Final Report

METABOLISM AND PHARMACOKINETICS OF TERTIARY BUTYL ALCOHOL IN MALE RATS: PILOT STUDY

SUBMITTED TO:

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

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SUMMARY

Eight male Fischer 344 rats were exposed to ^{14}C tertiary butyl alcohol in a nose-only inhalation system at a target concentration of 1750 ppm for 6 hours (actual concentration 1724 $\pm$ 85 ppm) . Following exposure, 4 rats were euthanized immediately and the radioactivity in the carcass was determined by digestion and scintillation counting (Group 1, Study A). The remaining four rats were transferred to all-glass metabolism cages, and urine, feces, exhaled CO_2 and exhaled volatiles were collected for seven days (Group 2, Study B). After seven days, the rats were euthanized and the radioactivity remaining in the carcass was determined by digestion and scintillation counting. Radioactivity was excreted primarily in urine (71 % of the recovered radioactivity). A lesser amount was recovered as exhaled volatiles (25% of the recovered radioactivity). Approximately 0.6% was recovered as $^{14}CO_2$, 1.7% in feces, and 0.6% in carcass at 7 days. Based on the recovered radioactivity, the estimated dose in the rats in Group 1 was 532 $\pm$ 38 mg/kg, and in Group 2 was 510 $\pm$ 40 mg/kg. Comparison of the exposure of rats to 1750 ppm [2-^{14}C]TBA/TBA euthanized immediately following exposure (532 $\pm$ 38 mg/kg) with that of rats euthanized following 7 days of collection of excreta and exhaled breath (510 $\pm$ 40 mg/kg) yielded values that were very similar. This suggested that there were no significant losses of radioactivity from the various sample types during processing of radioactivity.

Elimination of radioactivity was rapid, with 86% and 96% at of the radioactivity recovered within 24 h and 48 h, respectively, following the end of exposure. In urine collected at 12 h, three metabolites and ^{14}C TBA were detected by HPLC with radioactivity detection. By 24 hours, ^{14}C TBA was detected in 2/4 urine samples, and by 48 h was not detected. The three metabolites were tentatively identified as 2-methyl-1,2-propanediol, TBA glucuronide, and a glucuronide of α-hydroxyisobutyric acid. Unchanged TBA accounted for a major portion of the exhaled radioactivity.

Quality Assurance Statement

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Quality Assurance Statement

Study Title:	**Metabolism and Pharmacokinetics of Tertiary Butyl Alcohol in Male Rats: Pilot Study**
Sponsor:	**Section 211(b) Research Group, American Petroleum Institute**
Protocol Number:	**RTI-935**
Study Code:	**Rt05-935**

This study was audited by the Regulatory and Quality Assurance – 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	March 15-16, 21-23, 2006	March 24, 2006
Protocol Amendment 1 Audit	June 5, 2006	June 5, 2006
Dosing Inspection	June 1, 2006	June 7, 2006
Excreta Collection Inspection	June 2, 2006	June 7, 2006
Data and Report Audit	March 5-9, 13-14, 16, 19, 21-23 & 26, 2007	March 27, 2007
Report Audit – Updated Report	January 26-27, February 3, 6-7, 15-16, 21, & August 10, 13, 2012	August 13, 2012

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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 synthesis of 2-methyl-1,2-propanediol was not conducted under GLPs.

The method of synthesis of the test chemical tertiary butyl alcohol and [2-^{14}C]-tertiary butyl alcohol, or their location was not available at the time of conduct of the study. Information on the method of synthesis of [2-^{14}C]-tertiary butyl alcohol has been obtained from the vendor. However, the supplier of unlabeled tertiary butyl alcohol has been unable to provide information on the method of synthesis, despite extensive efforts on the part of the Study Director to obtain this information. The supplier was also not able to designate a place where this information may be stored. Since the unlabeled tertiary butyl alcohol was extensively characterized for identity and purity prior to conducting the study, it is unlikely to impact the outcome of the study.

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

Timothy R. Fennell 8/17/2012

Timothy R. Fennell, Ph.D. Date

Study Director

Russell D. White 8/16/2012

Russell White Date

American Petroleum Institute, Sponsor's Representative

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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 *tertiary* butyl alcohol (TBA) in rats. TBA is used as an oxygenate additive to gasoline.

Toxicity studies have been conducted in rats and mice (Mahler 1997). Alpha 2u-globulin nephropathy has been reported in rats exposed to TBA by inhalation (Borghoff et al., 2001). TBA has been demonstrated to bind to alpha 2u-globulin *in vivo* (Williams and Borghoff 2001).

Limited studies have been conducted on the metabolism and disposition of TBA. Baker et al. (1982) reported the metabolism of TBA to produce CO_2 and acetone *in vivo* in rats. The pharmacokinetics of TBA were evaluated in mice (Faulkner and Hussain 1989) and in rats (Poet et al., 1997).

2.0 OBJECTIVES AND PROTOCOL

The objectives of this pilot study were to:

Study A

1) conduct a nose-only exposure of rats to ^{14}C TBA/TBA (n=4; 1 backup unexposed).

2) analyze the amount of ^{14}C in whole body digests from rats exposed to ^{14}C TBA/TBA.

Study B

3) conduct a nose-only exposure of rats to ^{14}C TBA/TBA (n=4; 2 backup unexposed).

4) collect excreta from rats exposed to ^{14}C TBA/TBA.

5) analyze ^{14}C in whole body digests, urine, feces, exhaled CO_2, and exhaled volatiles from rats exposed to ^{14}C TBA/TBA.

6) develop an HPLC method for analysis of urinary metabolites.

7) analyze TBA in extracts from exhaled volatiles.

Copies of the approved protocol and protocol amendments for these studies are included in Appendix A.

3.0 MATERIALS AND METHODS

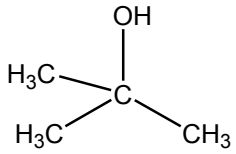
3.1 Test Substance

NAME: ***tertiary* Butyl Alcohol** (TBA; 2-methyl-2-propanol, CAS No. 75-65-0)

MOLECULAR FORMULA: $C_4H_{10}O$

MOLECULAR WEIGHT: 74.12

STRUCTURE:



3.1.1 Nonradiolabeled *tertiary* Butyl Alcohol

Source: Sigma-Aldrich, Milwaukee, WI

Product No: 36053-8

Lot No.: 01060AD

Purity: The chemical purity of ***tertiary* Butyl Alcohol** was characterized by Sigma-Aldrich at 99.6%.

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

3.1.2 [2-^{14}C]-labeled *tertiary* Butyl Alcohol

Position of radiolabel: **[2-^{14}C]-**

Source: Sigma Radiochemicals, prepared as a custom synthesis

Specific Activity: 7.3 mCi/mmol

Product No.: A8538-14C

Lot No.: 095K9406

Identity: The identity of [2-^{14}C]-*tertiary* Butyl Alcohol was confirmed by chromatographic comparison with unlabeled TBA using HPLC coupled with radioactivity detection and refractive index detection.

Purity: The radiochemical purity of [2-^{14}C]-*tertiary* Butyl Alcohol was determined chromatographically to be 98.2 %, using the HPLC system described in Section 3.8.

The labeled test material was characterized at RTI by HPLC coupled with radioactivity detection for purity and stability, and for identify by ^{1}H NMR spectroscopy and by HPLC coupled with radioactivity detection and refractive index detection. Details of the analysis are included in Appendix C.

3.2 Reference Substances

2-Hydroxyisobutyric acid (HBA) was obtained from Alfa Aesar. The purity specified by the Vendor's certificate of analysis was 98.9%. This material was assigned a chemical receipt number of BOC-B-0439. Identity of the material was verified by ^{1}H and ^{13}C NMR spectroscopy.

2-Methylpropane-1,2-diol was prepared at RTI using the method of Bernauer et al. (1998).

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 during the study for analysis of tertiary butyl alcohol, 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. Errors in sampling, shipping or receiving the first sample resulted in no data being obtained for the prestudy sample. In two subsequent samples tertiary amyl methyl ether, ethyl tertiary butyl ether, diisopropyl ether, and methyl tertiary butyl ether were below the limits of quantitation. Tertiary butyl alcohol was measured at 2.2 µg/L (with 0.5 µg/L as the reporting limit) in a sample collected during the in-life portion of the study. In a subsequent sample collected after the study, tertiary butyl alcohol was below the reporting limit of 1.0 µg/L. The California Office of Environmental Health Hazard Assessment (OEHHA) (CALEPA 1999) developed an interim drinking water level of 12 µg/L based on the incidence of kidney tumors reported in male rats. Although a single measurement of tertiary butyl alcohol was elevated, it is unlikely to have impacted the study, since the amount of TBA that could be consumed, assuming drinking water consumption of 0.028 L /day, would be equivalent to a total potential dose arising from drinking water of 0.4 µg TBA/rat over the course of the 7-day study. (Total consumption was calculated assuming a drinking water consumption rate of 0.028 L/ day (USEPA, 1988)). This would be insignificant compared to the total recovered dose, which ranged from 89 – 111 mg equiv. of TBA/rat.

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).

Following dosing, the animals were housed in individual glass metabolism chambers (for collection of blood, urine, feces, exhaled CO_2, and exhaled volatiles).

Quarantine: Rats were quarantined for a minimum of one week before use on a study. 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=4), Study B (n=4), Study C (n=2), and Study D (n=1).

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. The actual ranges observed during quarantine were 71.07–72.8 °F and 52.2–56.78 % relative humidity, and during the study were 71.1–75.09 °F, and 35.88–42.83 % relative humidity.

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 [2-^{14}C]TBA/TBA (Studies A and B), ^{14}C TBA was added to an appropriate amount of unlabeled TBA for generation of the exposure atmosphere. The amount of labeled and unlabeled TBA 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-^{14}C]TBA/TBA, [2-^{14}C]TBA was weighed into a tared flask with a Teflon faced screw cap. The weight of the labeled chemical added was recorded. Unlabeled TBA was added, and the weight added was recorded. The nominal specific activity of the TBA mixture will then be 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 TBA 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 TBA was then be calculated from the data obtained.

The exposure atmosphere concentration was monitored using a calibrated Miran IR detector. The concentration of TBA was monitored at the inlet to the tower, and at the outlet. The stability of TBA under the conditions of administration was monitored by sampling the inlet of the exposure tower at the end of the exposure. Samples of the exposure atmosphere were collected using a 10 ml 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 [2-^{14}C]TBA/TBA was conducted with a total of 8 rats for Study A (4 rats) and Study B (4 rats) on June 1st, 2006. The radiolabeled TBA was drawn into a clean syringe. The generation system consisted of a syringe containing the chemical with a syringe pump to deliver the chemical to the air supply of the exposure chamber. The TBA exposure atmosphere was generated by pumping liquid TBA 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 1750 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 TBA 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. 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 TBA 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 TBA 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, and that the port to port variability was within accepted limits. A report describing the preexposure characterization of the inhalation system is attached to this report as Appendix E, and a report characterizing the exposure of rats is attached as Appendix F.

3.6 Collection of Samples

3.6.1 Excreta

In Study B, 4 rats (2-05, 2-06, 2-07, and 2-08) were placed in all-glass metabolism cages for the separate collection of urine, feces, exhaled volatiles, and exhaled CO_2. Urine was collected over dry ice at 0–12, 12–24, 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). Expired $^{14}CO_2$ was collected in 1.0 N KOH at 0–1, 1–3, 3–5, 5–7, 7–12, 12–24, 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.

3.6.2 Carcass

For Study A, the amount of ^{14}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_2 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 ^{14}C (the sample is designated as **Exhaled VOC** for Group 1 animals), 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 was 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 ^{14}C contained in the rinse was determined by LSS. The Tedlar gas bag was rinsed with water, and the amount of ^{14}C contained in the rinse was determined by LSS.

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

3.7 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.

3.7.1 Excreta

Duplicate aliquots of urine (approximately 0.5 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_2O_2) prior to addition of scintillation cocktail. Control samples of urine and

feces were collected from unexposed animals and analyzed as described above to determine background counts.

3.7.2 Carcass

Carcasses were analyzed for total radioactivity following solubilization in 2N ethanolic NaOH. Duplicate samples of the solubilized carcass were analyzed. Solubilized samples were bleached (by adding approximately 125 µl of 70% perchloric acid, and then approximately 0.3 ml of 30% H_2O_2) prior to addition of scintillation cocktail and analysis by LSS. Control samples of carcass were collected from unexposed animals and analyzed as described above to determine background counts.

3.7.3 Exhaled Breath Traps

Aliquots of 1.0 N KOH from the exhaled breath trap for CO_2 were analyzed by LSS after addition of scintillation cocktail.

3.7.4 Exhaled Volatiles Traps

Each charcoal trap was extracted by elution with dimethylformamide (DMF) to determine absorbed radioactivity. To ensure that the samples with large amounts of radioactivity were thoroughly extracted, two extractions were performed on the VOC Trap 1 samples collected between 1 and 24 h. For a single extraction, charcoal was transferred to a preweighed centrifuge tube and weighed. DMF (4.0 ml) was added, and the sample weighed. After vortexing for approximately 30 seconds, the samples were centrifuged, and the supernatant was removed. Radioactivity in the DMF wash was measured by LSS of duplicate aliquots. For samples with two extractions, charcoal was transferred to a preweighed centrifuge tube and weighed. DMF (4.0 ml) was added, and the sample weighed. After vortexing for approximately 30 seconds, the samples were centrifuged, and the supernatant was removed, and transferred to a preweighed centrifuge tube labeled **Exhaled VOC Trap 1 Sample**. The sample was weighed, and duplicate aliquots were removed for analysis by LSS. Additional DMF (4.0 ml) was added to the tube containing the charcoal and residual DMF, and the extraction was repeated. The DMF was removed following centrifugation and radioactivity was determined in duplicate aliquots by LSS. For samples of VOC Trap 1 collected at 1 h, a third extraction was performed, and the recovery of radioactivity was evaluated prior to analysis of the remaining samples.

The sample designations used in the data tables correspond to the sample designations used in the Debra System, and are as follows:

Exhaled VOC Trap 1	**First extract of the first VOC trap in series**
VOC Trap 1 Extract 2	**Second extract of the first VOC trap in series**
Exhaled VOC Trap 2	**Extract of the second VOC trap in series**

3.8 HPLC Analysis of Urine Samples and Timed Fractions Collected from HPLC for Radioactivity

Urine samples containing greater than 5% of the recovered radioactivity were selected for analysis of metabolites by HPLC. Aliquots of the selected urine samples were transferred to glass centrifuge tubes, and the samples were centrifuged at 3000 rpm for 5 minutes to remove particulate matter. The supernatant was transferred to a 1.5 ml HPLC vial. Aliquots (30 µl) of urine were analyzed in duplicate directly by LSS to determine amount of radioactivity present in the 30 µl aliquot.

A 30-µl aliquot of urine was analyzed by HPLC/LSS as described below. The HPLC instrumentation consists of two Waters Model 515 Pumps, a Waters Model 717 plus Autosampler, an IN/US Systems β-RAM Model 3 radioactivity detector, and a Waters Model 410 refractive index (RI) detector.

HPLC was conducted using a Waters Atlantis dC18 column, 4.6 mm i.d. x 25 cm, 5 µ particle size, with a precolumn and an in-line solvent filter. The isocratic mobile phase consisted of 99 % water and 1% acetonitrile. The mobile phase flow rate through the column was 1.0 ml/min, and 100% of the flow passing through the 500-µl lithium glass solid-phase cell of the radioactivity detector before passing through the refractive index detector. HPLC column effluent was collected in scintillation vials containing scintillation cocktail, and quantitation of radioactivity was conducted using a Packard 1900CA liquid scintillation counter.

A standard solution including unlabeled TBA with metabolites of α-hydroxyisobutyric acid and 2-methyl-1-2-propanediol was used to conduct the performance verification for RI detector at the beginning and end of analysis of all urine samples.

Incubation of urine samples with β-glucuronidase was conducted by adding 15 mg of β-glucuronidase (Sigma-Aldrich) to each incubation tube, and adding 50 µl of 0.2 M sodium acetate buffer (pH 4.43) and 200 µl of urine. For one incubation, water was added instead of sodium acetate buffer, and for one tube the β-glucuronidase was boiled prior to addition of the urine. After addition of all components, the samples were incubated for 12 hours at 37°C. This was followed by analysis by HPLC with detection of radioactivity.

3.9 Determination of TBA in Exhaled Breath Trap Extracts.

Aliquots of the DMF extracts of Exhaled Breath Traps were analyzed by GC with FID detection,

Standard solutions were prepared at concentrations of 0.01, 0.1, 1 and 10 µg/µl. Analysis was conducted on an Agilent 6890 GC. Conditions for analysis were as follows:

Injection port	split/splitless
Temperature	200 ºC

Split ratio	1.0
Carrier gas	Helium
Flow rate	2.0 ml/min
Injection volume	1 µl
Detector	FID, 250 ºC
GC oven program	
Initial temperature	60 ºC
Initial time	0.5 min
Temperature rate	5 ºC/min
Final temperature	70 ºC
Final time	2 min
Ramp A	100 ºC/min
Final temperature A	200 ºC
Final time A	1.2 min
Column	DB 624 30m x 0.32 mm i.d. 1.8 um film thickness (Agilent technologies, Wilmington, DE)

The peak area of TBA was integrated. A linear regression equation (y= bx+a) was obtained using Prism software (version 4.03). The concentration TBA was back calculated by this equation.

Where: y= peak area of TBA
x = concentration of TBA
b = slope of the standard curve
a = y-intercept of the standard curve

3.10 Data Collection and Reporting

Study data was collected and reported in the Debra™ system version 5.5.10.72. 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 99.55 ± 0.18 %. Following completion of the study, test chemical purity was determined to be 99.71 ± 0.01 %. The radiochemical purity of 2-^{14}C TBA specified by the vendor was 98.994 % by HPLC, and was verified by HPLC at RTI prior to the inhalation exposure of rats as 98.16 ± 0.27% (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. The purity of the radiolabeled material was determined to be 94.6 ± 2.4 %. Analysis of the reference substances 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid is reported in Appendix D.

4.2 Test Chemical Formulation

The test chemical was prepared by mixing unlabeled TBA with 2-^{14}C labeled TBA, and used without further dilution. The specific activity of the formulation is provided in Table 2, and was measured as 18.57 µCi/mmol.

4.3 Inhalation exposure

The inhalation exposure system performance was evaluated and reported in Appendix E. Concentrations of TBA were measured at the inlet and the exhaust, and were measured in open ports, which represent the breathing zone of the rat. For the operation of the inhalation exposure system, which has a relatively short path length, the inlet port was considered to be a surrogate for the breathing zone of the rat. The concentration at the inlet and exhaust were found to be in good agreement with and within the acceptable range of variation specified in the study protocol of ≤ 10% of the nominal concentration of 1750 ppm (Appendix E, Table 5, 1681 ± 73 ppm at the inlet, and 1722 ± 36 ppm at the exhaust). Following equilibration, there was also good agreement at each timepoint between inlet and exhaust, implying no loss in the system. Port to port variability was verified to be within the acceptable range of ≤ 10% difference in concentration measurements taken at multiple locations (Appendix E, Table 3). To make the required measurements of port to port variability, to decrease the time required for the Miran to reach equilibrium, the air sampled at each port was diluted with house air, resulting in a lower concentration measured at the port. A total flow of 200 mL/min consisting of 150 mL/min from the port diluted with house air at 50 mL/min was delivered to the Miran detector. For measurement of within port variation, a single port, designated the “Home Port” (position 9), was sampled repeatedly. Other ports, numbered 1 through 8 were sampled once, with a sampling sequence of Home, 1, 2, 3, Home, 4, 5, 6, Home, 7,8, and Home, Repeated measurements at the Home Port produced a within port coefficient of variation of 1.16%, a between port coefficient of variation of 3.13%, and total port coefficient of variation of 3.34%. The measured port concentrations were less than the target concentration of 1750 ppm

because house air was used to dilute the sample air to reduce the time necessary to reach equilibrium. However, after correction for the dilution with house air, the port concentrations were 1766 ± 202 ppm, and were 99.4 ± 7.2 % of the inlet port concentration.

The data for a six-hour exposure of rats to a target concentration of 1750 ppm TBA is shown in Table 2. 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 Summary Report: ^{14}C-TBA/TBA Nose-Only Inhalation Exposure at RTI International. The actual exposure concentration was 1724 ± 85 ppm (Table 2). The design of the sampling at the inhalation tower inlet using the inlet Miran infrared spectrophotomer may have led to artificially low concentration readings for the first two timepoints (Appendix F, Table 4, 1447 ppm at 32 minutes, and 1689 ppm at 62 minutes). This was caused by the large volume of the Miran detector flow cell relative to the sampling flow rate to the inlet Miran of 200 ml/min. Excluding the first two timepoints from the calculation results in an estimated concentration of 1752 ± 7 ppm.

4.4 Radioactivity Retained Following Exposure

Four rats were euthanized immediately following inhalation exposure to [2-^{14}C]TBA/TBA (Study A, Group 1, rats 1-01, 1-02, 1-03, and 1-04). 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 3. The amount of radioactivity retained in Group 1 ranged from approximately 22 to 28 µCi. The estimated dose was 532 ± 38 mg/kg. This estimate of dose included radioactivity recovered in carcass, from washing the bag used to euthanize the rat with CO_2 (Transfer Bag Rinse), 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 (99%) of the radioactivity was recovered in the carcass (Table 4).

4.5 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-^{14}C]TBA/TBA (Study B, Group 2, Rats 2-05, 2-06, 2-07, and 2-08). 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. On the morning of the last day in metabolism cages, one rat was found dead, as a result of asphyxiation because of clogging of one of the sinters in the exhaled CO_2 traps, which had restricted air flow (Rat 2-08). Samples from this rat were collected, and analyzed for the recovery of radioactivity. The total recovery of

radioactivity from all of the samples collected from rats in group 2 is presented in Table 3, and ranged from approximately 22 to 28 µCi. The estimated dose was 510 ± 40 mg/kg (Table 3).

The amount of radioactivity in urine, feces, exhaled VOCs, and CO_2 expressed as a percentage of the total radioactivity recovered is presented in Table 5. Urinary excretion was the primary route of elimination, with 71% of the recovered radioactivity excreted in the urine, and 2% excreted in feces. Exhaled volatiles (VOC traps) accounted for the second largest fraction of the excreted radioactivity, with the majority of the VOC radioactivity retained on the first charcoal trap in a series of two. A second extract of the first trap recovered additional radioactivity, with approximately 25 % (the first and second extract combined) of the recovered radioactivity in the first trap. The recovery of the radioactivity from the exhaled VOC traps was investigated by extracting the first VOC traps from the 0-1 hr timepoint for rats 2-01, 2-02, 2-03, and 2-04 three times with successive aliquots of DMF. The weight of DMF added was recorded, and the weight of DMF extract removed was also recorded. The amount of radioactivity recovered in each extract was calculated based on the amount of radioactivity removed, and on the amount of radioactivity in the total volume of DMF. Compared with three successive extracts, it was determined that a single extract recovered approximately 82.5 ± 5.9 % of the radioactivity, and two extracts recovered 99.8 ± 1.1 % of the radioactivity. For the samples obtained at times up to 24 hours, two extracts were applied to each of the VOC trap 1 samples. For those samples of VOC trap 1 obtained after 24 hours, a single extraction with DMF was conducted. Exhaled $^{14}CO_2$ accounted for approximately 0.5 % of the recovered radioactivity.

Radioactivity in urine was excreted primarily in the first 48 hours (Table 6), with 37%, 22%, 9.3% and 1.9% excreted at 12, 24, 48, and 72 hours, respectively. Less than 1% of the radioactivity was recovered in urine at 96, 120, 144, and 168 hours. In feces (Table 7), approximately 0.9 % of the recovered activity was found in the 0-24 h samples, which contained the largest amount of fecal radioactivity.

Radioactivity in the first CO_2 trap was excreted primarily in the first 48 hours (Table 8). Many of the samples collected after 48 hours contained levels of radioactivity below 3 times background. Little radioactivity was recovered in the second CO_2 trap (Table 9). 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 10). For each of the VOC Trap 1 samples collected between 0 and 24 hours, a second extract was performed. Data from these analyses are presented in Table 11. For the cumulative total, approximately 14% was recovered in the first extract (0-168h), and 11 % in the second extract (0-24h). Very little of the dose was trapped in the VOC Trap 2 samples, with one exception. For rat 2-05, the 48-72 h VOC trap 2 sample contained 0.113 % of the radioactivity, whereas the 48-72 h VOC trap 1 sample contained only 0.000%. Reanalysis of these samples suggested that the traps were switched in order during collection.

The cumulative excretion of radioactivity (Table 13) shows that approximately 95% of the activity was recovered by 48 hours following exposure.

4.6 TBA in Exhaled VOC Traps

To evaluate the pulmonary excretion of TBA, DMF extracts of the charcoal exhaled VOC traps were analyzed by GC-FID for quantitation of TBA. This analysis was confined to the first VOC trap, since the amount of radioactivity recovered from VOC Trap 2 was extremely low. The concentration of TBA was highest in the samples collected at 1, 3, 5, 7, and 12 hours (Table 14). The concentration of TBA in the extracts fell rapidly by 24 hours, and was not detectable in all but one sample by 96 hours (Rat 2-08, Exhaled VOC Trap 1, 168 hr, 0.013 mg/g).

Comparison of the concentration of radioactivity (mg equiv. TBA/g DMF) vs. the concentration of TBA measured in each DMF extract provided an estimate of the percentage of TBA in the exhaled VOC trap. The percentage as TBA was high initially at 84 % in the first hour following exposure, dropping to a low of approximately 28 % at 48 hours.

4.7 Excretion of TBA and Metabolites in Urine

HPLC analysis of urine was limited to samples containing greater than 5% of the total recovered radioactivity. HPLC analysis of ^{14}C TBA metabolites in urine revealed that little of the radioactivity was due to TBA itself. Typical chromatograms of urine are shown in Figure 1. The retention times of TBA and standards for 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid were established by chromatography with refractive index detection (Figure 2). The percentage of the total radioactivity that was present as unchanged ^{14}C TBA in urine was extremely low at all time points measured (Table 16), with the highest levels observed at 12 h following exposure, with ^{14}C TBA accounting for 4.4% of the urinary radioactivity. At 24 hours, ^{14}C TBA was detected in urine from two of four rats, and by 48 hours none was detected in any of the urine samples of exposed animals. Three additional radiolabeled peaks (metabolites) were observed. One of these peaks (Peak 3) co-eluted with 2-methyl-1,2-propanediol. Peak 2 was removed after incubation with β-glucuronidase, producing TBA and a broad peak at a retention time of approximately 15 minutes (Figure 3). Incubation with acid caused movement of peaks 1 and 2 with the appearance of a new peak that was consistent with the retention time of α-methyl-isobutyric acid.

5.0 CONCLUSION

The objectives of the study were successfully met. A nose-only system for the inhalation exposure of rats to 14C TBA was implemented. Male rats were exposed to ^{14}C TBA/TBA, and the recovery of radioactivity was determined immediately following exposure and over a 7-day period with collection of urine, feces, exhaled volatiles and ^{14}C CO_2. Comparison of the exposure of rats to 1750 ppm [2-^{14}C]TBA/TBA euthanized immediately following exposure (532 $\pm$ 38 mg/kg) with that of rats euthanized

following 7 days of collection of excreta and exhaled breath (510 ± 40 mg/kg) yielded values that were very similar. This suggested that there were no significant losses of radioactivity from the various sample types during processing of radioactivity.

Urinary excretion was the major route of excretion, followed by exhalation as volatile components. Radioactivity was rapidly excreted in urine (71% of the recovered radioactivity, Table 6) and in exhaled breath as volatile components (25% of the recovered radioactivity, Tables 10 and 11). The cumulative excretion of radioactivity was 86% and 96% within 24 and 48 hours, respectively, of the end of the exposure. At seven days, only 0.6% was recovered in the carcass.

The determination of unchanged TBA and metabolites in urine and exhaled breath traps indicated that TBA is extensively metabolized. Unchanged TBA was detected as a substantial component in exhaled breath (ranging from mean values of 95 – 49% in the first 24 h following exposure), and as a minor component in urine at 12 (mean value of 4.4 %) and 24 hours (0.5 %, mean of two animals). In urine collected at 12 h, three metabolites and ^{14}C TBA were detected by HPLC with radioactivity detection. By 24 hours, ^{14}C TBA was detected in 2/4 urine samples, and by 48 h was not detected. The three metabolites were tentatively identified as 2-methyl-1,2-propanediol, TBA glucuronide, and a glucuronide of α-hydroxyisobutyric acid.

Analysis of the drinking water for the presence of oxygenates during the study revealed the presence of a single measurement of tertiary butyl alcohol (2.2 µg/L) that was above the reporting limit (1.0 µg/L). The detected concentration is considerably below the interim drinking water standard level of 12 µg/L developed by the California Office of Environmental Health Hazard Assessment (OEHHA) (Office of Environmental Health Hazard Assessment 1999). Estimation of the consumption of water from rats administered TBA (NTP 1995) in the drinking water (approximately 22 ml/day) suggests that the consumption of unlabeled TBA was at most 0.3388 µg (2.2 µg/L x 0.022 L/day x 7 days).

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

Upon acceptance of the audited draft report by the Sponsor, a final report will be issued.

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 and samples of nonradiolabeled and radiolabeled TBA will be stored at RTI in a secured area by the RTI project number for up to two 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

RTI International

Study Director:	Timothy R. Fennell, Ph.D.
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CIIT Centers for Health Research

Principal Investigator::	R Arden James.
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Table 1. Male Fischer 344 Rats Used on the Study

Group	Subject	Species	Strain	Subj Wt[a]
Group 1 Study A	1-01	Rat	Fischer 344	192.73 g
Group 1 Study A	1-02	Rat	Fischer 344	201.00 g
Group 1 Study A	1-03	Rat	Fischer 344	196.32 g
Group 1 Study A	1-04	Rat	Fischer 344	187.85 g
Group 2 Study B	2-05	Rat	Fischer 344	193.42 g
Group 2 Study B	2-06	Rat	Fischer 344	203.17 g
Group 2 Study B	2-07	Rat	Fischer 344	203.48 g
Group 2 Study B	2-08	Rat	Fischer 344	202.00 g
Group 3 Study B BKG[b]	3-09	Rat	Fischer 344	187.70 g
Group 3 Study B BKG	3-10	Rat	Fischer 344	206.45 g
Group 4 Study A BKG[b]	4-11	Rat	Fischer 344	195.31 g

[a]Body weight on the day of exposure

[b]Animals used in Group 3 and Group 4 were not exposed to [2-^{14}C]TBA/TBA, and provided samples for determination of background only.

Table 2. Exposure Conditions and Concentration of [2-^{14}C]TBA/TBA.

Analysis results

Treatment name: TBA 1750 Nose-Only Inhalation
Dpm/g of dose prep: 556338044.1 dpm/g
Molecular weight: 74.12
Specific Activity: 0.2506 µCi/mg

Exposure Summary

	Target (ppm)	1750
	Number of Open Ports	12
ACC[1] - Inlet (ppm)	**Mean**	**1724**
	Std Dev	85
	No. of Data Points[2]	13
ACC - Exhaust (ppm)	**Mean**	**1581**
	Std Dev	40
	No. of Data Points[2]	13
Exposure Temperature (°F)	**Mean**	**72.8**
	Std Dev	0.5
	No. of Data Points[2]	13
Exposure Relative Humidity (%)	**Mean**	**47.6**
	Std Dev	0.5
	No. of Data Points[2]	13
Exposure Static Pressure (in H_20)	**Mean**	**-0.05**
	Std Dev	0.00
	No. of Data Points[2]	13

[1]ACC Analytical Chamber Concentration

[2]Data points were collected during the 6-h inhalation exposure.

Table 3. Total Recovery of Radioactivity following Exposure to 1750 ppm [2-^{14}C]TBA/TBA

Rat	Total DPM Recovered	Total μCi	Dose (mg)	Bodyweight (g)	Dose (mg/kg)	Group Mean Dose (mg/kg)	SD
1-01	59,110,333.86	26.6263	106.25	192.73	551.3	531.68	38.15
1-02	60,334,744.16	27.1778	108.45	201	539.6		
1-03	61,168,290.20	27.5533	109.95	196.32	560.0		
1-04	49,730,780.50	22.4013	89.39	187.85	475.9		
2-05	49,473,192.23	22.2852	88.93	193.42	459.8	509.56	40.30
2-06	56,087,309.04	25.2646	100.82	203.17	496.2		
2-07	60,071,898.65	27.0594	107.98	203.48	530.7		
2-08	61,989,781.11	27.9233	111.42	202	551.6		

Table 4. Radioactivity in Group 1 Study A Rats Following Exposure to 1750 ppm [2-^{14}C]TBA/TBA, Expressed as a Percentage of the Total Radioactivity Recovered

	Group 1 Study A	Group 1 Study A	Group 1 Study A	Group 1 Study A	Group 1 Study A	Group 1 Study A
Sample	**1-01**	**1-02**	**1-03**	**1-04**	**Mean**	**SD**
Feces	0.216	0.041	0.244	1.670	0.543	0.757
Transfer Bag Rinse	0.032	* 0.005	0.561	0.033	0.158	0.269
Carcass Digest	99.469	99.623	98.765	96.918	98.694	1.241
Exhaled VOC[1]	0.002	0.001	0.004	0.016	0.006	0.007
Nose Only Tube Rinse	0.281	0.329	0.426	1.362	0.600	0.512
Total	100.00	100.00	100.00	100.00	100.000	0.000

* Value is below 3 x background for the sample.

[1] Exhaled VOC represents the charcoal trap attached to the Tedlar bag used for euthanizing the rats.

Table 5. Radioactivity in Excreta and Carcass following Exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
	Male	Male	Male	Male		
Sample	**2-05**	**2-06**	**2-07**	**2-08**	**Mean**	**SD**
Urine	74.113	70.499	69.858	69.479	70.987	2.126
Feces	1.293	1.850	1.264	2.550	1.739	0.604
CO2 Trap 1	0.571	0.540	0.570	0.537	0.555	0.019
CO2 Trap 2	0.020	0.032	0.043	0.051	0.037	0.013
Exhaled VOC Trap 1[1]	12.402	14.420	15.042	14.894	14.190	1.221
VOC Trap 1 Extract 2[2]	10.051	11.181	12.087	11.429	11.187	0.848
Exhaled VOC Trap 2	0.114	0.005	0.006	0.002	0.032	0.055
Nose Only Tube Rinse	0.698	0.247	0.237	0.184	0.342	0.239
Transfer Bag Rinse	0.018	0.032	*0.009	0.014	0.018	0.010
Carcass Digest	0.579	0.784	0.622	0.510	0.624	0.116
Cage Rinse	0.139	0.409	0.263	0.350	0.290	0.118
Total	100.00	100.00	100.00	100.00	100.000	0.000

* Value is below 3 x background for the sample.
[1]The first extract of the first VOC trap in series
[2]The second extract of the first VOC trap in series

Table 6. Radioactivity in Urine following Exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
Urine	0 - 12 h	46.743	34.991	29.371	36.637	36.936	7.240
Urine	12 h - 24 h	18.627	19.231	26.677	22.963	21.875	3.732
Urine	24 h - 48 h	6.700	12.137	10.677	7.662	9.294	2.542
Urine	48 h - 72 h	1.492	2.590	2.077	1.407	1.891	0.553
Urine	72 h - 96 h	0.284	0.548	0.580	0.479	0.473	0.133
Urine	96 h - 120 h	0.128	0.294	0.247	0.205	0.218	0.070
Urine	120 h - 144 h	0.085	0.387	0.159	0.066	0.174	0.147
Urine	144 h - 168 h	0.054	0.321	0.069	0.061	0.126	0.130
Total		74.113	70.499	69.858	69.479	70.987	2.126

* Value is below 3 x background for the sample.

Table 7. Radioactivity in Feces following Exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
Feces	**0 h**	0.126	0.266	0.091	1.034	0.379	0.443
Feces	**0 h - 24 h**	0.929	0.952	0.845	0.800	0.881	0.071
Feces	**24 h - 48 h**	0.134	0.206	0.221	0.233	0.199	0.044
Feces	**48 h - 72 h**	0.065	0.056	0.045	0.089	0.064	0.019
Feces	**72 h - 96 h**	*0.017	0.063	0.035	0.082	0.049	0.029
Feces	**96 h - 120 h**	*0.005	*0.007	0.013	*0.008	0.008	0.003
Feces	**120 h - 144 h**	*0.009	0.013	*0.008	0.262	0.073	0.126
Feces	**144 h - 168 h**	*0.008	0.286	*0.007	0.042	0.086	0.135
Total		1.293	1.850	1.264	2.550	1.739	0.604

* Value is below 3 x background for the sample.

Table 8. Radioactivity in CO2 Trap 1 following exposure to 1750 ppm ^{14}C TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
CO2 Trap 1	0 - 1 h	0.051	0.044	0.035	0.034	0.041	0.008
CO2 Trap 1	1 h - 3 h	0.061	0.057	0.053	0.050	0.055	0.005
CO2 Trap 1	3 h - 5 h	0.058	0.051	0.043	0.043	0.049	0.007
CO2 Trap 1	5 h - 7 h	0.042	0.045	0.047	0.042	0.044	0.003
CO2 Trap 1	7 h - 12 h	0.083	0.086	0.098	0.090	0.089	0.007
CO2 Trap 1	12 h - 24 h	0.163	0.141	0.205	0.192	0.175	0.029
CO2 Trap 1	24 h - 48 h	0.062	0.069	0.052	0.057	0.060	0.007
CO2 Trap 1	48 h - 72 h	*0.022	*0.023	*0.017	*0.006	0.017	0.008
CO2 Trap 1	72 h - 96 h	*0.013	*0.010	*0.008	*0.011	0.011	0.002
CO2 Trap 1	96 h - 120 h	*0.008	*0.009	*0.005	*0.006	0.007	0.002
CO2 Trap 1	120 h - 144 h	*0.003	*0.004	*0.002	*0.004	0.003	0.001
CO2 Trap 1	144 h - 168 h	*0.005	*0.002	*0.003	*0.002	0.003	0.002
Total		0.571	0.540	0.570	0.537	0.555	0.019

* Value is below 3 x background for the sample.

Table 9. Radioactivity in CO2 Trap 2 following exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
CO2 Trap 2	0 - 1 h	*0.001	*0.000	*0.003	*0.000	0.001	0.001
CO2 Trap 2	1 h - 3 h	*0.001	*0.002	*0.001	*0.000	0.001	0.001
CO2 Trap 2	3 h - 5 h	*0.000	*0.001	*0.003	*0.003	0.002	0.001
CO2 Trap 2	5 h - 7 h	*0.000	*0.003	*0.000	*0.000	0.001	0.001
CO2 Trap 2	7 h - 12 h	*0.000	*0.002	*0.000	*0.000	0.001	0.001
CO2 Trap 2	12 h - 24 h	*0.002	*0.002	*0.002	*0.003	0.002	0.000
CO2 Trap 2	24 h - 48 h	*0.009	*0.011	*0.019	*0.016	0.013	0.005
CO2 Trap 2	48 h - 72 h	*0.005	*0.006	*0.006	*0.020	0.009	0.007
CO2 Trap 2	72 h - 96 h	*0.000	*0.003	*0.003	*0.004	0.002	0.002
CO2 Trap 2	96 h - 120 h	*0.001	*0.000	*0.002	*0.003	0.001	0.001
CO2 Trap 2	120 h - 144 h	*0.002	*0.001	*0.003	*0.002	0.002	0.001
CO2 Trap 2	144 h - 168 h	*0.000	*0.001	*0.001	*0.001	0.001	0.000
Total		0.020	0.032	0.043	0.051	0.037	0.013

* Value is below 3 x background for the sample.

Table 10. Radioactivity in Exhaled VOC Trap 1 following exposure to 1750 ppm ^{14}C TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
Exhaled VOC Trap 1	**0 - 1 h**	2.159	2.688	2.415	3.147	2.602	0.423
Exhaled VOC Trap 1	**1 h - 3 h**	2.443	2.446	2.229	2.561	2.420	0.138
Exhaled VOC Trap 1	**3 h - 5 h**	2.719	2.479	2.287	2.601	2.521	0.185
Exhaled VOC Trap 1	**5 h - 7 h**	2.209	2.061	2.415	2.078	2.191	0.164
Exhaled VOC Trap 1	**7 h - 12 h**	2.129	3.011	3.730	3.072	2.986	0.657
Exhaled VOC Trap 1	**12 h - 24 h**	0.449	0.690	1.266	0.877	0.820	0.345
Exhaled VOC Trap 1	**24 h - 48 h**	0.184	0.757	0.560	0.222	0.431	0.275
Exhaled VOC Trap 1	**48 h - 72 h**	0.000	0.154	0.039	0.146	0.085	0.077
Exhaled VOC Trap 1	**72 h - 96 h**	0.036	0.046	0.030	0.055	0.042	0.011
Exhaled VOC Trap 1	**96 h - 120 h**	0.038	0.035	0.027	0.037	0.034	0.005
Exhaled VOC Trap 1	**120 h - 144 h**	0.019	0.027	0.025	0.029	0.025	0.005
Exhaled VOC Trap 1	**144 h - 168 h**	0.018	0.027	0.018	0.067	0.033	0.024
Total		12.402	14.420	15.042	14.894	14.190	1.221

Table 11. Radioactivity in VOC Trap 1 Extract 2 following Exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
VOC Trap 1 Extract 2	0 - 1 h	1.922	2.348	2.414	2.384	2.267	0.232
VOC Trap 1 Extract 2	1 h - 3 h	2.059	2.135	2.058	2.478	2.183	0.200
VOC Trap 1 Extract 2	3 h - 5 h	2.320	2.179	2.165	2.326	2.247	0.088
VOC Trap 1 Extract 2	5 h - 7 h	1.459	1.469	1.737	1.508	1.543	0.131
VOC Trap 1 Extract 2	7 h - 12 h	1.919	2.536	2.925	2.124	2.376	0.447
VOC Trap 1 Extract 2	12 h - 24 h	0.373	0.514	0.788	0.608	0.571	0.174
Total		10.051	11.181	12.087	11.429	11.187	0.848

* Value is below 3 x background for the sample.

Table 12. Radioactivity in Exhaled VOC Trap 2 following Exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

		Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
		Male	Male	Male	Male		
		2-05	2-06	2-07	2-08	Mean	SD
Exhaled VOC Trap 2	**0 - 1 h**	*0.000	*0.000	*0.000	*0.000	0.000	0.000
Exhaled VOC Trap 2	**1 h - 3 h**	*0.000	*0.000	0.000	*0.000	0.000	0.000
Exhaled VOC Trap 2	**3 h - 5 h**	*0.000	*0.000	*0.000	*0.000	0.000	0.000
Exhaled VOC Trap 2	**5 h - 7 h**	*0.000	*0.000	*0.000	*0.000	0.000	0.000
Exhaled VOC Trap 2	**7 h - 12 h**	*0.000	*0.000	*0.000	*0.000	0.000	0.000
Exhaled VOC Trap 2	**12 h - 24 h**	*0.000	*0.000	0.005	0.000	0.001	0.002
Exhaled VOC Trap 2	**24 h - 48 h**	0.001	0.001	*0.000	*0.000	0.000	0.000
Exhaled VOC Trap 2	**48 h - 72 h**	0.113	0.001	*0.000	*0.000	0.028	0.056
Exhaled VOC Trap 2	**72 h - 96 h**	*0.000	0.001	*0.000	0.001	0.000	0.001
Exhaled VOC Trap 2	**96 h - 120 h**	*0.000	0.001	*0.000	0.001	0.000	0.000
Exhaled VOC Trap 2	**120 h - 144 h**	0.000	0.001	0.000	0.000	0.000	0.000
Exhaled VOC Trap 2	**144 h - 168 h**	*0.000	*0.000	*0.000	*0.000	0.000	0.000
Total		0.114	0.005	0.006	0.002	0.032	0.055

* Value is below 3 x background for the sample.

Table 13. Cumulative Recovery of Radioactivity in Excreta and Carcass following Exposure to 1750 ppm [2-^{14}C]TBA/TBA (Group 2, Study B) Expressed as a Percentage of the Total Radioactivity Recovered

	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B	Group 2 Study B
	Male	Male	Male	Male		
Timepoint	2-05	2-06	2-07	2-08	Mean	SD
0 h	0.842	0.545	0.328	1.231	0.737	0.391
1 h	4.974	5.624	5.192	6.796	5.647	0.813
3 h	9.536	10.262	9.533	11.885	10.304	1.108
5 h	14.633	14.970	14.028	16.856	15.122	1.220
7 h	18.343	18.545	18.228	20.484	18.900	1.064
12 h	69.217	59.168	54.353	62.407	61.286	6.237
24 h	89.758	80.695	84.138	87.848	85.610	4.022
48 h	96.838	93.865	95.649	96.022	95.593	1.255
72 h	98.508	96.667	97.809	97.664	97.662	0.759
96 h	98.829	97.325	98.454	98.281	98.222	0.640
120 h	98.995	97.655	98.741	98.524	98.479	0.582
144 h	99.099	98.083	98.926	98.881	98.747	0.453
168 h	99.889	99.911	99.898	99.912	99.902	0.011
Total	99.889	99.911	99.898	99.912	99.902	0.011

Table 14. TBA Measured by GC in DMF Extracts of VOC Trap 1[1] following Exposure to 1750 ppm [2-^{14}C]TBA/TBA

		TBA Concentration (mg/g)					
Sample	**Timepoint**	**2-05**	**2-06**	**2-07**	**2-08**	**Mean**	**SD**
Exhaled VOC Trap 1	1 h	0.796	0.810	0.843	1.149	0.899	0.167
Exhaled VOC Trap 1	3 h	0.638	0.680	0.751	0.902	0.743	0.116
Exhaled VOC Trap 1	5 h	0.697	0.652	0.649	0.806	0.701	0.073
Exhaled VOC Trap 1	7 h	0.562	0.563	0.768	0.635	0.632	0.097
Exhaled VOC Trap 1	12 h	0.524	0.786	1.191	0.999	0.875	0.287
Exhaled VOC Trap 1	24 h	0.075	0.099	0.247	0.218	0.159	0.086
Exhaled VOC Trap 1	48 h	0.011	0.083	0.011	0.024	0.032	0.034
Exhaled VOC Trap 1	72 h	0.014	0.021	<LOQ	0.028	0.021	0.007
Exhaled VOC Trap 1	96 h	<LOQ	<LOQ	<LOQ	<LOQ	-[2]	-
Exhaled VOC Trap 1	120 h	<LOQ	<LOQ	<LOQ	<LOQ	-	-
Exhaled VOC Trap 1	144 h	NA	<LOQ	<LOQ	<LOQ	-	-
Exhaled VOC Trap 1	168 h	NA	<LOQ	NA	0.013	-	-
VOC Trap 1 Extract 2	1 h	0.299	0.431	0.465	0.465	0.415	0.079
VOC Trap 1 Extract 2	3 h	0.329	0.377	0.408	0.523	0.409	0.082
VOC Trap 1 Extract 2	5 h	0.344	0.371	0.414	0.482	0.403	0.060
VOC Trap 1 Extract 2	7 h	0.243	0.269	0.322	0.308	0.286	0.036
VOC Trap 1 Extract 2	12 h	0.310	0.474	0.569	0.430	0.446	0.108
VOC Trap 1 Extract 2	24 h	0.039	0.082	0.130	0.100	0.088	0.038

[1] VOC Trap 1 was extracted to give **Exhaled VOC Trap 1** for all samples, and a second extract **VOC Trap 1 Extract 2** for the samples collected between 1 and 24 h.
[2]- Mean and SD not calculated for values less than limit of quantitation (<LOQ)

Table 15. Percentage of Radioactivity as TBA in the DMF Extracts of VOC Trap 1[1] following Exposure to 1750 ppm [2-^{14}C]TBA/TBA

		% of Radioactivity as TBA					
Sample	**Timepoint**	**2-05**	**2-06**	**2-07**	**2-08**	**Mean**	**SD**
Exhaled VOC Trap 1	1 h	99.6	70.8	82. 5	83.1	84.0	11.8
Exhaled VOC Trap 1	3 h	75.0	72.2	77. 5	79.3	76.0	3.1
Exhaled VOC Trap 1	5 h	79.6	66.6	68.5	75.2	72.5	6.0
Exhaled VOC Trap 1	7 h	73.8	70.1	75.2	71.1	72.5	2.4
Exhaled VOC Trap 1	12 h	69.7	68.4	76.4	75.3	72.5	4.0
Exhaled VOC Trap 1	24 h	42.6	40.5	56.4	57.4	49.3	8.9
Exhaled VOC Trap 1	48 h	26.8	42.9	6.9	35.9	28.1	15.6
Exhaled VOC Trap 1	72 h	54.3	54.0	<LOQ	66.0	58.1	6.8
Exhaled VOC Trap 1	96 h	<LOQ	<LOQ	<LOQ	<LOQ	-[2]	-
Exhaled VOC Trap 1	120 h	<LOQ	<LOQ	<LOQ	<LOQ	-	-
Exhaled VOC Trap 1	144 h	NA[3]	<LOQ	<LOQ	<LOQ	-	-
Exhaled VOC Trap 1	168 h	NA	<LOQ	NA	69.9	-	-
VOC Trap 1 Extract 2	1 h	92.1	96.2	91.3	89.2	92.2	2.9
VOC Trap 1 Extract 2	3 h	92.9	92.9	97.6	96.3	94.9	2.4
VOC Trap 1 Extract 2	5 h	84.0	85.3	93.5	94.1	89.2	5.3
VOC Trap 1 Extract 2	7 h	94.7	91.1	88.1	91.8	91.4	2.7
VOC Trap 1 Extract 2	12 h	91.9	92.8	92.8	92.1	92.4	0.5
VOC Trap 1 Extract 2	24 h	61.2	75.6	76.7	75.8	72.3	7.4

[1] VOC Trap 1 was extracted to give **Exhaled VOC Trap 1** for all samples, and a second extract **VOC Trap 1 Extract 2** for the samples collected between 1 and 24 h
[2]- Mean and SD not calculated for values less than limit of quantitation (<LOQ).
[3]NA = not analyzed.

Table 16. Analysis of Radioactivity in Urine following Exposure to 1750 ppm [2-^{14}C]TBA/TBA

Time point	Animal	Percentage of radioactivity present (%[a])				Recovery[b] (%)
		Peak 1	Peak 2	Peak 3	Peak 4 (^{14}C TBA)	(HPLC)
12 hr	2-05	35.7	36.1	19.6	4.13	108
	2-06	33.3	38.0	20.3	4.69	96.1
	2-07	26.2	43.8	21.3	4.21	98.3
	2-08	30.3	41.8	19.2	4.58	98.2
	Mean	31.4	39.9	20.1	4.40	
	SD	4.10	3.51	0.920	0.274	
	CV%[c]	13.1%	8.78%	4.58%	6.23%	
24 hr	2-05	71.0	11.5	14.3	0.469	95.7
	2-06	65.3	13.7	17.7	0.524	106
	2-07	57.9	17.8	20.9	*	98.1
	2-08	63.5	15.8	17.2	*	94.9
	Mean	64.4	14.7	17.5	0.497	
	SD	5.40	2.71	2.70	-	
	CV%	8.38%	18.4%	15.4%	-	
48 hr	2-05	64.9	11.6	7.98	*	96.6
	2-06	71.9	10.4	12.5	*	96.4
	2-07	81.9	7.79	*	*	97.4
	2-08	76.2	8.82	*	*	96.9
	Mean	73.7	9.65	10.2	-	
	SD	7.17	1.68	-	-	
	CV%	9.72%	17.5%	-	-	

[a]Values represent (radioactivity detected in each peak/ total radioactivity recovered from the column)*100
[b] Total recovery of radioactivity in the collected eluent, as a percentage of the radioactivity injected.
[c] CV% = coefficient of variation, calculated as (SD/Mean)*100.
* Peak not detected with radioactivity detector.
- Values not calculated for less than three determinations.

Figure 1. Cumulative Excretion of Radioactivity in Urine and Feces.

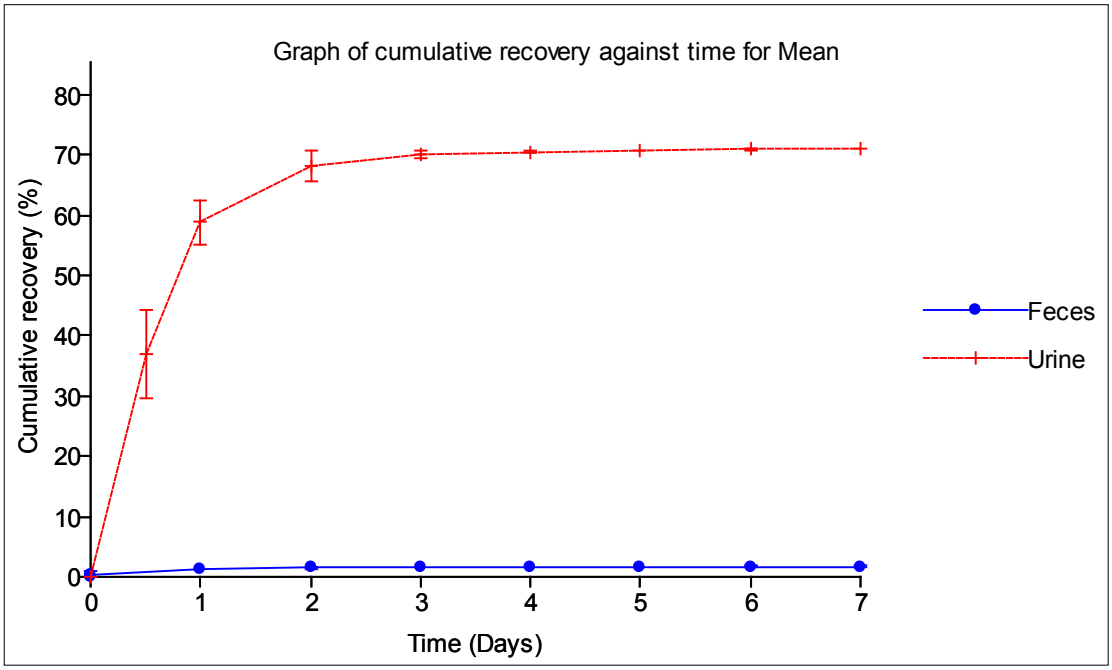


Figure 2. HPLC Analysis of Urinary Metabolites from Rat 2-06 Following Exposure to 1750 ppm [2-^{14}C]TBA/TBA.

Sample from Rat 2-06 obtained at 12 h (top) 24 h (middle) and 48 h (bottom). Chromatograms represent detection of radioactivity.

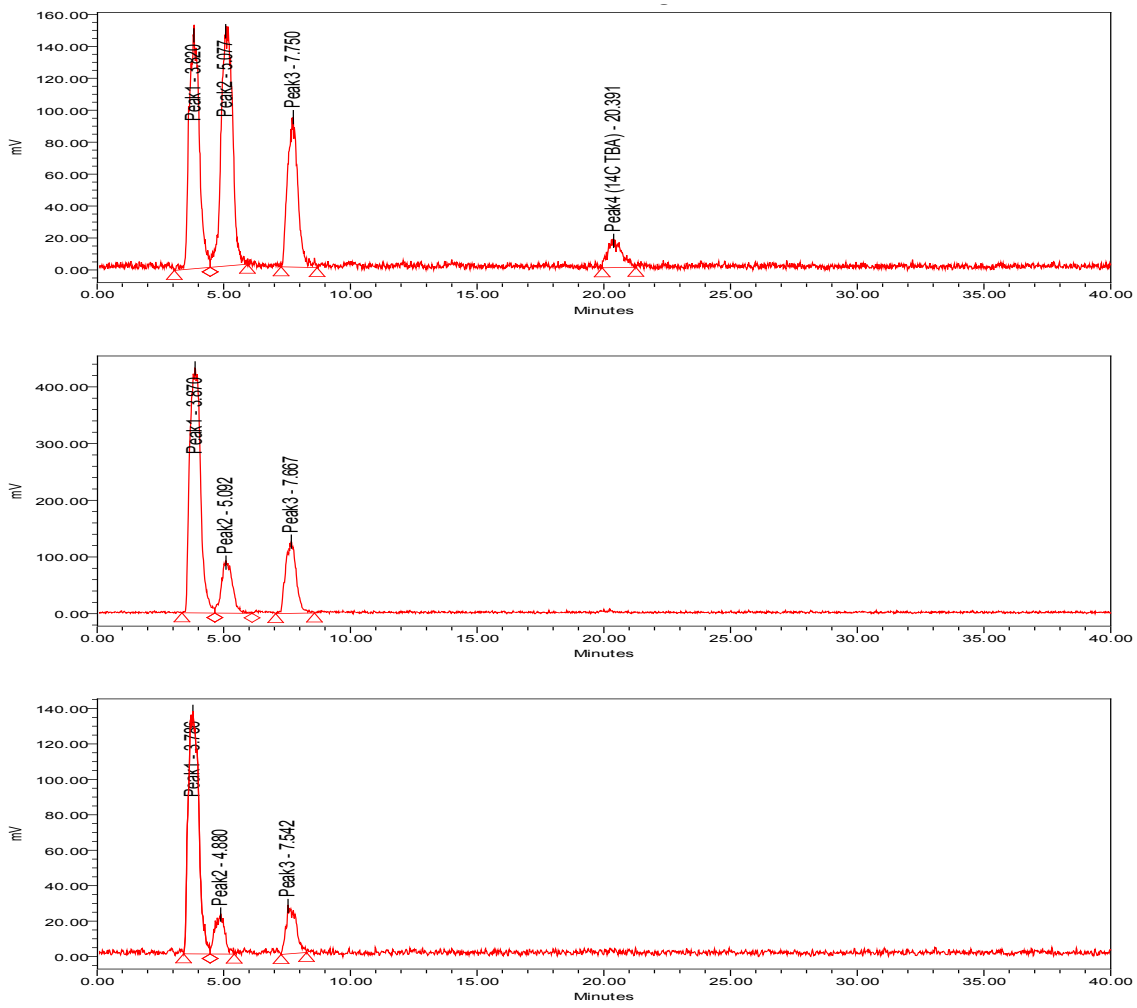


Figure 3. HPLC Separation of 2-Methyl-1,2-propanediol, α-Hydroxyisobutyric Acid, and TBA.

Unlabeled standard 2-methyl-1,2-propanediol and α-hydroxyisobutyric acid were chromatographed with labeled TBA to establish retention time in the HPLC system used for analysis of urinary metabolites. The eluted peaks were detected with refractive index detection.

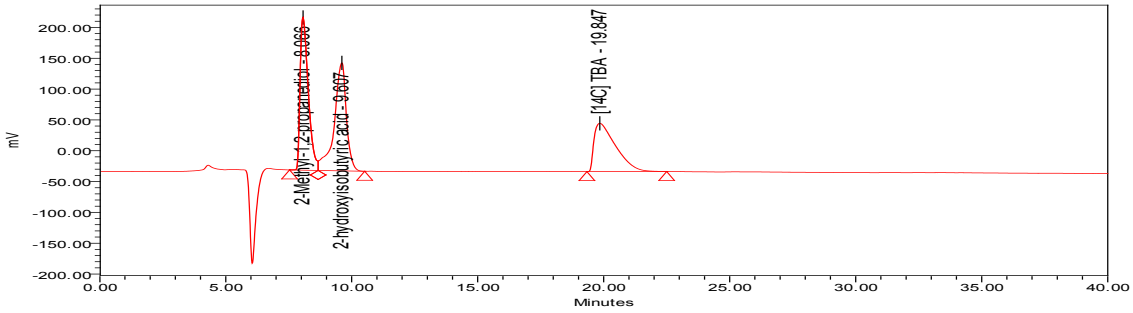
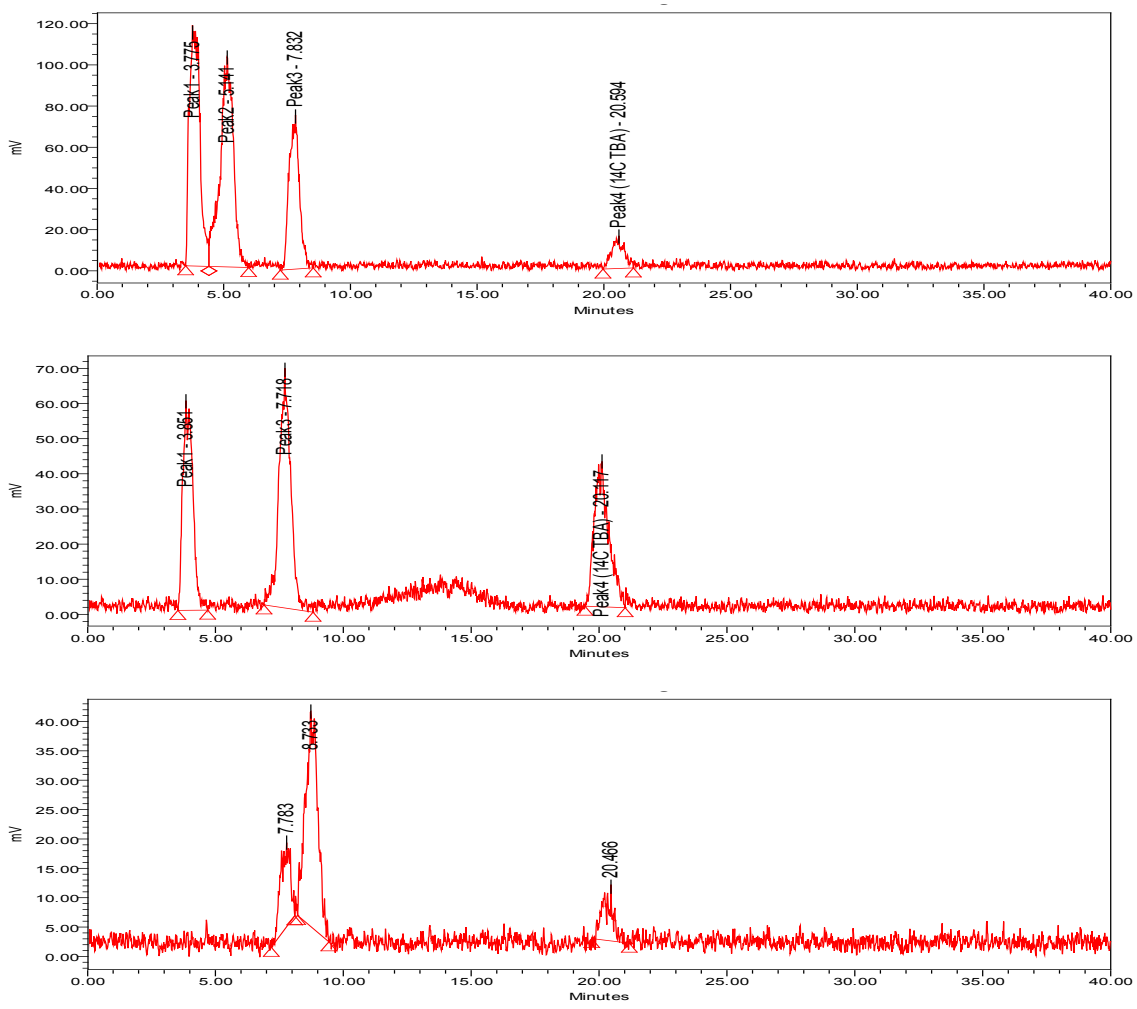


Figure 4. HPLC Analysis of Urinary Metabolites from Rat 2-06 after Incubation with β-Glucuronidase or Acid Treatment

Sample from Rat 2-06 12 h urine following exposure to 1750 ppm [2-^{14}C]TBA/TBA, incubated with buffer (top), with β-glucuronidase (middle) and with acid (bottom). Chromatograms represent detection of radioactivity.



Appendix A

Approved Study Protocol, Amendments, and Deviations

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TITLE: METABOLISM AND PHARMACOKINETICS OF TERTIARY BUTYL ALCOHOL IN MALE RATS: PILOT STUDY

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

TESTING FACILITY: Science and Engineering
RTI International*
3040 Cornwallis Road
Post Office Box 12194
Research Triangle Park, NC 27709

RTI PROJECT NO.: 0209408.004

RTI Study Code: Rt05-935

RTI STUDY DIRECTOR: Timothy R. Fennell

PROPOSED STUDY DATES: May 2006 – July 2006

AMENDMENTS:

No.	Date	Section	Pages
1			
2			
3			
4			

Thomas M. Gray
Sponsor's Representative
5/19/06
Date

Timothy R. Fennell, Ph.D.
Study Director
RTI International
05/22/2006
Date

R. Arden James
Principal Investigator
CIIT Centers for Health Research
05/22/2006
Date

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

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1.0 OBJECTIVES

The objectives of this pilot study on *tertiary* butyl alcohol (TBA) are to:

Study A

1) conduct a nose-only exposure of rats to ^{14}C TBA/TBA (n=4; 1 backup unexposed).

2) analyze the amount of ^{14}C in whole body digests from rats exposed to ^{14}C TBA/TBA.

Study B

3) conduct a nose-only exposure of rats to ^{14}C TBA/TBA (n=4; 2 backup unexposed).

4) collect excreta from rats exposed to ^{14}C TBA/TBA.

5) analyze ^{14}C in whole body digests, urine, feces, exhaled CO_2, and exhaled volatiles in rats exposed to ^{14}C TBA/TBA.

6) develop an HPLC method for analysis of metabolites in urine.

7) analyze TBA in extracts from exhaled volatiles.

2.0 STUDY ORGANIZATION

Testing Facility:

Science and Engineering
RTI International
3040 Cornwallis Road
PO Box 12194
Research Triangle Park, NC 27709

For purposes of this study, the terms "RTI", "RTI International", and "Research Triangle Institute" are synonymous.

Test Facility Management:

Alan Staple, Vice President
Health Sciences
Science and Engineering
RTI International
Phone: 919.485.5674

Study Director:

Timothy R. Fennell, Ph.D.
Health Sciences
Science and Engineering
RTI International
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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.

Lead Quality Assurance Contact:

Celia Keller, M.S.
Science and Engineering QA Unit
RTI International
Phone: (919) 541 7272
Email: cdk@rti.org

The lead Quality Assurance has overall responsibility for quality assurance for the entire study.

RTI Study Personnel

Timothy R. 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

Other personnel will be used as required. A full list of study participants will be included in the study report.

Test Site for the development of the inhalation exposure system:

CIIT Centers for Health Research
6 Davis Drive
P.O. Box 12137
Research Triangle Park
NC 27709

For the purposes of this study, the terms "CIIT Centers for Health Research", "CIIT CHR", and "CIIT" are synonymous.

CIIT Principal Investigator

R. Arden James
CIIT Centers for Health Research
Phone (919) 558 1279
Fax (919) 558 1300

	Email: james@ciit.org
CIIT Quality Assurance Contact	Patricia O'Brien Pomerleau CIIT Centers for Health Research Phone (919) 558 1341 Fax (919) 558 1300 Email: pomerleau@ciit.org
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

3.0 STUDY DESIGN

Prior to the exposure of rats to TBA, an inhalation exposure system will be set up and evaluated for the nose-only exposure of rats to a single exposure of TBA. The exposure system will consist of a 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 atmosphere for exposure of rodents to TBA for 6 hrs. The concentration of TBA on the exposure tower will be monitored using a Miran infrared analyzer (Foxboro, MA) or by gas chromatography (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 TBA at the exposure concentration to be used in the TBA pilot studies, with no animals on the exposure tower. The test exposure will be run for 6 hours, with sampling of air for TBA 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 between target and actual concentration on the tower, and agreement in concentration measurements taken at multiple locations on the tower. The criteria that will indicate successful performance of the system are agreement (≤10% difference) between target and actual concentration on the tower, and agreement (≤10% difference) in concentration measurements taken at multiple locations on the tower.

The stability of TBA concentrations during the exposure will be monitored by comparison of the TBA concentration measurements at the beginning and end of the exposures.

Study A

Four male F344 rats will be exposed for approximately 6 hr to 1750 ppm ^{14}C TBA/TBA via nose only inhalation. At the end of exposure, the 4 rats will be immediately euthanized by CO_2 asphyxiation. 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_2 asphyxiation for collection of a carcass sample that will be used for determination of background radioactivity.

Study B

Four male F344 rats will be exposed for approximately 6 hr to a mixture of 1750 ppm ^{14}C TBA/TBA via nose-only inhalation. They will be transferred to individual glass metabolism cages for the collection of urine, feces, expired volatiles, and expired CO_2. Urine and feces will be collected over dry ice at approximately 12 and 24 hr after exposure, and thereafter at approximately every 24 hr for up to 7 days or until 90% of the dose is eliminated. Expired volatiles and CO_2 will be collected at approximately 1, 3, 5, 7, 12 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 samples from the unexposed rats will be collected as described above for the exposed rats for a period of 48 hr, at which point they will be euthanized for collection of tissues.

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 samples will be stored at approximately -20°C or below until analyzed.

4.0 JUSTIFICATIONS

4.1 Animal Species

The present studies are designed to evaluate the uptake, distribution and excretion of TBA to provide information that will be used for safety assessments to humans. No *in vitro* techniques are

available that allow for adequate determination of uptake, distribution, and excretion of chemicals by mammals. Fischer 344 rats are an established animal species and strain for toxicological testing, and pharmacokinetic studies.

4.2 Numbers of Animals

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.

4.3 Routes of Administration and Dose Levels

The route of administration is an expected exposure route in humans (inhalation) and has been used in toxicity or safety assessment studies. The exposure concentration is the mid range concentration from a TBA inhalation toxicity study conducted on rats and mice exposed for 18 days, 6 hours per day, 5 days per week (Mahler, 1997), and is expected to be without significant toxicity. The exposure concentrations used were 450, 900, 1,750, 3,500, and 7,000 ppm for 6 hours per day, 5 days per week, for 12 exposure days. After a single exposure to 7000 ppm, all rats were sacrificed moribund. One 3,500 ppm male mouse died on day 3. Final mean body weights of 3,500 ppm male and female rats were significantly lower than those of the controls. In a 13-week inhalation study (Mahler, 1997), groups of 10 male and 10 female rats and mice were exposed to TBA at concentrations of 0, 135, 270, 540, 1,080, and 2,100 ppm for 6 hours per day, 5 days per week, for 13 weeks. There were no treatment-related gross findings in male or female rats or mice.

5.0 REGULATORY COMPLIANCE

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 prestudy exposure system method development will be completed at CIIT CHR and will not be conducted under GLPs. The synthesis of 2-methyl-1,2-propanediol will not be conducted under GLPs.

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 inspections were made, and the dates on which results of the inspections were reported to the Study Director and the Study Director's management.

The Quality Assurance Unit at the test site will prepare and sign a QA Statement to be included in the CIIT CHR report on the inhalation exposure. It will specify: the phases of the study that were inspected by CIIT CHR Quality Assurance Unit; the dates on which inspections were made; the dates on which the results of the inspections were reported to the CIIT CHR 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.

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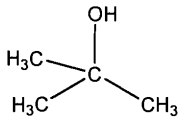
6.0 TEST SUBSTANCE

NAME: ***tertiary* Butyl Alcohol** (TBA; 2-methyl-2-propanol, CAS No. 75-65-0)

MOLECULAR FORMULA: $C_4H_{10}O$

MOLECULAR WEIGHT: 74.12

STRUCTURE:



SOURCE OF NON-RADIOLABELED TEST SUBSTANCE: The non-radiolabeled TBA was purchased from Sigma-Aldrich, Milwaukee, WI. The certificate of analysis from the vendor indicated purity of 99.6%.

PRODUCT NUMBER: 36053-8

LOT NUMBER: 01060AD

RADIOLABELED TEST SUBSTANCE: **[2-^{14}C]-labeled *tertiary* Butyl Alcohol**

SOURCE OF RADIOLABELED TEST SUBSTANCE: [2-^{14}C]-labeled TBA was obtained from Sigma Radiochemicals, as a custom synthesis. Product information from the vendor indicated purity of 99.0%. The specific activity indicated by the vendor is 7.3 mCi/mmol.

PRODUCT NUMBER: A8538-14C

LOT NUMBER: 095K9406

IDENTITY AND PURITY: The identity of the unlabeled TBA will be confirmed at RTI by ^{1}H and ^{13}C 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 [2-^{14}C] TBA will be confirmed by ^{1}H NMR spectroscopy, and by coelution of radioactivity with unlabeled 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.

STORAGE CONDITIONS: [2-^{14}C]TBA will be stored in the dark at approximately -20 °C. Nonradiolabeled TBA will be stored in the dark at room temperature.

STABILITY: TBA is expected to be stable for the duration of the study. A sample of the unlabeled TBA will be analyzed before the start of this study, periodically throughout the study, and after the study to confirm stability. Radiochemical purity of the [2-^{14}C]-TBA will be confirmed by HPLC before and after the animal exposures.

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7.0 ANIMALS

1. Species and Strains: Fischer 344 rats
2. Approximate Age: 8-9 weeks old at time of exposure
3. Approximate Weight: 200 g
4. Number/Sex:

   Study A: 5 Male rats.

   Study B: 6 Male rats.
5. Sources: Charles River (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 Harlan (Indianapolis, IN). The source(s) of all animals will be documented in the raw data, and included in the Final Report.

7.1 Husbandry

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.

7.1.1 Identification

Rats will be identified by individual ear tags. 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 ear tag number or to treatment group and animal number or to both.

7.1.2 Quarantine

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 Corporation, Montville, New Jersey).

7.1.3 Feed and Water

Animals will be provided Certified Purina Rodent Chow (5002) *ad libitum*, except during the periods of inhalation exposure. Water will be provided *ad libitum* 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, diisopropyl ether, and methyl tertiary butyl ether. The samples will be sent to Kiff Analytical (Davis, CA 95616) for analysis. Documentation of these analyses will be inspected by the Study Director and maintained in the study records. 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 the water will not affect the design, conduct or conclusions of this study.

7.1.4 Environmental

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.

7.1.5 Acclimation and Housing During Studies

During the course of Study B, rats will be placed in individual glass Roth-type metabolism cages for the collection of urine and feces. There will be no acclimation period.

At the end of exposure, the rats from Study A will be immediately euthanized by CO_2 exposure.

7.2 Randomization and Assignment of Animals to Treatment Groups

Animals will be specifically purchased for each study, and will not be assigned randomly to specific study groups.

7.3 Body Weights

Individual body weights will be measured during the quarantine period, the day of exposure, and at sacrifice.

7.4 Found Dead/Moribund Animals

The Study Director or the veterinarian 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.

7.5 Euthanasia

Rats will be euthanized by CO_2 exposure.

8.0 STUDY PROCEDURES

8.1 Test Chemical Preparation and Analysis

For inhalation exposure to ^{14}C TBA/TBA (Studies A and B), an exposure atmosphere will be generated using ^{14}C TBA added to an appropriate amount of unlabeled TBA. The amount of labeled and unlabeled TBA 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-^{14}C]TBA/TBA, [2-^{14}C]TBA will be weighed into a tared flask with a Teflon faced screw cap. The weight of the labeled chemical added will be recorded. Unlabeled TBA will be added, and the weight added will be recorded. The nominal specific activity of the TBA 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 TBA 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 TBA will then be calculated from the data obtained.

The exposure atmosphere concentration will be monitored using a calibrated analytical instrument (e.g. Miran IR detector). Concentrations of TBA will be monitored at strategic locations to document exposure concentrations on the tower. The stability of TBA 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.

8.2 Inhalation Exposure

The inhalation system including the generation and exposure systems will be constructed with materials that are chemically compatible with TBA to minimize chemical losses. The generation system will include a generator and delivery system to deliver a steady flow of TBA to the exposure tower air supply at appropriate flow rates to maintain the target concentration of 1750 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 TBA that may leak from the system.

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 TBA 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 test animals during inhalation exposures. The inhalation system will be strategically placed in a chemical hood to prevent any TBA from entering the laboratory.

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.

8.3 Ante mortem Observations and Functional Assessments

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.

8.4 Collection and Storage of Biological Samples

8.4.1 Excreta

In Study B, urine will be collected over dry ice at 0–12, 12–24, 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 $^{14}CO_2$ will be collected in 0.1 N KOH at 0–1, 1–3, 3–5, 5–7, 7–12, 12–24, 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 -20 °C in the dark.

8.4.2 Carcass

For Study A, the amount of ^{14}C 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 CO_2 will be pumped into the bag 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 ^{14}C, and the carcass will be digested with 2N ethanolic NaOH. After sample digestion, the amount of radioactivity in the carcass will be determined by LSS.

For Study B, the carcasses will be digested with 2N ethanolic NaOH. After sample digestion, the amount of radioactivity in the carcass will be determined by LSS.

8.5 Analysis of Samples for Total Radioactivity

8.5.1 Excreta

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 µl of 70% perchloric acid, and then approximately 0.3 ml of 30% H_2O_2) prior to addition of scintillation cocktail. Control samples of urine and feces will be collected prior to dosing on Study Day 1 and analyzed for radiochemical content to determine background counts.

8.5.2 Carcass

Carcasses will be analyzed for total radioactivity following solubilization in 2N ethanolic NaOH. Duplicate samples of the solubilized carcass will be analyzed. Solubilized samples may be bleached (by adding approximately 125 µl of 70% perchloric acid, and then approximately 0.3 ml of 30% H_2O_2) prior to addition of scintillation cocktail and analysis by LSS.

8.5.3 Exhaled Breath Traps

Aliquots of 0.1 N KOH from the exhaled breath trap for CO_2 will be analyzed by LSS after neutralization, and addition of scintillation cocktail.

8.5.4 Exhaled Volatiles Traps

Each charcoal trap will be extracted by elution with 2.0 ml of dimethylformamide (DMF). The radioactivity in the DMF wash will be measured by LSS of duplicate aliquots.

8.6 Found Dead/Moribund Animals

The Study Director or the veterinarian 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. The Sponsor will be notified as soon as possible if it is anticipated that the sacrifice may affect the integrity of the study.

8.7 Analysis of TBA and Metabolites in Selected Urine Samples

A method for the separation of urinary metabolites of TBA will be developed, using HPLC with radioflow detection. A standard of α-hydroxyisobutyric acid will be obtained from Sigma-Aldrich. 2-Methyl-1,2-propanediol will be synthesized by the procedure reported by Bernaur et al. (1998) by reduction of the ethyl ester of 2-hydroxyisobutyrate. Selected urine samples will be analyzed for the presence of TBA, α-hydroxyisobutyric acid, and 2-methyl-1,2-propanediol. Urine samples selected for analysis will contain ≥ 5 % of the total radioactivity recovered.

8.8 Analysis of TBA in Exhaled Volatiles Traps

A method for the quantitative analysis of TBA in DMF using GC will be developed. Standard solutions of TBA in DMF will be prepared at concentrations of 0.01, 0.1, 1, and 10 µg/ml. Aliquots of the DMF washes of each charcoal trap will be analyzed in duplicate for concentration of TBA.

9.0 DATA COLLECTION

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.

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 record.

10.0 STATISTICAL ANALYSIS

All data for carcass and excreta ^{14}C-content will be reported in tables as the mean ± standard deviation (SD).

11.0 RECORDS AND REPORT

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. Test chemical receipt, storage and use records
h. Balance calibration log references
i. Correspondences
j. All other raw data and documentation.

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:

a. Name and address of the facility performing the study, dates of study initiation and completion, and RTI study number.
b. A copy of the signed, dated and approved protocol and all deviations and authorized amendments to the original protocol.
c. A detailed description of all methods used, including the randomization method.
d. The lot number(s) of the test substances and details of the formulation of doses.
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.
f. Tabulated individual results for excreta.
g. Tabulated mean results for excreta.
h. Graphical presentation of disposition data for radioactivity.
i. 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.
j. 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 .
k. A compliance statement signed by the Study Director.

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:

Thomas M. Gray
Regulatory Analysis and Scientific
Affairs
American Petroleum Institute
1220 L Street NW
Washington, DC 20005
p: (202)682-8319
f: (202)682-8031

12.0 MAINTENANCE OF RECORDS AND RAW DATA

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 those of Good Laboratory Practices (GLPs) Standards for Inhalation Exposure Health Effects Testing, 40 CFR 79.60. Facility data will be maintained in the archives of RTI International and CIIT CHR, respectively. Documentation and raw data will be maintained in the Archives for a period of ten years from the 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 (after the QAU assures that discarding the samples does not negatively impact the integrity of the study).

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.

13.0 SAFETY PRECAUTIONS

This study will be conducted in accordance with Nuclear Regulatory Commission (NRC) regulations, North Carolina License #032-0131-1.

a. Precautions for laboratory personnel:

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 regulations for work with radiolabeled compounds.

b. All radioactive wastes will be disposed of in accordance with standard RTI safety policies.

14.0 PROTOCOL AMENDMENTS AND DEVIATIONS

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 of the Study Director's approval). Subsequently, a formal protocol amendment will be prepared for approval by the Study Director and the Sponsor's Representative.

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 on the outcome of the study will be explained and the Study Director will sign the document.

15.0 REFERENCES

Bernauer, U., Amberg, A., Scheutzow, D., and Dekant, W. (1998). Biotransformation of ^{12}C- and 2-^{13}C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: identification of metabolites in urine by ^{13}C nuclear magnetic resonance and gas chromatography/mass spectrometry. *Chem Res Toxicol* 11, 651-658.

Cannon, W. C., Blanton, E. F., and McDonald, K. E. (1983). The flow-past chamber: an improved nose-only exposure system for rodents. *Am. Ind. Hyg. Assoc. J.* **44**, 923-928.

Mahler, J. (1997). NTP technical report on toxicity studies of t-butyl alcohol (CAS No. 75-65-0). Administered by inhalation to F344/N rats and B6C3F1 mice. *Toxic Rep Ser*, 1-56, A51-D59.

National Research Council (1996). Guide for the Care and Use of Laboratory Animals. National Academy Press: Washington, DC.

PROTOCOL	RTI INTERNATIONAL * POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-935 Amendment No. 1 Page 1 of 5

AMENDMENT 1

TITLE: METABOLISM AND PHARMACOKINETICS OF TERTIARY BUTYL ALCOHOL IN MALE RATS: PILOT STUDY

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

TESTING FACILITY: Science and Engineering
RTI International*
3040 Cornwallis Road
Post Office Box 12194
Research Triangle Park, NC 27709

RTI PROJECT NO.: 0209408.004

RTI Study Code: Rt05-935

RTI STUDY DIRECTOR: Timothy R. Fennell

PROPOSED EXPERIMENTAL START DATE: June 1, 2006

PROPOSED EXPERIMENTAL TERMINATION DATE: July 15, 2006

APPROVED BY:

Thomas M. Gray — 6/9/2006 (Date)
Sponsor's Representative

Timothy R. Fennell, Ph.D. — 6-15-2006 (Date)
Study Director
RTI International

R. Arden James — 06/13/2006 (Date)
Principal Investigator
CIIT Centers for Health Research

RTI International is a tradename of Research Triangle Institute.

PROTOCOL	RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-935 Amendment No. 1 Page 2 of 5

Protocol Change No.: 1

Change (Title Page, Page 1):

PROPOSED STUDY DATES: May 2006 – July 2006

To:

PROPOSED EXPERIMENTAL START DATE: June 1, 2006

PROPOSED EXPERIMENTAL TERMINATION DATE: July 15, 2006

Reason for change:

To include proposed experimental start date and experimental termination date as required by GLP regulations.

Protocol Change No.: 2

Delete entire Table of Contents (Pages 2 and 3) and replace with:

Table of Contents

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3.0 STUDY DESIGN 6

4.0 JUSTIFICATIONS 7

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4.3 Routes of Administration and Dose Levels 8

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7.1 Husbandry 10

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7.1.3 Feed and Water 11

7.1.4 Environmental 11

7.1.5 Acclimation and Housing During Studies 11

PROTOCOL	RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-935 Amendment No. 1 Page 3 of 5

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7.3 Body Weights 11
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Reason for change:

The page numbers were incorrect.

PROTOCOL	RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-935 Amendment No. 1 Page 4 of 5

Protocol Change No.: 3

Change (Section 3.0 Study B, Page 7):

All samples will be stored at approximately -20°C or below until analyzed.

To:

All urine, feces, and expired volatile trap samples will be stored at approximately -20°C or below until analyzed. CO_2 trap samples will be stored at room temperature.

Reason for change:

Clarification.

Protocol Change No.: 4

Change (Section 4.3, Page 8):

The route of administration is an expected exposure route in humans

To:

The route of administration is an expected potential exposure route in humans

Reason for change:

Clarification.

Protocol Change No.: 5

Change (Section 8.4.1, Page 13):

0.1N KOH

To:

1.0N KOH

Reason for change:

The concentration of KOH to be used in the CO_2 traps was incorrectly listed.

PROTOCOL	RTI INTERNATIONAL POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709	RTI-935 Amendment No. 1 Page 5 of 5

Protocol Change No.: 6

Change (Section 8.5.3, Page 14):

Aliquots of 0.1 N KOH from the exhaled breath trap for CO_2 will be analyzed by LSS after neutralization, and addition of scintillation cocktail.

To:

Aliquots of 1.0 N KOH from the exhaled breath trap for CO_2 will be analyzed by LSS after addition of scintillation cocktail.

Reason for change:

Correction of KOH concentration, and clarification of procedure.

Protocol Change No.: 7

Change (Section 8.8, Page 15):

Standard solutions of TBA in DMF will be prepared at concentrations of 0.01, 0.1, 1, and 10 µg/ml. Aliquots of the DMF washes of each charcoal trap will be analyzed in duplicate for concentration of TBA.

To:

Standard solutions of TBA in DMF will be prepared at concentrations of 0.01, 0.1, 1, and 10 µg/µl. Aliquots of the DMF washes of each charcoal trap that contains sufficient radioactivity to permit analysis will be analyzed in duplicate for concentration of TBA by GC.

Reason for change:

Correction of concentration units. Clarification.

RTI Protocol No. RTI-935 (RTI Project No. 020908.004)

Deviations from the Approved Protocol

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

Deviation
Protocol section 8.5.1 indicated that control samples of urine and feces will be collected prior to dosing on study Day 1 and analyzed for radiochemical content to determine background counts. The protocol indicates that excreta are to be obtained from unexposed rats for a period of 48 hours (Section 3.0, Page 7).
Reason for the Deviation
Excreta were not collected prior to exposure for determination of background because two unexposed rats were used to generate background samples at the time of collection from the exposed animals.

Deviation
The stability of TBA was not monitored by sampling at the beginning of the exposure, but it was sampled at the end of the exposure by sampling at the top of the tower (inlet). The samples obtained (2) were taken for GC-MS and HPLC analysis.
Reason for the Deviation
This deviation was an oversight. The test chemical was sampled and purity and identity were checked in the preparation used for exposure. A sample was collected at the end of the exposure to verify purity.

Deviation
The concentration of potassium hydroxide (KOH) was 1.0 N instead of 0.1 N which was stated in Section 8.5.3 and 8.4.1 of the protocol.
Reason for the Deviation
The value of 0.1 N was a typographical error in the protocol. The concentration was intended to match the concentration used in previous studies investigating TAME previously conducted by the sponsor elsewhere. 1.0 N is the concentration used typically for trapping exhaled $^{14}CO_2$. The study protocol was amended to include this change.

Deviation
The protocol indicates that aliquots of 0.1 N KOH will be analyzed for radioactivity by LSS after neutralization, and addition of scintillation cocktail. Aliquots of the CO2 traps were analyzed without neutralization
Reason for the Deviation
Neutralization of KOH trap aliquots was an error in the protocol. The protocol was amended.

Deviation
The protocol does not specify that the animals were to be randomized and assigned into groups. The animals were randomized according to SOP DPK-HUS-001, and they were assigned to study groups using the randomization.

Reason for the Deviation

Section 7.2 indicated that animals would be specifically purchased for each study and would not be randomly assigned to groups. However, both Study A and Study B were conducted on the same day, so animals were randomly assigned to study A and B, and to unexposed animals.

Deviation
Charcoal in traps used to collect exhaled volatile organic compounds was removed from the traps and subjected to 4 mL extractions with dimethylformamide.
Reason for the Deviation
The original procedure stated in the protocol was not effective for extraction of volatile organic compounds collected in the traps.

Deviation
The protocol indicated that "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." Radiochemical purity was determined by high pressure liquid chromatography and not gas chromatography.

Reason for the Deviation
The radiochemical purity of the radiolabeled material was determined by high pressure liquid chromatography. RTI does not have the capability to determine the radiochemical purity by gas chromatography. This is an error in the development of the protocol.

Appendix B

Test Chemical Analysis Report

Test Chemical Analysis Report

Tertiary Butyl Alcohol

RTI Reference 12323-05

SUBMITTED TO:

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

TESTING FACILITY:

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

Timothy R. Fennell, Ph.D. 12-18-2007
Date

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

In October 2005, 500 ml of Tertiary Butyl Alcohol (TBA) was purchased from Sigma Aldrich (received on November 1, 2005) The material was 99+% A.C.S. Reagent, Product Number 36053-8, Lot Number 01060AD, Formula Weight 74.12. Purity from the Vendor Certificate of Analysis was 99.6% by GLC. No expiration date was indicated by the vendor.

RTI assigned this material Test Article Number of 12323-05. RTI confirmed the identity of the material using nuclear magnetic resonance spectroscopy, and mass spectrometry. RTI confirmed the purity of the material by gas chromatography.

Nuclear Magnetic Resonance Spectroscopy.
All NMR data were acquired on a 300 MHz Bruker spectrometer. The ^{1}H NMR spectra were acquired with a relaxation delay of 30 sec, a 6173 Hz sweep width, and an 8-µsec pulse. The sample was prepared in $CDCl_3$ (deuterochloroform) . The ^{1}H-decoupled ^{13}C NMR spectrum was acquired with a relaxation delay of 2 sec, a sweep width of 23810 Hz, and a 5.5-µsec pulse.

The ^{1}H NMR spectrum of the sample contained two singlets at 1.253 and 1.531 ppm (Figure 1). The singlet at 1.253 ppm are attributed to the CH_3 groups. The singlet at 1.531 is attributed to the hydroxyl proton. The ratio of integrals (8.5551:1.0000) is consistent with the expected ratio for 9 methyl protons, and 1 hydroxyl proton.

The ^{13}C NMR spectrum of the sample contained singlets at approximately 31.2 ppm and 69.2 ppm (Figure 2). The triplet at approximately 77.0 ppm is assigned to $CDCl_3$. The signal at 31.2 ppm is consistent with the *t*-butyl CH_3 groups, and the signal at 69.2 ppm is consistent with the quaternary carbon.

The ^{1}H and ^{13}C NMR data from the sample are consistent with the structure of TBA.

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 µm film thickness (J&W, Agilent Technologies, Wilmington, DE)

Injection port	split/splitless
Temperature	200 ºC

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

Split ratio	[1]100:1
Carrier gas	Helium
Flow rate	[1]1 ml/min
Injection volume	1 µl
Initial temperature	35 ºC
Initial time	[1]1 min,
Temperature rate	[1]5 ºC/min
Final temperature	220 ºC
Final time	[1]1 min

Analyses on August 24, 2006 were conducted as described above with the following exceptions:

Split ratio	50:1
Flow rate	4 ml/min
Initial time	0 min
Temperature rate	10 ºC/min
Final time	2 min

Purity of the material was determined by injection of 3 1-µl samples onto the GC column. The initial purity determined on 11/14/2005 was 99.55 %, with a standard deviation of 0.18 %. Figure 3 shows a typical chromatogram.

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 ºC
Split ratio	5:1
Carrier gas	Helium
Flow rate	1.7 ml/min
Injection volume	1 µl
Initial temperature	30 ºC
Initial time	3 min
Temperature rate	5 ºC/min
Final temperature	80 ºC

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

Final time	0
Ramp	100 ºC/min
Final temperature	200 ºC
Final time	1 min

5973 MSD

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

Identity of the material was verified by GC-MS analysis. A 10-µl sample was dissolved in 20 ml of methanol, and 1 µl was injected.

The total ion chromatogram of the sample showed a single peak at approximately 3.7 min (Figure 4, upper panel). The mass spectrum of this peak (Figure 4, lower panel) did not show a molecular ion. The absence of a molecular ion is expected for a tertiary alcohol. The major fragment ion at *m/z* 59 is consistent with the expected fragmentation of TBA (M-CH_3). A library search indicated a match with the spectrum of TBA (Figure 5).

Determination of Stability
The purity of the tertiary butyl alcohol 12323-05 was determined on several occasions following receipt. The purity and dates of analyses are indicated in Table 1. The initial analysis in November 2005 indicated a purity of 99.55%. The most recent analysis in August 2006 indicated a purity of 99.70%, indicating that the tertiary butyl alcohol is stable.

Conclusion
The test substance 12323-05 tertiary butyl alcohol obtained from Sigma Aldrich was found to be consistent with the expected NMR and mass spectra for tertiary butyl alcohol. The purity of the test substance was greater than 99% by GC-FID, and repeated analysis indicated that the test substance was stable.

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

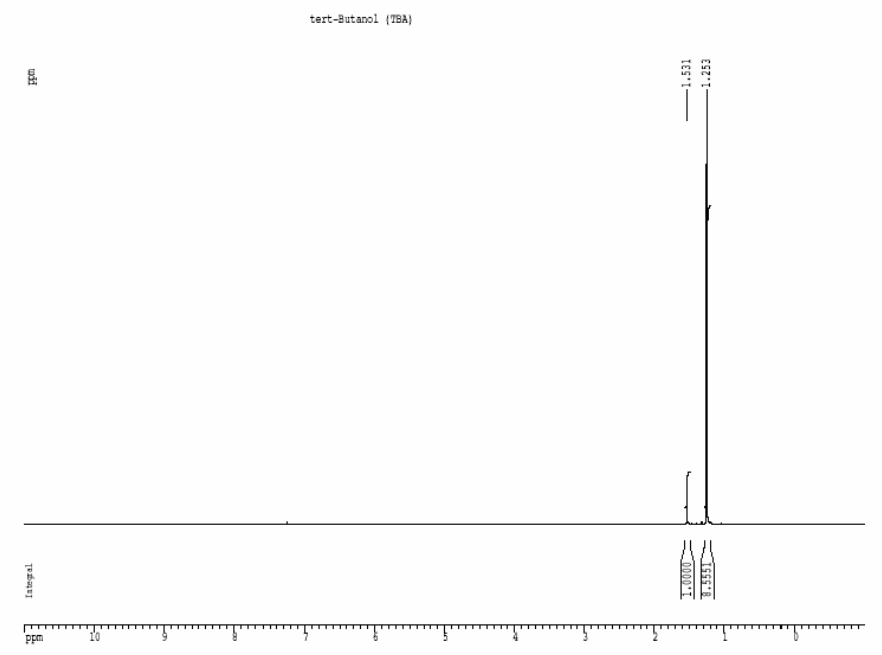
Table 1. Stability of Tertiary Butyl Alcohol 12323-05 determined by repeated GC-FID analysis.

Date of Analysis	Analyst	Mean Purity (%) [1]	Std. Dev.[1]
11-14-2005	Y. Hong	99.55	0.18
01-06-2006	Y. Hong	99.72	0.03
03-03-2006	Y. Hong	99.20	0.03
05-25-2006	Y. Hong	99.23	0.07
08-24-2006	Y. Hong	99.70	0.00

[1] Values represent mean and standard deviation of three determinations.

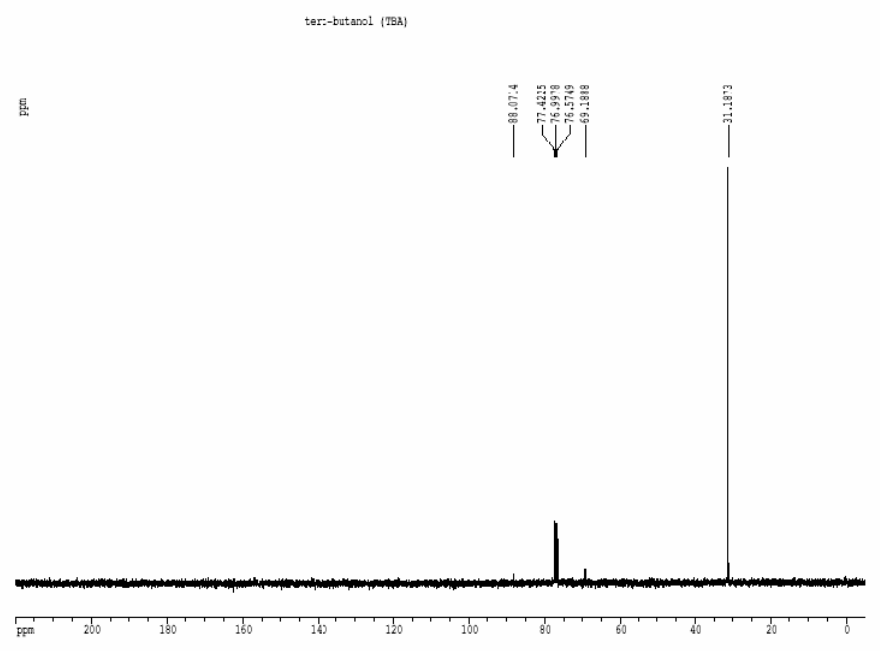
Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

Figure 1. 300 MHz ^{1}H NMR of Vendor-Supplied TBA (Analyzed on 11-09-05 by J. Burgess)



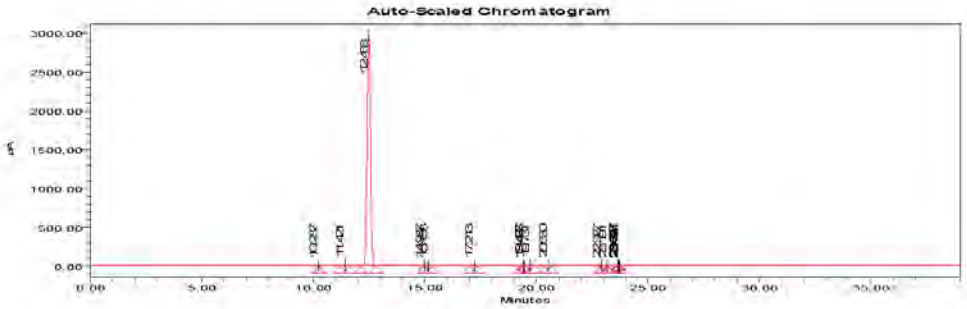
Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

Figure 2. 75 MHz ^{13}C NMR of Vendor-Supplied TBA (Analyzed on 11-09-05 by J. Burgess)



Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

Figure 3. GC-FID Chromatogram of Vendor-Supplied TBA. (Analyzed by Y. Hong on 11/14/2005)

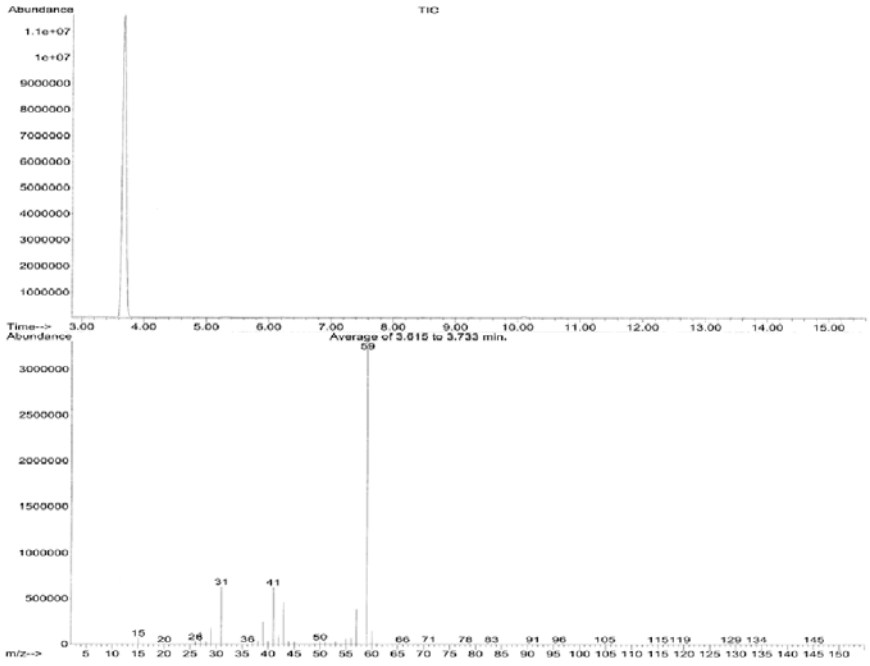


Unknown Peak Results

	Peak Type	RT	Area	% Area	Height
1	Unknown	10.237	0	0.00	0
2	Unknown	11.421	77	0.24	8
3	Unknown	12.488	31487	99.45	2965
4	Unknown	14.987	0	0.00	0
5	Unknown	15.153	10	0.03	1
6	Unknown	17.213	18	0.05	1
7	Unknown	19.427	2	0.01	0
8	Unknown	19.466	2	0.01	0
9	Unknown	19.731	56	0.18	5
10	Unknown	20.550	9	0.03	1
11	Unknown	22.927	0	0.00	0
12	Unknown	23.191	0	0.00	0
13	Unknown	23.647	0	0.00	0
14	Unknown	23.687	0	0.00	0
15	Unknown	23.697	0	0.00	0
16	Unknown	23.724	0	0.00	0

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

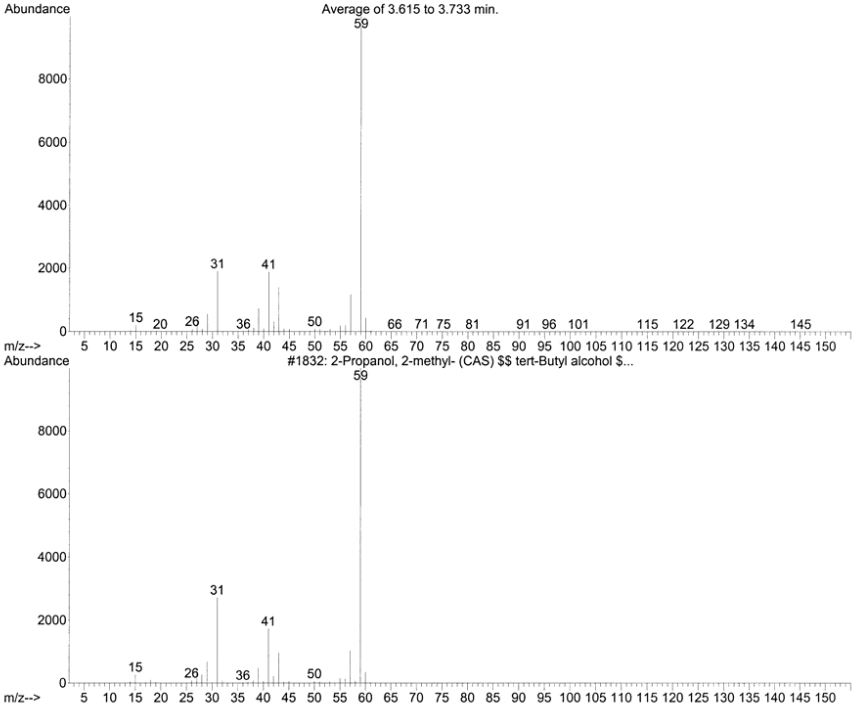
Figure 4. Total Ion Chromatogram (top) and Mass Spectrum (bottom) of Vendor-Supplied TBA (Analyzed by Y. Hong on 11-10-2005)



Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-05

Figure 5. EI Mass Spectrum of Vendor-Supplied TBA (top) and Library Search (bottom) (Analyzed by Y. Hong on 11-10-2005)

```
Library Searched : C:\Database\Wiley275.L
Quality          : 90
ID               : 2-Propanol, 2-methyl- (CAS) $$ tert-Butyl alcohol $$ t-Butanol $$ t
                   ert-Butanol $$ Trimethylmethanol $$ Trimethylcarbinol $$ 1,1-Dimeth
                   ylethanol $$ 2-Methyl-2-propanol $$ Ethanol, 1,1-dimethyl- $$ Potas
                   sium 1,1-dimethylethoxide $$ 2-methyl 2-propanol $$ te
```



Appendix C

Test Chemical Analysis Report: 2-^{14}C Tertiary Butyl Alcohol

Test Chemical Analysis Report

[2-^{14}C] Tertiary Butyl Alcohol

RTI Reference 12323-11

SUBMITTED TO:

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

TESTING FACILITY:

Science and Engineering
RTI International*
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P.O. Box 12194
Research Triangle Park, NC 27709-2194

Timothy R. Fennell, Ph.D. 12-18-2007
Date

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-11

In February 2006, 20 mCi of t-Butanol-2-^{14}C (TBA) was purchased from Sigma Chemical Co. (St. Louis, MO) (received on February 13th 2006). The material was obtained as a custom synthesis. The specific activity indicated by the vendor is 7.3 mCi/mmol. The product number is A8538-14C. The lot number is 095K9406

Purity from the Vendor Certificate of Analysis was 98.994 % by HPLC. No expiration date was indicated by the vendor.

RTI assigned this material Test Article Number of 12323-11. RTI confirmed the identity of the material using ^{1}H nuclear magnetic resonance spectroscopy. RTI confirmed the purity of the material by HPLC with radiochemical detection.

Nuclear Magnetic Resonance Spectroscopy.

All NMR data were acquired on a 300 MHz Bruker spectrometer. The ^{1}H NMR spectra were acquired with a relaxation delay of 1 sec, a 6173 Hz sweep width, and an 5.5-µsec pulse. The sample was prepared in $CDCl_3$ (deuterochloroform). Approximately 2 µl of the 2-^{14}C TBA was dissolved in approximately 800 µl $CDCl_3$.

The ^{1}H NMR spectrum of the sample contained two singlets at 1.274 and 1.495 ppm (Figure 1). The singlet at 1.274 ppm are attributed to the CH_3 groups. The singlet at 1.495 is attributed to the hydroxyl proton.

The ^{1}H NMR data from the sample are consistent with the structure of TBA.

HPLC Analysis

For HPLC analysis a sample of the radiolabeled TBA was prepared by a series of dilutions in distilled water. A sample of 2-^{14}C TBA was prepared by transferring approximately 5 µl to a 10 ml volumetric flask, and diluting to a total volume of 10 ml with distilled water. For analysis, 0.35 ml of this solution was diluted further to a total volume of 3 ml.

HPLC Analysis of ^{14}C-TBA was conducted on a Waters Atlantis dC18 column, 4.6 mm i.d. x 25 cm, 5 µ particle size. The mobile phase consisted of 98 % water and 2% acetonitrile. Chromatography was conducted using a system that consisted of 2X Waters 515 Pumps, Waters 717 Plus Autoinjector, with a ABI 759A UV detector, and a β-RAM 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 215 nm.

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-11

After injection of 30-µl aliquot of the 2-^{14}C TBA 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 radioactive from the column, triplicate aliquots of the 2-14C TBA 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 22 minutes (Figure 2). The purity was 98.16 %, (Std. Dev. 0.27%). The recovery from the column was 96.9% (Std. Dev. 2.9%).

We were unable to detect unlabeled TBA by UV absorbance, and thus have not been able to establish that the 14C peak co-elutes from the HPLC column with unlabeled TBA.

Conclusion

The test substance 12323-11 t-butanol-2-^{14}C obtained from Sigma Aldrich was NMR spectrum that was consistent with that expected for tertiary butyl alcohol. The purity of the test substance was 98.16% by HPLC with detection of radioactivity. This was similar to the Vendor-supplied purity of the test chemical of 99.0%, and indicates that the radiolabeled material is stable.

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-11

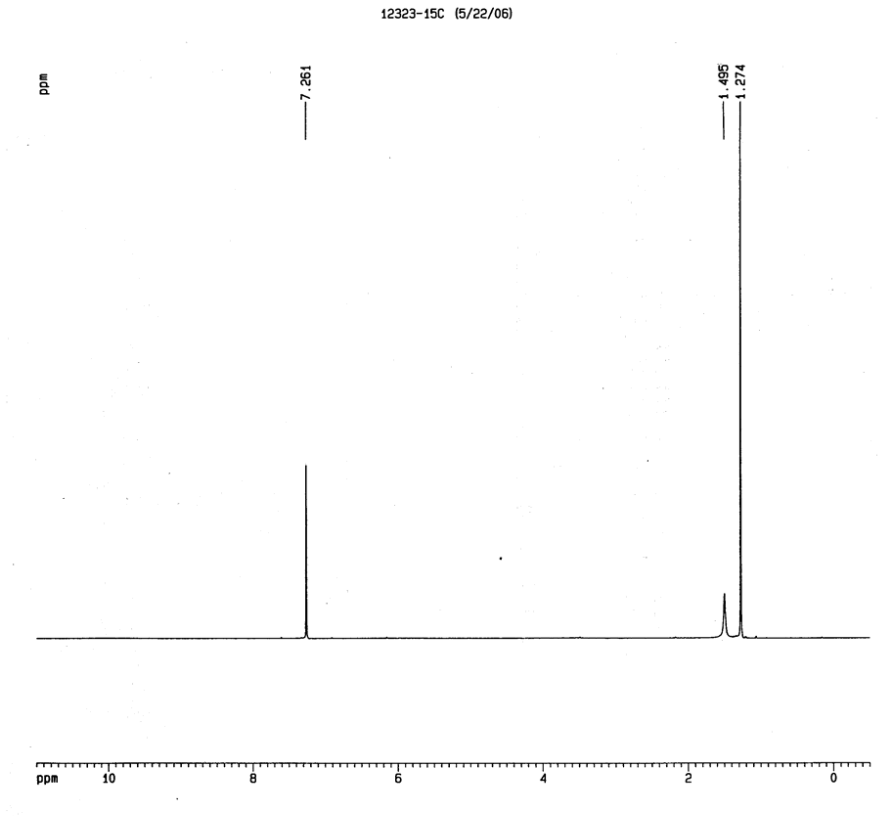

Figure 1. 300 MHz ^{1}H NMR of Vendor-Supplied 2-^{14}C TBA (Analyzed on 05-22-06 by J. Burgess)

Test Chemical Analysis Report **Tertiary Butyl Alcohol**
RTI Reference 12323-11

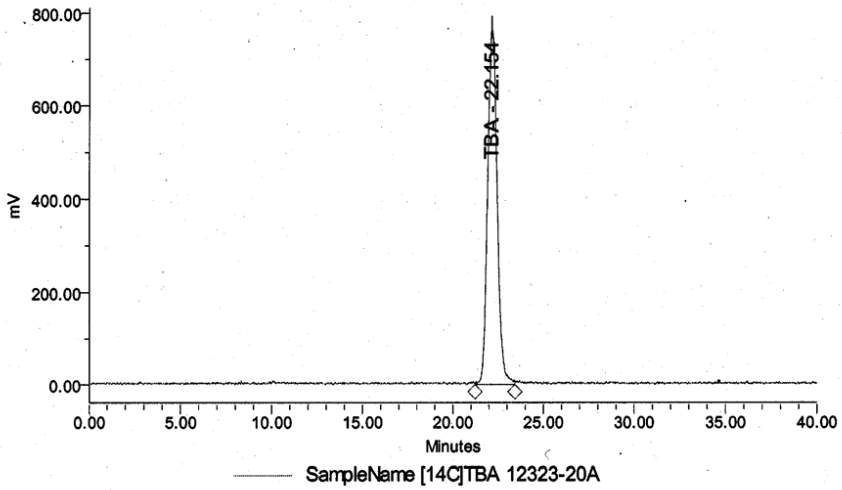

Figure 2. HPLC Radiochromatogram of 2-^{14}CTBA. (Analyzed by N. Gaudette on 05-25-2006)

Appendix D

Reference Standards Analysis Report

Reference Standards Analysis Report

2-Methylpropane-1,2-diol and 2-Hydroxyisobutyric Acid

SUBMITTED TO:

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TESTING FACILITY:

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Timothy R. Fennell, Ph.D. 12-18-2007 Date

Reference Standards Analysis Report **Tertiary Butyl Alcohol**

2-Hydroxyisobutyric Acid

2-Hydroxyisobutyric acid (HBA) was obtained from Alfa Aesar on February 4th, 2005. The catalog number was A13146, Lot Number J5164C, 25 g, with a nominal purity specified by the Vendor as 99%. The CAS number specified was 594-61-6. The purity specified by the Vendor's certificate of analysis was 98.9%,

This material was assigned a chemical receipt number of BOC-B-0439.

Nuclear Magnetic Resonance Spectroscopy.

All NMR data were acquired on a 300 MHz Bruker spectrometer. The ^{1}H NMR spectra were acquired with a relaxation delay of 1 sec, a 6173 Hz sweep width, and a 5.5-µsec pulse. The ^{1}H-decoupled ^{13}C NMR spectrum was acquired with a relaxation delay of 2 sec, a sweep width of 23810 Hz, and a 5.5-µsec pulse. The sample for analysis was prepared by dissolving 22.7 mg of the HBA in approximately 750 µl deuterated water (D_2O).

The ^{1}H NMR spectrum of the sample of HBA contained one singlet at 1.304 ppm (Figure 1). The singlet at 4.678 ppm is attributed to water The ^{1}H NMR data from the sample are consistent with the structure of 2-hydroxyisobutyric acid, which contains only methyl groups as non-exchangeable protons. The absence of other signals in the ^{1}H spectrum is consistent with the purity specified by the vendor.

The ^{13}C NMR spectrum of the sample of HBA (Figure 2) contains three signals. The singlet at 26.555 ppm is assigned to the two methyl groups of HBA. The singlet at 72.746 ppm is consistent with that expected for the 2-carbon. The singlet at 180.529 ppm is consistent with that expected for the carboxyl group.

2-Methylpropanediol

Synthesis

2-Methylpropane diol was prepared at RTI using the method of Bernauer et al. (1998).

A mixture containing 20 mmol of sodium borohydride ($NaBH_4$) (755 mg), 60 ml isopropanol and 50 mmol of ethyl hydroxyisobutyrate (Alfa Aesar, 99%, Lot H3426A) (6.9 ml) was stirred overnight at room temperature. Then, 2N hydrochloric acid was added to reaction in order to dissolve the precipitated solid. The clear solution was extracted with ethyl ether in triplicates. The organic phase was combined, dried over potassium carbonate and filtered. To remove solvents, ethyl ether was removed by rotary evaporator at room temperature, and 2-

propanol was removed at 45ºC under vacuum with water aspirator. The remaining material was purified by repeated fractional distillation under vacuum to isolate 2-methyl-1,2-propanediol (50ºC at 1.3 mm Hg).

Gas chromatograhic analysis of 2-Methyl-1,2-Propanediol

For gas chromatographic analysis, 10 µl of 2-methyl-1,2-propanediol was mixed with 100 µl of acetone. The purity was accessed using a Hewlett-Packard 6890 gas chromatograph equipped with a split inlet, a DB-624 column (30m x 0.32mm i.d. 1.8-µm film thickness, J&W) and a flame ionization detector. Injection (1 µl) was made with helium gas as the carrier gas (9.2 psi, 2 ml/min flow) with split ratio (50:1) at injection temperature of 200ºC. The oven temperature was held at 35ºC for 1 min, raised to 230ºC at 10 ºC/min. The temperature, hydrogen and air flow rates to the detector were 250ºC, 30 ml/min and 300 ml/min, respectively.

A single peak major peak with a retention time of approximately 9.5 minutes was observed. Excluding the peak associated with acetone used as solvent, the purity of the 2-methyl-1,2-propanediol as 96.7 %.

NMR Analysis

All NMR data were acquired on a 300 MHz Bruker spectrometer. The ^{1}H NMR spectra were acquired with a relaxation delay of 1 sec, a 6173 Hz sweep width, and a 5.5-µsec pulse. The ^{1}H-decoupled ^{13}C NMR spectrum was acquired with a relaxation delay of 2 sec, a sweep width of 23810 Hz, and a 5.5-µsec pulse. The sample for analysis was prepared by dissolving 17.8 mg of the 2-methyl-1,2-propanediol in approximately 750 µl deuterated water (D_2O).

The ^{1}H NMR spectrum of the sample of 2-methyl-1,2-propanediol contained one six-proton singlet at 1.076 ppm (Figure 3), and a second two-proton singlet at 3.298 ppm. The singlet at 4.683 ppm is attributed to water The ^{1}H NMR data from the sample are consistent with the structure of 2-methyl-1,2-propanediol, which contains two identical methyl groups and a methylene group as non-exchangeable protons.

The ^{13}C NMR spectrum of the sample of 2-methyl-1,2-propanediol (Figure 4) contains three signals. The large singlet at 24.8987 ppm is assigned to the two methyl groups of 2-methyl-1,2-propanediol. The singlet at 70.0777 ppm is consistent with that expected for the 1-carbon. The singlet at 71.7239 ppm is consistent with that expected for the 2-carbon.

Conclusion

NMR analysis of the 2-hydroxyisobutyric acid standard obtained produced spectra that was consistent with expectations. The NMR analysis of the

synthesized 2-methyl-1,2-propanediol was consistent with that reported previously by Bernaur et al. (1998).

References

Bernauer, U., Amberg, A., Scheutzow, D., and Dekant, W. (1998). Biotransformation of ^{12}C- and 2-^{13}C-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and tert-butyl alcohol in rats: identification of metabolites in urine by ^{13}C nuclear magnetic resonance and gas chromatography/mass spectrometry. *Chem Res Toxicol* 11, 651-658.

Reference Standards Analysis Report **Tertiary Butyl Alcohol**

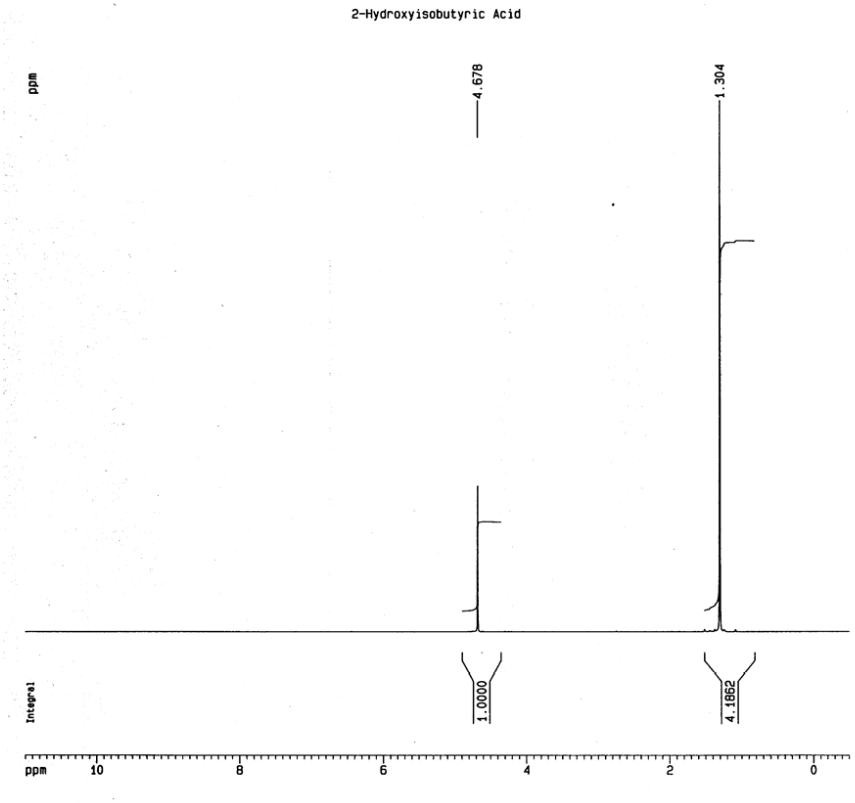

Figure 1. 300 MHz ^{1}H NMR of Vendor-Supplied HBA (Analyzed on 05-23-06 by J. Burgess)

Reference Standards Analysis Report **Tertiary Butyl Alcohol**

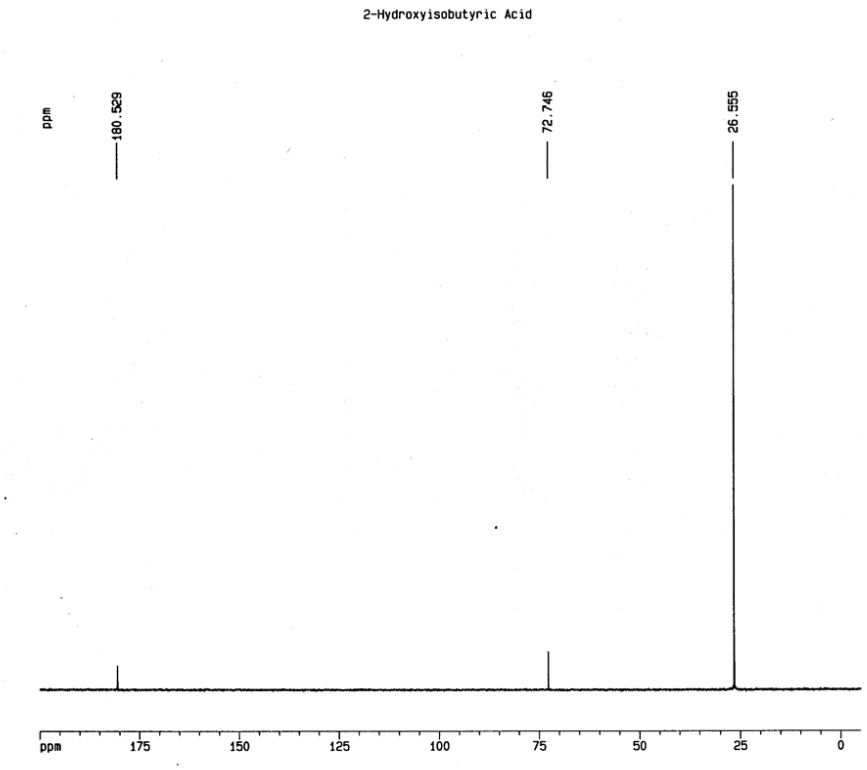

Figure 2. 75 MHz ^{1}H-Decoupled ^{13}C NMR spectrum of Vendor-Supplied HBA (Analyzed on 05-23-06 by J. Burgess)

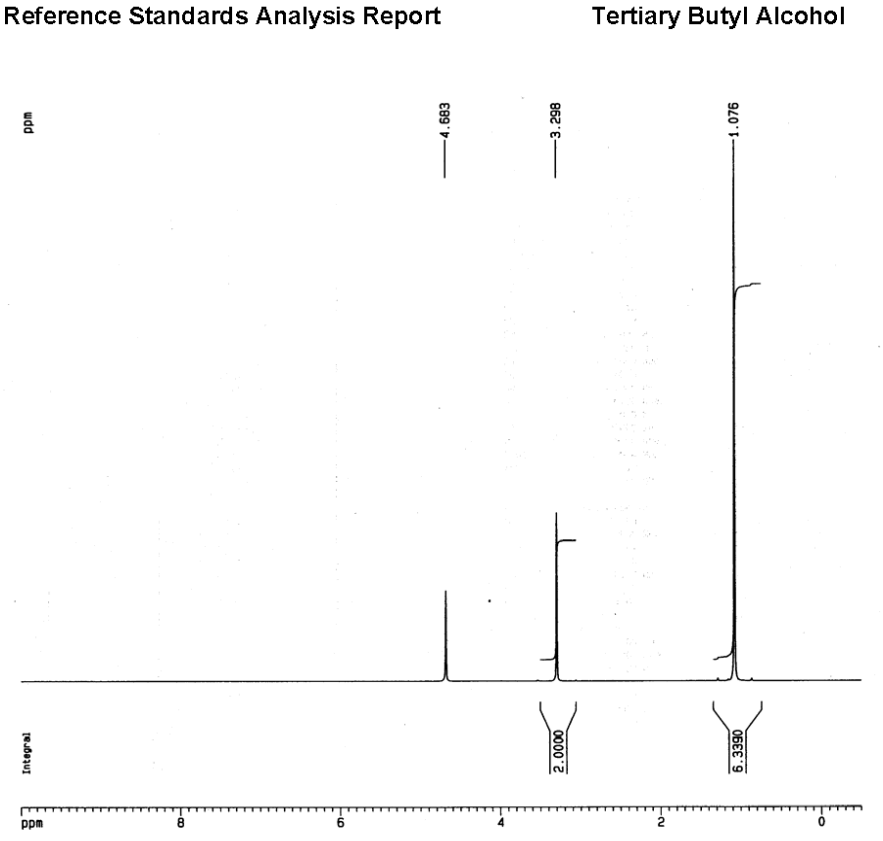

Figure 3. 300 MHz ^{1}H NMR of synthesized 2-methylpropane-1,2-diol (Analyzed on 05-24-06 by J. Burgess)

Reference Standards Analysis Report **Tertiary Butyl Alcohol**

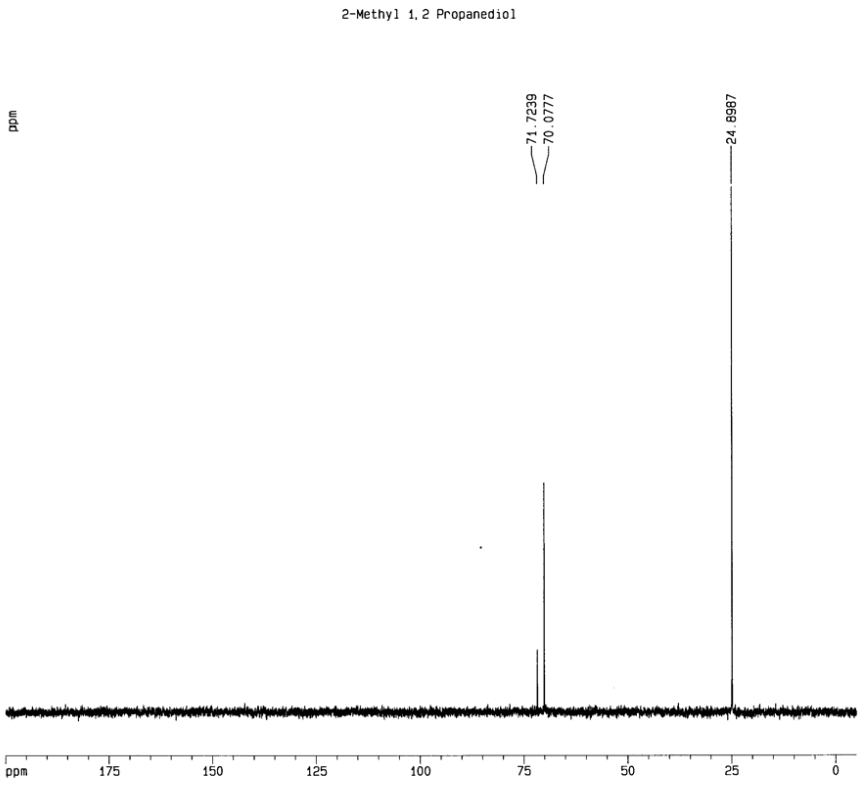

Figure 4. 75 MHz ^{1}H-Decoupled ^{13}C NMR spectrum of synthesized 2-methylpropane-1,2-diol (Analyzed on 05-24-06 by J. Burgess)

Appendix E

Pre-Study Inhalation Report

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Protocol Title

Metabolism and Pharmacokinetics of *Tertiary* Butyl Alcohol (TBA) in Male Rats: Pilot Study

Appendix Title

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

Study Protocol

RTI-935

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RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

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Research Associate

R. Arden James 07/11/2006
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Report Reviewed and Approved by:

Brian L Wong 7/13/2006
Dr. Brian Wong Date
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Report Reviewed by:

Timothy R. Fennell II 12/20/2007
Dr. Tim Fennell Date
RTI Study Director

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Inhalation Summary Report Version Information

Version		Page No.	Table No.	Change	Reason	Results
1	Original	N/A	N/A	N/A	N/A	N/A
2	Amended 11-Jul-06	12	Table 2	Calibration Curve Slope for MIRAN 4121 from y = 0.0423x to y = 0.0426x	Typing Error	Did not change data
		15	Table 5	Exhaust ACC (ppm) at 125 minutes from 1725 ppm to 1703 ppm	Recording Error	Table 4 (page 14) ACC – Exhaust (ppm) Daily mean from 1724 ppm to 1722 ppm

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RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Statement of Quality Assurance

This setup and evaluation of the nose only inhalation exposure system for *tertiary* butyl alcohol 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 nose only inhalation setup in January 2006 and the data and inhalation summary report in April 2006.

Patricia O'Brien Pomerleau 07/15/2006

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

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Introduction

The purpose of this study was to develop a nose-only inhalation exposure system for exposure of male rats to *tertiary* butyl alcohol (TBA). 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. Acceptable system verification at RTI was completed between October of 2005 and April of 2006.

Materials and Methods

Chemical

All liquid *tertiary* butyl alcohol (CAS No. 75-65-0) 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 TBA from a gas tight syringe which was surrounded by a heating block. The heating block was heated to approximately 145 deg F. Using a syringe pump (Harvard Apparatus, Model 956, Holliston, MA) liquid TBA was metered into a stainless steel tee where the liquid mixed with approximately 50 mL/min of nitrogen. The TBA 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 ($\geq$-0.20 in H_20) 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, nonrebreathing 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

TBA 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 TBA 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

TBA 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 (≤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 exhaust MIRAN sampled the total exhaust flow of the nose only tower, which varied, dependent upon the total number of open ports. 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 TBA into a closed loop, using a metal bellows pump to circulate the test chemical vapor. 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 during the system verifications completed at CIIT-CHR. System verification at RTI indicated that the calibrations remained unchanged. 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.

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[a]:

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

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): 0.78 g/ml
Molecular Volume (MV): 24.5 L/mole
Molecular Weight (MW): 74.12 g/mole

[a] Reference: Moss, OR: Calibration of gas and vapor samplers, in Sampling Instruments, 8th 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 *tertiary* butyl alcohol 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 4% for all of the sampling sites.

Generation and Chamber Concentration

Table 4 shows the summary data for TBA generation and characterization for the system verification. The mean and standard deviation of the values for analytical concentration (ACC), exposure temperature, exposure relative humidity and exposure static pressure are shown.

The individual analytical chamber concentration readings during system verification are shown in Table 5. The mean (with standard deviation) represents the average of the daily mean.

Deviations

Exposure chambers

There were no deviations.

SOP

There were no deviations.

Conclusion

The target concentration of TBA 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 April 2006.

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Table 1. MIRAN [1] Operating Conditions at RTI.

Instrument	MIRAN	MIRAN	MIRAN
Serial No.	3064	4121	4159
Sampling Location:	Inlet	Exhaust	Room Air
Pathlength:	15.75 meters	15.75 meters	15.75 meters
Wavelength:	9.9 microns	9.75 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:	4	10	4
Calibration Range: Low	0 – 686 ppm	0 – 686 ppm	0 – 686 ppm
Calibration Range: Mid	686 – 1600 ppm	686 – 1600 ppm	686 – 1600 ppm
Calibration Range: High	1600 – 2286 ppm	1600 – 2286 ppm	1600 – 2286 ppm
Curve Fit	Piecewise	Piecewise	Piecewise

[1] MIRAN is an Infrared Spectrophotometer

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 are the X variable and the chart divisions are the Y variable.

	MIRAN 3064	MIRAN 4121	MIRAN 4159
Lowest Readable Chart Division	0.1	0.1	0.1
Calibration Curve Slope[1]	y = 0.0435x	y = 0.0426x	y = 0.0442x
Calibration Curve Y Intercept[1]	0.0	0.0	0.0
ELOD (ppm)	2	2	2

[1]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.

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

Exposure Target Concentration	1750 ppm
Sample Position	(13 Open Ports)
Home [1]	1306
1	1196
2	1232
3	1220
4	1315
5	1291
6	1268
7	1303
8	1279
TPCV (%)[2]	3.34
WPCV (%)[3]	1.16
BPCV (%)[4]	3.13

[1] Average of sample position 9 MIRAN readings. Sample position 9 was the home port.
[2] TPCV = Total Port Measurements Coefficient of Variation: (St Dev TP/Average TP) * 100
[3] WPCV = Within Port Measurements Coefficient of Variation: (St Dev WP/Average WP) * 100
[4] BPCV = Between Port Measurements Coefficient of Variation: SqRt [$(TPCV)^2 - (WPCV)^2$]

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 50 mL/min of house air to decrease the time needed to fill the MIRAN cell and reach a stable concentration reading.

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

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

	Target (ppm)	1750
	Number of Open Ports	13
ACC - Inlet (ppm)	**Daily mean**	**1681**
	Std Dev	73
	No. of Data Points	14
ACC - Exhaust (ppm)	**Daily mean**	**1722**
	Std Dev	36
	No. of Data Points	14
Exposure Temperature (°F)	**Daily mean**	**72.8**
	Std Dev	0.2
	No. of Data Points	14
Exposure Relative Humidity (%)	**Daily mean**	**47.6**
	Std Dev	0.2
	No. of Data Points	14
Exposure Static Pressure (in H_20)	**Daily mean**	**-0.08**
	Std Dev	0.01
	No. of Data Points	14

ACC: Analytical Chamber Concentration

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Table 5. Nose only System Verification Data with 13 Open Ports.

These data represent the individual concentration readings during system verification.

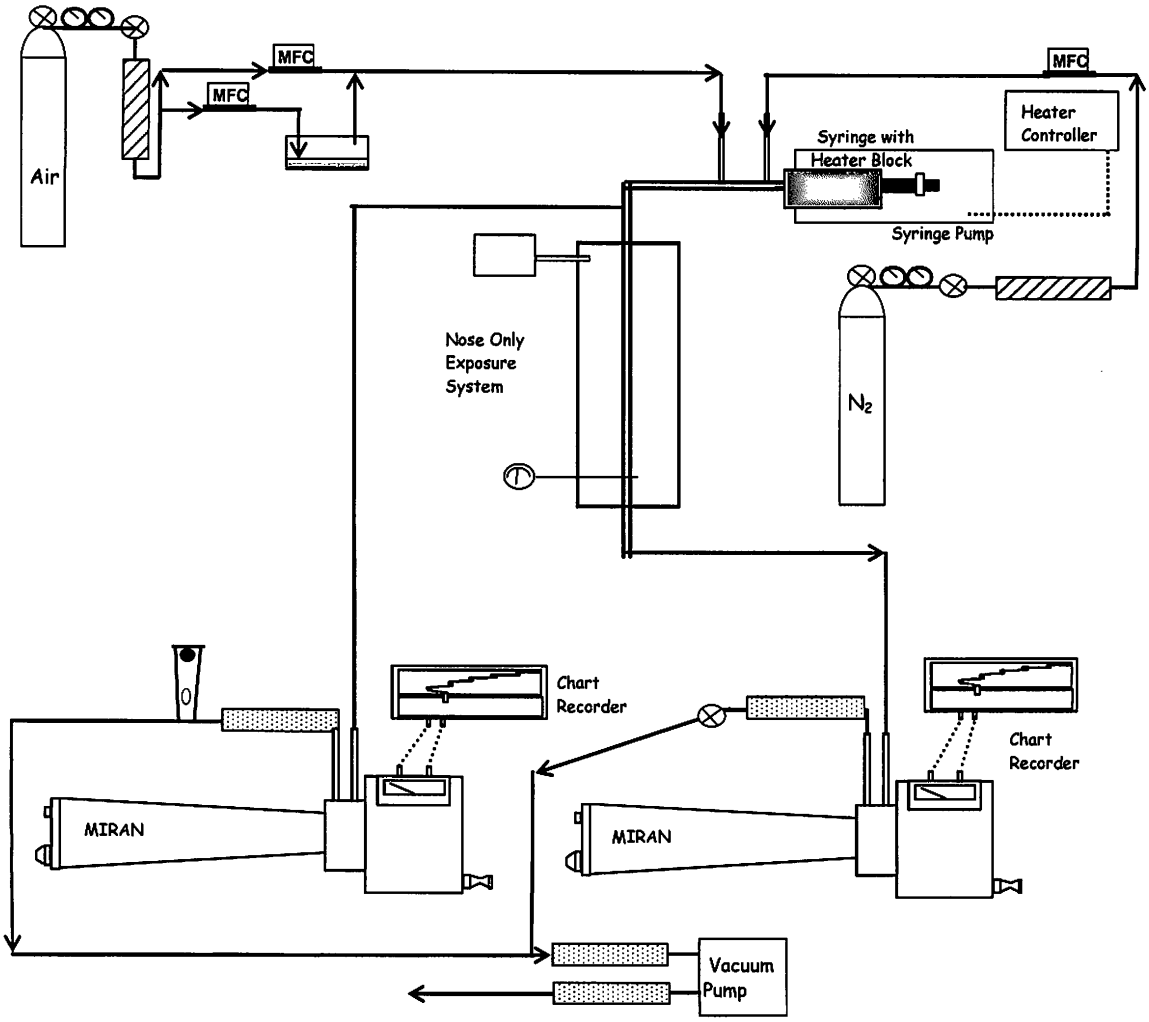
Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)
35	1447	1784
65	1646	1725
95	1660	1695
125	1703	1703
155	1703	1725
185	1703	1710
225	1689	1695
245	1689	1695
275	1689	1695
315	1689	1680
335	1689	1695
365	1732	1784
395	1746	1770
425	1746	1755
Mean	**1681**	**1722**
St Dev	73	36
No of Data Points	14	14

ACC: Analytical Chamber Concentration

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Figure 1: Example of Diagram of Exposure System.

MFC	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 Report

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Protocol Title

Metabolism and Pharmacokinetics of *Tertiary* Butyl Alcohol in Male Rats: Pilot Study

Appendix Title

Inhalation Summary Report: ^{14}C-TBA/TBA Nose-Only Inhalation Exposure at RTI International [1].

Study Protocol

RTI-935

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RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

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Report Reviewed by:

Timothy R. Fennell 12/20/2007
Dr. Tim Fennell Date
RTI Study Director

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RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

CIIT Centers for Health Research

QUALITY ASSURANCE STATEMENT

RTI Study Number: RTI-935 CIIT Principal Investigator: R. Arden James

RTI Protocol Title: Metabolism and Pharmacokinetics of Tertiary Butyl Alcohol in Male Rats: Pilot Study

Inhalation Portion: ^{14}C-TBA Nose-Only Inhalation Exposure at RTI International

Protocol No.: RTI-935 (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 CIIT Centers for Health Research (CIIT) staff. Phase inspections, data and inhalation summary report reviews were performed by the CIIT 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 CIIT Quality Assurance Unit inspections and the dates the results were reported to the CIIT Principal Investigator, CIIT Management, the Testing Facility's Study Director and Testing Facility's Management are noted below.

Phase(s)	**Inspection** Date(s)	Date Reported to CIIT Principal Investigator/Management	Date Reported to Testing Facility's Study Director/Management
	(MM/DD/YY)	**(MM/DD/YY)**	**(MM/DD/YY)**
Protocol	05/24/2006	05/25/2006	05/30/2006
Inhalation Phase	06/01/2006	06/02/2006	09/28/2006
Protocol Amendment, Draft Inhalation Summary Report, and Data	09/29/2006, 10/03/2006 and 10/04/2006	10/05/2006	11/29/06
Final Inhalation Summary Report	11/28/06	11/28/06	11/29/06

Patricia O'Brien Powers-Clem 11/29/2006
Quality Assurance Director Date
CIIT Centers for Health Research

R. Arden James 11/29/2006
R. Arden James, B.A. Date
Principal Investigator
Inhalation Manager

Brian D Wong 11/29/2006
Dr. Brian Wong Date
Senior Research Investigator

Inhalation Summary Report GLP Compliance Statement for CIIT Protocol No. RTI-935

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.

Only one sample for the stability of the ^{14}C-TBA/TBA under the conditions of administration was taken during the inhalation exposure instead of two as specified in the protocol. The sample was taken between the 322 to 327 minute mark near the end of the exposure. This deviation was discussed with the Study Director and it was decided that the deviation had no overall effect on the results of the study. The protocol deviation was written and distributed on June 01, 2006 by Study Director, Timothy R. Fennell.

R. Arden James, B.A.
Principal Investigator (CIIT Centers for Health Research)

11/29/2006
Date

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

12/20/2007
Date

RTI Inhalation Summary Report
Protocol RTI-935
Tertiary Butyl Alcohol

Introduction

The purpose of this study was to conduct a single nose-only inhalation exposure of male F-344 rats to ^{14}C *tertiary* butyl alcohol (TBA) and evaluate the effects. This report describes the inhalation portion of the exposure which was completed at RTI.

Summary

Rats were exposed nose-only to 1724 (±85) ppm ^{14}C-TBA/TBA for 6 hours. The concentration of ^{14}C-TBA/TBA 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 01-Jun-06 at RTI.

Materials and Methods

Chemical

All liquid ^{14}C *tertiary* butyl alcohol (CAS No. 75-65-0) and unlabeled TBA (^{14}C-TBA/TBA) used during the exposure were obtained from RTI personnel.

The test chemical mixture was received from the principal investigator as a solid contained within a flask with a teflon faced screw cap. The flask was placed within a Dri-Block heater (Dri-Block, MODEL DB-3, Arthur H. Thomas Co., Philadelphia, PA) at approximately 33 deg C, until in liquid form, at which time the test chemical mixture was loaded into the generation syringe.

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 once 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 1750 ppm ^{14}C-TBA/TBA. Exposure atmospheres were generated by metering liquid ^{14}C-TBA/TBA from a gas tight syringe which was surrounded by a heating block. The heating block was heated to approximately 145 deg F. Using a syringe pump (Harvard Apparatus, Model 956, Holliston, MA) liquid ^{14}C-TBA/TBA 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 ^{14}C-TBA/TBA 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 (≤-0.20 in H_20) static pressure.

The exposure was conducted using a Cannon-style nose-only exposure system (Lab Products, Seaford, DE) with 8 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, nonrebreathing system. The components of the generation system and delivery line were composed of glass, stainless steel, or teflon. These are materials that are chemically compatible

with ^{14}C-TBA/TBA 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.

Analytical System

The ^{14}C-TBA/TBA 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 ^{14}C-TBA/TBA 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}C-TBA/TBA 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 TBA into a closed loop, using a metal bellows pump to circulate the test chemical vapor. 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 2.3, 2.3, and 2.3 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 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 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

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 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[a]:

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

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):	0.78 g/ml
Molecular Volume (MV):	24.5 L/mole
Molecular Weight (MW):	74.12 g/mole

[a] Reference: Moss, OR: Calibration of gas and vapor samplers, in Sampling Instruments, 8th 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 ^{14}C-*tertiary* butyl alcohol was evaluated and will be reported by RTI. The stability of ^{14}C-TBA/TBA 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 (Version 2.0, Issued 11-Jul-06). The relative standard deviation was less than 4% for all of the sampling sites.

Generation and Chamber Concentration

Table 3 shows the summary data for the ^{14}C-TBA/TBA 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 1724 (±85) ppm, ACC-Outlet 1581 (±40) ppm; temperature, 72.8 (±0.5) deg F; relative humidity, 47.6 (±0.5) %, and static pressure, -0.05 (±0.00) inches of H_20. The mean concentration of 1724 (±85) ppm ^{14}C-TBA/TBA 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 3.53 L/min and remained unchanged through out the exposure. Approximately 11 mL of ^{14}C-TBA/TBA was loaded into the syringe. The syringe pump was set to deliver a nominal flow rate of 0.027 mL/min and remained unchanged through out the exposure. Animals were loaded onto the nose-only tower at 3 to 5 minute intervals beginning at 9:58 am with the last animal being loaded at 10:25 am. Each animal was exposed for a total of 6 hours. The first animal was removed from the nose-only tower at 3:58 pm and the last animal removed at 4:25 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 6 hours and 26 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 1972 ppm ^{14}C-TBA/TBA. The ratio of analytical concentration to nominal concentration was 87%.

Deviations

Protocol

The stability of the ^{14}C-TBA/TBA under the conditions of administration was sampled only once during the exposure. 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 CIIT personnel while conducting the study will be retained within CIIT-CHR Archive for ten years following issuance of the final report.

Conclusion

Rats were exposed nose-only to 1724 (±85) ppm ^{14}C-TBA/TBA for 6 hours. The concentration of ^{14}C-TBA/TBA 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 01-Jun-06 at RTI.

Table 1. MIRAN [1] Operating Conditions at RTI.

Instrument	MIRAN	MIRAN	MIRAN
Serial No.	3064	4121	4159
Sampling Location:	Inlet	Exhaust	Room Air
Pathlength:	15.75 meters	15.75 meters	15.75 meters
Wavelength:	9.9 microns	9.75 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:	4	10	4
Calibration Range: Low	0 – 686 ppm	0 – 686 ppm	0 – 686 ppm
Linear Regression	y = 0.0433x + 0	y = 0.0426x + 0	y = 0.0442x +0
Calibration Range: Mid	686 – 1600 ppm	686 – 1600 ppm	686 – 1600 ppm
Linear Regression	y = 0.0396x + 2.6816	y = 0.038x + 2.8348	y = 0.0393x + 3.595
Calibration Range: High	1600 – 2286 ppm	1600 – 2286 ppm	1600 – 2286 ppm
Linear Regression	y = 0.0351x + 9.7207	y = 0.0336x + 10.041	y = 0.0337x + 12.437
Curve Fit [2]	Linear	Linear	Linear

[1] MIRAN is an Infrared Spectrophotometer

[2]The calibration curve for each MIRAN was broken into 3 segments and a linear regression was calculated for each segment.

Table 2. Estimated Limit of Detection for each MIRAN [1].

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 3064	MIRAN 4121	MIRAN 4159
Lowest Readable Chart Division	0.1	0.1	0.1
Calibration Curve Slope[2]	y = 0.0433x	y = 0.0426x	y = 0.0442x
Calibration Curve Y Intercept[2]	0.0	0.0	0.0
ELOD (ppm)	2.3	2.3	2.3

[1] MIRAN is an infrared spectrophotometer

[2]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. Summary of Data for ^{14}C-TBA/TBA Exposure at RTI.

	Target (ppm)	1750
	Number of Open Ports	12
ACC - Inlet (ppm)	**Daily mean**	**1724**
	Std Dev	85
	No. of Data Points	13
ACC - Exhaust (ppm)	**Daily mean**	**1581**
	Std Dev	40
	No. of Data Points	13
Exposure Temperature (°F)	**Daily mean**	**72.8**
	Std Dev	0.5
	No. of Data Points	13
Exposure Relative Humidity (%)	**Daily mean**	**47.6**
	Std Dev	0.5
	No. of Data Points	13
Exposure Static Pressure (in H_20)	**Daily mean**	**-0.05**
	Std Dev	0.00
	No. of Data Points	13

ACC: Analytical Chamber Concentration
TBA: *Tertiary* Butyl Alcohol

Table 4. ^{14}C-TBA/TBA Nose-Only Exposure Data.

These data represent the individual readings during exposure.

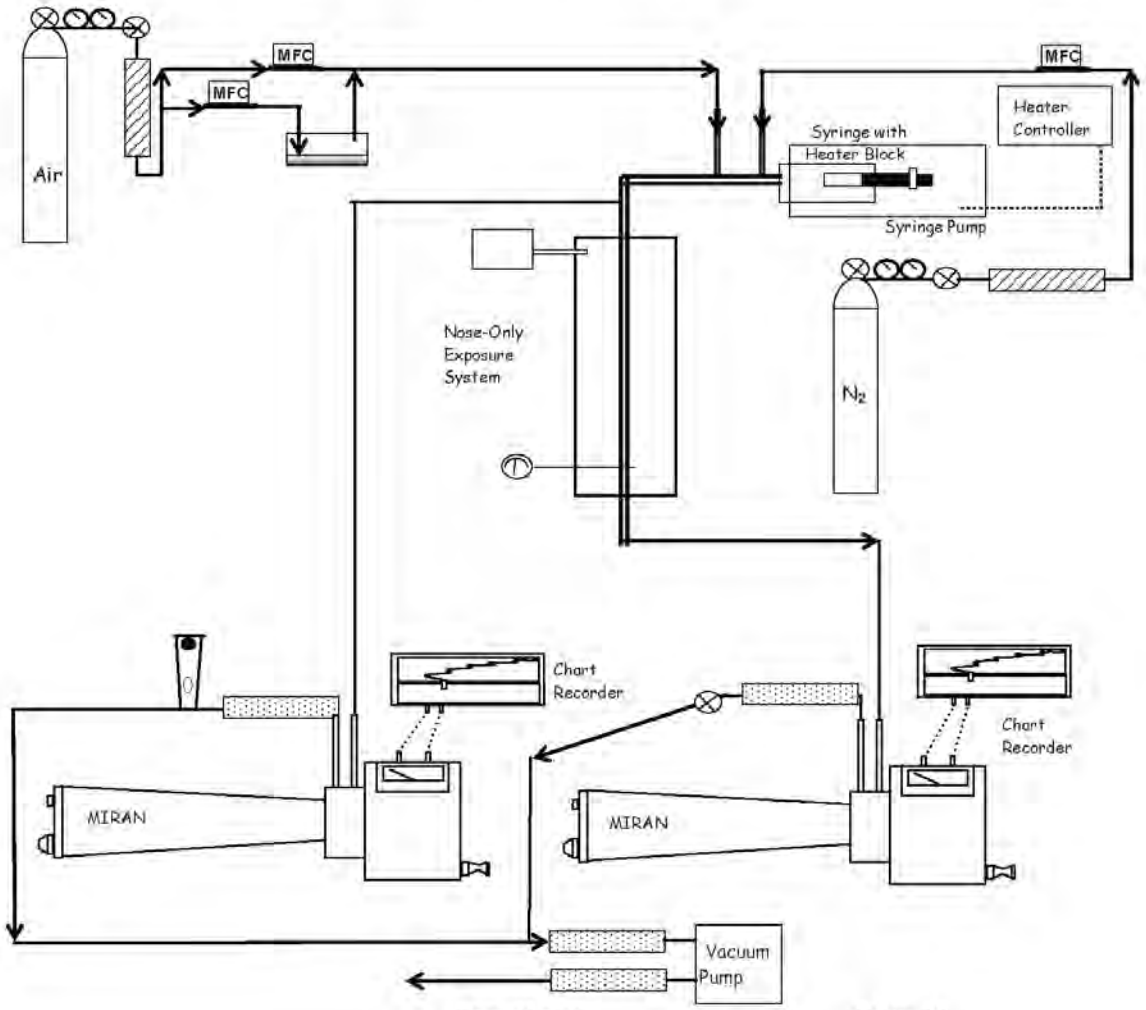
Exposure Minutes	Inlet ACC (ppm)	Exhaust ACC (ppm)	Temperature (deg F)	Relative Humidity (%)	Static Pressure (in of H_20)
32	1447 [1]	1531	72.7	47.8	-0.05
62	1689	1544	72.2	48.3	-0.05
92	1746	1583	72.1	48.4	-0.05
122	1760	1570	72.3	48.2	-0.05
152	1760	1583	72.5	47.9	-0.05
182	1760	1557	72.7	47.7	-0.05
212	1746	1557	72.7	47.7	-0.05
242	1746	1583	72.9	47.5	-0.05
272	1746	1570	73.0	47.4	-0.05
302	1746	1583	73.2	47.1	-0.05
332	1760	1583	73.4	47.0	-0.05
362	1746	1610	73.4	47.0	-0.05
387	1760	1695	73.4	47.1	-0.05
Mean	**1724**	**1581**	**72.8**	**47.6**	**-0.05**
St Dev	85	40	0.5	0.5	0.00
No of Data Points	13	13	13	13	13

ACC: Analytical Chamber Concentration
TBA: *Tertiary* Butyl Alcohol

[1] 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.

Figure 1. Example of Diagram of Exposure System.

MFC	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 G

Example Calculations

Formulae for Calculations

Formulae used in the Debra system for calculations are shown below:

Aliquot dpm/g is calculated by:

dpm/g = aliquot corrected count (dpm) / aliquot weight (g)

The mean of these aliquot figures for the sample is calculated by:

Mean dpm/g = Sum of all aliquot dpm/g / 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):

Weight of the sample = sample weight – 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_2 trap, Cage Wash) then:

Effective weight = weight of the sample

If processing type uses homogenate (e.g. for feces and carcass) then:

Effective weight = Homogenate weight

To calculate the total dpm in the sample, Debra uses the previously calculated figures;

Total Dpm = Mean dpm/g * 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:

Recovery = 100* (Total Dpm/ Dose Dpm)

Concentration is calculated by:

Concentration = (Total dpm/ effective weight) / (Specific activity)

Results are expressed in terms of the arithmetic mean ± 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_2 trap, and cage rinse made in the Debra™ system are shown below:

Subject = 2-05
Tissue = Urine
Timepoint = 24 h
Aliquot 1 dpm/g = 1472068.215/0.5091
= 2891510.9310548 dpm/g
Aliquot 2 dpm/g = 1496832.465/0.5175
= 2892429.88405797 dpm/g
Sum of dpm/g = 5783940.81511277 dpm/g
Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots
= 5783940.81511277 / 2
= 2891970.40755639 dpm/g

Recovery Effective Wt
= Sample weight
= 3.1866g

Concentration Effective Weight
= Sample weight
= 3.1866g

Total dpm = Dpm/g x Recovery effective weight
= 2891970.40755639 x 3.1866
= 9215552.90071918 dpm

Dose dpm (using Exposure Dose)
= sum of all dpm recovered
= 49473192.2325294 dpm

Recovery = 100 x (Total dpm / Dose dpm)
= 100 x (9215552.90071918 / 49473192.2325294)
= 100 x (0.186273666300025)
= 18.6273666300025%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)
= (9215552.90071918 / 3.1866) / (0.2506030761 µCi/mg)
= (2891970.40755639 dpm/g) / (556338.828942 dpm/mg)
= 5.19821780740363 mg/g

Subject = 2-05
Tissue = Feces
Timepoint = 24 h
Aliquot 1 dpm/g = 3912.08625/0.1122
= 34867.0788770053 dpm/g
Aliquot 2 dpm/g = 3094.52625/0.0848
= 36492.0548349057 dpm/g
Sum of dpm/g = 71359.133711911 dpm/g
Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots
= 71359.133711911 / 2
= 35679.5668559555 dpm/g

Recovery Effective Weight
= Homogenate weight
= 12.8805g

Concentration Effective Weight
= Sample weight

= 6.3064g

Total dpm = Dpm/g x Recovery effective weight
= 35679.5668559555 x 12.8805
= 459570.660888135 dpm

Dose dpm (using Exposure Dose)
= sum of all dpm recovered
= 49473192.2325294 dpm

Recovery = 100 x (Total dpm / Dose dpm)
= 100 x (459570.660888135 / 49473192.2325294)
= 100 x (0.00928928658430009)
= 0.928928658430009%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)
= (459570.660888135 / 6.3064) / (0.2506030761 µCi/mg)
= (72873.6935316718 dpm/g) / (556338.828942 dpm/mg)
= 0.130987969454257 mg/g

Subject = 2-05
Tissue = Carcass Digest
Timepoint = 168 h
Aliquot 1 dpm/g = 412.47/0.8479
= 486.460667531549 dpm/g
Aliquot 2 dpm/g = 451.32/0.9093
= 496.337842296272 dpm/g
Sum of dpm/g = 982.79850982782 dpm/g
Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots
= 982.79850982782 / 2
= 491.39925491391 dpm/g

Recovery Effective Weight
= Homogenate weight
= 583.38g

Concentration Effective Weight
= Sample weight
= 214.6g

Total dpm = Dpm/g x Recovery effective weight
= 491.39925491391 x 583.38
= 286672.497331677 dpm

Dose dpm (using Exposure Dose)
= sum of all dpm recovered
= 49473192.2325294 dpm

Recovery = 100 x (Total dpm / Dose dpm)
= 100 x (286672.497331677 / 49473192.2325294)
= 100 x (0.00579450171689518)
= 0.579450171689518%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)
= (286672.497331677 / 214.6) / (0.2506030761 µCi/mg)
= (1335.84574711872 dpm/g) / (556338.828942 dpm/mg)
= 0.0024011370007359 mg/g

Subject = 2-05
Tissue = CO2 Trap 1
Timepoint = 48 h
Aliquot 1 dpm/g = 52.13875/1.0448
= 49.9030915007657 dpm/g
Aliquot 2 dpm/g = 68.56875/1.0425
= 65.773381294964 dpm/g

Sum of dpm/g = 115.67647279573 dpm/g
Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots
= 115.67647279573 / 2
= 57.8382363978649 dpm/g

Recovery Effective Wt
= Sample weight
= 529.19g

Concentration Effective Weight
= Sample weight
= 529.19g

Total dpm = Dpm/g x Recovery effective weight
= 57.8382363978649 x 529.19
= 30607.4163193861 dpm

Dose dpm (using Exposure Dose)
= sum of all dpm recovered
= 49473192.2325294 dpm

Recovery = 100 x (Total dpm / Dose dpm)
= 100 x (30607.4163193861 / 49473192.2325294)
= 100 x (0.000618666694793575)
= 0.061866694793575%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)
= (30607.4163193861 / 529.19) / (0.2506030761 µCi/mg)
= (57.8382363978649 dpm/g) / (556338.828942 dpm/mg)
= 0.000103962249961695 mg/g

Subject = 2-05
Tissue = Exhaled VOC Trap 1
Timepoint = 48 h
Aliquot 1 dpm/g = 2184.66857142857/0.0935
= 23365.4392666157 dpm/g
Aliquot 2 dpm/g = 2312.18857142857/0.0971
= 23812.4466676475 dpm/g
Sum of dpm/g = 47177.8859342632 dpm/g
Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots
= 47177.8859342632 / 2
= 23588.9429671316 dpm/g

Recovery Effective Wt
= Sample weight
= 3.8622g

Concentration Effective Weight
= Sample weight
= 3.8622g

Total dpm = Dpm/g x Recovery effective weight
= 23588.9429671316 x 3.8622
= 91105.2155276557 dpm

Dose dpm (using Exposure Dose)
= sum of all dpm recovered
= 49473192.2325294 dpm

Recovery = 100 x (Total dpm / Dose dpm)
= 100 x (91105.2155276557 / 49473192.2325294)
= 100 x (0.00184150671134079)
= 0.184150671134079%

Concentration = (Total dpm/Concentration effective weight) / (Specific activity)
= (91105.2155276557 / 3.8622) / (0.2506030761 μCi/mg)
= (23588.9429671316 dpm/g) / (556338.828942 dpm/mg)
= 0.0424003174683873 mg/g

Subject = 2-05
Tissue = Cage Rinse
Timepoint = 168 h
Aliquot 1 dpm/g = 295.995/0.892
= 331.832959641256 dpm/g
Aliquot 2 dpm/g = 316.605/0.8937
= 354.263175562269 dpm/g
Sum of dpm/g = 686.096135203525 dpm/g
Number of aliquots = 2

Mean dpm/g = Sum of dpm/g / Number of aliquots
= 686.096135203525 / 2
= 343.048067601762 dpm/g

Recovery Effective Wt
= Sample weight
= 200.08g

Concentration Effective Weight
= Sample weight
= 200.08g

Total dpm = Dpm/g x Recovery effective weight
= 343.048067601762 x 200.08
= 68637.0573657606 dpm

Dose dpm (using Exposure Dose)
= sum of all dpm recovered
= 49473192.2325294 dpm

Recovery = 100 x (Total dpm / Dose dpm)
= 100 x (68637.0573657606 / 49473192.2325294)
= 100 x (0.00138735857276318)
= 0.138735857276318%
Concentration = (Total dpm/Concentration effective weight) / (Specific activity)
= (68637.0573657606 / 200.08) / (0.2506030761 μCi/mg)
= (343.048067601762 dpm/g) / (556338.828942 dpm/mg)
= 0.00061661715802606 mg/g

The concentrations of TBA determined in DMF extracts of Exhaled VOC Traps by gas chromatography were calculated based on a calibration curve, with a linear regression (y=bx+a) of slope 1280, intercept 2.856, and linear correlation coefficient of 0.992. Concentrations were measured as μg TBA/μl DMF. Using the regression equation, concentrations for samples were calculated from peak areas:

Concentration = (Peak Area – intercept)/Slope

Concentrations measured as μg TBA/μl DMF were converted to units of μg TBA/mg DMF (or mg TBA/g DMF) for comparison with values measured for radioactivity using the density of DMF for conversion, as follows

Concentration (μg TBA/mg DMF) = Concentration (μg TBA/μl DMF)/0.949

Appendix H

Raw Data Tables

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 DPM value is less than Bkg DPM, then the sample is flagged with an *

Column Heading	Source	Explanation
LOQ	Calculated	Limit of Quantitation. If Orig DPM value is less than 3 x Bkg DPM, 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 ^{14}C TBA Formulation (Feedstock) for Inhalation Exposure

[^{14}C] TBA Test Material			
Specific activity	7.3	mCi/mmol	(A)
Nonradiolabeled TBA Test Material			
Molecular weight	74.12	g/mol or mg/mmol	(B)
Density	0.781	mg/μL	(C)
Formulation (Feedstock)			
Nonradiolabeled TBA	8.9693	g	(D
[14C] TBA	0.0309	g	(E)
Net Feedstock Weight	9.0002	g	(F)=(D+E)
Total TBA present in the formulation (by weight)	9.0002	g	(F)=(D+E)
Total TBA present in the formulation (by weight)	9000.2	mg	(G)=(D+E)*100
Nominal Specific Activity			
Activity present	3.043308149	mCi	(H)=((E)*1000/(B))*(A)
[14C]TBA and nonradiolabeled TBA	9000.2	mg	(G)
Nominal Specific Activity	0.000338138	mCi/mg	(I)=(H)/(G)
Nominal Specific Activity	0.338137836	μCi/mg	(J)=(I)*1000
Nominal Specific Activity	25.06277638	μCi/mmol	(K)=(((H)*1000)/(G))*(B)
Formulation Dilution			
Weight of Feedstock	0.0121	g	(L)
Weight of Water	10.1036	g	(M)
Total weight of solution	10.1157	g	(N)=(L)+(M)

Dose Formulation Dilution Aliquots

Aliq #	Aliquot Wt (g)	DPM	DPM/g
1	0.1186	78813.9	664535.4
2	0.1237	82635.8	668034
3	0.1288	85502.5	663839.3
Mean dilution DPM/g			665469.6 (O)

Total Radioactivity in the Dilution	6731690.334	DPM	(P)=(M)*(O)
Radiochemical Concentration of the Feedstock	556338044.1	DPM/g	(Q)=(P)/(L)
Radiochemical Concentration of the Feedstock	250.6027226	μCi/g	(R)=(Q)/2220000
Specific Activity (μCi/mg TBA)	0.250602723	μCi/mg	(S)=(R)*(F)/(G)
Specific Activity (μCi/mmol TBA)	18.5746738	μCi/mmol	(T)=(R)*(B)/1000

Table 2. Recovery of Radioactivity in Samples from Group 1.

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	Recovery (%)
1-01	Feces	0 h	17.1491 g	19.1633 g	2.0142 g	21.3519 g	4.2028 g	0.0775 g 0.0589 g	2355.72 1813.94	19.27 19.27	2336.455 1794.675			30147.806 30469.864	127381.9731 dpm	0.215
1-01	Carcass Digest	0 h	107.92 g	293.96 g	186.04 g	783.61 g	675.69 g	0.4277 g 0.4578 g	37178.83 39886.08	4.03 4.03	37174.800 39882.050			86917.933 87116.754	58796748.8875 dpm	99.469
1-01	Exhaled VOC	0 h	15.1571 g	18.8776 g	3.7205 g	N.A.	N.A.	0.0905 g 0.0898 g	28.69 26.47	0.67 0.67	28.025 25.805			309.669 287.361	1110.6238 dpm	0.002
1-01	Nose Only Tube Rinse	0 h	81.9423 g	125.5502 g	43.6079 g	N.A.	N.A.	0.1507 g 0.1582 g	578.23 602.02	1.70 1.70	576.535 600.325			3825.713 3794.722	166155.5883 dpm	0.281
1-01	Transfer Bag Rinse	0 h	287.67 g	398.52 g	110.85 g	N.A.	N.A.	0.1642 g 0.1678 g	26.19 32.67	1.04 1.04	25.150 31.630			153.167 188.498	18936.7872 dpm	0.032
1-02	Feces	0 h	16.8063 g	17.2823 g	0.4760 g	18.4583 g	1.6520 g	0.0763 g 0.0668 g	1175.29 1021.04	19.27 19.27	1156.025 1001.775			15151.048 14996.632	24901.9839 dpm	0.041
1-02	Carcass Digest	0 h	107.28 g	300.18 g	192.90 g	769.27 g	661.99 g	0.4304 g 0.4431 g	39197.21 40119.34	4.03 4.03	39193.180 40115.310			91062.221 90533.311	60107213.0993 dpm	99.623
1-02	Exhaled VOC	0 h	15.0588 g	18.9152 g	3.8564 g	N.A.	N.A.	0.0923 g 0.0934 g	19.81 19.35	0.67 0.67	19.145 18.685			207.421 200.054	785.6933 dpm	0.001
1-02	Nose Only Tube Rinse	0 h	81.6788 g	129.1637 g	47.4849 g	N.A.	N.A.	0.1634 g 0.1525 g	684.85 640.58	1.70 1.70	683.155 638.885			4180.875 4189.410	198731.0728 dpm	0.329
1-02	Transfer Bag Rinse	0 h	288.36 g	528.87 g	240.51 g	N.A.	N.A.	0.1668 g 0.1601 g	1.45 4.79	1.04 1.04	0.410 3.750		* *	2.458 23.423	3112.3069 dpm	0.005
1-03	Feces	0 h	17.2105 g	19.0064 g	1.7959 g	22.0264 g	4.8159 g	0.0420 g 0.0532 g	1321.20 1669.08	19.27 19.27	1301.935 1649.815			30998.452 31011.560	149317.0097 dpm	0.244
1-03	Carcass Digest	0 h	107.93 g	296.94 g	189.01 g	836.10 g	728.17 g	0.4344 g 0.4404 g	36033.02 36553.10	4.03 4.03	36028.990 36549.070			82939.664 82990.622	60412728.2023 dpm	98.765
1-03	Exhaled VOC	0 h	15.1591 g	19.0830 g	3.9239 g	N.A.	N.A.	0.0910 g 0.0907 g	62.28 53.18	0.67 0.67	61.615 52.515			677.088 578.997	2464.3752 dpm	0.004
1-03	Nose Only Tube Rinse	0 h	80.3060 g	126.4599 g	46.1539 g	N.A.	N.A.	0.1481 g 0.1304 g	845.49 730.93	1.70 1.70	843.795 729.235			5697.468 5592.293	260533.2472 dpm	0.426
1-03	Transfer Bag Rinse	0 h	286.08 g	443.75 g	157.67 g	N.A.	N.A.	0.1773 g 0.1554 g	382.59 343.23	1.04 1.04	381.550 342.190			2152.002 2201.995	343247.3620 dpm	0.561
1-04	Feces	0 h	17.2253 g	20.6504 g	3.4251 g	23.7856 g	6.5603 g	0.1027 g 0.0603 g	13092.03 7614.69	19.27 19.27	13072.765 7595.425			127290.798 125960.614	830702.6192 dpm	1.670
1-04	Carcass Digest	0 h	107.69 g	286.22 g	178.53 g	763.68 g	655.99 g	0.4276 g 0.4506 g	31375.46 33159.92	4.03 4.03	31371.430 33155.890			73366.300 73581.647	48198191.8677 dpm	96.918
1-04	Exhaled VOC	0 h	15.2184 g	18.9558 g	3.7374 g	N.A.	N.A.	0.0899 g 0.0937 g	196.10 200.53	0.67 0.67	195.435 199.865			2173.915 2133.031	8048.3908 dpm	0.016
1-04	Nose Only Tube Rinse	0 h	77.6745 g	123.5568 g	45.8823 g	N.A.	N.A.	0.1296 g 0.1284 g	1935.39 1876.51	1.70 1.70	1933.695 1874.815			14920.486 14601.363	677265.1671 dpm	1.362
1-04	Transfer Bag Rinse	0 h	287.65 g	536.46 g	248.81 g	N.A.	N.A.	0.1486 g 0.1565 g	13.04 9.25	1.04 1.04	12.000 8.210			80.754 52.460	16572.4584 dpm	0.033

* 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 1.

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Recovery (%)
1-01	Feces	0 h	127381.9731 dpm	59110333.860 dpm	0.215
1-01	Carcass Digest	0 h	58796748.8875 dpm		99.469
1-01	Exhaled VOC	0 h	1110.6238 dpm		0.002
1-01	Nose Only Tube Rinse	0 h	166155.5883 dpm		0.281
1-01	Transfer Bag Rinse	0 h	18936.7872 dpm		0.032
1-02	Feces	0 h	24901.9839 dpm	60334744.156 dpm	0.041
1-02	Carcass Digest	0 h	60107213.0993 dpm		99.623
1-02	Exhaled VOC	0 h	785.6933 dpm		0.001
1-02	Nose Only Tube Rinse	0 h	198731.0728 dpm		0.329
1-02	Transfer Bag Rinse	0 h	3112.3069 dpm		0.005
1-03	Feces	0 h	149317.0097 dpm	61168290.196 dpm	0.244
1-03	Carcass Digest	0 h	60412728.2023 dpm		98.765
1-03	Exhaled VOC	0 h	2464.3752 dpm		0.004
1-03	Nose Only Tube Rinse	0 h	260533.2472 dpm		0.426
1-03	Transfer Bag Rinse	0 h	343247.3620 dpm		0.561
1-04	Feces	0 h	830702.6192 dpm	49730780.503 dpm	1.670
1-04	Carcass Digest	0 h	48198191.8677 dpm		96.918
1-04	Exhaled VOC	0 h	8048.3908 dpm		0.016
1-04	Nose Only Tube Rinse	0 h	677265.1671 dpm		1.362
1-04	Transfer Bag Rinse	0 h	16572.4584 dpm		0.033

Table 4. 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 (%)
2-05	Urine	12 h	48.4777 g	55.5796 g	7.1019 g	0.5018 g 0.5113 g	1637519.88 1661278.13	12.42 12.42	1637507.465 1661265.715			3263267.168 3249101.731	23125096.3422 dpm	46.743
2-05	Urine	24 h	49.0173 g	52.2039 g	3.1866 g	0.5091 g 0.5175 g	1472080.63 1496844.88	12.42 12.42	1472068.215 1496832.465			2891510.931 2892429.884	9215552.9007 dpm	18.627
2-05	Urine	48 h	48.9178 g	57.6047 g	8.6869 g	0.5031 g 0.5104 g	191790.38 194940.59	12.42 12.42	191777.965 194928.175			381192.536 381912.569	3314508.8677 dpm	6.700
2-05	Urine	72 h	48.3855 g	64.8394 g	16.4539 g	0.4994 g 0.4997 g	23677.51 21161.84	12.42 12.42	23665.095 21149.425			47387.054 42324.245	738050.3714 dpm	1.492
2-05	Urine	96 h	48.2028 g	57.1882 g	8.9854 g	0.5040 g 0.5043 g	7878.68 7930.01	12.42 12.42	7866.265 7917.595			15607.669 15700.169	140656.7202 dpm	0.284
2-05	Urine	120 h	48.3716 g	56.8512 g	8.4796 g	0.5059 g 0.5137 g	3846.39 3810.20	14.99 14.99	3831.400 3795.210			7573.433 7387.989	63433.4395 dpm	0.128
2-05	Urine	144 h	49.8184 g	58.5489 g	8.7305 g	0.5027 g 0.5109 g	2421.95 2505.26	14.99 14.99	2406.960 2490.270			4788.064 4874.281	42178.5521 dpm	0.085
2-05	Urine	168 h	48.2023 g	56.5844 g	8.3821 g	0.5082 g 0.5107 g	1601.91 1664.44	8.59 8.59	1593.320 1655.850			3135.222 3242.314	26728.5757 dpm	0.054
2-06	Urine	12 h	49.8359 g	55.9201 g	6.0842 g	0.5025 g 0.5088 g	1622134.25 1639983.00	12.42 12.42	1622121.835 1639970.585			3228103.154 3223212.628	19625547.7404 dpm	34.991
2-06	Urine	24 h	48.2608 g	50.4130 g	2.1522 g	0.4959 g 0.5164 g	2472429.75 2601387.25	12.42 12.42	2472417.335 2601374.835			4985717.554 5037519.045	10786004.9045 dpm	19.231
2-06	Urine	48 h	49.2520 g	57.9015 g	8.6495 g	0.5058 g 0.5120 g	398530.56 402483.47	12.42 12.42	398518.145 402471.055			787896.688 786076.279	6807039.5921 dpm	12.137
2-06	Urine	72 h	48.3592 g	60.0249 g	11.6657 g	0.5019 g 0.5042 g	64208.31 61098.18	12.42 12.42	64195.895 61085.765			127905.748 121153.838	1452727.2057 dpm	2.590
2-06	Urine	96 h	48.5784 g	57.9105 g	9.3321 g	0.5000 g 0.5052 g	16424.76 16722.69	12.42 12.42	16412.345 16710.275			32824.690 33076.554	307498.4988 dpm	0.548
2-06	Urine	120 h	48.8772 g	58.1885 g	9.3113 g	0.5009 g 0.5066 g	8874.07 8999.24	14.99 14.99	8859.080 8984.250			17686.325 17734.406	164906.5238 dpm	0.294
2-06	Urine	144 h	48.2892 g	57.1652 g	8.8760 g	0.4986 g 0.5010 g	12200.45 12256.26	14.99 14.99	12185.460 12241.270			24439.350 24433.673	216898.4753 dpm	0.387
2-06	Urine	168 h	49.6696 g	61.0100 g	11.3404 g	0.5019 g 0.5053 g	7996.96 8034.14	8.59 8.59	7988.370 8025.550			15916.258 15882.743	180306.6963 dpm	0.321
2-07	Urine	12 h	48.5781 g	53.3809 g	4.8028 g	0.5125 g 0.5121 g	1864389.63 1899674.88	12.42 12.42	1864377.215 1899662.465			3637809.200 3709553.730	17643957.3395 dpm	29.371
2-07	Urine	24 h	48.0274 g	51.5810 g	3.5536 g	0.5038 g 0.5159 g	2287319.75 2310785.50	12.42 12.42	2287307.335 2310773.085			4540109.835 4479110.457	16025350.6161 dpm	26.677
2-07	Urine	48 h	48.2920 g	58.3811 g	10.0891 g	0.5178 g 0.5093 g	328525.50 324468.25	12.42 12.42	328513.085 324455.835			634440.102 637062.311	6414157.4994 dpm	10.677
2-07	Urine	72 h	48.4478 g	59.1004 g	10.6526 g	0.5099 g 0.5128 g	61115.38 58673.90	12.42 12.42	61102.965 58661.485			119833.232 114394.472	1247567.0173 dpm	2.077
2-07	Urine	96 h	48.3720 g	58.1982 g	9.8262 g	0.5051 g 0.5094 g	17897.08 18100.37	12.42 12.42	17884.665 18087.955			35408.167 35508.353	348419.9528 dpm	0.580
2-07	Urine	120 h	48.3381 g	58.3222 g	9.9841 g	0.5070 g 0.5124 g	7531.42 7617.89	14.99 14.99	7516.430 7602.900			14825.306 14837.822	148079.8168 dpm	0.247
2-07	Urine	144 h	48.6293 g	56.4096 g	7.7803 g	0.5056 g 0.5131 g	6264.36 6306.39	14.99 14.99	6249.370 6291.400			12360.305 12261.547	95782.6977 dpm	0.159
2-07	Urine	168 h	48.5092 g	54.1272 g	5.6180 g	0.5096 g 0.5153 g	3792.09 3814.00	8.59 8.59	3783.500 3805.410			7424.451 7384.844	41599.3078 dpm	0.069

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 (%)
2-08	Urine	12 h	48.3010 g	54.7531 g	6.4521 g	0.5011 g 0.5119 g	1765224.25 1800520.50	12.42 12.42	1765211.835 1800508.085			3522673.788 3517304.327	22711321.3969 dpm	36.637
2-08	Urine	24 h	50.5195 g	54.1668 g	3.6473 g	0.5020 g 0.5119 g	1958559.38 1998603.88	12.42 12.42	1958546.965 1998591.465			3901487.978 3904261.506	14234955.0469 dpm	22.963
2-08	Urine	48 h	50.3854 g	61.6526 g	11.2672 g	0.4996 g 0.5061 g	210063.56 213895.67	12.42 12.42	210051.145 213883.255			420438.641 422610.660	4749402.5413 dpm	7.662
2-08	Urine	72 h	50.5831 g	63.2441 g	12.6610 g	0.4988 g 0.5047 g	34314.61 34828.00	12.42 12.42	34302.195 34815.585			68769.437 68982.732	872040.1056 dpm	1.407
2-08	Urine	96 h	50.7012 g	61.7412 g	11.0400 g	0.4969 g 0.5048 g	13373.21 13565.48	12.42 12.42	13360.795 13553.065			26888.297 26848.385	296626.4898 dpm	0.479
2-08	Urine	120 h	50.3520 g	61.9769 g	11.6249 g	0.4969 g 0.5047 g	5473.85 5483.55	14.99 14.99	5458.860 5468.560			10985.832 10835.268	126834.0564 dpm	0.205
2-08	Urine	144 h	47.8162 g	54.3558 g	6.5396 g	0.4967 g 0.5065 g	3119.05 3174.78	14.99 14.99	3104.060 3159.790			6249.366 6238.480	40832.7575 dpm	0.066
2-08	Urine	168 h	48.0251 g	54.4967 g	6.4716 g	0.4964 g 0.4989 g	2912.59 2923.72	8.59 8.59	2904.000 2915.130			5850.121 5843.115	37836.9722 dpm	0.061

Table 5. Recovery of Radioactivity in Feces from Group 2.

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 (%)
2-05	Feces	0 h	17.3561 g	18.3092 g	0.9531 g	19.5465 g	2.1904 g	0.0793 g 0.1011 g	2266.02 2918.78	19.27 19.27	2246.755 2899.515			28332.346 28679.674	62439.5633 dpm	0.126
2-05	Feces	24 h	50.4950 g	56.8014 g	6.3064 g	63.3755 g	12.8805 g	0.1122 g 0.0848 g	3930.69 3113.13	18.60 18.60	3912.086 3094.526			34867.079 36492.055	459570.6609 dpm	0.929
2-05	Feces	48 h	50.3150 g	56.4899 g	6.1749 g	63.5808 g	13.2658 g	0.0781 g 0.0719 g	408.38 379.13	18.60 18.60	389.776 360.526			4990.733 5014.273	66362.2065 dpm	0.134
2-05	Feces	72 h	48.1692 g	56.7115 g	8.5423 g	66.3532 g	18.1840 g	0.0726 g 0.0642 g	156.50 124.90	18.60 18.60	137.896 106.296			1899.397 1655.705	32322.9893 dpm	0.065
2-05	Feces	96 h	48.0192 g	54.7298 g	6.7106 g	60.2640 g	12.2448 g	0.0583 g 0.0743 g	52.03 76.80	18.60 18.60	33.426 58.196		* *	573.349 783.260	8305.7059 dpm	0.017
2-05	Feces	120 h	50.2988 g	55.6046 g	5.3058 g	60.6441 g	10.3453 g	0.0778 g 0.1007 g	34.16 45.39	18.60 18.60	15.556 26.786		* *	199.952 266.000	2410.2081 dpm	0.005
2-05	Feces	144 h	48.3341 g	55.1847 g	6.8506 g	60.8989 g	12.5648 g	0.0788 g 0.0799 g	51.75 42.43	18.60 18.60	33.146 23.826		* *	420.638 298.201	4516.0314 dpm	0.009
2-05	Feces	168 h	48.9074 g	57.3788 g	8.4714 g	67.9759 g	19.0685 g	0.0800 g 0.0582 g	34.75 29.87	18.60 18.60	16.146 11.266		* *	201.828 193.578	3769.9026 dpm	0.008
2-06	Feces	0 h	17.3441 g	19.0451 g	1.7010 g	21.2880 g	3.9439 g	0.0858 g 0.0749 g	3228.24 2885.64	19.27 19.27	3208.975 2866.375			37400.641 38269.359	149217.4568 dpm	0.266
2-06	Feces	24 h	48.2585 g	53.0876 g	4.8291 g	60.3116 g	12.0531 g	0.0742 g 0.1289 g	3367.54 5616.20	18.60 18.60	3348.936 5597.596			45133.912 43425.882	533710.0277 dpm	0.952
2-06	Feces	48 h	48.3841 g	54.1529 g	5.7688 g	60.4651 g	12.0810 g	0.1018 g 0.0844 g	1050.80 779.79	18.60 18.60	1032.196 761.186			10139.452 9018.794	115725.3897 dpm	0.206
2-06	Feces	72 h	50.3396 g	56.5580 g	6.2184 g	67.0466 g	16.7070 g	0.0913 g 0.0632 g	183.38 143.76	18.60 18.60	164.776 125.156			1804.778 1980.320	31618.8213 dpm	0.056
2-06	Feces	96 h	49.6338 g	56.1027 g	6.4689 g	62.1870 g	12.5532 g	0.0745 g 0.0714 g	230.57 216.53	18.60 18.60	211.966 197.926			2845.185 2772.076	35257.2997 dpm	0.063
2-06	Feces	120 h	50.3651 g	56.6967 g	6.3316 g	64.9455 g	14.5804 g	0.0971 g 0.0901 g	44.40 45.00	18.60 18.60	25.796 26.396		* *	265.667 292.966	4072.5462 dpm	0.007
2-06	Feces	144 h	49.9713 g	55.7063 g	5.7350 g	61.2773 g	11.3060 g	0.0815 g 0.0628 g	71.02 60.68	18.60 18.60	52.416 42.076			643.144 670.004	7423.2265 dpm	0.013
2-06	Feces	168 h	50.2069 g	59.8452 g	9.6383 g	70.8147 g	20.6078 g	0.0523 g 0.0538 g	462.19 399.94	18.60 18.60	443.586 381.336			8481.573 7088.034	160427.6740 dpm	0.286
2-07	Feces	0 h	16.9432 g	17.9306 g	0.9874 g	19.5061 g	2.5629 g	0.0875 g 0.0804 g	1912.96 1709.77	19.27 19.27	1893.695 1690.505			21642.229 21026.182	54677.4342 dpm	0.091
2-07	Feces	24 h	49.7696 g	57.4228 g	7.6532 g	64.6886 g	14.9190 g	0.0893 g 0.0924 g	3121.24 3092.33	18.60 18.60	3102.636 3073.726			34743.967 33265.436	507316.1385 dpm	0.845
2-07	Feces	48 h	50.0818 g	57.4051 g	7.3233 g	64.6862 g	14.6044 g	0.0844 g 0.0664 g	782.14 623.68	18.60 18.60	763.536 605.076			9046.638 9112.594	132602.3451 dpm	0.221
2-07	Feces	72 h	49.7447 g	55.7840 g	6.0393 g	64.0307 g	14.2860 g	0.0552 g 0.0752 g	124.64 156.25	18.60 18.60	106.036 137.646			1920.947 1830.402	26795.8846 dpm	0.045
2-07	Feces	96 h	50.3259 g	59.1257 g	8.7998 g	66.9389 g	16.6130 g	0.0886 g 0.0866 g	132.74 126.04	18.60 18.60	114.136 107.436			1288.220 1240.603	21005.6672 dpm	0.035
2-07	Feces	120 h	50.1903 g	58.6582 g	8.4679 g	68.3520 g	18.1617 g	0.0881 g 0.1038 g	61.66 56.44	18.60 18.60	43.056 37.836			488.720 364.511	7748.0653 dpm	0.013
2-07	Feces	144 h	49.7925 g	56.2451 g	6.4526 g	61.8678 g	12.0753 g	0.0659 g 0.0882 g	44.45 51.73	18.60 18.60	25.846 33.126		* *	392.204 375.581	4635.6181 dpm	0.008
2-07	Feces	168 h	49.8721 g	57.0401 g	7.1680 g	68.1028 g	18.2307 g	0.0790 g 0.0661 g	38.40 32.72	18.60 18.60	19.796 14.116		* *	250.585 213.559	4230.8391 dpm	0.007

* 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 (contd). Recovery of Radioactivity in Feces from Group 2.

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 (%)
2-08	Feces	0 h	17.0588 g	20.5817 g	3.5229 g	22.8737 g	5.8149 g	0.0717 g 0.0673 g	7982.10 7380.21	19.27 19.27	7962.835 7360.945			111057.671 109375.111	640897.2929 dpm	1.034
2-08	Feces	24 h	50.1918 g	56.3884 g	6.1966 g	63.7060 g	13.5142 g	0.1163 g 0.0825 g	4358.94 2992.43	18.60 18.60	4340.336 2973.826			37320.174 36046.379	495745.1346 dpm	0.800
2-08	Feces	48 h	49.7597 g	55.3926 g	5.6329 g	62.3769 g	12.6172 g	0.0817 g 0.0752 g	947.34 887.74	18.60 18.60	928.736 869.136			11367.641 11557.663	144626.5707 dpm	0.233
2-08	Feces	72 h	49.5965 g	56.8954 g	7.2989 g	65.3529 g	15.7564 g	0.0789 g 0.0590 g	303.33 219.56	18.60 18.60	284.726 200.956			3608.698 3406.038	55263.4920 dpm	0.089
2-08	Feces	96 h	50.7319 g	57.9331 g	7.2012 g	65.3341 g	14.6022 g	0.0976 g 0.0802 g	366.41 292.37	18.60 18.60	347.806 273.766			3563.589 3413.544	50940.7450 dpm	0.082
2-08	Feces	120 h	49.9121 g	55.6105 g	5.6984 g	64.0965 g	14.1844 g	0.1097 g 0.0973 g	55.32 54.72	18.60 18.60	36.716 36.116		* *	334.697 371.184	5006.2519 dpm	0.008
2-08	Feces	144 h	50.2497 g	64.5511 g	14.3014 g	72.7015 g	22.4518 g	0.0970 g 0.0640 g	698.30 495.70	18.60 18.60	679.696 477.096			7007.178 7454.629	162346.7963 dpm	0.262
2-08	Feces	168 h	50.2561 g	54.2132 g	3.9571 g	58.9658 g	8.7097 g	0.0770 g 0.0869 g	241.07 284.19	18.60 18.60	222.466 265.586			2889.172 3056.228	25891.3274 dpm	0.042

* 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_2 Trap 1 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 (%)
2-05	CO2 Trap 1	1 h	0.00 g	504.89 g	504.89 g	1.0380 g	71.16	15.65	55.509			53.477	25312.6590 dpm	0.051
						1.0373 g	64.19	15.65	48.539			46.793		
2-05	CO2 Trap 1	3 h	0.00 g	567.25 g	567.25 g	1.0353 g	72.03	15.65	56.379			54.456	29938.9474 dpm	0.061
						1.0381 g	68.70	15.65	53.049			51.102		
2-05	CO2 Trap 1	5 h	0.00 g	486.09 g	486.09 g	1.0369 g	72.99	15.65	57.339			55.298	28709.8064 dpm	0.058
						1.0352 g	80.69	15.65	65.039			62.827		
2-05	CO2 Trap 1	7 h	0.00 g	383.70 g	383.70 g	1.0380 g	70.06	15.65	54.409			52.417	20676.0218 dpm	0.042
						1.0362 g	73.01	15.65	57.359			55.355		
2-05	CO2 Trap 1	12 h	0.00 g	487.96 g	487.96 g	1.0383 g	98.92	15.65	83.269			80.197	41007.3838 dpm	0.083
						1.0372 g	106.80	15.65	91.149			87.880		
2-05	CO2 Trap 1	24 h	0.00 g	520.19 g	520.19 g	1.0343 g	170.80	15.65	155.149			150.004	80693.9335 dpm	0.163
						1.0346 g	181.44	15.65	165.789			160.244		
2-05	CO2 Trap 1	48 h	0.00 g	529.19 g	529.19 g	1.0448 g	67.79	15.65	52.139			49.903	30607.4163 dpm	0.062
						1.0425 g	84.22	15.65	68.569			65.773		
2-05	CO2 Trap 1	72 h	0.00 g	574.29 g	574.29 g	1.0440 g	30.11	15.65	14.459		*	13.849	11034.1810 dpm	0.022
						1.0452 g	41.34	15.65	25.689		*	24.578		
2-05	CO2 Trap 1	96 h	0.00 g	504.54 g	504.54 g	1.0474 g	32.48	16.48	16.002		*	15.278	6345.6999 dpm	0.013
						1.0451 g	26.80	16.48	10.322		*	9.877		
2-05	CO2 Trap 1	120 h	0.00 g	620.97 g	620.97 g	1.0540 g	20.76	16.48	4.282		*	4.063	3879.4523 dpm	0.008
						1.0486 g	25.32	16.48	8.842		*	8.432		
2-05	CO2 Trap 1	144 h	0.00 g	501.17 g	501.17 g	1.0445 g	20.41	16.36	4.047		*	3.874	1729.9618 dpm	0.003
						1.0420 g	19.52	16.36	3.157		*	3.029		
2-05	CO2 Trap 1	168 h	0.00 g	551.93 g	551.93 g	1.0472 g	22.45	19.30	3.147		*	3.005	2677.0845 dpm	0.005
						1.0464 g	26.31	19.30	7.007		*	6.696		
2-06	CO2 Trap 1	1 h	0.00 g	456.18 g	456.18 g	1.0363 g	70.49	15.65	54.839			52.918	24431.0438 dpm	0.044
						1.0370 g	71.85	15.65	56.199			54.194		
2-06	CO2 Trap 1	3 h	0.00 g	424.39 g	424.39 g	1.0375 g	88.73	15.65	73.079			70.437	31814.5020 dpm	0.057
						1.0363 g	98.03	15.65	82.379			79.493		
2-06	CO2 Trap 1	5 h	0.00 g	477.89 g	477.89 g	1.0375 g	75.97	15.65	60.319			58.139	28364.8291 dpm	0.051
						1.0363 g	78.42	15.65	62.769			60.570		
2-06	CO2 Trap 1	7 h	0.00 g	523.01 g	523.01 g	1.0369 g	61.50	15.65	45.849			44.217	25273.3452 dpm	0.045
						1.0330 g	69.81	15.65	54.159			52.429		
2-06	CO2 Trap 1	12 h	0.00 g	556.94 g	556.94 g	1.0352 g	112.68	15.65	97.029			93.729	48016.0344 dpm	0.086
						1.0352 g	97.12	15.65	81.469			78.699		
2-06	CO2 Trap 1	24 h	0.00 g	444.82 g	444.82 g	1.0367 g	194.94	15.65	179.289			172.942	78910.1092 dpm	0.141
						1.0339 g	203.67	15.65	188.019			181.854		
2-06	CO2 Trap 1	48 h	0.00 g	603.68 g	603.68 g	1.0487 g	84.60	15.65	68.949			65.747	38684.0640 dpm	0.069
						1.0459 g	80.93	15.65	65.279			62.414		
2-06	CO2 Trap 1	72 h	0.00 g	483.60 g	483.60 g	1.0467 g	45.46	15.65	29.809		*	28.479	13010.2157 dpm	0.023
						1.0439 g	42.09	15.65	26.439		*	25.327		
2-06	CO2 Trap 1	96 h	0.00 g	529.65 g	529.65 g	1.0446 g	28.46	16.48	11.982		*	11.470	5881.9861 dpm	0.010
						1.0467 g	27.72	16.48	11.242		*	10.740		
2-06	CO2 Trap 1	120 h	0.00 g	489.55 g	489.55 g	1.0438 g	27.97	16.48	11.492		*	11.010	5013.1972 dpm	0.009
						1.0455 g	26.38	16.48	9.902		*	9.471		
2-06	CO2 Trap 1	144 h	0.32 g	492.44 g	492.12 g	1.0417 g	23.14	16.36	6.777		*	6.505	2136.6406 dpm	0.004
						1.0407 g	18.63	16.36	2.267		*	2.178		
2-06	CO2 Trap 1	168 h	0.00 g	457.71 g	457.71 g	1.0441 g	22.24	19.30	2.937		*	2.813	1337.1844 dpm	0.002
						1.0450 g	22.47	19.30	3.167		*	3.030		

* 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 (contd). Recovery of Radioactivity in CO_2 Trap 1 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 (%)
2-07	CO2 Trap 1	1 h	0.00 g	476.12 g	476.12 g	1.0407 g	62.23	15.65	46.579			44.757	20952.4919 dpm	0.035
						1.0382 g	60.56	15.65	44.909			43.256		
2-07	CO2 Trap 1	3 h	0.00 g	416.65 g	416.65 g	1.0365 g	100.15	15.65	84.499			81.523	32054.4486 dpm	0.053
						1.0360 g	90.60	15.65	74.949			72.344		
2-07	CO2 Trap 1	5 h	0.00 g	467.01 g	467.01 g	1.0370 g	69.60	15.65	53.949			52.024	26005.3543 dpm	0.043
						1.0351 g	77.08	15.65	61.429			59.346		
2-07	CO2 Trap 1	7 h	0.00 g	421.22 g	421.22 g	1.0356 g	84.77	15.65	69.119			66.743	28318.4957 dpm	0.047
						1.0337 g	85.65	15.65	69.999			67.717		
2-07	CO2 Trap 1	12 h	0.01 g	420.44 g	420.43 g	1.0430 g	150.88	15.65	135.229			129.654	58927.0911 dpm	0.098
						1.0364 g	171.80	15.65	156.149			150.665		
2-07	CO2 Trap 1	24 h	0.00 g	486.98 g	486.98 g	1.0452 g	291.66	15.65	276.009			264.073	123337.9000 dpm	0.205
						1.0419 g	268.28	15.65	252.629			242.469		
2-07	CO2 Trap 1	48 h	0.00 g	498.31 g	498.31 g	1.0480 g	79.07	15.65	63.419			60.514	31395.8570 dpm	0.052
						1.0454 g	84.12	15.65	68.469			65.495		
2-07	CO2 Trap 1	72 h	0.00 g	468.55 g	468.55 g	1.0466 g	39.58	15.65	23.929		*	22.863	9927.4072 dpm	0.017
						1.0429 g	36.00	15.65	20.349		*	19.512		
2-07	CO2 Trap 1	96 h	0.00 g	569.14 g	569.14 g	1.0477 g	28.50	11.62	16.880		*	16.111	5077.6325 dpm	0.008
						1.0406 g	18.28	16.48	1.802		*	1.732		
2-07	CO2 Trap 1	120 h	0.00 g	548.87 g	548.87 g	1.0413 g	21.44	16.48	4.962		*	4.765	3262.5011 dpm	0.005
						1.0434 g	23.91	16.48	7.432		*	7.123		
2-07	CO2 Trap 1	144 h	0.01 g	458.50 g	458.49 g	1.0351 g	16.14	16.36	0.000	*		0.000	1297.3772 dpm	0.002
						1.0384 g	22.24	16.36	5.877	*		5.659		
2-07	CO2 Trap 1	168 h	0.00 g	550.13 g	550.13 g	1.0447 g	24.04	19.30	4.737		*	4.534	1962.0883 dpm	0.003
						1.0452 g	22.02	19.30	2.717		*	2.599		
2-08	CO2 Trap 1	1 h	0.00 g	425.31 g	425.31 g	1.0379 g	67.81	15.65	52.159			50.254	20930.6686 dpm	0.034
						1.0315 g	65.34	15.65	49.689			48.171		
2-08	CO2 Trap 1	3 h	0.00 g	435.55 g	435.55 g	1.0382 g	85.62	15.65	69.969			67.394	30695.6456 dpm	0.050
						1.0351 g	91.79	15.65	76.139			73.557		
2-08	CO2 Trap 1	5 h	0.00 g	419.30 g	419.30 g	1.0374 g	74.10	15.65	58.449			56.342	26931.3325 dpm	0.043
						1.0358 g	90.35	15.65	74.699			72.117		
2-08	CO2 Trap 1	7 h	0.00 g	506.98 g	506.98 g	1.0386 g	67.77	15.65	52.119			50.182	25999.1264 dpm	0.042
						1.0339 g	69.81	15.65	54.159			52.383		
2-08	CO2 Trap 1	12 h	0.00 g	498.68 g	498.68 g	1.0375 g	132.60	15.65	116.949			112.722	55496.9970 dpm	0.090
						1.0421 g	130.13	15.65	114.479			109.854		
2-08	CO2 Trap 1	24 h	0.00 g	426.07 g	426.07 g	1.0495 g	321.69	15.65	306.039			291.604	118996.9798 dpm	0.192
						1.0441 g	294.40	15.65	278.749			266.975		
2-08	CO2 Trap 1	48 h	0.00 g	443.14 g	443.14 g	1.0501 g	105.05	15.65	89.399			85.134	35345.0111 dpm	0.057
						1.0464 g	93.49	15.65	77.839			74.387		
2-08	CO2 Trap 1	72 h	0.00 g	473.10 g	473.10 g	1.0459 g	18.62	15.65	2.969		*	2.838	3776.5574 dpm	0.006
						1.0352 g	29.24	15.65	13.589		*	13.127		
2-08	CO2 Trap 1	96 h	0.00 g	457.10 g	457.10 g	1.0393 g	34.75	16.48	18.272		*	17.581	6904.9372 dpm	0.011
						1.0373 g	29.58	16.48	13.102		*	12.631		
2-08	CO2 Trap 1	120 h	0.00 g	480.46 g	480.46 g	1.0427 g	23.24	16.48	6.762		*	6.485	3745.6389 dpm	0.006
						1.0412 g	25.96	16.48	9.482		*	9.107		
2-08	CO2 Trap 1	144 h	0.00 g	373.05 g	373.05 g	1.0407 g	22.88	16.36	6.517		*	6.262	2743.1002 dpm	0.004
						1.0417 g	25.16	16.36	8.797		*	8.445		
2-08	CO2 Trap 1	168 h	0.00 g	521.68 g	521.68 g	1.0356 g	22.93	19.30	3.627		*	3.502	1200.8666 dpm	0.002
						1.0316 g	20.44	19.30	1.137		*	1.102		

* 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_2 Trap 2 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 (%)
2-05	CO2 Trap 2	1 h	0.00 g	586.33 g	586.33 g	1.0367 g	16.80	15.65	1.149		*	1.108	522.5057 dpm	0.001
						1.0364 g	16.35	15.65	0.699		*	0.674		
2-05	CO2 Trap 2	3 h	0.00 g	447.27 g	447.27 g	1.0349 g	15.00	15.65	0.000	*		0.000	444.9252 dpm	0.001
						1.0348 g	17.71	15.65	2.059	*		1.990		
2-05	CO2 Trap 2	5 h	0.00 g	505.17 g	505.17 g	1.0359 g	16.12	15.65	0.469	*		0.453	114.2960 dpm	0.000
						1.0343 g	14.54	15.65	0.000	*		0.000		
2-05	CO2 Trap 2	7 h	0.00 g	362.03 g	362.03 g	1.0343 g	12.07	15.65	0.000	*		0.000	245.3675 dpm	0.000
						1.0319 g	17.05	15.65	1.399	*		1.356		
2-05	CO2 Trap 2	12 h	0.00 g	490.26 g	490.26 g	1.0317 g	13.20	15.65	0.000	*		0.000	166.0539 dpm	0.000
						1.0315 g	16.35	15.65	0.699	*		0.677		
2-05	CO2 Trap 2	24 h	0.00 g	430.42 g	430.42 g	1.0251 g	19.52	15.65	3.869	*		3.774	812.2073 dpm	0.002
						1.0225 g	8.91	15.65	0.000	*		0.000		
2-05	CO2 Trap 2	48 h	0.00 g	439.74 g	439.74 g	1.0392 g	25.84	15.65	10.189		*	9.804	4217.3753 dpm	0.009
						1.0386 g	25.39	15.65	9.739		*	9.377		
2-05	CO2 Trap 2	72 h	0.00 g	429.90 g	429.90 g	1.0380 g	20.21	15.65	4.559		*	4.392	2249.7045 dpm	0.005
						1.0388 g	17.93	11.62	6.310		*	6.074		
2-05	CO2 Trap 2	96 h	0.00 g	515.61 g	515.61 g	0.6031 g	7.42	16.48	0.000	*		0.000	0.0000 dpm	0.000
						0.7338 g	12.60	16.48	0.000	*		0.000		
2-05	CO2 Trap 2	120 h	0.01 g	474.72 g	474.71 g	1.0404 g	16.95	16.48	0.472		*	0.454	420.3863 dpm	0.001
						1.0414 g	17.85	16.48	1.372		*	1.317		
2-05	CO2 Trap 2	144 h	0.00 g	488.66 g	488.66 g	1.0345 g	14.11	16.36	0.000	*		0.000	905.0133 dpm	0.002
						1.0358 g	20.20	16.36	3.837	*		3.704		
2-05	CO2 Trap 2	168 h	0.00 g	465.37 g	465.37 g	1.0371 g	18.62	19.30	0.000	*		0.000	0.0000 dpm	0.000
						1.0372 g	13.88	19.30	0.000	*		0.000		
2-06	CO2 Trap 2	1 h	0.00 g	539.72 g	539.72 g	1.0373 g	15.90	15.65	0.249	*		0.240	64.7138 dpm	0.000
						1.0358 g	15.00	15.65	0.000	*		0.000		
2-06	CO2 Trap 2	3 h	0.00 g	481.65 g	481.65 g	1.0340 g	17.93	15.65	2.279		*	2.204	1220.5133 dpm	0.002
						1.0330 g	18.61	15.65	2.959		*	2.864		
2-06	CO2 Trap 2	5 h	0.00 g	445.61 g	445.61 g	1.0358 g	13.20	15.65	0.000	*		0.000	544.8391 dpm	0.001
						1.0341 g	18.18	15.65	2.529	*		2.445		
2-06	CO2 Trap 2	7 h	0.00 g	495.89 g	495.89 g	1.0362 g	22.22	15.65	6.569	*		6.339	1571.7899 dpm	0.003
						1.0318 g	12.08	15.65	0.000	*		0.000		
2-06	CO2 Trap 2	12 h	0.00 g	457.67 g	457.67 g	1.0412 g	11.40	15.65	0.000	*		0.000	1000.6984 dpm	0.002
						1.0379 g	20.19	15.65	4.539	*		4.373		
2-06	CO2 Trap 2	24 h	0.00 g	469.49 g	469.49 g	1.0344 g	18.84	15.65	3.189		*	3.083	1400.2508 dpm	0.002
						1.0300 g	18.62	15.65	2.969		*	2.882		
2-06	CO2 Trap 2	48 h	0.00 g	415.80 g	415.80 g	1.0400 g	31.48	15.65	15.829		*	15.220	6107.0738 dpm	0.011
						1.0377 g	30.34	15.65	14.689		*	14.155		
2-06	CO2 Trap 2	72 h	0.00 g	473.05 g	473.05 g	1.0390 g	22.47	15.65	6.819		*	6.563	3515.8090 dpm	0.006
						1.0382 g	24.27	15.65	8.619		*	8.302		
2-06	CO2 Trap 2	96 h	0.00 g	537.81 g	537.81 g	1.0375 g	21.91	16.48	5.432		*	5.236	1645.0503 dpm	0.003
						1.0341 g	17.39	16.48	0.912		*	0.882		
2-06	CO2 Trap 2	120 h	0.00 g	570.30 g	570.30 g	1.0361 g	16.26	16.48	0.000	*		0.000	63.8683 dpm	0.000
						1.0358 g	16.71	16.48	0.232	*		0.224		
2-06	CO2 Trap 2	144 h	0.00 g	435.00 g	435.00 g	1.0372 g	19.07	16.36	2.707		*	2.610	615.2279 dpm	0.001
						1.0348 g	16.59	16.36	0.227		*	0.219		
2-06	CO2 Trap 2	168 h	0.00 g	503.78 g	503.78 g	1.0371 g	18.19	19.30	0.000	*		0.000	436.9899 dpm	0.001
						1.0414 g	21.11	19.30	1.807	*		1.735		

* 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 (contd). Recovery of Radioactivity in CO_2 Trap 2 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 (%)
2-07	CO2 Trap 2	1 h	0.00 g	490.69 g	490.69 g	1.0364 g	19.74	15.65	4.089		*	3.945	1884.2059 dpm	0.003
						1.0359 g	19.52	15.65	3.869		*	3.735		
2-07	CO2 Trap 2	3 h	0.00 g	448.09 g	448.09 g	1.0371 g	17.27	15.65	1.619	*		1.561	349.6990 dpm	0.001
						1.0339 g	13.87	15.65	0.000	*		0.000		
2-07	CO2 Trap 2	5 h	0.00 g	446.02 g	446.02 g	1.0355 g	22.44	15.65	6.789		*	6.556	1858.2873 dpm	0.003
						1.0349 g	17.49	15.65	1.839		*	1.777		
2-07	CO2 Trap 2	7 h	0.01 g	392.82 g	392.81 g	1.0367 g	15.68	15.65	0.029	*		0.028	5.4467 dpm	0.000
						1.0333 g	14.09	15.65	0.000	*		0.000		
2-07	CO2 Trap 2	12 h	0.00 g	482.78 g	482.78 g	1.0339 g	12.30	15.65	0.000	*		0.000	6.7209 dpm	0.000
						1.0326 g	15.68	15.65	0.029	*		0.028		
2-07	CO2 Trap 2	24 h	0.00 g	445.59 g	445.59 g	1.0368 g	19.53	15.65	3.879		*	3.741	1424.4095 dpm	0.002
						1.0326 g	18.39	15.65	2.739		*	2.652		
2-07	CO2 Trap 2	48 h	0.00 g	429.69 g	429.69 g	1.0422 g	42.31	15.65	26.659		*	25.579	11132.7684 dpm	0.019
						1.0427 g	43.01	15.65	27.359		*	26.238		
2-07	CO2 Trap 2	72 h	0.00 g	459.73 g	459.73 g	1.0402 g	25.17	15.65	9.519		*	9.151	3863.7279 dpm	0.006
						1.0393 g	23.61	15.65	7.959		*	7.658		
2-07	CO2 Trap 2	96 h	0.00 g	446.79 g	446.79 g	1.0356 g	19.41	16.48	2.932		*	2.831	1557.7036 dpm	0.003
						1.0363 g	20.77	16.48	4.292		*	4.142		
2-07	CO2 Trap 2	120 h	0.00 g	487.53 g	487.53 g	1.0364 g	17.16	16.48	0.682		*	0.658	1276.2660 dpm	0.002
						1.0381 g	21.23	16.48	4.752		*	4.578		
2-07	CO2 Trap 2	144 h	0.00 g	445.83 g	445.83 g	1.0269 g	17.94	16.36	1.577		*	1.535	1845.1343 dpm	0.003
						1.0363 g	23.35	16.36	6.987		*	6.742		
2-07	CO2 Trap 2	168 h	0.00 g	417.56 g	417.56 g	1.0445 g	18.18	19.30	0.000	*		0.000	361.5065 dpm	0.001
						1.0434 g	21.11	19.30	1.807	*		1.732		
2-08	CO2 Trap 2	1 h	0.00 g	532.29 g	532.29 g	1.0377 g	13.65	15.65	0.000	*		0.000	0.0000 dpm	0.000
						1.0347 g	12.06	15.65	0.000	*		0.000		
2-08	CO2 Trap 2	3 h	0.00 g	414.39 g	414.39 g	1.0341 g	15.45	15.65	0.000	*		0.000	142.1170 dpm	0.000
						1.0333 g	16.36	15.65	0.709	*		0.686		
2-08	CO2 Trap 2	5 h	0.00 g	460.63 g	460.63 g	1.0365 g	19.74	15.65	4.089		*	3.945	1619.0815 dpm	0.003
						1.0336 g	18.84	15.65	3.189		*	3.085		
2-08	CO2 Trap 2	7 h	0.00 g	440.05 g	440.05 g	1.0366 g	11.62	15.65	0.000	*		0.000	153.4922 dpm	0.000
						1.0303 g	16.37	15.65	0.719	*		0.698		
2-08	CO2 Trap 2	12 h	0.00 g	465.49 g	465.49 g	1.0314 g	13.65	15.65	0.000	*		0.000	0.0000 dpm	0.000
						1.0288 g	14.78	15.65	0.000	*		0.000		
2-08	CO2 Trap 2	24 h	0.00 g	415.97 g	415.97 g	1.0388 g	23.35	15.65	7.699		*	7.411	1728.9196 dpm	0.003
						1.0413 g	16.59	15.65	0.939		*	0.902		
2-08	CO2 Trap 2	48 h	0.00 g	470.23 g	470.23 g	1.0409 g	43.19	15.65	27.539		*	26.457	9895.3037 dpm	0.016
						1.0402 g	31.91	15.65	16.259		*	15.630		
2-08	CO2 Trap 2	72 h	0.01 g	483.77 g	483.76 g	1.0368 g	44.76	15.65	29.109		*	28.076	12320.9789 dpm	0.020
						1.0370 g	39.36	15.65	23.709		*	22.863		
2-08	CO2 Trap 2	96 h	0.00 g	479.91 g	479.91 g	1.0323 g	26.86	16.48	10.382		*	10.057	2520.5821 dpm	0.004
						1.0330 g	16.94	16.48	0.462		*	0.447		
2-08	CO2 Trap 2	120 h	0.00 g	493.02 g	493.02 g	1.0325 g	21.89	16.48	5.412		*	5.242	1565.1681 dpm	0.003
						1.0310 g	17.62	16.48	1.142		*	1.108		
2-08	CO2 Trap 2	144 h	0.00 g	357.30 g	357.30 g	1.0382 g	15.69	16.36	0.000	*		0.000	1515.3066 dpm	0.002
						1.0371 g	25.16	16.36	8.797	*		8.482		
2-08	CO2 Trap 2	168 h	0.00 g	495.43 g	495.43 g	1.0350 g	17.72	19.30	0.000	*		0.000	438.5894 dpm	0.001
						1.0317 g	21.13	19.30	1.827	*		1.771		

* 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 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 (%)
2-05	Exhaled VOC Trap 1	1 h	16.6587 g	19.0611 g	2.4024 g	0.0952 g 0.0915 g	42609.00 40409.26	0.56 0.56	42608.445 40408.705			447567.700 441625.191	1068098.5005 dpm	2.159
2-05	Exhaled VOC Trap 1	3 h	5.5949 g	8.1508 g	2.5559 g	0.0962 g 0.0964 g	45589.39 45475.99	1.37 1.37	45588.017 45474.617			473887.907 471728.389	1208450.3452 dpm	2.443
2-05	Exhaled VOC Trap 1	5 h	5.6200 g	8.3826 g	2.7626 g	0.0953 g 0.0949 g	46341.34 46268.63	1.37 1.37	46339.967 46267.257			486253.585 487536.951	1345096.8679 dpm	2.719
2-05	Exhaled VOC Trap 1	7 h	6.2892 g	8.8687 g	2.5795 g	0.0944 g 0.0967 g	40170.29 40778.50	3.11 3.11	40167.180 40775.390			425499.788 421668.976	1092635.9138 dpm	2.209
2-05	Exhaled VOC Trap 1	12 h	6.3941 g	8.9155 g	2.5214 g	0.0949 g 0.0957 g	39733.92 39890.12	3.11 3.11	39730.810 39887.010			418659.747 416792.163	1053254.2231 dpm	2.129
2-05	Exhaled VOC Trap 1	24 h	6.3346 g	8.6143 g	2.2797 g	0.0942 g 0.0965 g	9206.38 9380.16	3.11 3.11	9203.270 9377.050			97699.257 97171.503	222123.4352 dpm	0.449
2-05	Exhaled VOC Trap 1	48 h	15.4945 g	19.3567 g	3.8622 g	0.0935 g 0.0971 g	2185.72 2313.24	1.05 1.05	2184.669 2312.189			23365.439 23812.447	91105.2155 dpm	0.184
2-05	Exhaled VOC Trap 1	72 h	15.3148 g	19.1512 g	3.8364 g	0.0937 g 0.0948 g	5.56 4.23	1.05 1.05	4.509 3.179			48.117 33.529	156.6140 dpm	0.000
2-05	Exhaled VOC Trap 1	96 h	15.4770 g	19.4526 g	3.9756 g	0.0942 g 0.0946 g	426.16 427.73	1.05 1.05	425.109 426.679			4512.830 4510.344	17936.2656 dpm	0.036
2-05	Exhaled VOC Trap 1	120 h	15.5003 g	19.2707 g	3.7704 g	0.0946 g 0.0946 g	465.75 483.35	1.05 1.05	464.699 482.299			4912.247 5098.294	18871.8712 dpm	0.038
2-05	Exhaled VOC Trap 1	144 h	15.4025 g	19.2635 g	3.8610 g	0.0935 g 0.0920 g	233.14 215.81	1.05 1.05	232.089 214.759			2482.231 2334.332	9298.3749 dpm	0.019
2-05	Exhaled VOC Trap 1	168 h	15.5671 g	19.5384 g	3.9713 g	0.0952 g 0.0981 g	214.17 217.05	1.05 1.05	213.119 215.999			2238.640 2201.820	8817.2009 dpm	0.018
2-06	Exhaled VOC Trap 1	1 h	16.5047 g	18.8733 g	2.3686 g	0.0928 g 0.0950 g	59116.26 60404.46	0.56 0.56	59115.705 60403.905			637022.683 635830.579	1507440.1183 dpm	2.688
2-06	Exhaled VOC Trap 1	3 h	5.4573 g	8.0748 g	2.6175 g	0.0962 g 0.0956 g	50487.18 50036.97	1.37 1.37	50485.807 50035.597			524800.485 523384.902	1371812.6259 dpm	2.446
2-06	Exhaled VOC Trap 1	5 h	5.4917 g	8.0427 g	2.5510 g	0.0968 g 0.0945 g	52829.12 51430.03	1.37 1.37	52827.747 51428.657			545741.185 544218.589	1390243.6913 dpm	2.479
2-06	Exhaled VOC Trap 1	7 h	6.2921 g	8.8802 g	2.5881 g	0.0943 g 0.0959 g	42269.10 42669.73	3.11 3.11	42265.990 42666.620			448207.741 444907.404	1155735.6531 dpm	2.061
2-06	Exhaled VOC Trap 1	12 h	6.4080 g	9.0483 g	2.6403 g	0.0965 g 0.0975 g	61918.35 62157.82	3.11 3.11	61915.240 62154.710			641608.705 637484.205	1688594.5049 dpm	3.011
2-06	Exhaled VOC Trap 1	24 h	6.3212 g	9.1878 g	2.8666 g	0.0981 g 0.0959 g	13209.38 12974.59	3.11 3.11	13206.270 12971.480			134620.489 135260.480	386820.3928 dpm	0.690
2-06	Exhaled VOC Trap 1	48 h	15.5030 g	19.4476 g	3.9446 g	0.0939 g 0.0961 g	10079.20 10378.35	1.05 1.05	10078.149 10377.299			107328.526 107984.376	424661.6370 dpm	0.757
2-06	Exhaled VOC Trap 1	72 h	15.3767 g	19.3583 g	3.9816 g	0.0948 g 0.0928 g	2070.28 2010.22	1.05 1.05	2069.229 2009.169			21827.306 21650.523	86555.6620 dpm	0.154
2-06	Exhaled VOC Trap 1	96 h	15.4565 g	19.1471 g	3.6906 g	0.0936 g 0.0947 g	655.83 667.72	1.05 1.05	654.779 666.669			6995.498 7039.795	25899.3251 dpm	0.046
2-06	Exhaled VOC Trap 1	120 h	15.4861 g	19.2296 g	3.7435 g	0.0949 g 0.0945 g	494.01 507.58	1.05 1.05	492.959 506.529			5194.505 5360.091	19755.5654 dpm	0.035
2-06	Exhaled VOC Trap 1	144 h	15.4536 g	19.4112 g	3.9576 g	0.0937 g 0.0966 g	359.16 384.58	1.05 1.05	358.109 383.529			3821.863 3970.275	15419.0830 dpm	0.027
2-06	Exhaled VOC Trap 1	168 h	15.5544 g	19.2828 g	3.7284 g	0.0935 g 0.0938 g	381.27 376.35	1.05 1.05	380.219 375.299			4066.509 4001.051	15039.5447 dpm	0.027

* 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 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 (%)
2-07	Exhaled VOC Trap 1	1 h	16.4649 g	19.0175 g	2.5526 g	0.0963 g 0.0961 g	54791.73 54575.29	0.56 0.56	54791.175 54574.735			568963.396 567895.265	1450972.7090 dpm	2.415
2-07	Exhaled VOC Trap 1	3 h	5.5508 g	8.0311 g	2.4803 g	0.0965 g 0.0952 g	52033.66 51447.91	1.37 1.37	52032.287 51446.537			539194.680 540404.797	1338865.2919 dpm	2.229
2-07	Exhaled VOC Trap 1	5 h	5.6245 g	8.2297 g	2.6052 g	0.0962 g 0.0940 g	50508.80 49786.18	1.37 1.37	50507.427 49784.807			525025.225 529625.603	1373788.1686 dpm	2.287
2-07	Exhaled VOC Trap 1	7 h	6.2672 g	8.8190 g	2.5518 g	0.0958 g 0.0944 g	54592.50 53545.45	3.11 3.11	54589.390 53542.340			569826.618 567185.805	1450714.1506 dpm	2.415
2-07	Exhaled VOC Trap 1	12 h	6.3533 g	8.9389 g	2.5856 g	0.0956 g 0.0944 g	82855.93 81814.11	3.11 3.11	82852.820 81811.000			866661.297 866641.949	2240814.4367 dpm	3.730
2-07	Exhaled VOC Trap 1	24 h	6.3045 g	9.4339 g	3.1294 g	0.0971 g 0.0956 g	23596.90 23228.77	3.11 3.11	23593.790 23225.660			242984.449 242946.234	760335.7402 dpm	1.266
2-07	Exhaled VOC Trap 1	48 h	15.3679 g	19.0664 g	3.6985 g	0.0948 g 0.0941 g	8604.37 8587.18	1.05 1.05	8603.319 8586.129			90752.306 91244.724	336558.0078 dpm	0.560
2-07	Exhaled VOC Trap 1	72 h	15.5164 g	19.2441 g	3.7277 g	0.0934 g 0.0960 g	601.52 589.66	1.05 1.05	600.469 588.609			6429.000 6131.339	23410.5878 dpm	0.039
2-07	Exhaled VOC Trap 1	96 h	15.3215 g	19.1944 g	3.8729 g	0.0944 g 0.0960 g	435.31 455.73	1.05 1.05	434.259 454.679			4600.197 4736.235	18079.5335 dpm	0.030
2-07	Exhaled VOC Trap 1	120 h	15.3647 g	19.3883 g	4.0236 g	0.0971 g 0.0941 g	395.20 386.89	1.05 1.05	394.149 385.839			4059.203 4100.304	16415.2946 dpm	0.027
2-07	Exhaled VOC Trap 1	144 h	15.4907 g	19.2182 g	3.7275 g	0.0936 g 0.0932 g	384.42 377.23	1.05 1.05	383.369 376.179			4095.818 4036.251	15156.1433 dpm	0.025
2-07	Exhaled VOC Trap 1	168 h	15.3582 g	19.2050 g	3.8468 g	0.0947 g 0.0956 g	263.70 283.70	1.05 1.05	262.649 282.649			2773.480 2956.575	11021.1881 dpm	0.018
2-08	Exhaled VOC Trap 1	1 h	16.1723 g	18.7103 g	2.5380 g	0.0960 g 0.0912 g	73812.57 70099.53	0.56 0.56	73812.015 70098.975			768875.156 768629.112	1951092.9162 dpm	3.147
2-08	Exhaled VOC Trap 1	3 h	5.5698 g	8.0776 g	2.5078 g	0.0954 g 0.0967 g	60185.21 61414.21	1.37 1.37	60183.837 61412.837			630857.827 635086.212	1587367.2297 dpm	2.561
2-08	Exhaled VOC Trap 1	5 h	5.4883 g	8.1929 g	2.7046 g	0.0951 g 0.0956 g	56775.29 56915.98	1.37 1.37	56773.917 56914.607			596991.763 595341.074	1612391.6954 dpm	2.601
2-08	Exhaled VOC Trap 1	7 h	6.2917 g	8.8823 g	2.5906 g	0.0969 g 0.0949 g	47799.45 47567.88	3.11 3.11	47796.340 47564.770			493254.283 501209.378	1288128.7802 dpm	2.078
2-08	Exhaled VOC Trap 1	12 h	6.3609 g	8.9430 g	2.5821 g	0.0967 g 0.0962 g	71343.36 70947.23	3.11 3.11	71340.250 70944.120			737748.190 737464.865	1904573.8148 dpm	3.072
2-08	Exhaled VOC Trap 1	24 h	6.3546 g	8.9252 g	2.5706 g	0.0952 g 0.0952 g	20182.22 20086.95	3.11 3.11	20179.110 20083.840			211965.441 210964.706	543592.1180 dpm	0.877
2-08	Exhaled VOC Trap 1	48 h	15.5288 g	19.2240 g	3.6952 g	0.0938 g 0.0944 g	3510.65 3513.97	1.05 1.05	3509.599 3512.919			37415.763 37213.120	137884.3251 dpm	0.222
2-08	Exhaled VOC Trap 1	72 h	15.4359 g	19.2739 g	3.8380 g	0.0949 g 0.0945 g	2259.23 2220.83	1.05 1.05	2258.179 2219.779			23795.348 23489.720	90740.0471 dpm	0.146
2-08	Exhaled VOC Trap 1	96 h	15.3461 g	19.3510 g	4.0049 g	0.0941 g 0.0936 g	793.48 814.51	1.05 1.05	792.429 813.459			8421.133 8690.797	34265.7827 dpm	0.055
2-08	Exhaled VOC Trap 1	120 h	15.3140 g	19.0620 g	3.7480 g	0.0935 g 0.0945 g	589.99 568.73	1.05 1.05	588.939 567.679			6298.808 6007.181	23061.4232 dpm	0.037
2-08	Exhaled VOC Trap 1	144 h	15.3891 g	19.2218 g	3.8327 g	0.0938 g 0.0955 g	441.41 451.52	1.05 1.05	440.359 450.469			4694.654 4716.948	18035.9248 dpm	0.029
2-08	Exhaled VOC Trap 1	168 h	15.4982 g	19.4540 g	3.9558 g	0.0935 g 0.0935 g	1000.56 975.18	1.05 1.05	999.509 974.129			10689.931 10418.487	41750.3412 dpm	0.067

* 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 1 Extract 2 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 (%)
2-05	VOC Trap 1 Extract 2	1 h	15.2429 g	20.5005 g	5.2576 g	0.0930 g 0.0935 g	16563.82 17160.06	0.00 0.00	16563.820 17160.060			178105.591 183530.053	950667.7832 dpm	1.922
2-05	VOC Trap 1 Extract 2	3 h	15.5891 g	20.7659 g	5.1768 g	0.0941 g 0.0947 g	18552.55 18597.69	0.15 0.15	18552.403 18597.543			197156.252 196383.773	1018639.0021 dpm	2.059
2-05	VOC Trap 1 Extract 2	5 h	15.3572 g	20.4054 g	5.0482 g	0.0945 g 0.0960 g	21425.14 21882.97	0.15 0.15	21424.993 21882.823			226719.506 227946.076	1147621.3969 dpm	2.320
2-05	VOC Trap 1 Extract 2	7 h	15.3069 g	20.3657 g	5.0588 g	0.0937 g 0.0941 g	13415.99 13385.55	0.89 0.89	13415.100 13384.660			143170.758 142238.682	721914.6375 dpm	1.459
2-05	VOC Trap 1 Extract 2	12 h	15.5266 g	20.5913 g	5.0647 g	0.0935 g 0.0941 g	17599.39 17574.75	0.89 0.89	17598.500 17573.860			188219.251 186757.279	949571.8178 dpm	1.919
2-05	VOC Trap 1 Extract 2	24 h	15.4645 g	20.6807 g	5.2162 g	0.0939 g 0.0942 g	3319.49 3330.14	0.89 0.89	3318.600 3329.250			35341.853 35342.357	184351.4874 dpm	0.373
2-06	VOC Trap 1 Extract 2	1 h	15.0710 g	20.3594 g	5.2884 g	0.0933 g 0.0913 g	23194.21 22777.92	0.00 0.00	23194.210 22777.920			248598.178 249484.337	1317029.7869 dpm	2.348
2-06	VOC Trap 1 Extract 2	3 h	15.4822 g	20.7871 g	5.3049 g	0.0940 g 0.0934 g	21261.65 21039.99	0.15 0.15	21261.503 21039.843			226186.206 225265.989	1197454.3726 dpm	2.135
2-06	VOC Trap 1 Extract 2	5 h	15.5796 g	20.6246 g	5.0450 g	0.0943 g 0.0929 g	22811.83 22526.41	0.15 0.15	22811.683 22526.263			241905.444 242478.615	1221858.7879 dpm	2.178
2-06	VOC Trap 1 Extract 2	7 h	15.6275 g	20.6480 g	5.0205 g	0.0947 g 0.0946 g	15575.44 15493.96	0.89 0.89	15574.550 15493.070			164461.985 163774.524	823955.6980 dpm	1.469
2-06	VOC Trap 1 Extract 2	12 h	15.5558 g	20.5583 g	5.0025 g	0.0946 g 0.0944 g	26917.67 26820.97	0.89 0.89	26916.780 26820.080			284532.558 284111.017	1422319.7422 dpm	2.536
2-06	VOC Trap 1 Extract 2	24 h	15.7434 g	20.4903 g	4.7469 g	0.0937 g 0.0941 g	5683.56 5728.98	0.89 0.89	5682.670 5728.090			60647.492 60872.370	288421.3160 dpm	0.514
2-07	VOC Trap 1 Extract 2	1 h	15.2044 g	20.3277 g	5.1233 g	0.0932 g 0.0957 g	26396.38 27060.66	0.00 0.00	26396.380 27060.660			283222.961 282765.517	1449864.3862 dpm	2.414
2-07	VOC Trap 1 Extract 2	3 h	15.4117 g	20.7273 g	5.3156 g	0.0947 g 0.0938 g	21999.11 21845.64	0.15 0.15	21998.963 21845.493			232301.619 232894.385	1236397.9404 dpm	2.058
2-07	VOC Trap 1 Extract 2	5 h	15.3420 g	20.6164 g	5.2744 g	0.0942 g 0.0939 g	23272.73 23102.99	0.15 0.15	23272.583 23102.843			247055.025 246036.670	1300381.4180 dpm	2.165
2-07	VOC Trap 1 Extract 2	7 h	15.3742 g	20.5050 g	5.1308 g	0.0939 g 0.0958 g	19077.29 19512.60	0.89 0.89	19076.400 19511.710			203156.550 203671.294	1043676.1507 dpm	1.737
2-07	VOC Trap 1 Extract 2	12 h	15.4368 g	20.5857 g	5.1489 g	0.0943 g 0.0945 g	32103.48 32335.74	0.89 0.89	32102.590 32334.850			340430.435 342167.725	1757314.8321 dpm	2.925
2-07	VOC Trap 1 Extract 2	24 h	15.3879 g	20.4294 g	5.0415 g	0.0944 g 0.0925 g	8933.41 8608.51	0.89 0.89	8932.520 8607.620			94624.153 93055.351	473093.1094 dpm	0.788
2-08	VOC Trap 1 Extract 2	1 h	14.8992 g	19.9990 g	5.0998 g	0.0925 g 0.0935 g	26759.04 27139.56	0.00 0.00	26759.040 27139.560			289286.919 290262.674	1477793.5065 dpm	2.384
2-08	VOC Trap 1 Extract 2	3 h	15.4964 g	20.5783 g	5.0819 g	0.0948 g 0.0929 g	28609.10 28134.04	0.15 0.15	28608.953 28133.893			301782.208 302840.617	1536316.3680 dpm	2.478
2-08	VOC Trap 1 Extract 2	5 h	15.5654 g	20.6280 g	5.0626 g	0.0937 g 0.0945 g	26714.79 26889.67	0.15 0.15	26714.643 26889.523			285108.253 284545.220	1441963.8381 dpm	2.326
2-08	VOC Trap 1 Extract 2	7 h	15.3876 g	20.3979 g	5.0103 g	0.0948 g 0.0938 g	17776.98 17412.43	0.89 0.89	17776.090 17411.540			187511.498 185624.094	934760.6276 dpm	1.508
2-08	VOC Trap 1 Extract 2	12 h	15.3812 g	20.4511 g	5.0699 g	0.0936 g 0.0936 g	24270.62 24348.38	0.89 0.89	24269.730 24347.490			259291.987 260122.756	1316690.4043 dpm	2.124
2-08	VOC Trap 1 Extract 2	24 h	15.4964 g	20.6431 g	5.1467 g	0.0948 g 0.0946 g	6953.84 6925.89	0.89 0.89	6952.950 6925.000			73343.354 73202.960	377114.9578 dpm	0.608

* 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 Exhaled VOC Trap 2 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 (%)
2-05	Exhaled VOC Trap 2	1 h	15.3426 g	19.3568 g	4.0142 g	0.0933 g	4.67	4.33	0.340	*		3.644	7.3142 dpm	0.000
						0.0948 g	2.22	4.33	0.000	*		0.000		
2-05	Exhaled VOC Trap 2	3 h	15.4097 g	19.2040 g	3.7943 g	0.0936 g	0.56	0.79	0.000	*		0.000	13.5438 dpm	0.000
						0.0928 g	1.45	0.79	0.663	*		7.139		
2-05	Exhaled VOC Trap 2	5 h	15.4169 g	19.3380 g	3.9211 g	0.0937 g	3.23	0.79	2.443	*		26.067	51.1061 dpm	0.000
						0.0940 g	0.11	0.79	0.000	*		0.000		
2-05	Exhaled VOC Trap 2	7 h	15.2511 g	19.1544 g	3.9033 g	0.0933 g	4.56	0.79	3.773	*		40.434	78.9132 dpm	0.000
						0.0930 g	0.00	0.79	0.000	*		0.000		
2-05	Exhaled VOC Trap 2	12 h	15.4542 g	19.3548 g	3.9006 g	0.0958 g	0.00	0.79	0.000	*		0.000	0.0000 dpm	0.000
						0.0958 g	0.00	0.79	0.000	*		0.000		
2-05	Exhaled VOC Trap 2	24 h	15.6718 g	19.5417 g	3.8699 g	0.0943 g	0.00	0.79	0.000	*		0.000	9.0865 dpm	0.000
						0.0921 g	1.22	0.79	0.433	*		4.696		
2-05	Exhaled VOC Trap 2	48 h	15.4354 g	19.3379 g	3.9025 g	0.0941 g	6.34	0.79	5.553			59.006	275.8430 dpm	0.001
						0.0917 g	8.34	0.79	7.553			82.361		
2-05	Exhaled VOC Trap 2	72 h	15.3993 g	19.2778 g	3.8785 g	0.0941 g	1367.72	0.79	1366.933			14526.382	55849.4086 dpm	0.113
						0.0951 g	1358.16	0.79	1357.373			14273.107		
2-05	Exhaled VOC Trap 2	96 h	15.5076 g	19.3758 g	3.8682 g	0.0937 g	1.67	0.79	0.883	*		9.418	18.2160 dpm	0.000
						0.0943 g	0.33	0.79	0.000	*		0.000		
2-05	Exhaled VOC Trap 2	120 h	15.5718 g	19.5836 g	4.0118 g	0.0943 g	0.00	0.79	0.000	*		0.000	0.0000 dpm	0.000
						0.0946 g	0.00	0.79	0.000	*		0.000		
2-05	Exhaled VOC Trap 2	144 h	15.5200 g	19.3984 g	3.8784 g	0.0942 g	4.79	0.79	4.003			42.489	146.7416 dpm	0.000
						0.0935 g	3.89	0.79	3.103			33.182		
2-05	Exhaled VOC Trap 2	168 h	15.5800 g	19.4838 g	3.9038 g	0.0942 g	0.00	0.79	0.000	*		0.000	0.0000 dpm	0.000
						0.0931 g	0.56	0.79	0.000	*		0.000		
2-06	Exhaled VOC Trap 2	1 h	15.3245 g	19.3504 g	4.0259 g	0.0929 g	0.00	4.33	0.000	*		0.000	0.0000 dpm	0.000
						0.0955 g	1.56	4.33	0.000	*		0.000		
2-06	Exhaled VOC Trap 2	3 h	15.3156 g	19.0605 g	3.7449 g	0.0928 g	6.34	0.79	5.553	*		59.833	112.0343 dpm	0.000
						0.0931 g	0.00	0.79	0.000	*		0.000		
2-06	Exhaled VOC Trap 2	5 h	15.1946 g	19.1061 g	3.9115 g	0.0936 g	4.34	0.79	3.553	*		37.954	74.2287 dpm	0.000
						0.0922 g	0.56	0.79	0.000	*		0.000		
2-06	Exhaled VOC Trap 2	7 h	15.2829 g	19.1896 g	3.9067 g	0.0931 g	1.89	0.79	1.103	*		11.842	23.1318 dpm	0.000
						0.0952 g	0.11	0.79	0.000	*		0.000		
2-06	Exhaled VOC Trap 2	12 h	15.5104 g	19.4298 g	3.9194 g	0.0941 g	3.67	0.79	2.883	*		30.632	60.0301 dpm	0.000
						0.0933 g	0.00	0.79	0.000	*		0.000		
2-06	Exhaled VOC Trap 2	24 h	15.5566 g	19.4764 g	3.9198 g	0.0935 g	0.78	0.79	0.000	*		0.000	86.2723 dpm	0.000
						0.0907 g	4.78	0.79	3.993	*		44.019		
2-06	Exhaled VOC Trap 2	48 h	15.5687 g	19.5009 g	3.9322 g	0.0936 g	11.90	0.79	11.113			118.723	423.4122 dpm	0.001
						0.0943 g	9.90	0.79	9.113			96.633		
2-06	Exhaled VOC Trap 2	72 h	15.5236 g	19.4145 g	3.8909 g	0.0930 g	14.35	0.79	13.563			145.833	402.1725 dpm	0.001
						0.0948 g	6.56	0.79	5.773			60.891		
2-06	Exhaled VOC Trap 2	96 h	15.4612 g	19.4204 g	3.9592 g	0.0940 g	17.02	0.79	16.233			172.686	669.9382 dpm	0.001
						0.0939 g	16.35	0.79	15.563			165.735		
2-06	Exhaled VOC Trap 2	120 h	15.5035 g	19.4640 g	3.9605 g	0.0940 g	11.68	0.79	10.893			115.878	517.1124 dpm	0.001
						0.0933 g	14.34	0.79	13.553			145.257		
2-06	Exhaled VOC Trap 2	144 h	15.4627 g	19.3511 g	3.8884 g	0.0942 g	11.24	0.79	10.453			110.961	350.1237 dpm	0.001
						0.0932 g	7.23	0.79	6.443			69.126		

* 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 (contd). Recovery of Radioactivity in Exhaled VOC Trap 2 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 (%)
2-06	Exhaled VOC Trap 2	168 h	15.4423 g	19.3899 g	3.9476 g	0.0936 g 0.0913 g	5.23 0.78	0.79 0.79	4.443 0.000	* *		47.463 0.000	93.6817 dpm	0.000
2-07	Exhaled VOC Trap 2	1 h	15.0724 g	19.1245 g	4.0521 g	0.0935 g 0.0956 g	2.00 1.11	4.33 4.33	0.000 0.000	* *		0.000 0.000	0.0000 dpm	0.000
2-07	Exhaled VOC Trap 2	3 h	15.4542 g	19.4454 g	3.9912 g	0.0934 g 0.0935 g	4.34 8.78	0.79 0.79	3.553 7.993			38.035 85.481	246.4898 dpm	0.000
2-07	Exhaled VOC Trap 2	5 h	15.2801 g	19.1518 g	3.8717 g	0.0937 g 0.0959 g	0.11 5.23	0.79 0.79	0.000 4.443	* *		0.000 46.324	89.6769 dpm	0.000
2-07	Exhaled VOC Trap 2	7 h	15.1926 g	19.1153 g	3.9227 g	0.0928 g 0.0940 g	0.00 2.34	0.79 0.79	0.000 1.553	* *		0.000 16.516	32.3936 dpm	0.000
2-07	Exhaled VOC Trap 2	12 h	15.5111 g	19.4129 g	3.9018 g	0.0938 g 0.0935 g	0.00 5.23	0.79 0.79	0.000 4.443	* *		0.000 47.513	92.6938 dpm	0.000
2-07	Exhaled VOC Trap 2	24 h	15.4917 g	19.4203 g	3.9286 g	0.0943 g 0.0931 g	71.33 61.29	0.79 0.79	70.543 60.503			748.065 649.866	2745.9547 dpm	0.005
2-07	Exhaled VOC Trap 2	48 h	15.4092 g	19.3010 g	3.8918 g	0.0940 g 0.0940 g	0.00 5.00	0.79 0.79	0.000 4.213	* *		0.000 44.814	87.2032 dpm	0.000
2-07	Exhaled VOC Trap 2	72 h	15.4296 g	19.3698 g	3.9402 g	0.0939 g 0.0946 g	0.00 4.79	0.79 0.79	0.000 4.003	* *		0.000 42.310	83.3544 dpm	0.000
2-07	Exhaled VOC Trap 2	96 h	15.3534 g	19.2494 g	3.8960 g	0.0940 g 0.0947 g	0.00 1.00	0.79 0.79	0.000 0.213	* *		0.000 2.244	4.3712 dpm	0.000
2-07	Exhaled VOC Trap 2	120 h	15.2014 g	18.9873 g	3.7859 g	0.0932 g 0.0962 g	0.00 0.00	0.79 0.79	0.000 0.000	* *		0.000 0.000	0.0000 dpm	0.000
2-07	Exhaled VOC Trap 2	144 h	15.4584 g	19.3407 g	3.8823 g	0.0936 g 0.0941 g	2.56 5.23	0.79 0.79	1.773 4.443			18.937 47.210	128.4020 dpm	0.000
2-07	Exhaled VOC Trap 2	168 h	15.4710 g	19.3587 g	3.8877 g	0.0941 g 0.0970 g	2.78 0.00	0.79 0.79	1.993 0.000	* *		21.174 0.000	41.1596 dpm	0.000
2-08	Exhaled VOC Trap 2	1 h	15.3109 g	19.3126 g	4.0017 g	0.0934 g 0.0906 g	0.89 1.56	4.33 4.33	0.000 0.000	* *		0.000 0.000	0.0000 dpm	0.000
2-08	Exhaled VOC Trap 2	3 h	15.3969 g	19.3144 g	3.9175 g	0.0933 g 0.0942 g	1.00 3.00	0.79 0.79	0.213 2.213		* *	2.278 23.487	50.4669 dpm	0.000
2-08	Exhaled VOC Trap 2	5 h	15.3218 g	19.1980 g	3.8762 g	0.0930 g 0.0947 g	1.67 1.45	0.79 0.79	0.883 0.663		* *	9.489 6.996	31.9496 dpm	0.000
2-08	Exhaled VOC Trap 2	7 h	15.2221 g	19.1040 g	3.8819 g	0.0927 g 0.0913 g	0.00 1.67	0.79 0.79	0.000 0.883	* *		0.000 9.666	18.7611 dpm	0.000
2-08	Exhaled VOC Trap 2	12 h	15.4036 g	19.3119 g	3.9083 g	0.0936 g 0.0938 g	0.00 0.00	0.79 0.79	0.000 0.000	* *		0.000 0.000	0.0000 dpm	0.000
2-08	Exhaled VOC Trap 2	24 h	15.6988 g	19.6286 g	3.9298 g	0.0947 g 0.0945 g	2.78 4.34	0.79 0.79	1.993 3.553			21.040 37.593	115.2074 dpm	0.000
2-08	Exhaled VOC Trap 2	48 h	15.3274 g	19.2230 g	3.8956 g	0.0941 g 0.0970 g	4.34 1.22	0.79 0.79	3.553 0.433		* *	37.752 4.459	82.2189 dpm	0.000
2-08	Exhaled VOC Trap 2	72 h	15.4340 g	19.4207 g	3.9867 g	0.0937 g 0.0935 g	4.78 1.44	0.79 0.79	3.993 0.653		* *	42.609 6.979	98.8462 dpm	0.000
2-08	Exhaled VOC Trap 2	96 h	15.4458 g	19.3784 g	3.9326 g	0.0934 g 0.0936 g	7.67 9.90	0.79 0.79	6.883 9.113			73.688 97.356	336.3242 dpm	0.001
2-08	Exhaled VOC Trap 2	120 h	15.4225 g	19.3224 g	3.8999 g	0.0935 g 0.0961 g	11.23 14.13	0.79 0.79	10.443 13.343			111.684 138.840	488.5097 dpm	0.001
2-08	Exhaled VOC Trap 2	144 h	15.4391 g	19.3232 g	3.8841 g	0.0938 g 0.0928 g	5.00 8.12	0.79 0.79	4.213 7.333			44.909 79.014	240.6654 dpm	0.000
2-08	Exhaled VOC Trap 2	168 h	15.2489 g	19.1267 g	3.8778 g	0.0939 g 0.0937 g	1.00 1.67	0.79 0.79	0.213 0.883		* *	2.263 9.418	22.6491 dpm	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 11. Recovery of Radioactivity in Nose Only Tube Rinse 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 (%)
2-05	Nose Only Tube Rinse	0 h	83.8656 g	134.9639 g	51.0983 g	0.1686 g 0.1655 g	1122.26 1138.91	1.70 1.70	1120.565 1137.215			6646.293 6871.390	345365.3037 dpm	0.698
2-06	Nose Only Tube Rinse	0 h	79.5942 g	129.8091 g	50.2149 g	0.1474 g 0.1488 g	405.76 415.32	1.70 1.70	404.065 413.625			2741.282 2779.738	138618.7368 dpm	0.247
2-07	Nose Only Tube Rinse	0 h	84.2557 g	127.9618 g	43.7061 g	0.1409 g 0.1264 g	455.13 419.69	1.70 1.70	453.435 417.995			3218.133 3306.922	142592.3728 dpm	0.237
2-08	Nose Only Tube Rinse	0 h	78.3760 g	125.9693 g	47.5933 g	0.1332 g 0.1302 g	331.08 302.35	1.70 1.70	329.385 300.655			2472.860 2309.178	113796.4976 dpm	0.184

Table 12. Recovery of Radioactivity in Transfer Bag Rinse 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 (%)
2-05	Transfer Bag Rinse	0 h	290.66 g	496.78 g	206.12 g	0.1516 g 0.1404 g	8.80 5.90	1.04 1.04	7.760 4.860			51.187 34.615	8842.8283 dpm	0.018
2-06	Transfer Bag Rinse	0 h	287.30 g	583.63 g	296.33 g	0.1317 g 0.1336 g	8.35 9.91	1.04 1.04	7.310 8.870			55.505 66.392	18060.8914 dpm	0.032
2-07	Transfer Bag Rinse	0 h	288.07 g	603.49 g	315.42 g	0.1212 g 0.1244 g	1.22 5.23	1.04 1.04	0.180 4.190		* *	1.485 33.682	5546.1593 dpm	0.009
2-08	Transfer Bag Rinse	0 h	287.34 g	580.22 g	292.88 g	0.1193 g 0.1251 g	3.67 5.46	1.04 1.04	2.630 4.420			22.045 35.332	8402.2877 dpm	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 13. Recovery of Radioactivity in Cage Rinse 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 (%)
2-05	Cage Rinse	168 h	169.96 g	370.04 g	200.08 g	0.8920 g 0.8937 g	298.38 318.99	2.39 2.39	295.995 316.605			331.833 354.263	68637.0574 dpm	0.139
2-06	Cage Rinse	168 h	168.90 g	358.74 g	189.84 g	0.8893 g 0.9284 g	1075.24 1128.62	2.39 2.39	1072.855 1126.235			1206.404 1213.092	229658.5917 dpm	0.409
2-07	Cage Rinse	168 h	169.17 g	353.03 g	183.86 g	0.9166 g 0.9270 g	793.43 795.41	2.39 2.39	791.045 793.025			863.021 855.475	157981.3002 dpm	0.263
2-08	Cage Rinse	168 h	169.62 g	386.55 g	216.93 g	0.9011 g 0.9239 g	890.18 939.75	2.39 2.39	887.795 937.365			985.235 1014.574	216909.2616 dpm	0.350

Table 14. Recovery of Radioactivity in Carcass Digest from Group 2.

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 (%)
2-05	Carcass Digest	168 h	107.75 g	322.35 g	214.60 g	691.13 g	583.38 g	0.8479 g 0.9093 g	413.43 452.28	0.96 0.96	412.470 451.320			486.461 496.338	286672.4973 dpm	0.579
2-06	Carcass Digest	168 h	107.74 g	324.03 g	216.29 g	724.91 g	617.17 g	0.8404 g 0.8884 g	617.09 615.51	0.96 0.96	616.130 614.550			733.139 691.749	439699.1232 dpm	0.784
2-07	Carcass Digest	168 h	107.66 g	327.39 g	219.73 g	677.63 g	569.97 g	0.8519 g 0.9302 g	567.26 601.26	0.96 0.96	566.300 600.300			664.749 645.345	373357.2728 dpm	0.622
2-08	Carcass Digest	168 h	107.91 g	314.63 g	206.72 g	859.98 g	752.07 g	0.8656 g 0.9196 g	375.01 377.41	0.96 0.96	374.050 376.450			432.128 409.363	316429.9817 dpm	0.510

Table 15. Total Recovery of Radioactivity in Samples from Group 2.

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Recovery (%)
2-05	Urine	12 h	23125096.3422 dpm	49473192.233 dpm	46.743
2-05	Urine	24 h	9215552.9007 dpm		18.627
2-05	Urine	48 h	3314508.8677 dpm		6.700
2-05	Urine	72 h	738050.3714 dpm		1.492
2-05	Urine	96 h	140656.7202 dpm		0.284
2-05	Urine	120 h	63433.4395 dpm		0.128
2-05	Urine	144 h	42178.5521 dpm		0.085
2-05	Urine	168 h	26728.5757 dpm		0.054
2-05	Feces	0 h	62439.5633 dpm		0.126
2-05	Feces	24 h	459570.6609 dpm		0.929
2-05	Feces	48 h	66362.2065 dpm		0.134
2-05	Feces	72 h	32322.9893 dpm		0.065
2-05	Feces	96 h	8305.7059 dpm		0.017
2-05	Feces	120 h	2410.2081 dpm		0.005
2-05	Feces	144 h	4516.0314 dpm		0.009
2-05	Feces	168 h	3769.9026 dpm		0.008
2-05	Carcass Digest	168 h	286672.4973 dpm		0.579
2-05	CO2 Trap 1	1 h	25312.6590 dpm		0.051
2-05	CO2 Trap 1	3 h	29938.9474 dpm		0.061
2-05	CO2 Trap 1	5 h	28709.8064 dpm		0.058
2-05	CO2 Trap 1	7 h	20676.0218 dpm		0.042
2-05	CO2 Trap 1	12 h	41007.3838 dpm		0.083
2-05	CO2 Trap 1	24 h	80693.9335 dpm		0.163
2-05	CO2 Trap 1	48 h	30607.4163 dpm		0.062
2-05	CO2 Trap 1	72 h	11034.1810 dpm		0.022
2-05	CO2 Trap 1	96 h	6345.6999 dpm		0.013
2-05	CO2 Trap 1	120 h	3879.4523 dpm		0.008
2-05	CO2 Trap 1	144 h	1729.9618 dpm		0.003
2-05	CO2 Trap 1	168 h	2677.0845 dpm		0.005
2-05	CO2 Trap 2	1 h	522.5057 dpm		0.001
2-05	CO2 Trap 2	3 h	444.9252 dpm		0.001
2-05	CO2 Trap 2	5 h	114.2960 dpm		0.000
2-05	CO2 Trap 2	7 h	245.3675 dpm		0.000
2-05	CO2 Trap 2	12 h	166.0539 dpm		0.000
2-05	CO2 Trap 2	24 h	812.2073 dpm		0.002
2-05	CO2 Trap 2	48 h	4217.3753 dpm		0.009
2-05	CO2 Trap 2	72 h	2249.7045 dpm		0.005
2-05	CO2 Trap 2	96 h	0.0000 dpm		0.000
2-05	CO2 Trap 2	120 h	420.3863 dpm		0.001
2-05	CO2 Trap 2	144 h	905.0133 dpm		0.002
2-05	CO2 Trap 2	168 h	0.0000 dpm		0.000
2-05	Exhaled VOC Trap 1	1 h	1068098.5005 dpm		2.159
2-05	Exhaled VOC Trap 1	3 h	1208450.3452 dpm		2.443
2-05	Exhaled VOC Trap 1	5 h	1345096.8679 dpm		2.719
2-05	Exhaled VOC Trap 1	7 h	1092635.9138 dpm		2.209
2-05	Exhaled VOC Trap 1	12 h	1053254.2231 dpm		2.129
2-05	Exhaled VOC Trap 1	24 h	222123.4352 dpm		0.449
2-05	Exhaled VOC Trap 1	48 h	91105.2155 dpm		0.184
2-05	Exhaled VOC Trap 1	72 h	156.6140 dpm		0.000
2-05	Exhaled VOC Trap 1	96 h	17936.2656 dpm		0.036
2-05	Exhaled VOC Trap 1	120 h	18871.8712 dpm		0.038
2-05	Exhaled VOC Trap 1	144 h	9298.3749 dpm		0.019
2-05	Exhaled VOC Trap 1	168 h	8817.2009 dpm		0.018
2-05	Exhaled VOC Trap 2	1 h	7.3142 dpm		0.000
2-05	Exhaled VOC Trap 2	3 h	13.5438 dpm		0.000
2-05	Exhaled VOC Trap 2	5 h	51.1061 dpm		0.000
2-05	Exhaled VOC Trap 2	7 h	78.9132 dpm		0.000
2-05	Exhaled VOC Trap 2	12 h	0.0000 dpm		0.000
2-05	Exhaled VOC Trap 2	24 h	9.0865 dpm		0.000
2-05	Exhaled VOC Trap 2	48 h	275.8430 dpm		0.001
2-05	Exhaled VOC Trap 2	72 h	55849.4086 dpm		0.113
2-05	Exhaled VOC Trap 2	96 h	18.2160 dpm		0.000
2-05	Exhaled VOC Trap 2	120 h	0.0000 dpm		0.000
2-05	Exhaled VOC Trap 2	144 h	146.7416 dpm		0.000
2-05	Exhaled VOC Trap 2	168 h	0.0000 dpm		0.000

Table 15 (contd). Total Recovery of Radioactivity in Samples from Group 2.

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Recovery (%)
2-05	Cage Rinse	168 h	68637.0574 dpm		0.139
2-05	Nose Only Tube Rinse	0 h	345365.3037 dpm		0.698
2-05	Transfer Bag Rinse	0 h	8842.8283 dpm		0.018
2-05	VOC Trap 1 Extract 2	1 h	950667.7832 dpm		1.922
2-05	VOC Trap 1 Extract 2	3 h	1018639.0021 dpm		2.059
2-05	VOC Trap 1 Extract 2	5 h	1147621.3969 dpm		2.320
2-05	VOC Trap 1 Extract 2	7 h	721914.6375 dpm		1.459
2-05	VOC Trap 1 Extract 2	12 h	949571.8178 dpm		1.919
2-05	VOC Trap 1 Extract 2	24 h	184351.4874 dpm		0.373
2-06	Urine	12 h	19625547.7404 dpm	56087309.043 dpm	34.991
2-06	Urine	24 h	10786004.9045 dpm		19.231
2-06	Urine	48 h	6807039.5921 dpm		12.137
2-06	Urine	72 h	1452727.2057 dpm		2.590
2-06	Urine	96 h	307498.4988 dpm		0.548
2-06	Urine	120 h	164906.5238 dpm		0.294
2-06	Urine	144 h	216898.4753 dpm		0.387
2-06	Urine	168 h	180306.6963 dpm		0.321
2-06	Feces	0 h	149217.4568 dpm		0.266
2-06	Feces	24 h	533710.0277 dpm		0.952
2-06	Feces	48 h	115725.3897 dpm		0.206
2-06	Feces	72 h	31618.8213 dpm		0.056
2-06	Feces	96 h	35257.2997 dpm		0.063
2-06	Feces	120 h	4072.5462 dpm		0.007
2-06	Feces	144 h	7423.2265 dpm		0.013
2-06	Feces	168 h	160427.6740 dpm		0.286
2-06	Carcass Digest	168 h	439699.1232 dpm		0.784
2-06	CO2 Trap 1	1 h	24431.0438 dpm		0.044
2-06	CO2 Trap 1	3 h	31814.5020 dpm		0.057
2-06	CO2 Trap 1	5 h	28364.8291 dpm		0.051
2-06	CO2 Trap 1	7 h	25273.3452 dpm		0.045
2-06	CO2 Trap 1	12 h	48016.0344 dpm		0.086
2-06	CO2 Trap 1	24 h	78910.1092 dpm		0.141
2-06	CO2 Trap 1	48 h	38684.0640 dpm		0.069
2-06	CO2 Trap 1	72 h	13010.2157 dpm		0.023
2-06	CO2 Trap 1	96 h	5881.9861 dpm		0.010
2-06	CO2 Trap 1	120 h	5013.1972 dpm		0.009
2-06	CO2 Trap 1	144 h	2136.6406 dpm		0.004
2-06	CO2 Trap 1	168 h	1337.1844 dpm		0.002
2-06	CO2 Trap 2	1 h	64.7138 dpm		0.000
2-06	CO2 Trap 2	3 h	1220.5133 dpm		0.002
2-06	CO2 Trap 2	5 h	544.8391 dpm		0.001
2-06	CO2 Trap 2	7 h	1571.7899 dpm		0.003
2-06	CO2 Trap 2	12 h	1000.6984 dpm		0.002
2-06	CO2 Trap 2	24 h	1400.2508 dpm		0.002
2-06	CO2 Trap 2	48 h	6107.0738 dpm		0.011
2-06	CO2 Trap 2	72 h	3515.8090 dpm		0.006
2-06	CO2 Trap 2	96 h	1645.0503 dpm		0.003
2-06	CO2 Trap 2	120 h	63.8683 dpm		0.000
2-06	CO2 Trap 2	144 h	615.2279 dpm		0.001
2-06	CO2 Trap 2	168 h	436.9899 dpm		0.001
2-06	Exhaled VOC Trap 1	1 h	1507440.1183 dpm		2.688
2-06	Exhaled VOC Trap 1	3 h	1371812.6259 dpm		2.446
2-06	Exhaled VOC Trap 1	5 h	1390243.6913 dpm		2.479
2-06	Exhaled VOC Trap 1	7 h	1155735.6531 dpm		2.061
2-06	Exhaled VOC Trap 1	12 h	1688594.5049 dpm		3.011
2-06	Exhaled VOC Trap 1	24 h	386820.3928 dpm		0.690
2-06	Exhaled VOC Trap 1	48 h	424661.6370 dpm		0.757
2-06	Exhaled VOC Trap 1	72 h	86555.6620 dpm		0.154
2-06	Exhaled VOC Trap 1	96 h	25899.3251 dpm		0.046
2-06	Exhaled VOC Trap 1	120 h	19755.5654 dpm		0.035
2-06	Exhaled VOC Trap 1	144 h	15419.0830 dpm		0.027
2-06	Exhaled VOC Trap 1	168 h	15039.5447 dpm		0.027
2-06	Exhaled VOC Trap 2	1 h	0.0000 dpm		0.000
2-06	Exhaled VOC Trap 2	3 h	112.0343 dpm		0.000

Table 15 (contd). Total Recovery of Radioactivity in Samples from Group 2.

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Recovery (%)
2-06	Exhaled VOC Trap 2	5 h	74.2287 dpm		0.000
2-06	Exhaled VOC Trap 2	7 h	23.1318 dpm		0.000
2-06	Exhaled VOC Trap 2	12 h	60.0301 dpm		0.000
2-06	Exhaled VOC Trap 2	24 h	86.2723 dpm		0.000
2-06	Exhaled VOC Trap 2	48 h	423.4122 dpm		0.001
2-06	Exhaled VOC Trap 2	72 h	402.1725 dpm		0.001
2-06	Exhaled VOC Trap 2	96 h	669.9382 dpm		0.001
2-06	Exhaled VOC Trap 2	120 h	517.1124 dpm		0.001
2-06	Exhaled VOC Trap 2	144 h	350.1237 dpm		0.001
2-06	Exhaled VOC Trap 2	168 h	93.6817 dpm		0.000
2-06	Cage Rinse	168 h	229658.5917 dpm		0.409
2-06	Nose Only Tube Rinse	0 h	138618.7368 dpm		0.247
2-06	Transfer Bag Rinse	0 h	18060.8914 dpm		0.032
2-06	VOC Trap 1 Extract 2	1 h	1317029.7869 dpm		2.348
2-06	VOC Trap 1 Extract 2	3 h	1197454.3726 dpm		2.135
2-06	VOC Trap 1 Extract 2	5 h	1221858.7879 dpm		2.178
2-06	VOC Trap 1 Extract 2	7 h	823955.6980 dpm		1.469
2-06	VOC Trap 1 Extract 2	12 h	1422319.7422 dpm		2.536
2-06	VOC Trap 1 Extract 2	24 h	288421.3160 dpm		0.514
2-07	Urine	12 h	17643957.3395 dpm	60071898.654 dpm	29.371
2-07	Urine	24 h	16025350.6161 dpm		26.677
2-07	Urine	48 h	6414157.4994 dpm		10.677
2-07	Urine	72 h	1247567.0173 dpm		2.077
2-07	Urine	96 h	348419.9528 dpm		0.580
2-07	Urine	120 h	148079.8168 dpm		0.247
2-07	Urine	144 h	95782.6977 dpm		0.159
2-07	Urine	168 h	41599.3078 dpm		0.069
2-07	Feces	0 h	54677.4342 dpm		0.091
2-07	Feces	24 h	507316.1385 dpm		0.845
2-07	Feces	48 h	132602.3451 dpm		0.221
2-07	Feces	72 h	26795.8846 dpm		0.045
2-07	Feces	96 h	21005.6672 dpm		0.035
2-07	Feces	120 h	7748.0653 dpm		0.013
2-07	Feces	144 h	4635.6181 dpm		0.008
2-07	Feces	168 h	4230.8391 dpm		0.007
2-07	Carcass Digest	168 h	373357.2728 dpm		0.622
2-07	CO2 Trap 1	1 h	20952.4919 dpm		0.035
2-07	CO2 Trap 1	3 h	32054.4486 dpm		0.053
2-07	CO2 Trap 1	5 h	26005.3543 dpm		0.043
2-07	CO2 Trap 1	7 h	28318.4957 dpm		0.047
2-07	CO2 Trap 1	12 h	58927.0911 dpm		0.098
2-07	CO2 Trap 1	24 h	123337.9000 dpm		0.205
2-07	CO2 Trap 1	48 h	31395.8570 dpm		0.052
2-07	CO2 Trap 1	72 h	9927.4072 dpm		0.017
2-07	CO2 Trap 1	96 h	5077.6325 dpm		0.008
2-07	CO2 Trap 1	120 h	3262.5011 dpm		0.005
2-07	CO2 Trap 1	144 h	1297.3772 dpm		0.002
2-07	CO2 Trap 1	168 h	1962.0883 dpm		0.003
2-07	CO2 Trap 2	1 h	1884.2059 dpm		0.003
2-07	CO2 Trap 2	3 h	349.6990 dpm		0.001
2-07	CO2 Trap 2	5 h	1858.2873 dpm		0.003
2-07	CO2 Trap 2	7 h	5.4467 dpm		0.000
2-07	CO2 Trap 2	12 h	6.7209 dpm		0.000
2-07	CO2 Trap 2	24 h	1424.4095 dpm		0.002
2-07	CO2 Trap 2	48 h	11132.7684 dpm		0.019
2-07	CO2 Trap 2	72 h	3863.7279 dpm		0.006
2-07	CO2 Trap 2	96 h	1557.7036 dpm		0.003
2-07	CO2 Trap 2	120 h	1276.2660 dpm		0.002
2-07	CO2 Trap 2	144 h	1845.1343 dpm		0.003
2-07	CO2 Trap 2	168 h	361.5065 dpm		0.001

Table 15 (contd). Total Recovery of Radioactivity in Samples from Group 2.

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Recovery (%)
2-07	Exhaled VOC Trap 1	1 h	1450972.7090 dpm		2.415
2-07	Exhaled VOC Trap 1	3 h	1338865.2919 dpm		2.229
2-07	Exhaled VOC Trap 1	5 h	1373788.1686 dpm		2.287
2-07	Exhaled VOC Trap 1	7 h	1450714.1506 dpm		2.415
2-07	Exhaled VOC Trap 1	12 h	2240814.4367 dpm		3.730
2-07	Exhaled VOC Trap 1	24 h	760335.7402 dpm		1.266
2-07	Exhaled VOC Trap 1	48 h	336558.0078 dpm		0.560
2-07	Exhaled VOC Trap 1	72 h	23410.5878 dpm		0.039
2-07	Exhaled VOC Trap 1	96 h	18079.5335 dpm		0.030
2-07	Exhaled VOC Trap 1	120 h	16415.2946 dpm		0.027
2-07	Exhaled VOC Trap 1	144 h	15156.1433 dpm		0.025
2-07	Exhaled VOC Trap 1	168 h	11021.1881 dpm		0.018
2-07	Exhaled VOC Trap 2	1 h	0.0000 dpm		0.000
2-07	Exhaled VOC Trap 2	3 h	246.4898 dpm		0.000
2-07	Exhaled VOC Trap 2	5 h	89.6769 dpm		0.000
2-07	Exhaled VOC Trap 2	7 h	32.3936 dpm		0.000
2-07	Exhaled VOC Trap 2	12 h	92.6938 dpm		0.000
2-07	Exhaled VOC Trap 2	24 h	2745.9547 dpm		0.005
2-07	Exhaled VOC Trap 2	48 h	87.2032 dpm		0.000
2-07	Exhaled VOC Trap 2	72 h	83.3544 dpm		0.000
2-07	Exhaled VOC Trap 2	96 h	4.3712 dpm		0.000
2-07	Exhaled VOC Trap 2	120 h	0.0000 dpm		0.000
2-07	Exhaled VOC Trap 2	144 h	128.4020 dpm		0.000
2-07	Exhaled VOC Trap 2	168 h	41.1596 dpm		0.000
2-07	Cage Rinse	168 h	157981.3002 dpm		0.263
2-07	Nose Only Tube Rinse	0 h	142592.3728 dpm		0.237
2-07	Transfer Bag Rinse	0 h	5546.1593 dpm		0.009
2-07	VOC Trap 1 Extract 2	1 h	1449864.3862 dpm		2.414
2-07	VOC Trap 1 Extract 2	3 h	1236397.9404 dpm		2.058
2-07	VOC Trap 1 Extract 2	5 h	1300381.4180 dpm		2.165
2-07	VOC Trap 1 Extract 2	7 h	1043676.1507 dpm		1.737
2-07	VOC Trap 1 Extract 2	12 h	1757314.8321 dpm		2.925
2-07	VOC Trap 1 Extract 2	24 h	473093.1094 dpm		0.788
2-08	Urine	12 h	22711321.3969 dpm	61989781.106 dpm	36.637
2-08	Urine	24 h	14234955.0469 dpm		22.963
2-08	Urine	48 h	4749402.5413 dpm		7.662
2-08	Urine	72 h	872040.1056 dpm		1.407
2-08	Urine	96 h	296626.4898 dpm		0.479
2-08	Urine	120 h	126834.0564 dpm		0.205
2-08	Urine	144 h	40832.7575 dpm		0.066
2-08	Urine	168 h	37836.9722 dpm		0.061
2-08	Feces	0 h	640897.2929 dpm		1.034
2-08	Feces	24 h	495745.1346 dpm		0.800
2-08	Feces	48 h	144626.5707 dpm		0.233
2-08	Feces	72 h	55263.4920 dpm		0.089
2-08	Feces	96 h	50940.7450 dpm		0.082
2-08	Feces	120 h	5006.2519 dpm		0.008
2-08	Feces	144 h	162346.7963 dpm		0.262
2-08	Feces	168 h	25891.3274 dpm		0.042
2-08	Carcass Digest	168 h	316429.9817 dpm		0.510
2-08	CO2 Trap 1	1 h	20930.6686 dpm		0.034
2-08	CO2 Trap 1	3 h	30695.6456 dpm		0.050
2-08	CO2 Trap 1	5 h	26931.3325 dpm		0.043
2-08	CO2 Trap 1	7 h	25999.1264 dpm		0.042
2-08	CO2 Trap 1	12 h	55496.9970 dpm		0.090
2-08	CO2 Trap 1	24 h	118996.9798 dpm		0.192
2-08	CO2 Trap 1	48 h	35345.0111 dpm		0.057
2-08	CO2 Trap 1	72 h	3776.5574 dpm		0.006
2-08	CO2 Trap 1	96 h	6904.9372 dpm		0.011
2-08	CO2 Trap 1	120 h	3745.6389 dpm		0.006
2-08	CO2 Trap 1	144 h	2743.1002 dpm		0.004
2-08	CO2 Trap 1	168 h	1200.8666 dpm		0.002

Table 15 (contd). Total Recovery of Radioactivity in Samples from Group 2.

Subject	Sample	Time	Sample DPM	Sample DPM Sum	Recovery (%)
2-08	CO2 Trap 2	1 h	0.0000 dpm		0.000
2-08	CO2 Trap 2	3 h	142.1170 dpm		0.000
2-08	CO2 Trap 2	5 h	1619.0815 dpm		0.003
2-08	CO2 Trap 2	7 h	153.4922 dpm		0.000
2-08	CO2 Trap 2	12 h	0.0000 dpm		0.000
2-08	CO2 Trap 2	24 h	1728.9196 dpm		0.003
2-08	CO2 Trap 2	48 h	9895.3037 dpm		0.016
2-08	CO2 Trap 2	72 h	12320.9789 dpm		0.020
2-08	CO2 Trap 2	96 h	2520.5821 dpm		0.004
2-08	CO2 Trap 2	120 h	1565.1681 dpm		0.003
2-08	CO2 Trap 2	144 h	1515.3066 dpm		0.002
2-08	CO2 Trap 2	168 h	438.5894 dpm		0.001
2-08	Exhaled VOC Trap 1	1 h	1951092.9162 dpm		3.147
2-08	Exhaled VOC Trap 1	3 h	1587367.2297 dpm		2.561
2-08	Exhaled VOC Trap 1	5 h	1612391.6954 dpm		2.601
2-08	Exhaled VOC Trap 1	7 h	1288128.7802 dpm		2.078
2-08	Exhaled VOC Trap 1	12 h	1904573.8148 dpm		3.072
2-08	Exhaled VOC Trap 1	24 h	543592.1180 dpm		0.877
2-08	Exhaled VOC Trap 1	48 h	137884.3251 dpm		0.222
2-08	Exhaled VOC Trap 1	72 h	90740.0471 dpm		0.146
2-08	Exhaled VOC Trap 1	96 h	34265.7827 dpm		0.055
2-08	Exhaled VOC Trap 1	120 h	23061.4232 dpm		0.037
2-08	Exhaled VOC Trap 1	144 h	18035.9248 dpm		0.029
2-08	Exhaled VOC Trap 1	168 h	41750.3412 dpm		0.067
2-08	Exhaled VOC Trap 2	1 h	0.0000 dpm		0.000
2-08	Exhaled VOC Trap 2	3 h	50.4669 dpm		0.000
2-08	Exhaled VOC Trap 2	5 h	31.9496 dpm		0.000
2-08	Exhaled VOC Trap 2	7 h	18.7611 dpm		0.000
2-08	Exhaled VOC Trap 2	12 h	0.0000 dpm		0.000
2-08	Exhaled VOC Trap 2	24 h	115.2074 dpm		0.000
2-08	Exhaled VOC Trap 2	48 h	82.2189 dpm		0.000
2-08	Exhaled VOC Trap 2	72 h	98.8462 dpm		0.000
2-08	Exhaled VOC Trap 2	96 h	336.3242 dpm		0.001
2-08	Exhaled VOC Trap 2	120 h	488.5097 dpm		0.001
2-08	Exhaled VOC Trap 2	144 h	240.6654 dpm		0.000
2-08	Exhaled VOC Trap 2	168 h	22.6491 dpm		0.000
2-08	Cage Rinse	168 h	216909.2616 dpm		0.350
2-08	Nose Only Tube Rinse	0 h	113796.4976 dpm		0.184
2-08	Transfer Bag Rinse	0 h	8402.2877 dpm		0.014
2-08	VOC Trap 1 Extract 2	1 h	1477793.5065 dpm		2.384
2-08	VOC Trap 1 Extract 2	3 h	1536316.3680 dpm		2.478
2-08	VOC Trap 1 Extract 2	5 h	1441963.8381 dpm		2.326
2-08	VOC Trap 1 Extract 2	7 h	934760.6276 dpm		1.508
2-08	VOC Trap 1 Extract 2	12 h	1316690.4043 dpm		2.124
2-08	VOC Trap 1 Extract 2	24 h	377114.9578 dpm		0.608

Table 16. Concentration (mg equiv./g) of Radioactivity in Exhaled VOC Trap Extracts from Group 2.

Subject	Sample	Time	Conc (mg/g)	LOD	LOQ
2-05	Exhaled VOC Trap 1	1 h	0.7991		
2-05	Exhaled VOC Trap 1	3 h	0.8499		
2-05	Exhaled VOC Trap 1	5 h	0.8752		
2-05	Exhaled VOC Trap 1	7 h	0.7614		
2-05	Exhaled VOC Trap 1	12 h	0.7508		
2-05	Exhaled VOC Trap 1	24 h	0.1751		
2-05	Exhaled VOC Trap 1	48 h	0.0424		
2-05	Exhaled VOC Trap 1	72 h	0.0001		
2-05	Exhaled VOC Trap 1	96 h	0.0081		
2-05	Exhaled VOC Trap 1	120 h	0.0090		
2-05	Exhaled VOC Trap 1	144 h	0.0043		
2-05	Exhaled VOC Trap 1	168 h	0.0040		
2-05	Exhaled VOC Trap 2	1 h	0.0000	*	
2-05	Exhaled VOC Trap 2	3 h	0.0000	*	
2-05	Exhaled VOC Trap 2	5 h	0.0000	*	
2-05	Exhaled VOC Trap 2	7 h	0.0000	*	
2-05	Exhaled VOC Trap 2	12 h	0.0000	*	
2-05	Exhaled VOC Trap 2	24 h	0.0000	*	
2-05	Exhaled VOC Trap 2	48 h	0.0001		
2-05	Exhaled VOC Trap 2	72 h	0.0259		
2-05	Exhaled VOC Trap 2	96 h	0.0000	*	
2-05	Exhaled VOC Trap 2	120 h	0.0000	*	
2-05	Exhaled VOC Trap 2	144 h	0.0001		
2-05	Exhaled VOC Trap 2	168 h	0.0000	*	
2-05	VOC Trap 1 Extract 2	1 h	0.3250		
2-05	VOC Trap 1 Extract 2	3 h	0.3537		
2-05	VOC Trap 1 Extract 2	5 h	0.4086		
2-05	VOC Trap 1 Extract 2	7 h	0.2565		
2-05	VOC Trap 1 Extract 2	12 h	0.3370		
2-05	VOC Trap 1 Extract 2	24 h	0.0635		
2-06	Exhaled VOC Trap 1	1 h	1.1440		
2-06	Exhaled VOC Trap 1	3 h	0.9420		
2-06	Exhaled VOC Trap 1	5 h	0.9796		
2-06	Exhaled VOC Trap 1	7 h	0.8027		
2-06	Exhaled VOC Trap 1	12 h	1.1496		
2-06	Exhaled VOC Trap 1	24 h	0.2426		
2-06	Exhaled VOC Trap 1	48 h	0.1935		
2-06	Exhaled VOC Trap 1	72 h	0.0391		
2-06	Exhaled VOC Trap 1	96 h	0.0126		
2-06	Exhaled VOC Trap 1	120 h	0.0095		
2-06	Exhaled VOC Trap 1	144 h	0.0070		
2-06	Exhaled VOC Trap 1	168 h	0.0073		
2-06	Exhaled VOC Trap 2	1 h	0.0000	*	
2-06	Exhaled VOC Trap 2	3 h	0.0001	*	
2-06	Exhaled VOC Trap 2	5 h	0.0000	*	
2-06	Exhaled VOC Trap 2	7 h	0.0000	*	
2-06	Exhaled VOC Trap 2	12 h	0.0000	*	
2-06	Exhaled VOC Trap 2	24 h	0.0000	*	
2-06	Exhaled VOC Trap 2	48 h	0.0002		
2-06	Exhaled VOC Trap 2	72 h	0.0002		
2-06	Exhaled VOC Trap 2	96 h	0.0003		

* 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 16 (contd). Concentration (mg equiv./g) of Radioactivity in Exhaled VOC Trap Extracts from Group 2.

Subject	Sample	Time	Conc (mg/g)	LOD	LOQ
2-06	Exhaled VOC Trap 2	120 h	0.0002		
2-06	Exhaled VOC Trap 2	144 h	0.0002		
2-06	Exhaled VOC Trap 2	168 h	0.0000	*	
2-06	VOC Trap 1 Extract 2	1 h	0.4476		
2-06	VOC Trap 1 Extract 2	3 h	0.4057		
2-06	VOC Trap 1 Extract 2	5 h	0.4353		
2-06	VOC Trap 1 Extract 2	7 h	0.2950		
2-06	VOC Trap 1 Extract 2	12 h	0.5111		
2-06	VOC Trap 1 Extract 2	24 h	0.1092		
2-07	Exhaled VOC Trap 1	1 h	1.0217		
2-07	Exhaled VOC Trap 1	3 h	0.9703		
2-07	Exhaled VOC Trap 1	5 h	0.9478		
2-07	Exhaled VOC Trap 1	7 h	1.0219		
2-07	Exhaled VOC Trap 1	12 h	1.5578		
2-07	Exhaled VOC Trap 1	24 h	0.4367		
2-07	Exhaled VOC Trap 1	48 h	0.1636		
2-07	Exhaled VOC Trap 1	72 h	0.0113		
2-07	Exhaled VOC Trap 1	96 h	0.0084		
2-07	Exhaled VOC Trap 1	120 h	0.0073		
2-07	Exhaled VOC Trap 1	144 h	0.0073		
2-07	Exhaled VOC Trap 1	168 h	0.0051		
2-07	Exhaled VOC Trap 2	1 h	0.0000	*	
2-07	Exhaled VOC Trap 2	3 h	0.0001		
2-07	Exhaled VOC Trap 2	5 h	0.0000	*	
2-07	Exhaled VOC Trap 2	7 h	0.0000	*	
2-07	Exhaled VOC Trap 2	12 h	0.0000	*	
2-07	Exhaled VOC Trap 2	24 h	0.0013		
2-07	Exhaled VOC Trap 2	48 h	0.0000	*	
2-07	Exhaled VOC Trap 2	72 h	0.0000	*	
2-07	Exhaled VOC Trap 2	96 h	0.0000	*	
2-07	Exhaled VOC Trap 2	120 h	0.0000	*	
2-07	Exhaled VOC Trap 2	144 h	0.0001		
2-07	Exhaled VOC Trap 2	168 h	0.0000	*	
2-07	VOC Trap 1 Extract 2	1 h	0.5087		
2-07	VOC Trap 1 Extract 2	3 h	0.4181		
2-07	VOC Trap 1 Extract 2	5 h	0.4432		
2-07	VOC Trap 1 Extract 2	7 h	0.3656		
2-07	VOC Trap 1 Extract 2	12 h	0.6135		
2-07	VOC Trap 1 Extract 2	24 h	0.1687		
2-08	Exhaled VOC Trap 1	1 h	1.3818		
2-08	Exhaled VOC Trap 1	3 h	1.1377		
2-08	Exhaled VOC Trap 1	5 h	1.0716		
2-08	Exhaled VOC Trap 1	7 h	0.8938		
2-08	Exhaled VOC Trap 1	12 h	1.3258		
2-08	Exhaled VOC Trap 1	24 h	0.3801		
2-08	Exhaled VOC Trap 1	48 h	0.0671		
2-08	Exhaled VOC Trap 1	72 h	0.0425		
2-08	Exhaled VOC Trap 1	96 h	0.0154		
2-08	Exhaled VOC Trap 1	120 h	0.0111		
2-08	Exhaled VOC Trap 1	144 h	0.0085		
2-08	Exhaled VOC Trap 1	168 h	0.0190		

* 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 16 (contd). Concentration (mg equiv./g) of Radioactivity in Exhaled VOC Trap Extracts from Group 2.

Subject	Sample	Time	Conc (mg/g)	LOD	LOQ
2-08	Exhaled VOC Trap 2	1 h	0.0000	*	
2-08	Exhaled VOC Trap 2	3 h	0.0000		*
2-08	Exhaled VOC Trap 2	5 h	0.0000		*
2-08	Exhaled VOC Trap 2	12 h	0.0000	*	
2-08	Exhaled VOC Trap 2	24 h	0.0001		
2-08	Exhaled VOC Trap 2	48 h	0.0000		*
2-08	Exhaled VOC Trap 2	72 h	0.0000		*
2-08	Exhaled VOC Trap 2	96 h	0.0002		
2-08	Exhaled VOC Trap 2	120 h	0.0002		
2-08	Exhaled VOC Trap 2	144 h	0.0001		
2-08	Exhaled VOC Trap 2	168 h	0.0000		*
2-08	VOC Trap 1 Extract 2	1 h	0.5209		
2-08	VOC Trap 1 Extract 2	3 h	0.5434		
2-08	VOC Trap 1 Extract 2	5 h	0.5120		
2-08	VOC Trap 1 Extract 2	7 h	0.3353		
2-08	VOC Trap 1 Extract 2	12 h	0.4668		
2-08	VOC Trap 1 Extract 2	24 h	0.1317		

* 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 17. Concentration of TBA in Exhaled VOC Trap Extracts from Group 2.

Sample I.D	injection	Peak Area	Conc. (µg/µl)	Mean (µg/µl)	Mean (µg/mg)
2-05 VOC Trap 1 1 h	1	751	0.584	0.755	0.796
2-05 VOC Trap 1 1 h	2	1188	0.926		
2-06 VOC Trap 1 1 h	1	808	0.629	0.769	0.811
2-06 VOC Trap 1 1 h	2	1167	0.909		
2-07 VOC Trap 1 1 h	1	997	0.777	0.800	0.843
2-07 VOC Trap 1 1 h	2	1057	0.824		
2-08 VOC Trap 1 1 h	1	1430	1.11	1.09	1.149
2-08 VOC Trap 1 1 h	2	1366	1.06		
2-05 VOC Trap 1 3 h	1	797	0.620	0.605	0.638
2-05 VOC Trap 1 3 h	2	758	0.590		
2-06 VOC Trap 1 3 h	1	780	0.607	0.645	0.679
2-06 VOC Trap 1 3 h	2	876	0.682		
2-07 VOC Trap 1 3 h	1	945	0.736	0.713	0.751
2-07 VOC Trap 1 3 h	2	885	0.689		
2-08 VOC Trap 1 3 h	1	1086	0.846	0.856	0.902
2-08 VOC Trap 1 3 h	2	1112	0.867		
2-05 VOC Trap 1 5 h	1	788	0.613	0.661	0.696
2-05 VOC Trap 1 5 h	2	909	0.708		
2-06 VOC Trap 1 5 h	1	777	0.605	0.619	0.652
2-06 VOC Trap 1 5 h	2	813	0.633		
2-07 VOC Trap 1 5 h	1	701	0.545	0.616	0.649
2-07 VOC Trap 1 5 h	2	881	0.686		
2-08 VOC Trap 1 5 h	1	1031	0.803	0.765	0.806
2-08 VOC Trap 1 5 h	2	934	0.727		
2-05 VOC Trap 1 7 h	1	730	0.568	0.533	0.561
2-05 VOC Trap 1 7 h	2	639	0.497		
2-06 VOC Trap 1 7 h	1	764	0.595	0.534	0.563
2-06 VOC Trap 1 7 h	2	609	0.474		
2-07 VOC Trap 1 7 h	1	946	0.737	0.729	0.768
2-07 VOC Trap 1 7 h	2	925	0.720		
2-08 VOC Trap 1 7 h	1	838	0.652	0.603	0.635
2-08 VOC Trap 1 7 h	2	711	0.553		
2-05 VOC Trap 1 12 h	1	627	0.488	0.497	0.524
2-05 VOC Trap 1 12 h	2	652	0.507		
2-06 VOC Trap 1 12 h	1	1059	0.825	0.746	0.786
2-06 VOC Trap 1 12 h	2	857	0.667		
2-07 VOC Trap 1 12 h	1	1445	1.13	1.13	1.196
2-07 VOC Trap 1 12 h	2	1466	1.14		
2-08 VOC Trap 1 12 h	1	1197	0.933	0.948	0.999
2-08 VOC Trap 1 12 h	2	1235	0.963		

Table 17 (contd). Concentration of TBA in Exhaled VOC Trap Extracts from Group 2.

Sample I.D	injection	Peak Area	Conc. (µg/µl)	Mean (µg/µl)	Mean (µg/mg)
2-05 VOC Trap 1 24 h	1	88	0.0665	0.0708	0.075
2-05 VOC Trap 1 24 h	2	99	0.0751		
2-06 VOC Trap 1 24 h	1	116	0.0884	0.0935	0.098
2-06 VOC Trap 1 24 h	2	129	0.0986		
2-07 VOC Trap 1 24 h	1	291	0.225	0.234	0.247
2-07 VOC Trap 1 24 h	2	314	0.243		
2-08 VOC Trap 1 24 h	1	258	0.199	0.207	0.218
2-08 VOC Trap 1 24 h	2	277	0.214		
2-05 VOC Trap 1 48 h	1	15	0.00949	0.0107	0.011
2-05 VOC Trap 1 48 h	2	18	0.0118		
2-06 VOC Trap 1 48 h	1	110	0.0837	0.0790	0.083
2-06 VOC Trap 1 48 h	2	98	0.0743		
2-07 VOC Trap 1 48 h	1	17	0.0111	0.0107	0.011
2-07 VOC Trap 1 48 h	2	16	0.0103		
2-08 VOC Trap 1 48 h	1	29	0.0204	0.0228	0.024
2-08 VOC Trap 1 48 h	2	35	0.0251		
2-05 VOC Trap 1 72 h	1	n/a	n/a	n/a	n/a
2-05 VOC Trap 1 72 h	2	n/a	n/a		
2-06 VOC Trap 1 72 h	1	26	0.0181	0.0200	0.021
2-06 VOC Trap 1 72 h	2	31	0.0220		
2-07 VOC Trap 1 72 h	1	6	below LOQ	below LOQ	below LOQ
2-07 VOC Trap 1 72 h	2	6	below LOQ		
2-08 VOC Trap 1 72 h	1	38	0.0275	0.0263	0.028
2-08 VOC Trap 1 72 h	2	35	0.0251		
2-05 VOC Trap 1 96 h	1	6	below LOQ	below LOQ	below LOQ
2-05 VOC Trap 1 96 h	2	6	below LOQ		
2-06 VOC Trap 1 96 h	1	8	below LOQ	below LOQ	below LOQ
2-06 VOC Trap 1 96 h	2	8	below LOQ		
2-07 VOC Trap 1 96 h	1	6	below LOQ	below LOQ	below LOQ
2-07 VOC Trap 1 96 h	2	6	below LOQ		
2-08 VOC Trap 1 96 h	1	12	below LOQ	below LOQ	below LOQ
2-08 VOC Trap 1 96 h	2	11	below LOQ		
2-05 VOC Trap 1 120 h	1	7	below LOQ	below LOQ	below LOQ
2-05 VOC Trap 1 120 h	2	8	below LOQ		
2-06 VOC Trap 1 120 h	1	7	below LOQ	below LOQ	below LOQ
2-06 VOC Trap 1 120 h	2	6	below LOQ		
2-07 VOC Trap 1 120 h	1	6	below LOQ	below LOQ	below LOQ
2-07 VOC Trap 1 120 h	2	5	below LOQ		
2-08 VOC Trap 1 120 h	1	9	below LOQ	below LOQ	below LOQ
2-08 VOC Trap 1 120 h	2	9	below LOQ		

Table 17 (contd). Concentration of TBA in Exhaled VOC Trap Extracts from Group 2.

Sample I.D	injection	Peak Area	Conc. (µg/µl)	Mean (µg/µl)	Mean (µg/mg)
2-05 VOC Trap 1 144 h	1	n/a	n/a	n/a	n/a
2-05 VOC Trap 1 144 h	2	n/a	n/a		
2-06 VOC Trap 1 144 h	1	5	below LOQ	below LOQ	below LOQ
2-06 VOC Trap 1 144 h	2	n/a	n/a		
2-07 VOC Trap 1 144 h	1	6	below LOQ	below LOQ	below LOQ
2-07 VOC Trap 1 144 h	2	6	below LOQ		
2-08 VOC Trap 1 144 h	1	7	below LOQ	below LOQ	below LOQ
2-08 VOC Trap 1 144 h	2	7	below LOQ		
2-05 VOC Trap 1 168 h	1	n/a	n/a	n/a	n/a
2-05 VOC Trap 1 168 h	2	n/a	n/a		
2-06 VOC Trap 1 168 h	1	7	below LOQ	below LOQ	below LOQ
2-06 VOC Trap 1 168 h	2	6	below LOQ		
2-07 VOC Trap 1 168 h	1	n/a	n/a	n/a	n/a
2-07 VOC Trap 1 168 h	2	n/a	n/a		
2-08 VOC Trap 1 168 h	1	18	0.0118	0.0126	0.013
2-08 VOC Trap 1 168 h	2	20	0.0134		
2-05 VOC 1 EX2 1 h	1	368	0.285	0.284	0.300
2-05 VOC 1 EX2 1 h	2	366	0.284		
2-06 VOC 1 EX2 1 h	1	536	0.417	0.409	0.431
2-06 VOC 1 EX2 1 h	2	516	0.401		
2-07 VOC 1 EX2 1 h	1	549	0.427	0.441	0.464
2-07 VOC 1 EX2 1 h	2	585	0.455		
2-08 VOC 1 EX2 1 h	1	603	0.469	0.441	0.464
2-08 VOC 1 EX2 1 h	2	531	0.413		
2-05 VOC Trap 2 72 h	1	21	0.0142	0.0134	0.014
2-05 VOC Trap 2 72 h	2	19	0.0126		

Table 17 (contd). Concentration of TBA in Exhaled VOC Trap Extracts from Group 2.

Sample I.D	injection	Peak Area	Conc. (µg/µl)	Mean (µg/µl)	Mean (µg/mg)
2-05 VOC 1 EX2 3 h	1	397	0.308	0.312	0.329
2-05 VOC 1 EX2 3 h	2	407	0.316		
2-06 VOC 1 EX2 3 h	1	462	0.359	0.358	0.377
2-06 VOC 1 EX2 3 h	2	459	0.356		
2-07 VOC 1 EX2 3 h	1	517	0.402	0.387	0.408
2-07 VOC 1 EX2 3 h	2	479	0.372		
2-08 VOC 1 EX2 3 h	1	623	0.484	0.496	0.523
2-08 VOC 1 EX2 3 h	2	653	0.508		
2-05 VOC 1 EX2 5 h	1	471	0.366	0.326	0.343
2-05 VOC 1 EX2 5 h	2	369	0.286		
2-06 VOC 1 EX2 5 h	1	496	0.385	0.392	0.413
2-06 VOC 1 EX2 5 h	2	512	0.398		
2-07 VOC 1 EX2 5 h	1	502	0.390	0.393	0.414
2-07 VOC 1 EX2 5 h	2	509	0.395		
2-08 VOC 1 EX2 5 h	1	581	0.452	0.457	0.482
2-08 VOC 1 EX2 5 h	2	595	0.463		
2-05 VOC 1 EX2 7 h	1	301	0.233	0.231	0.243
2-05 VOC 1 EX2 7 h	2	296	0.229		
2-06 VOC 1 EX2 7 h	1	338	0.262	0.255	0.269
2-06 VOC 1 EX2 7 h	2	321	0.249		
2-07 VOC 1 EX2 7 h	1	431	0.334	0.306	0.322
2-07 VOC 1 EX2 7 h	2	358	0.277		
2-08 VOC 1 EX2 7 h	1	361	0.280	0.292	0.308
2-08 VOC 1 EX2 7 h	2	392	0.304		
2-05 VOC 1 EX2 12 h	1	392	0.304	0.294	0.310
2-05 VOC 1 EX2 12 h	2	366	0.284		
2-06 VOC 1 EX2 12 h	1	572	0.445	0.450	0.474
2-06 VOC 1 EX2 12 h	2	586	0.456		
2-07 VOC 1 EX2 12 h	1	669	0.520	0.540	0.569
2-07 VOC 1 EX2 12 h	2	720	0.560		
2-08 VOC 1 EX2 12 h	1	553	0.430	0.408	0.430
2-08 VOC 1 EX2 12 h	2	497	0.386		
2-05 VOC 1 EX2 24 h	1	51	0.0376	0.0372	0.039
2-05 VOC 1 EX2 24 h	2	50	0.0368		
2-06 VOC 1 EX2 24 h	1	101	0.0767	0.0782	0.082
2-06 VOC 1 EX2 24 h	2	105	0.0798		
2-07 VOC 1 EX2 24 h	1	162	0.124	0.123	0.129
2-07 VOC 1 EX2 24 h	2	158	0.121		
2-08 VOC 1 EX2 24 h	1	124	0.0946	0.0950	0.100
2-08 VOC 1 EX2 24 h	2	125	0.0954		