717	n/a

**Table 8 (continued). Isopropanol Blood Concentration Analysis for Group A.**

Rat.	Time point	Isopropanol Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AF 04	Predose	3375	1876350	0.00180	below LOQ	0.1832	n/a
	10 min	125400	1738855	0.0721	below LOQ	0.1888	n/a
	30 min	342561	1460313	0.235	7.02	0.2115	3.32
	2 hr	1216382	1711152	0.711	17.2	0.1532	11.2
	6 hr	3624672	1348619	2.69	59.2	0.1843	32.1
	6.5 hr	2949339	1514401	1.95	43.5	0.1705	25.5
	7.33 hr	1503455	1442100	1.04	24.2	0.1716	14.1
	10 hr	192882	1468611	0.131	below LOQ	0.1712	n/a
	24 hr	0	1176006	0.000	below LOQ	0.1802	n/a
AF 05	Predose	5016	2179453	0.00230	below LOQ	0.1491	n/a
	10 min	124944	1623682	0.0770	below LOQ	0.2031	n/a
	30 min	264987	1780808	0.149	5.20	0.1657	3.14
	2 hr	1128969	1597459	0.707	17.1	0.1658	10.3
	6 hr	3437636	1295821	2.65	58.5	0.1947	30.0
	6.5 hr	2758250	1565204	1.76	39.5	0.1667	23.7
	7.33 hr	1522278	1340694	1.14	26.2	0.1825	14.3
	10 hr	261068	1319854	0.198	6.24	0.1838	3.39
	24 hr	0	1163884	0.000	below LOQ	0.1821	n/a
AF 06	Predose	5826	2016276	0.00289	below LOQ	0.1692	n/a
	10 min	137916	2246791	0.0614	below LOQ	0.1329	n/a
	30 min	379115	1386373	0.273	7.85	0.2223	3.53
	2 hr	1220027	1416451	0.861	20.4	0.1890	10.8
	6 hr	3606894	1404703	2.57	56.6	0.1733	32.7
	6.5 hr	3314092	1551243	2.14	47.5	0.1721	27.6
	7.33 hr	2032056	1060293	1.92	42.8	0.2513	17.0
	10 hr	281055	1761284	0.160	5.43	0.1525	3.56
	24 hr	0	1062345	0.000	below LOQ	0.1854	n/a

**Table 9. Acetone Blood Concentration Analysis for Group A.**

Rat.	Time point	Acetone Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AM 01	Predose	270086	2153513	0.125	below LOQ	0.1715	n/a
	5 min	375279	2380795	0.158	6.37	0.1451	4.39
	15 min	1029684	1766360	0.583	23.6	0.1815	13.0
	1 hr	3296086	1573428	2.09	84.7	0.1883	45.0
	4hr	14568860	1352656	10.8	435	0.1980	220
	6.25 hr	21514425	1647935	13.1	528	0.1646	321
	7 hr	20743258	1461713	14.2	574	0.1698	338
	8 hr	19230404	1621117	11.9	480	0.1497	320
	16 hr	4501539	1437329	3.13	127	0.1602	79.0
AM 02	Predose	180456	2146112	0.0841	3.39	0.1612	2.11
	5 min	549846	1654781	0.332	13.4	0.2109	6.37
	15 min	798701	1543333	0.518	20.9	0.2103	9.95
	1 hr	3979523	1028884	3.87	156	0.3015	51.9
	4hr	12062123	1579939	7.63	309	0.1649	187
	6.25 hr	19050666	1712280	11.1	450	0.1585	284
	7 hr	19021029	1450585	13.1	530	0.1769	300
	8 hr	17189719	1617537	10.6	430	0.1489	289
	16 hr	3481263	1301098	2.68	108	0.1800	60.1
AM 03	Predose	184997	1854237	0.0998	4.03	0.1952	2.06
	5 min	455526	1978677	0.230	9.30	0.1710	5.44
	15 min	1147140	1495362	0.767	31.0	0.2109	14.7
	1 hr	3857093	1319785	2.92	118	0.2168	54.5
	4hr	13229788	1272284	10.4	420	0.2073	203
	6.25 hr	19661195	1717442	11.4	463	0.1584	292
	7 hr	19369795	1501241	12.9	522	0.1740	300
	8 hr	21034680	1594458	13.2	533	0.1798	297
	16 hr	3466818	1468074	2.36	95.5	0.1570	60.8

**Table 9 (continued). Acetone Blood Concentration Analysis for Group A.**

Rat.	Time point	Acetone Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AM 04	Predose	151802	2106071	0.0721	2.91	0.1625	1.79
	10 min	681027	1980354	0.344	13.9	0.1804	7.70
	30 min	1652770	1750458	0.944	38.2	0.1694	22.5
	2 hr	6621080	1492442	4.44	179	0.1877	95.6
	6 hr	22100456	1210042	18.3	738	0.2212	334
	6.5 hr	20006287	1428055	14.0	566	0.1876	302
	7.33 hr	19441867	1579500	12.3	498	0.1697	293
	10 hr	14971899	1529728	9.79	396	0.1727	229
	24 hr	117779	1413218	0.0833	3.36	0.1752	1.92
AM 05	Predose	143411	1933430	0.0742	2.99	0.1819	1.65
	10 min	661144	1511261	0.437	17.7	0.2252	7.85
	30 min	1950469	1478297	1.32	53.3	0.2180	24.5
	2 hr	7122622	1479577	4.81	195	0.1854	105
	6 hr	10322687	2805396	3.68	149	0.0745	200
	6.5 hr	21299372	1433356	14.9	601	0.1826	329
	7.33 hr	20830982	1321156	15.8	637	0.1936	329
	10 hr	15006981	1449282	10.4	419	0.1845	227
	24 hr	88945	1314654	0.0677	2.73	0.1801	1.52
AM 06	Predose	181028	1923945	0.0941	3.80	0.1844	2.06
	10 min	898106	1319405	0.681	27.5	0.2635	10.4
	30 min	2197824	1338377	1.64	66.4	0.2273	29.2
	2 hr	6285399	1535211	4.09	166	0.1726	95.9
	6 hr	22661455	1155596	19.6	793	0.2390	332
	6.5 hr	21938553	1319920	16.6	672	0.2054	327
	7.33 hr	20723658	1401146	14.8	598	0.1852	323
	10 hr	15227985	1404476	10.8	438	0.1872	234
	24 hr	88171	1290696	0.0683	2.76	0.1835	1.50

**Table 9 (continued). Acetone Blood Concentration Analysis for Group A.**

Rat.	Time point	Acetone Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. ( $\mu\text{g/ml}$ )	Sample weight (g)	Corrected Conc. ( $\mu\text{g/ml}$ )
AF 01	Predose	131976	1870330	0.0706	2.85	0.2480	1.15
	5 min	327787	1646387	0.199	8.04	0.2088	3.85
	15 min	617501	1669197	0.370	15.0	0.1851	8.08
	1 hr	2489512	1418766	1.75	70.9	0.2037	34.8
	4hr	8926147	1323597	6.74	273	0.2003	136
	6.25 hr	14273540	1254062	11.4	460	0.2162	213
	7 hr	12104797	1447194	8.36	338	0.1763	192
	8 hr	11243122	1430873	7.86	318	0.1733	183
	16 hr	111294	1258028	0.0885	3.57	0.1920	1.86
AF 02	Predose	164632	1867624	0.0882	3.56	0.1865	1.91
	5 min	567774	1462557	0.388	15.7	0.2403	6.53
	15 min	821344	1488895	0.552	22.3	0.2115	10.5
	1 hr	2599217	1405743	1.85	74.8	0.2138	35.0
	4hr	8146237	1340890	6.08	246	0.1890	130
	6.25 hr	12949810	1421113	9.11	368	0.1821	202
	7 hr	12856118	1442899	8.91	360	0.1753	205
	8 hr	10485038	1535827	6.83	276	0.1622	170
	16 hr	111861	1518048	0.0737	2.97	0.1533	1.94
AF 03	Predose	147114	2114181	0.0696	2.81	0.1581	1.78
	5 min	194811	2478779	0.0786	3.17	0.1241	2.56
	15 min	511492	1929727	0.265	10.7	0.1540	6.96
	1 hr	2669939	1339129	1.99	80.6	0.2102	38.3
	4hr	11791675	1194444	9.87	399	0.2261	177
	6.25 hr	14015764	1691576	8.29	335	0.1531	219
	7 hr	14710448	1498467	9.82	397	0.1741	228
	8 hr	16129545	1245271	13.0	524	0.2131	246
	16 hr	79578	1376268	0.0578	below LOQ	0.1717	n/a

**Table 9 (continued). Acetone Blood Concentration Analysis for Group A.**

Rat.	Time point	Acetone Peak Area	1-Propanol Peak Area	Peak Area Ratio	Cal. Conc. (µg/ml)	Sample weight (g)	Corrected Conc. (µg/ml)
AF 04	Predose	144203	1876350	0.0769	3.10	0.1832	1.69
	10 min	354288	1738855	0.204	8.23	0.1888	4.36
	30 min	1293392	1460313	0.886	35.8	0.2115	16.9
	2 hr	4690259	1711152	2.74	111	0.1532	72.3
	6 hr	13313353	1348619	9.87	399	0.1843	217
	6.5 hr	12520755	1514401	8.27	334	0.1705	196
	7.33 hr	11564909	1442100	8.02	324	0.1716	189
	10 hr	6852970	1468611	4.67	189	0.1712	110
	24 hr	60026	1176006	0.0510	below LOQ	0.1802	n/a
AF 05	Predose	116074	2179453	0.0533	below LOQ	0.1491	n/a
	10 min	396178	1623682	0.244	9.86	0.2031	4.85
	30 min	889165	1780808	0.499	20.2	0.1657	12.2
	2 hr	4274073	1597459	2.68	108	0.1658	65.2
	6 hr	12281058	1295821	9.48	383	0.1947	197
	6.5 hr	10953458	1565204	7.00	283	0.1667	170
	7.33 hr	10930515	1340694	8.15	330	0.1825	181
	10 hr	7119040	1319854	5.39	218	0.1838	119
	24 hr	68095	1163884	0.0585	below LOQ	0.1821	n/a
AF 06	Predose	167418	2016276	0.0830	3.35	0.1692	1.98
	10 min	429890	2246791	0.191	7.73	0.1329	5.82
	30 min	1618253	1386373	1.17	47.2	0.2223	21.2
	2 hr	4960176	1416451	3.50	142	0.1890	74.9
	6 hr	12933193	1404703	9.21	372	0.1733	215
	6.5 hr	13552770	1551243	8.74	353	0.1721	205
	7.33 hr	14730115	1060293	13.9	562	0.2513	224
	10 hr	7809518	1761284	4.43	179	0.1525	118
	24 hr	132375	1062345	0.125	5.03	0.1854	2.71

Appendix J  
Noncompartmental Pharmacokinetic Analysis

## Kinetics of DIPE in Male and Female Rats exposed to 3600 ppm DIPE by inhalation.

Input File: Data - [A:\DIPEPK~1.XLS]  
Sex=Female

Start Time: 20:22:01 07-26-2013  
End Time: 20:22:02 07-26-2013

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM (V01.5A)  
Core Version 29Oct97

Listing of input commands

TITLE 1  
DIPE inhalation 3600 ppm  
MODEL 200  
NVARIABLES 5  
NPOINTS 100  
XNUMBER 1  
YNUMBER 5  
DTIME 0  
NCONSTANTS 1  
CONSTANTS 300  
METHOD 2 'Linear trapezoidal'  
NCATRANS  
MISSING 'Missing'  
NOBSERVATIONS 14  
DATA 'WINNLIN.DAT'  
BEGIN

DIPE inhalation 3600 ppm

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Noncompartmental Analysis for Extravascular Administration

Linear Trapezoidal Rule Used to Compute AUC, AUMC

X	Y	Pred.	Res.	AUC	AUMC	WEIGHT
.0000	.0000			.0000	.0000	
.8330E-01	20.22			.8421	.7015E-01	
.1670	29.69			2.931	.3482	
.2500	31.60			5.474	.8818	
.5000	42.05			14.68	4.497	
1.000	47.61			37.09	21.66	
2.000	60.34			91.07	105.8	
4.000	60.56			212.0	468.7	
6.000	65.61			338.1	1105.	
6.250	18.67			348.7	1168.	
6.500	12.06			352.5	1193.	
7.000	* 6.130	5.693	.4370	357.1	1223.	1.000
7.330	* 4.409	4.211	.1971	358.8	1236.	1.000
8.000	* 1.923	2.283	-.3608	360.9	1252.	1.000
10.00	* .3870	.3673	.1972E-01	363.2	1271.	1.000

\*) Starred values were included in estimation of Lambda\_z.

@) Note - the concentration at time zero (DTIME) was added for extrapolation purposes

Dosing_time	.0000
Rsq	.9913
Rsq(adjusted)	.9869
Corr(x:y)	-.9956
Tlag	.0000
Tmax	6.0000
Cmax	65.6099
No._points_Lambda_z	4
Tlast	10.0000
Clast	.3870
AUClast	363.2418
Lambda_z	.9136
Lambda_z_lower	7.0000
Lambda_z_upper	10.0000
t1/2_Lambda_z	.7587
AUCall	363.2418
AUCINF (observed)	363.6654
AUCINF (observed) /D	1.2122
AUC_%Extrap(obs.)	.1165
Vz (observed) /F	.9029
Cl (observed) /F	.8249
AUCINF (predicted)	363.6438
AUCINF (predicted) /D	1.2121
AUC_%Extrap(pred.)	.1106
Vz (predicted) /F	.9030
Cl (predicted) /F	.8250
AUMClast	1270.7918
AUMCINF (observed)	1275.4915
AUMC_%Extrap(obs.)	.3685
AUMCINF (predicted)	1275.2520
AUMC_%Extrap(pred.)	.3497
MRTlast	3.4985
MRTINF (observed)	3.5073
MRTINF (predicted)	3.5069

NORMAL ENDING

Input File: Data - [A:\DIPEPK~1.XLS]  
Sex=Male

Start Time: 20:22:02 07-26-2013  
End Time: 20:22:03 07-26-2013

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM (V01.5A)  
Core Version 29Oct97

Listing of input commands

TITLE 1  
DIPE inhalation 3600 ppm  
MODEL 200  
N VARIABLES 5  
NPOINTS 100  
XNUMBER 1  
YNUMBER 5  
D TIME 0  
NCONSTANTS 1  
CONSTANTS 300  
METHOD 2 'Linear trapezoidal'  
NCATTRANS  
MISSING 'Missing'  
NOBSERVATIONS 14  
DATA 'WINNLIN.DAT'  
BEGIN

DIPE inhalation 3600 ppm

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Noncompartmental Analysis for Extravascular Administration

Linear Trapezoidal Rule Used to Compute AUC, AUMC

X	Y	Pred.	Res.	AUC	AUMC	WEIGHT
.0000	.0000			.0000	.0000	
.8330E-01	19.30			.8038	.6695E-01	
.1670	25.21			2.667	.3104	
.2500	31.75			5.031	.8146	
.5000	37.95			13.74	4.179	
1.000	44.55			34.37	20.06	
2.000	48.72			81.01	91.06	
4.000	55.71			185.4	411.3	
6.000	47.56			288.7	919.5	
6.250	17.94			296.9	969.2	
6.500	10.34			300.4	991.6	
7.000	7.899			305.0	1022.	
7.330	* 3.841	3.765	.7544E-01	306.9	1036.	1.000
8.000	* 2.209	2.268	-.5928E-01	308.9	1051.	1.000
10.00	* .5031	.4998	.3333E-02	311.7	1074.	1.000

\*) Starred values were included in estimation of Lambda\_z.

@) Note - the concentration at time zero (DTIME) was added for extrapolation purposes

Dosing_time	.0000
Rsq	.9995
Rsq(adjusted)	.9990
Corr(x:y)	-.9997
Tlag	.0000
Tmax	4.0000
Cmax	55.7082
No._points_Lambda_z	3
Tlast	10.0000
Clast	.5031
AUClast	311.6541
Lambda_z	.7563
Lambda_z_lower	7.3300
Lambda_z_upper	10.0000
t1/2_Lambda_z	.9165
AUCall	311.6541
AUCINF (observed)	312.3194
AUCINF (observed) /D	1.0411
AUC_%Extrap(obs.)	.2130
Vz (observed) /F	1.2701
Cl (observed) /F	.9606
AUCINF (predicted)	312.3150
AUCINF (predicted) /D	1.0410
AUC_%Extrap(pred.)	.2116
Vz (predicted) /F	1.2701
Cl (predicted) /F	.9606
AUMClast	1074.0417
AUMCINF (observed)	1081.5742
AUMC_%Extrap(obs.)	.6964
AUMCINF (predicted)	1081.5243
AUMC_%Extrap(pred.)	.6919
MRTlast	3.4463
MRTINF (observed)	3.4630
MRTINF (predicted)	3.4629

NORMAL ENDING

## Kinetics of Isopropanol in Male and Female Rats exposed to 3600 ppm DIPE by inhalation.

Input File: Data - [A:\DIPEPK~1.XLS]  
Sex=Female

Start Time: 20:20:27 07-26-2013  
End Time: 20:20:28 07-26-2013

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM (V01.5A)  
Core Version 29Oct97

Listing of input commands

```
MODEL 200
N VARIABLES 5
NPOINTS 100
XNUMBER 1
YNUMBER 4
DTIME 0
NCONSTANTS 1
CONSTANTS 300
METHOD 2 'Linear trapezoidal'
NCATRANS
MISSING 'Missing'
NOBSERVATIONS 11
DATA 'WINNLIN.DAT'
BEGIN
```

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Noncompartmental Analysis for Extravascular Administration

Linear Trapezoidal Rule Used to Compute AUC, AUMC

X	Y	Pred.	Res.	AUC	AUMC	WEIGHT
.0000	.0000			.0000	.0000	
.5000	3.329			.8323	.4162	
1.000	5.146			2.951	2.119	
2.000	10.75			10.90	15.44	
4.000	21.48			43.13	122.9	
6.000	31.61			96.21	398.4	
6.250	35.71			104.6	450.0	
6.500	25.59			112.3	498.7	
7.000	25.37			125.0	584.7	
7.330	* 15.16	16.02	-.8632	131.7	632.3	1.000
8.000	* 11.81	10.97	.8418	140.8	701.2	1.000
10.00	* 3.477	3.542	-.6510E-01	156.0	830.5	1.000

\*) Starred values were included in estimation of Lambda<sub>z</sub>.

@) Note - the concentration at time zero (DTIME) was added for extrapolation purposes

```
Dosing_time .0000
Rsq .9929
```

Rsq (adjusted)	.9857
Corr (x:y)	-.9964
Tlag	.0000
Tmax	6.2500
Cmax	35.7084
No._points_Lambda_z	3
Tlast	10.0000
Clast	3.4767
AUClast	156.0429
Lambda_z	.5653
Lambda_z_lower	7.3300
Lambda_z_upper	10.0000
t1/2_Lambda_z	1.2261
AUCall	156.0429
AUCINF (observed)	162.1929
AUCINF (observed) /D	.5406
AUC_%Extrap (obs.)	3.7918
Vz (observed) /F	3.2718
Cl (observed) /F	1.8496
AUCINF (predicted)	162.3080
AUCINF (predicted) /D	.5410
AUC_%Extrap (pred.)	3.8600
Vz (predicted) /F	3.2695
Cl (predicted) /F	1.8483
AUMClast	830.4762
AUMCINF (observed)	902.8544
AUMC_%Extrap (obs.)	8.0166
AUMCINF (predicted)	904.2096
AUMC_%Extrap (pred.)	8.1545
MRTlast	5.3221
MRTINF (observed)	5.5665
MRTINF (predicted)	5.5709

NORMAL ENDING

Input File: Data - [A:\DIPEPK~1.XLS]  
Sex=Male

Start Time: 20:20:29 07-26-2013  
End Time: 20:20:30 07-26-2013

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM (V01.5A)  
Core Version 29Oct97

Listing of input commands

```
MODEL 200
N VARIABLES 5
NPOINTS 100
XNUMBER 1
YNUMBER 4
DTIME 0
NCONSTANTS 1
CONSTANTS 300
METHOD 2 'Linear trapezoidal'
NCATRANS
MISSING 'Missing'
NOBSERVATIONS 13
DATA 'WINNLIN.DAT'
BEGIN
```

## WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

## Noncompartmental Analysis for Extravascular Administration

## Linear Trapezoidal Rule Used to Compute AUC, AUMC

X	Y	Pred.	Res.	AUC	AUMC	WEIGHT
.0000	.0000			.0000	.0000	
.1670	2.655			.2217	.3702E-01	
.2500	3.340			.4705	.9008E-01	
.5000	5.876			1.623	.5617	
1.000	11.66			6.008	4.212	
2.000	22.37			23.02	32.41	
4.000	42.30			87.69	246.4	
6.000	66.18			196.2	812.7	
6.250	63.39			212.4	911.8	
6.500	63.39			228.2	1013.	
7.000	50.95			256.8	1205.	
7.330	* 43.93	45.46	-1.528	272.5	1317.	1.000
8.000	* 31.88	30.46	1.423	297.9	1510.	1.000
10.00	* 9.113	9.218	-.1050	338.9	1857.	1.000

\*) Starred values were included in estimation of Lambda\_z.

@) Note - the concentration at time zero (DTIME) was added for extrapolation purposes

Dosing_time	.0000
Rsq	.9976
Rsq(adjusted)	.9951
Corr(x:y)	-.9988
Tlag	.0000
Tmax	6.0000
Cmax	66.1823
No._points_Lambda_z	3
Tlast	10.0000
Clast	9.1135
AUClast	338.8580
Lambda_z	.5976
Lambda_z_lower	7.3300
Lambda_z_upper	10.0000
t1/2_Lambda_z	1.1598
AUCall	338.8580
AUCINF(observed)	354.1077
AUCINF(observed)/D	1.1804
AUC_%Extrap(obs.)	4.3065
Vz(observed)/F	1.4176
Cl(observed)/F	.8472
AUCINF(predicted)	354.2833
AUCINF(predicted)/D	1.1809
AUC_%Extrap(pred.)	4.3539
Vz(predicted)/F	1.4169
Cl(predicted)/F	.8468
AUMClast	1856.5314
AUMCINF(observed)	2034.5451
AUMC_%Extrap(obs.)	8.7496

AUMCINF (predicted)	2036.5957
AUMC_%Extrap(pred.)	8.8414
MRTlast	5.4788
MRTINF (observed)	5.7456
MRTINF (predicted)	5.7485

NORMAL ENDING

## Kinetics of Acetone in Male and Female Rats exposed to 3600 ppm DIPE by inhalation.

Input File: Data - [A:\DIPEPK~1.XLS]  
Sex=Female

Start Time: 20:34:13 07-26-2013  
End Time: 20:34:14 07-26-2013

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM (V01.5A)  
Core Version 29Oct97

## Listing of input commands

TITLE 1  
ACETONE  
MODEL 200  
N VARIABLES 5  
NPOINTS 100  
XNUMBER 1  
YNUMBER 3  
D TIME 0  
NCONSTANTS 1  
CONSTANTS 0  
METHOD 2 'Linear trapezoidal'  
NCATrans  
MISSING 'Missing'  
NOBSERVATIONS 16  
DATA 'WINNLIN.DAT'  
BEGIN

ACETONE

## WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

## Noncompartmental Analysis for Extravascular Administration

## Linear Trapezoidal Rule Used to Compute AUC, AUMC

X	Y	Pred.	Res.	AUC	AUMC	WEIGHT
.0000	.0000			.0000	.0000	
.8330E-01	4.313			.1796	.1496E-01	
.1670	5.011			.5698	.6502E-01	
.2500	8.525			1.132	.1882	
.5000	16.78			4.295	1.503	
1.000	36.05			17.50	12.61	
2.000	70.83			70.94	101.5	
4.000	147.5			289.3	833.3	
6.000	* 209.4	238.2	-28.85	646.2	2680.	1.000
6.250	* 211.3	221.2	-9.884	698.8	3002.	1.000
6.500	* 190.3	205.4	-15.05	749.0	3322.	1.000
7.000	* 208.4	177.1	31.35	848.7	3996.	1.000
7.330	* 197.7	160.6	37.13	915.7	4476.	1.000
8.000	* 199.7	131.6	68.12	1049.	5496.	1.000
10.00	* 115.5	72.72	42.75	1364.	8249.	1.000

16.00	*	1.900	12.26	-10.36	1716.	.1180E+05	1.000
24.00	*	2.714	1.143	1.572	1735.	.1219E+05	1.000

---

\*) Starred values were included in estimation of Lambda\_z.

@) Note - the concentration at time zero (DTIME) was added for extrapolation purposes

Dosing_time	.0000
Rsq	.8462
Rsq(adjusted)	.8242
Corr(x:y)	-.9199
Tlag	.0000
Tmax	6.2500
Cmax	211.3271
No._points_Lambda_z	9
Tlast	24.0000
Clast	2.7144
AUClast	1734.6584
Lambda_z	.2967
Lambda_z_lower	6.0000
Lambda_z_upper	24.0000
t1/2_Lambda_z	2.3365
AUCall	1734.6584
AUCINF(observed)	1743.8079
AUC_%Extrap(obs.)	.5247
Vz(observed)/F	.0000
Cl(observed)/F	.0000
AUCINF(predicted)	1738.5099
AUC_%Extrap(pred.)	.2215
Vz(predicted)/F	.0000
Cl(predicted)/F	.0000
AUMClast	12186.3930
AUMCINF(observed)	12436.8235
AUMC_%Extrap(obs.)	2.0136
AUMCINF(predicted)	12291.8109
AUMC_%Extrap(pred.)	.8576
MRTlast	7.0252
MRTINF(observed)	7.1320
MRTINF(predicted)	7.0703

NORMAL ENDING

Input File: Data - [A:\DIPEPK~1.XLS]  
Sex=Male

Start Time: 20:34:14 07-26-2013  
End Time: 20:34:15 07-26-2013

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM (V01.5A)  
Core Version 29Oct97

Listing of input commands

TITLE 1  
ACETONE  
MODEL 200  
NVARIABLES 5  
NPOINTS 100  
XNUMBER 1  
YNUMBER 3  
DTIME 0  
NCONSTANTS 1  
CONSTANTS 0  
METHOD 2 'Linear trapezoidal'  
NCATRANS  
MISSING 'Missing'  
NOBSERVATIONS 16  
DATA 'WINNLIN.DAT'  
BEGIN

ACETONE

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM

Noncompartmental Analysis for Extravascular Administration

Linear Trapezoidal Rule Used to Compute AUC, AUMC

X	Y	Pred.	Res.	AUC	AUMC	WEIGHT
.0000	.0000			.0000	.0000	
.8330E-01	5.399			.2249	.1873E-01	
.1670	8.666			.8135	.9812E-01	
.2500	12.54			1.694	.2883	
.5000	25.40			6.437	2.268	
1.000	50.45			25.40	18.06	
2.000	98.81			100.0	142.1	
4.000	203.3			402.2	1153.	
6.000	288.4			893.9	3697.	
6.250	298.9			967.3	4147.	
6.500	319.4			1045.	4640.	
7.000	* 312.5	434.3	-121.8	1203.	5705.	1.000
7.330	* 315.1	393.3	-78.20	1306.	6447.	1.000
8.000	* 301.9	321.7	-19.86	1513.	8030.	1.000
10.00	* 230.1	176.6	53.50	2045.	.1275E+05	1.000
16.00	* 66.65	29.19	37.46	2935.	.2285E+05	1.000
24.00	* 1.646	2.649	-1.003	3208.	.2727E+05	1.000

\*) Starred values were included in estimation of Lambda\_z.

@) Note - the concentration at time zero (DTIME) was added for extrapolation purposes

Dosing_time	.0000
Rsq	.9471
Rsq(adjusted)	.9339
Corr(x:y)	-.9732
Tlag	.0000
Tmax	6.5000
Cmax	319.3774
No._points_Lambda_z	6
Tlast	24.0000
Clast	1.6460
AUClast	3208.0971
Lambda_z	.3000
Lambda_z_lower	7.0000
Lambda_z_upper	24.0000
t1/2_Lambda_z	2.3107
AUCall	3208.0971
AUCINF(observed)	3213.5841
AUC_%Extrap(obs.)	.1707
Vz(observed)/F	.0000
Cl(observed)/F	.0000
AUCINF(predicted)	3216.9267
AUC_%Extrap(pred.)	.2745
Vz(predicted)/F	.0000
Cl(predicted)/F	.0000
AUMClast	27270.9342
AUMCINF(observed)	27420.9130
AUMC_%Extrap(obs.)	.5470
AUMCINF(predicted)	27512.2765
AUMC_%Extrap(pred.)	.8772
MRTlast	8.5007
MRTINF(observed)	8.5328
MRTINF(predicted)	8.5523

NORMAL ENDING