

# IND Enabling Preclinical Studies of [<sup>11</sup>C]COU, a Trapped Metabolite Radiotracer for Monoamine Oxidase-B

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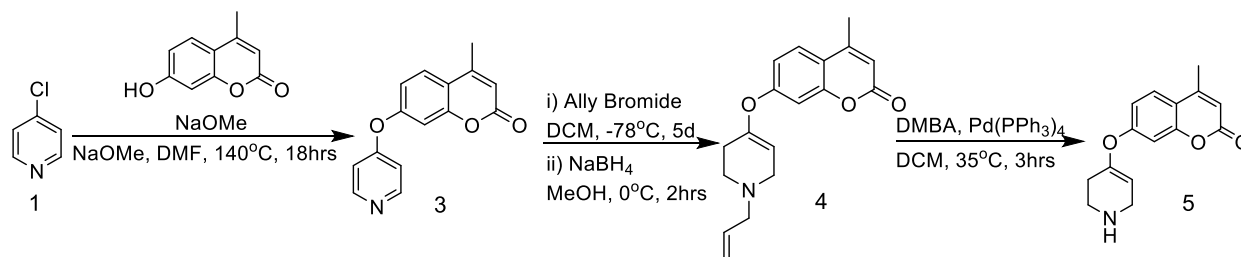
## 1. Chemistry Validation

### 1.1. General Considerations

All the chemicals were purchased from commercially available suppliers and used without purification. Automated flash chromatography was performed with Biotage Isolera Prime system. High-performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a

Bioscan B-FC-1000 radiation detector.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra: Varian 400 apparatus (400 MHz for  $^1\text{H}$  NMR and 101 MHz for  $^{13}\text{C}$  NMR), in DMSO- $d_6$  or  $\text{CDCl}_3$  unless otherwise indicated,  $\delta$  in ppm rel. to tetramethylsilane ( $\delta = 0$ ), J in Hz. Mass spectra were measured on an Agilent Q-TOF HPLC-MS or VG (Micromass) 70-250-S Magnetic sector mass spectrometer employing the electrospray ionization (ESI) method.

## 1.2. Large Scale Synthesis of COU precursor



### Supplemental Figure 1.

#### 4-Methyl-7-(pyridin-4-yloxy)-2H-chromen-2-one (3):

To a round bottom flask, 4-methylumbelliferone (12 mmol, 1.2 eq) was dissolved in DMF (16 mL). This was then heated to 140°C for 15 minutes. 4-chloropyridine [1] (1.5 g, 1 eq) was added portion-wise to the reaction mixture. Reaction was allowed to stir at 140°C overnight. Next day, the reaction was quenched with sat. K<sub>2</sub>CO<sub>3</sub> and subsequently extracted with ethyl acetate and water. Organic fraction was dried over Na<sub>2</sub>SO<sub>4</sub>. Product was purified by silica gel flash column chromatography (hexanes: ethyl acetate gradient).

Product collected in 12% yield.

$^1\text{H}$  NMR (400 MHz;  $\text{CDCl}_3$ )/ $\delta$  (ppm): 7.60 (1H, d, J = 8.7 Hz), 6.94 (1H, dd, J = 8.7, 2.4 Hz), 6.84 (1H, d, J = 2.4), 6.09 (1H, s), 5.28 (1H, s), 3.49 (2H, s), 5.24 (1H, s), 3.39 (2H, d, J = 3.1 Hz), 3.05 (2H, t, J = 5.9 Hz), 2.35 (3H, s)

$^{13}\text{C}$  NMR (100 MHz;  $\text{CD}_3\text{OD}$ )/ $\delta$  (ppm): 161.43, 159.41, 154.60, 153.75, 150.34, 126.20, 114.96, 114.56, 111.85, 108.55, 104.80, 47.44, 47.01, 42.48, 42.07, 25.93, 17.38

HRMS: calculated for [M]<sup>+</sup> (M = C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>), 258.1085, found 258.1125.

HPLC Column: Gemini NX 5 $\mu$  C18 110 A 250 x 4.6 mm

HPLC Buffer: 50 mM NH<sub>4</sub>HCO<sub>3</sub> 50% MeCN pH 10

Flow rate: 1.5 mL/min

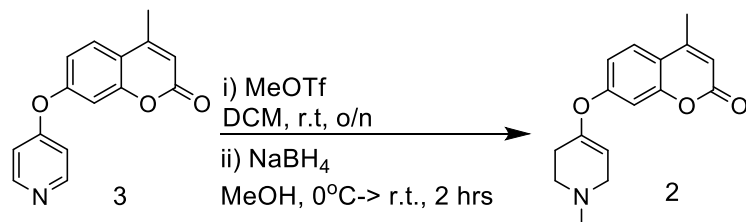
#### 7-((1-Allyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-4-methyl-2H-chromen-2-one (4):

Prepared as previously described by Brooks, et. al., 2020.<sup>1</sup>

#### 4-Methyl-7-((1,2,3,6-tetrahydropyridin-4-yl)-oxy)-2H-chromen-2-one (COU) (5):

Prepared as previously described by Brooks, et. al., 2020.<sup>1</sup>

## 1.3. Large Scale Synthesis of COU standard



**Supplemental Figure 2.**

#### **4-Methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one**

4-Methyl-7-(pyridin-4-yloxy)-2H-chromen-2-one (1.0 g, 3.95 mmol) was dissolved in DCM (20 mL). Methyltriflate (1.36 mL, 11.85 mmol) was added to the reaction dropwise and stirred overnight. Next day, DCM was evaporated. Residue was dissolved in MeOH (20 mL) and cooled at 0 °C. Sodium borohydride (597.3 mg, 15.8 mmol) was added and stirred for 2 hrs. Next day, the reaction was quenched with sat. K<sub>2</sub>CO<sub>3</sub> and subsequently extracted with ethyl acetate and water. Organic fraction was dried over Na<sub>2</sub>SO<sub>4</sub>. Product was purified by silica gel flash column chromatography (DCM: Methanol gradient).

Product collected in 35.3% yield (377.6 mg).

<sup>1</sup>H NMR (400 MHz; CDCl<sub>3</sub>)/δ (ppm): 7.54 (1H, d, J = 8.6 Hz), 7.04-6.96 (2H, m), 6.18 (1H, d, J = 1.3 Hz), 5.15-5.09 (1H, m), 3.30-3.22 (2H, m), 2.92 (2H, t, J = 5.9), 2.58 (2H, s), 2.49 (2H, s), 2.40 (3H, s)

<sup>13</sup>C NMR (100 MHz; CDCl<sub>3</sub>)/δ (ppm): 160.92, 158.73, 154.83, 152.32, 150.59, 125.91, 115.61, 115.33, 113.18, 106.53, 52.50, 51.74, 44.68, 26.41, 18.78

HRMS: calculated for [M]<sup>+</sup> (M = C<sub>18</sub>H<sub>19</sub>NO<sub>3</sub>), 272.1242, found 272.1281.

HPLC Column: Gemini NX 5μ C18 110 A 250 x 4.6 mm

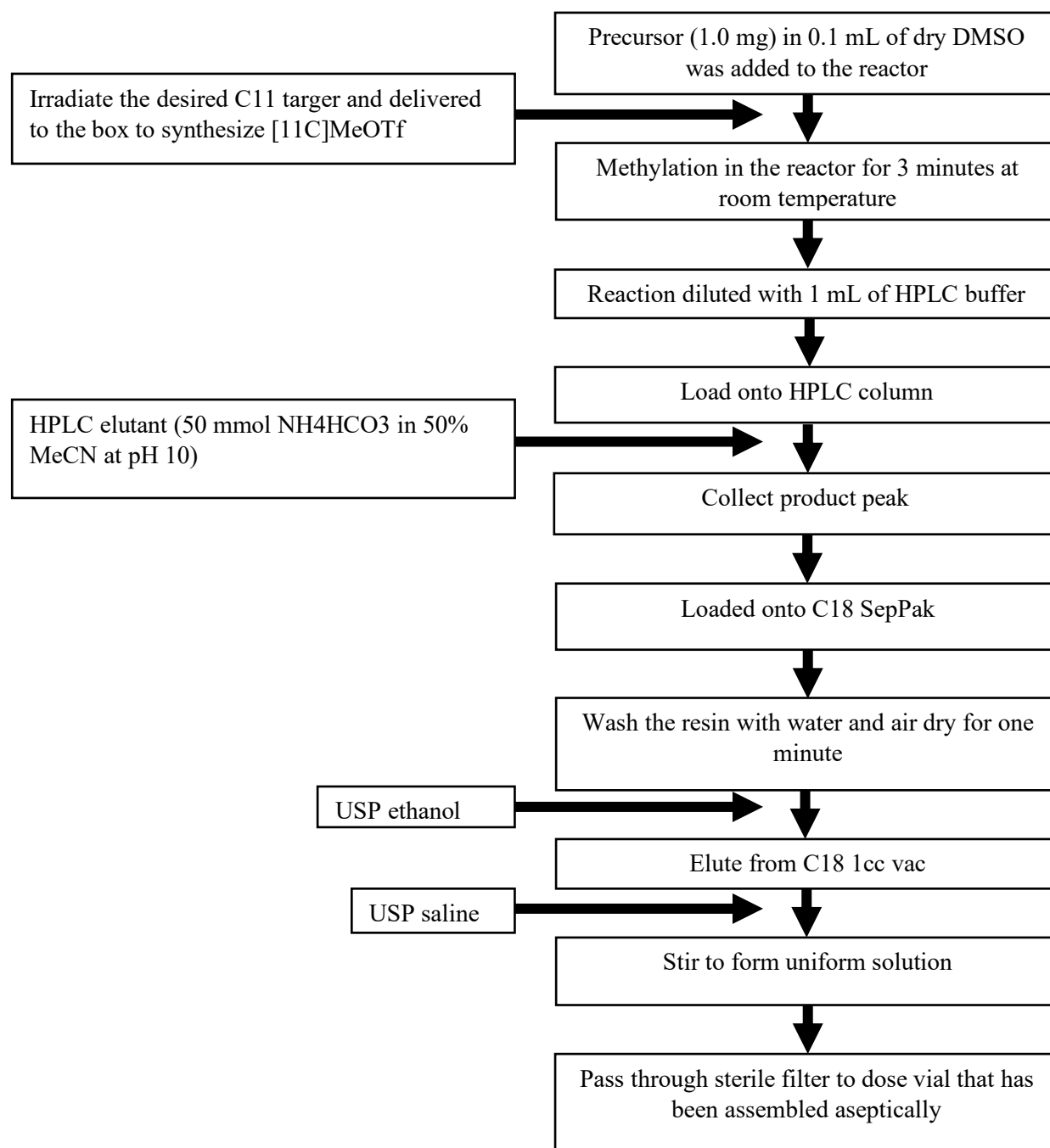
HPLC Buffer: 50 mM NH<sub>4</sub>HCO<sub>3</sub> 50% MeCN pH 10

Flow rate: 1.5 mL/min

Manufacturing Process for [<sup>11</sup>C]COU for Injection (API + Excipients) PET Drug Product

Unless otherwise stated, reagents and solvents were commercially available and used without further purification. Ethanol (200 proof, USP) was purchased from Decon Laboratories, Inc. Sodium chloride 0.9%, USP was sourced from Hospira. Other synthesis components were obtained as follows: Sterile vials were obtained from Hollister-Stier; C18 Sep-Pak's were purchased from Waters. Prior to use C18 Sep-Pak's were flushed with ethanol (10 mL) and sterile water (10 mL). Acetonitrile was purchased from Alfa Aesar.

**Supplemental Figure 3: COU Manufacturing Flow Diagram**



## 2. In Vivo Dosimetry

### 2.1. Biodistribution

For each timepoint, male (n=2) and female (n=2) Sprague Dawley (SD) rats weighing 161–251 g were anesthetized using isoflurane and subsequently injected with 150–500  $\mu\text{Ci}$  of  $[^{11}\text{C}]\text{COU}$  via femoral vein.<sup>2</sup> Pressure was applied to the injection site to stop bleeding. The rats were allowed to

recover from the anesthetic and resume normal activity. Each animal was sacrificed by decapitation at either 10, 20, 60, or 120 min post injection. Tissues and fluids were excised, homogenized, and counted for radioactivity in an auto-gamma counter to determine dosimetry and biodistribution. Brain samples included striatum, whole cerebral cortex, hippocampus, hypothalamus, and cerebellum. The results were corrected for radioactive decay and expressed in terms of percent-injected dose per gram of tissue (%ID/g) (Supplemental Table 1). Each data point represents the mean values from the four animals used for the experiment.

**Supplemental Table 1. [ $^{11}\text{C}$ ]COU biodistribution in rodents shown as %ID/g of tissue (average of n=4 per timepoint).**

| ORGANS                      | 10 min | 20 min | 60 min | 120 min |
|-----------------------------|--------|--------|--------|---------|
| BRAIN                       | 0.155  | 0.101  | 0.068  | 0.079   |
| EYEBALLS                    | 0.109  | 0.056  | 0.036  | 0.029   |
| HEART                       | 0.102  | 0.081  | 0.057  | 0.070   |
| LUNG                        | 0.195  | 0.107  | 0.049  | 0.035   |
| LIVER                       | 0.626  | 0.603  | 0.085  | 0.039   |
| PANCREAS                    | 0.212  | 0.172  | 0.072  | 0.067   |
| SPLEEN                      | 0.211  | 0.185  | 0.098  | 0.088   |
| ADRENAL                     | 0.252  | 0.354  | 0.211  | 0.218   |
| KIDNEY                      | 1.546  | 0.475  | 0.124  | 0.096   |
| ADIPOSE                     | 0.022  | 0.026  | 0.017  | 0.008   |
| STOMACH                     | 0.368  | 0.217  | 0.075  | 0.024   |
| CONTENTS OF STOMACH         | 0.284  | 0.211  | 0.568  | 0.013   |
| SMALL INTESTINE             | 0.819  | 0.777  | 0.939  | 0.219   |
| CONTENTS OF SMALL INTESTINE | 0.130  | 0.167  | 0.933  | 0.821   |
| CAECUM                      | 0.095  | 0.068  | 0.062  | 0.151   |
| CONTENTS OF CAECUM          | 0.012  | 0.004  | 0.014  | 0.576   |
| LARGE INTESTINE             | 0.126  | 0.114  | 0.069  | 0.052   |
| CONTENTS OF LARGE INTESTINE | 0.015  | 0.015  | 0.013  | 0.013   |
| OVARY                       | 0.168  | 0.229  | 0.084  | 0.059   |
| UTERUS                      | 0.129  | 0.084  | 0.060  | 0.058   |
| MUSCLE                      | 0.021  | 0.020  | 0.017  | 0.012   |
| BONE                        | 0.051  | 0.051  | 0.028  | 0.022   |
| BLOOD                       | 0.033  | 0.030  | 0.015  | 0.009   |
| TESTES                      | 0.101  | 0.052  | 0.037  | 0.025   |

## 2.2. Human Dosimetry Estimates

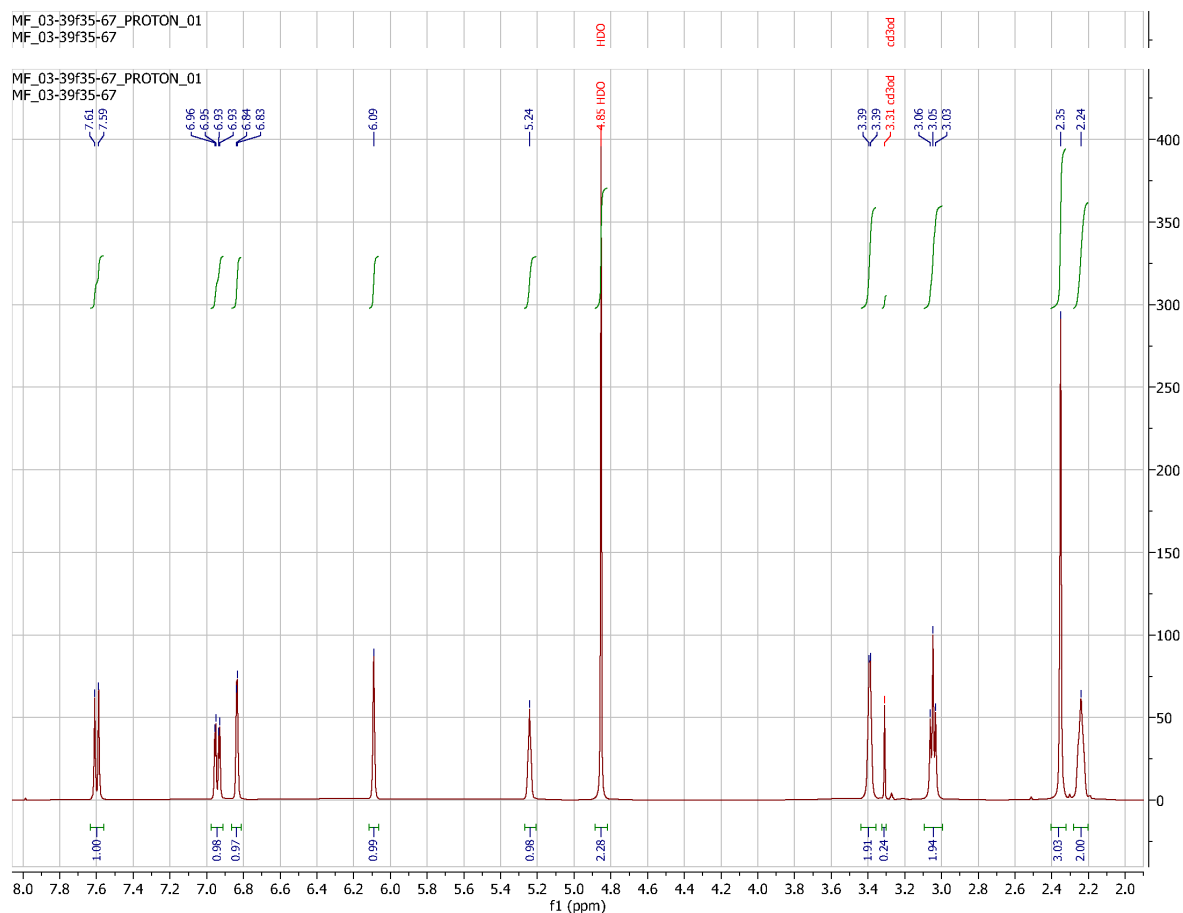
The biodistribution data was used to estimate human internal radiation dosimetry, calculated from rat biodistribution using the MIRD formalism Table 2.<sup>3</sup> Consistent with the short half-life of carbon-11 (20 min), dosimetry burden is estimated to be low. The bladder wall was estimated to receive the largest dose, 0.168 rem/mCi. Applying the guidelines of RDRCs operating in the United States, the dose limit for adults is defined as follows: 30 mSv per single injection for the whole body, lens of the eyes, red marrow, and gonads; 50 mSv per year for the whole body, lens of the eyes, red marrow, and gonads; 50 mSv per single injection for all other organs; and 150 mSv per year for all other organs (FDA 21 CFR 361.1). The estimated dosimetry for [<sup>11</sup>C]COU allows administration of up to 2 x 18 mCi doses of [<sup>11</sup>C]COU with an effective dose of 3.06 mSv, this falls well within the acceptable limits established by the RDRC.

## 3. References

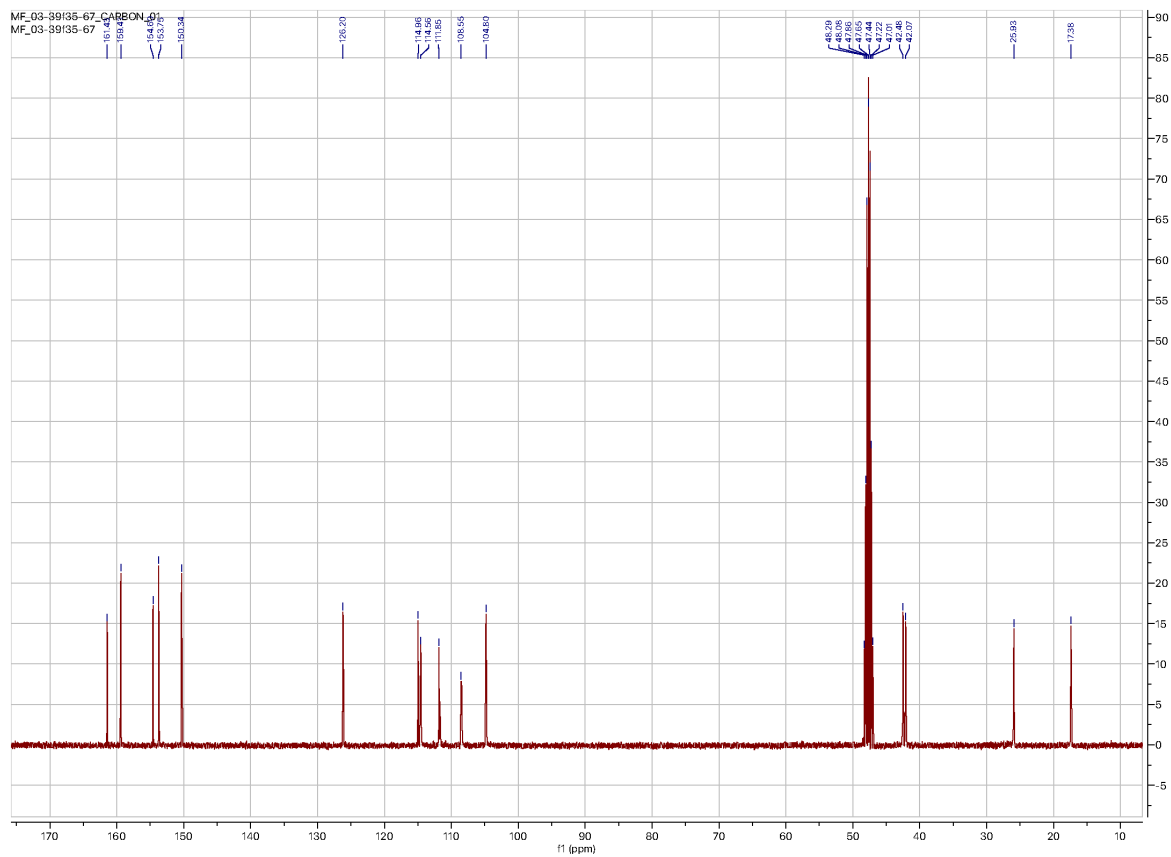
- (1) Brooks, A. F.; Mufarreh, A. J.; Shao, X.; Kaur, T.; Stauff, J.; Arteaga, J.; Kilbourn, M. R.; Scott, P. J. H. Improved Synthesis of [<sup>11</sup>C]COU and [<sup>11</sup>C]PHXY, Evaluation of Neurotoxicity, and Imaging of MAOs in Rodent Heart. *ACS Med. Chem. Lett.* **2020**, 11 (11), 2300–2304.
- (2) Stabin, M. G.; Siegel, J. A. Physical Models and Dose Factors for Use in Internal Dose Assessment. *Health Phys.* **2003**, 85 (3), 294–310.
- (3) Stabin, M. G. OLINDA/EXM 2—The Next-Generation Personal Computer Software for Internal Dose Assessment in Nuclear Medicine. *Health Phys.* **2023**, 124 (5), 397.  
<https://doi.org/10.1097/HP.0000000000001682>.

#### 4. $^1\text{H}$ NMR, $^{13}\text{C}$ NMR, HRMS, and HPLC spectra

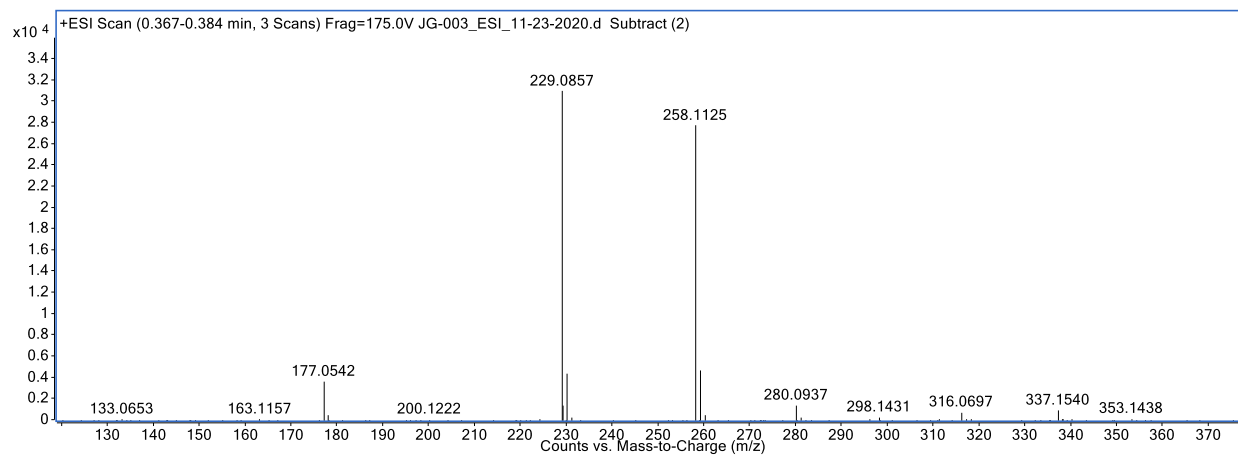
##### 4.1. 4-methyl-7-((1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one



**Supplemental Figure 4.**  $^1\text{H}$  NMR of 4-methyl-7-((1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one

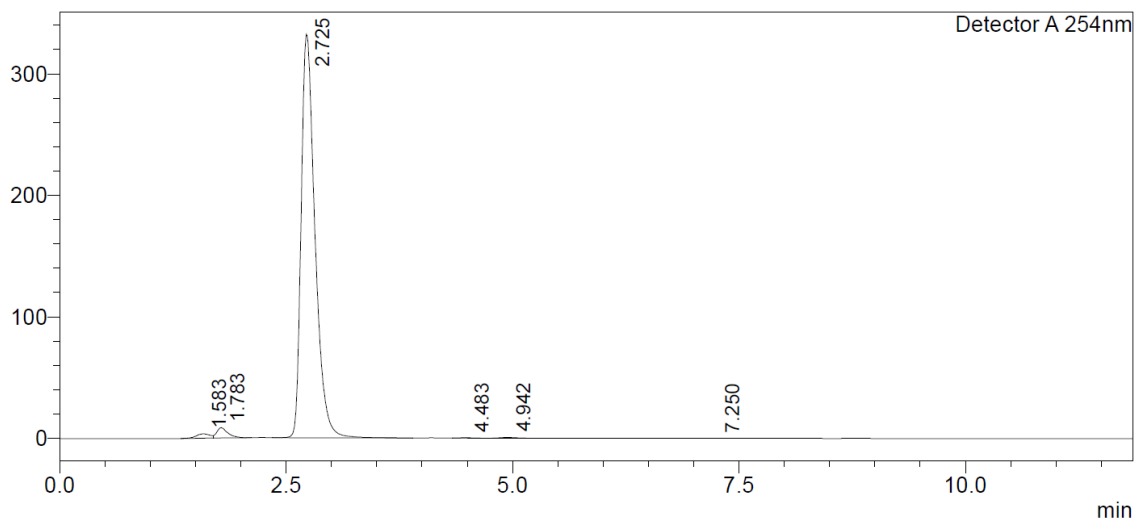


**Supplemental Figure 5.**  $^{13}\text{C}$  NMR of 4-methyl-7-((1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one



**Supplemental Figure 6.** HRMS of 4-methyl-7-((1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one

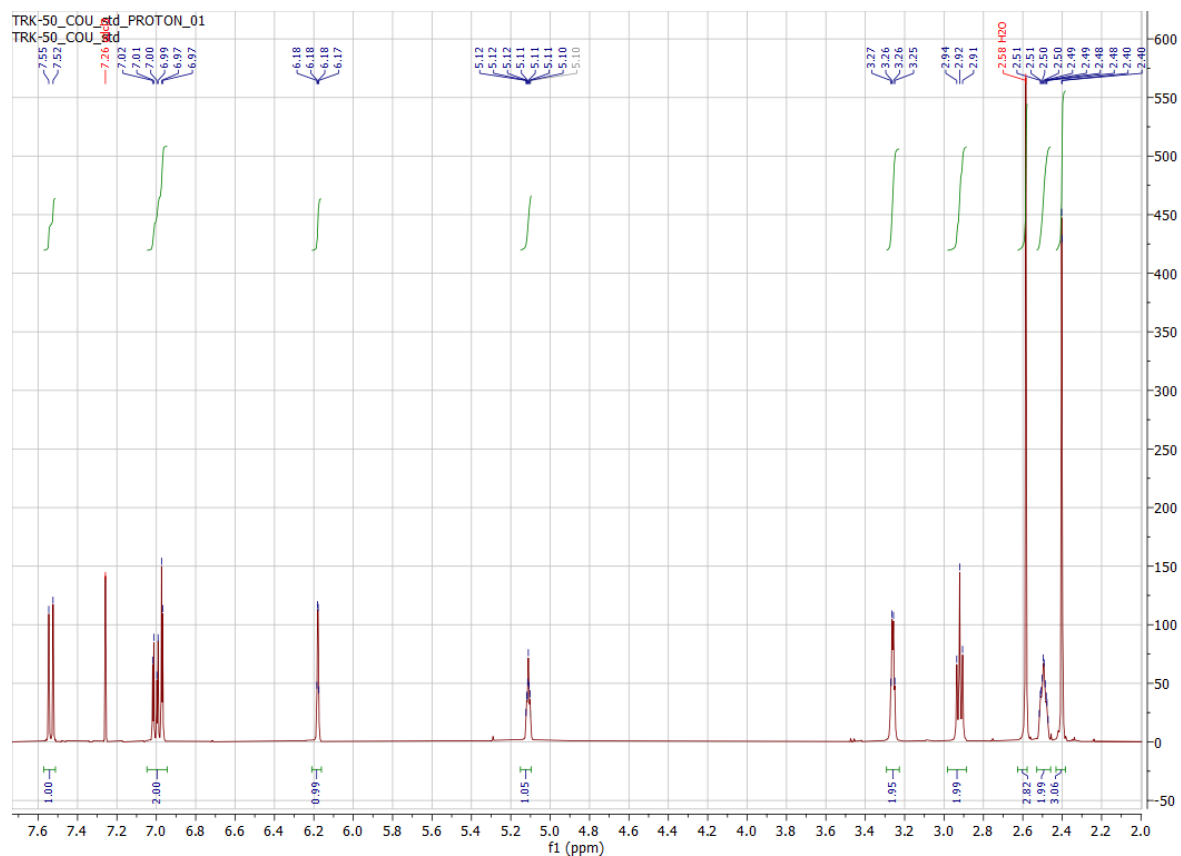
mV



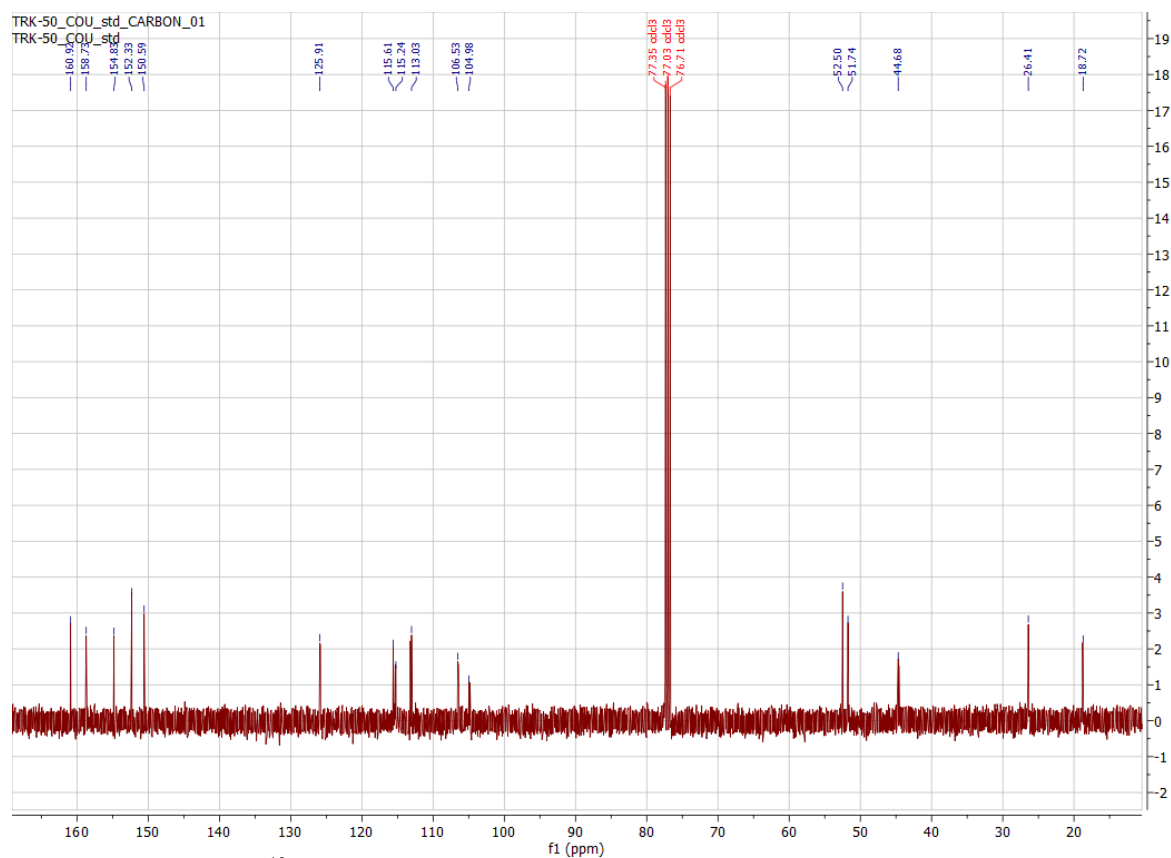
| Peak# | Ret. Time | Area    | Height | Area%   |
|-------|-----------|---------|--------|---------|
| 1     | 1.583     | 37100   | 3567   | 1.016   |
| 2     | 1.783     | 72642   | 8520   | 1.990   |
| 3     | 2.725     | 3524666 | 332042 | 96.567  |
| 4     | 4.483     | 3054    | 319    | 0.084   |
| 5     | 4.942     | 8452    | 644    | 0.232   |
| 6     | 7.250     | 4054    | 127    | 0.111   |
| Total |           | 3649969 | 345219 | 100.000 |

**Supplemental Figure 7.** Analytical HPLC of 4-methyl-7-((1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one

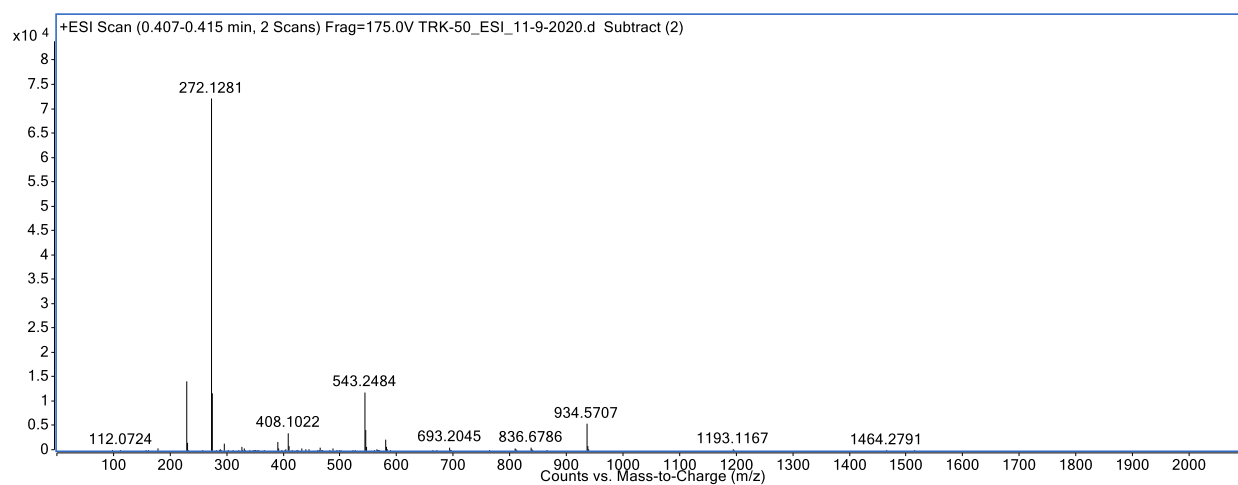
## 4.2 4-methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one



**Supplemental Figure 8.**  $^1\text{H}$  NMR of 4-methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one

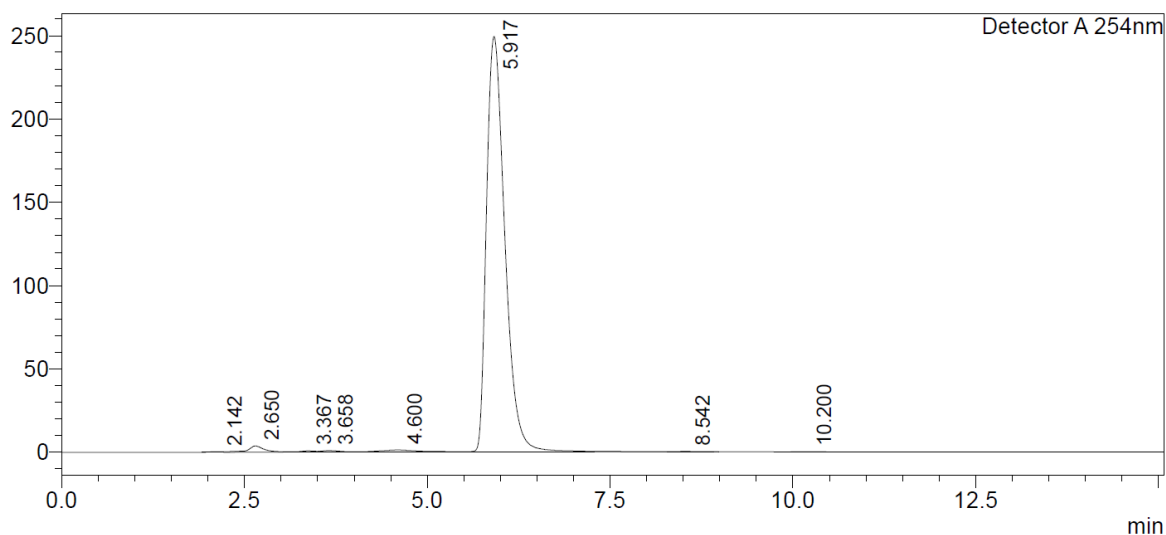


**Supplemental Figure 9.**  $^{13}\text{C}$  NMR of 4-methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one



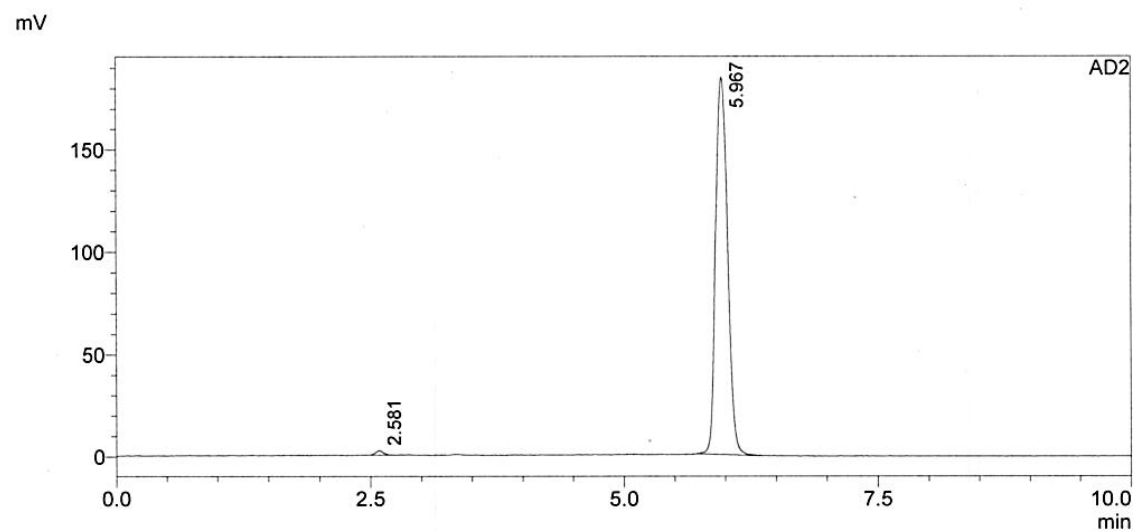
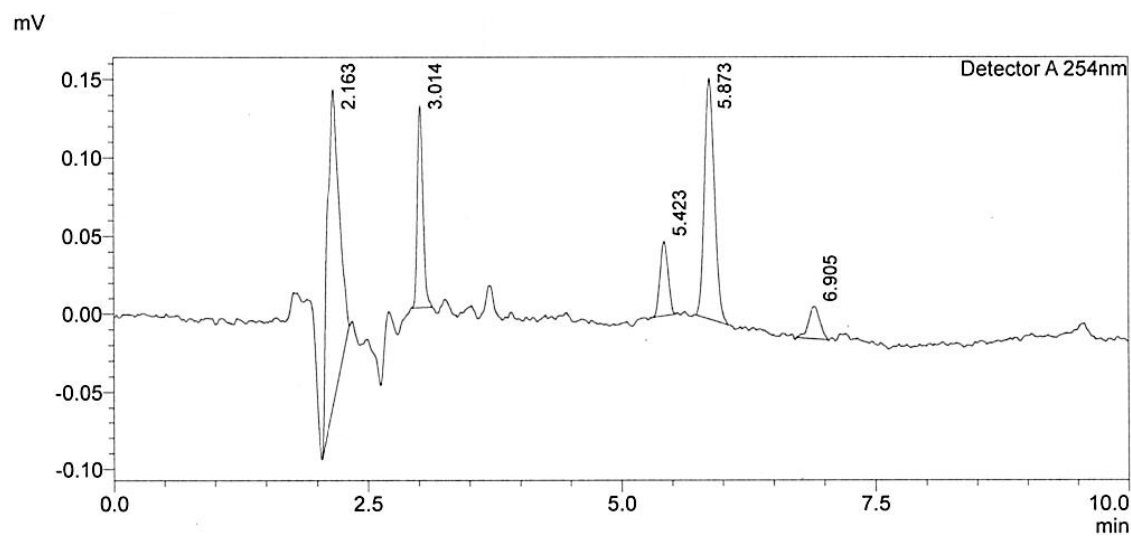
**Supplemental Figure 10.** HRMS of 4-methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one

mV



| Peak# | Ret. Time | Area    | Height | Area%   |
|-------|-----------|---------|--------|---------|
| 1     | 2.142     | 3933    | 359    | 0.087   |
| 2     | 2.650     | 61184   | 3768   | 1.353   |
| 3     | 3.367     | 10725   | 751    | 0.237   |
| 4     | 3.658     | 16730   | 956    | 0.370   |
| 5     | 4.600     | 50855   | 1264   | 1.125   |
| 6     | 5.917     | 4370750 | 249435 | 96.687  |
| 7     | 8.542     | 2947    | 161    | 0.065   |
| 8     | 10.200    | 3388    | 165    | 0.075   |
| Total |           | 4520511 | 256858 | 100.000 |

**Supplemental Figure 11.** Analytical HPLC of 4-methyl-7-((1-methyl-1,2,3,6-tetrahydropyridin-4-yl)oxy)-2H-chromen-2-one

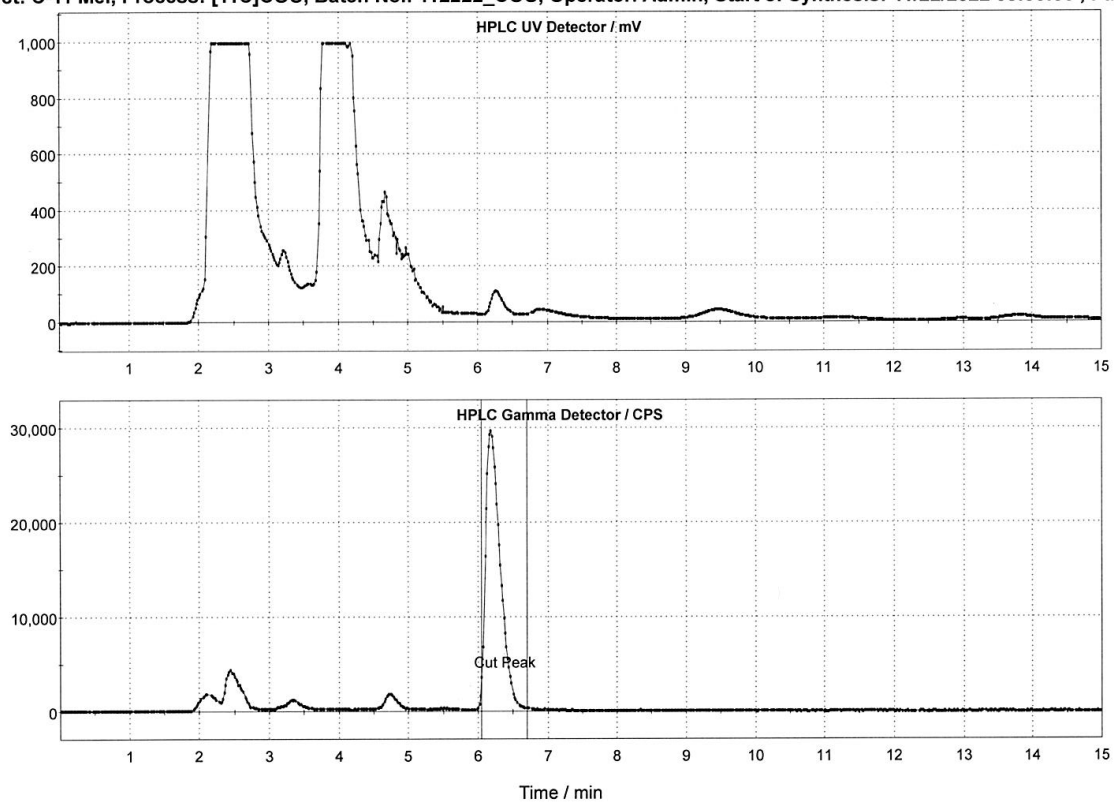


| Peak# | Ret. Time | Area | Height | Conc.  | Area%   |
|-------|-----------|------|--------|--------|---------|
| 1     | 2.163     | 1664 | 200    | 45.922 | 45.922  |
| 2     | 3.014     | 495  | 129    | 13.668 | 13.668  |
| 3     | 5.423     | 259  | 48     | 7.159  | 7.159   |
| 4     | 5.873     | 1044 | 154    | 28.806 | 28.806  |
| 5     | 6.905     | 161  | 21     | 4.445  | 4.445   |
| Total |           | 3624 | 551    |        | 100.000 |

AD2

| Peak# | Ret. Time | Area    | Height | Conc. | Area%   |
|-------|-----------|---------|--------|-------|---------|
| 1     | 2.581     | 11323   | 2140   | 0.000 | 0.747   |
| 2     | 5.967     | 1503911 | 184207 | 0.000 | 99.253  |
| Total |           | 1515234 | 186347 |       | 100.000 |

**Supplemental Figure 12.** Analytical HPLC of [ $^{11}\text{C}$ ]COU



**Supplemental Figure 13.** Semi-preparative HPLC of [11C]COU

# 5. In Vivo Pharmacology and Toxicology Reports

Report for Study 2021101

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**Single Dose IV UM\_COU Rat Study**  
**Study Number: 2021101**

**Michigan State University In Vivo Facility**  
**East Lansing, Michigan 48824**

**Sponsor:** Peter J. H. Scott, PhD  
Division of Nuclear Medicine / Department of Radiology  
University of Michigan Medical School

**Sponsor Contact:** Peter J.H. Scott, PhD

**APPROVAL SIGNATURES**

The following individuals confirm that this report accurately represents their interpretation of the data:



Date: 2022.03.15  
18:48:07 -04'00'

3/15/2022

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Teresa Krieger-Burke, DVM, PhD

Date

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## Single Dose IV UM\_COU Rat Study

### 1. OBJECTIVE

The objective of this study was to investigate the toleration of a single intravenous dose of UM\_COU in male and female rats.

### 2. MATERIALS AND METHODS

#### 2.1. Test Article

|                                             |                                    |
|---------------------------------------------|------------------------------------|
| Compound:                                   | UM_COU                             |
| Lot Number:                                 | not applicable                     |
| Active Moiety (used for dose calculations): | 100% assumed                       |
| Vehicle:                                    | not applicable                     |
| Dose:                                       | 86 µg/kg                           |
| Dose Volume:                                | 0.86 mL/kg                         |
| Dose Route of Administration:               | intravenous                        |
| Storage Conditions:                         | refrigerated, protected from light |

#### 2.2. Dosing Formulation Preparation

Dosing formulation was provided by the Sponsor ready-to-dose. The formulation was stored under refrigerated conditions, protected from light, until the time of use. Formulations were used within 10 days of delivery.

#### 2.3. Animal Information

Ten (10) male and 10 female Sprague-Dawley rats were obtained from Charles River Laboratories and weighed 184-241 grams (males) or 200-229 grams (females) at the time of dosing initiation. Animals were individually housed in solid bottom cages with aspen bedding for the duration of the study. All animals had free access to standard rodent chow and fresh water throughout the study. Animals were maintained on an automated 12/12 hour light/dark cycle with 7:00am as the start of the light phase.

This study was conducted in accordance with the current guidelines for animal welfare (Guide for the Care and Use of Laboratory Animals, 8th Ed., 2011). The procedures used in this study have been reviewed and approved by the Institutional Animal Care and Use Committee.

## 2.4. Experimental Schedule

Animals were assigned to an experimental schedule according to Table 1 below.

Table 1. Treatment Schedule

| Group | No. of Animals | Animal Numbers | Sex | Treatment       | Scheduled Necropsy Day |
|-------|----------------|----------------|-----|-----------------|------------------------|
| 1     | 5              | 101-105        | M   | UM_COU 86 µg/kg | Day 15                 |
| 2     | 5              | 201-205        | M   | UM_COU 86 µg/kg | Day 4                  |
| 3     | 5              | 301-305        | F   | UM_COU 86 µg/kg | Day 15                 |
| 4     | 5              | 401-405        | F   | UM_COU 86 µg/kg | Day 4                  |

Test Subject Arrival Date: June 29, 2021  
In-Life Start Date (Study Day 1): July 6, 2021  
Monitor End Date (Completion of In-Life Phase): July 20, 2021  
Data Collection Rooms: B-23 and B-346, Life Sciences building, MSU

Clinical Observations: pre-dose and daily thereafter  
Body Weights: prior to dosing and at time of necropsy  
Food Consumption: Study Day -1 (baseline), and Days 3, 7, and 15  
Serum Biochemistry and Complete Blood Counts (CBC): prior to dosing (baseline) and at scheduled necropsy (Day 4: Groups 2 & 4; Day 15: Groups 1 & 3)  
Organs Collected and Weighed: Heart, liver, kidney, spleen, and brain  
Disposition of Animals at Study Completion: Euthanized at the time of the final blood collection and carcasses incinerated

## 2.5. Description of Experimental Procedure

Ten (10) male and 10 female Sprague-Dawley rats were used on study. Animals were received from the supplier and housed in room B-23 in the vivarium of the Life Sciences building at Michigan State University. Animals were individually housed in plastic solid bottom cages with aspen bedding throughout the duration of the study. Animals had free access to standard rodent chow and fresh water and were maintained on an automated 12/12 hour light/dark cycle with 7:00am as the start of the light phase.

Animals were acclimated at least 1 week. The study was conducted in two cohorts to facilitate timing of blood sample delivery to the Veterinary Diagnostic Laboratory on Day 1 (dosing day). Groups 1 and 3 were dosed on July 6, 2021. Groups 2 and 4 were dosed on July 12, 2021. The day of dosing of each individual animal was considered Study Day 1 for that animal. Animals were lightly anesthetized using isoflurane in oxygen on Study Day 1. Blood (~1mL total) was collected into K<sub>3</sub>EDTA and serum tubes, and processed for respective complete blood count (CBC) and clinical chemistry baseline measurements. The test article formulation was then delivered as a single intravenous bolus and animals were recovered from anesthesia.

Clinical observations were recorded daily. Food consumption was measured on Day -1 (baseline) and on Days 3, 7, and 15.

Groups 2 and 4 were euthanized on Day 4 and Groups 1 and 3 on Day 15. Terminal body weights were recorded. Terminal blood samples were collected for hematology and biochemistry analyses. Animals were euthanized by thoracotomy immediately following the final blood collection. A limited gross necropsy was performed, and heart, liver, kidneys, spleen, and brain removed, weighed, and fixed in 10% neutral-buffered formalin (see Gross Pathology & Histopathology section below). Slides were prepared, stained with standard Hematoxylin & Eosin, and reviewed by a veterinary pathologist.

### 2.5.1. Hematology

Blood samples containing K<sub>3</sub>EDTA as an anticoagulant were analyzed by the MSU Veterinary Diagnostic Laboratory (VDL) for hematology parameters including:

|                                           |                             |
|-------------------------------------------|-----------------------------|
| red cell count                            | red cell distribution width |
| hemoglobin                                | platelet count              |
| hematocrit                                | mean platelet volume        |
| mean corpuscular volume                   | white cell count            |
| mean corpuscular hemoglobin               | white cell differential     |
| mean corpuscular hemoglobin concentration |                             |

### 2.5.2. Biochemistry

Blood samples were collected into clot tubes. Serum was analyzed by the MSU VDL for biochemistry parameters including:

|                            |                       |                     |
|----------------------------|-----------------------|---------------------|
| alanine aminotransferase   | chloride              | phosphorus          |
| albumin                    | cholesterol           | potassium           |
| alkaline phosphatase       | creatinine            | protein (total)     |
| aspartate aminotransferase | globulin (calculated) | sodium              |
| bilirubin (total)          | glucose               | urea nitrogen (BUN) |
| calcium                    | magnesium             |                     |

### 2.5.3. Gross Pathology and Histopathology

The following tissues were evaluated grossly in all animals:

|         |                         |                 |
|---------|-------------------------|-----------------|
| adrenal | large & small intestine | spleen          |
| brain   | liver                   | stomach         |
| heart   | lung                    | thymus          |
| kidney  | pancreas                | urinary bladder |

Representative samples of the following tissues were collected from all rats, weighed, fixed in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin:

|       |        |       |                               |       |
|-------|--------|-------|-------------------------------|-------|
| Heart | Spleen | Liver | Kidney (L&R weighed together) | Brain |
|-------|--------|-------|-------------------------------|-------|

## 2.6. Statistical Analyses

Data were collected in Excel. Graphic interpretation of the data was performed using GraphPad Prism 9.2.0. Statistical comparisons of body weight, organ weights, hematology, and clinical chemistry parameters were made using Kruskal-Wallis one-way analysis of variance followed by post-hoc testing using Dunn's multiple comparisons test. In all cases, a  $p < 0.05$  was considered statistically significant. Numbers comparing groups on graphs indicate the corresponding p-values.

## 3. RESULTS

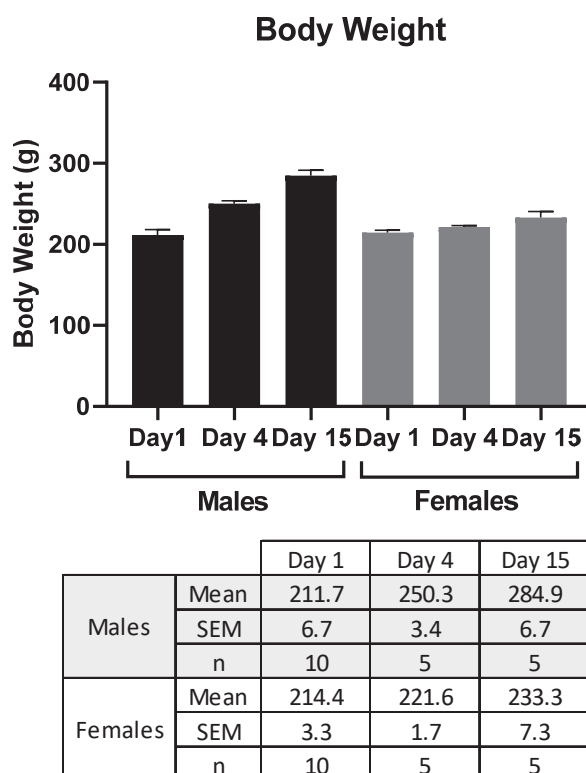
### 3.1. Clinical Observations

There were no drug-related clinical findings. All animals remained within normal limits throughout the study.

### 3.2. Body Weight

There were no adverse drug-related effects on body weight gain in males or females (Figure 1).

Figure 1. Mean Body Weight over Time



### 3.3. Food Consumption

Average daily food consumption of male animals remained similar over time as did average daily food consumption in females (Table 2).

Table 2. Average Daily Food Consumption (grams) over Time

|                          | Animal No.  | Baseline    | Day 3       | Day 7       | Day 15      |
|--------------------------|-------------|-------------|-------------|-------------|-------------|
| Males<br>Day 4 cohort    | 201         | 19.9        | 21.6        | NA          | NA          |
|                          | 202         | 21.7        | 24.6        | NA          | NA          |
|                          | 203         | 20.7        | 23.1        | NA          | NA          |
|                          | 204         | 20.7        | 20.7        | NA          | NA          |
|                          | 205         | 23.5        | 24.8        | NA          | NA          |
|                          | <b>Mean</b> | <b>21.3</b> | <b>23.0</b> |             |             |
|                          | <b>SEM</b>  | <b>0.6</b>  | <b>0.8</b>  |             |             |
| Males<br>Day 15 cohort   | 101         | 21.7        | 21.2        | 24.1        | 25.4        |
|                          | 102         | 19.3        | 20.6        | 23.1        | 22.9        |
|                          | 103         | 19.2        | 20.5        | 22.1        | 23.1        |
|                          | 104         | 19.8        | 19.9        | 23.6        | 23.2        |
|                          | 105         | 20.5        | 19.0        | 22.8        | 24.3        |
|                          | <b>Mean</b> | <b>20.1</b> | <b>20.2</b> | <b>23.1</b> | <b>23.8</b> |
|                          | <b>SEM</b>  | <b>0.5</b>  | <b>0.4</b>  | <b>0.3</b>  | <b>0.5</b>  |
| Females<br>Day 4 cohort  | 401         | 16.9        | 16.6        | NA          | NA          |
|                          | 402         | 17.4        | 17.9        | NA          | NA          |
|                          | 403         | 16.4        | 16.6        | NA          | NA          |
|                          | 404         | 16.4        | 19.6        | NA          | NA          |
|                          | 405         | 18.2        | 15.9        | NA          | NA          |
|                          | <b>Mean</b> | <b>17.1</b> | <b>17.3</b> |             |             |
|                          | <b>SEM</b>  | <b>0.3</b>  | <b>0.6</b>  |             |             |
| Females<br>Day 15 cohort | 301         | 16.1        | 19.8        | 19.7        | 15.5        |
|                          | 302         | 17.2        | 12.9        | 18.4        | 18.6        |
|                          | 303         | 19.0        | 16.7        | 19.7        | 18.6        |
|                          | 304         | 14.7        | 15.6        | 16.8        | 16.3        |
|                          | 305         | 17.8        | 15.2        | 18.5        | 17.3        |
|                          | <b>Mean</b> | <b>17.0</b> | <b>16.0</b> | <b>18.6</b> | <b>17.3</b> |
|                          | <b>SEM</b>  | <b>0.7</b>  | <b>1.1</b>  | <b>0.5</b>  | <b>0.6</b>  |

### 3.4. Organ Weights and Necropsy Gross Findings

There were no significant gross necropsy findings.

Organ weights were comparable within each sex between Day 4 and Day 15 (Tables 3 to 5).

Table 3. Terminal Organ Weights

| Organ Weight (g) |      |       |       |        |        |                    |
|------------------|------|-------|-------|--------|--------|--------------------|
| Group            |      | Brain | Heart | Liver  | Spleen | Kidneys (combined) |
| Males, Day 4     | Mean | 1.733 | 0.983 | 9.570  | 0.681  | 1.752              |
|                  | SEM  | 0.048 | 0.052 | 0.339  | 0.045  | 0.038              |
| Males, Day 15    | Mean | 1.737 | 1.059 | 11.250 | 0.629  | 2.089              |
|                  | SEM  | 0.019 | 0.018 | 0.320  | 0.016  | 0.072              |
| Females, Day 4   | Mean | 1.849 | 0.926 | 7.802  | 0.604  | 1.471              |
|                  | SEM  | 0.069 | 0.028 | 0.211  | 0.044  | 0.038              |
| Females, Day 15  | Mean | 1.789 | 0.920 | 8.025  | 0.552  | 1.496              |
|                  | SEM  | 0.028 | 0.087 | 0.293  | 0.036  | 0.060              |

Table 4. Terminal Organ Weight to Body Weight Ratios

| Organ Weight:Body Weight |      |                |                |                  |                 |                |
|--------------------------|------|----------------|----------------|------------------|-----------------|----------------|
| Group                    |      | Brain:BW Ratio | Heart:BW Ratio | Kidneys:BW Ratio | Spleen:BW Ratio | Liver:BW Ratio |
| Males, Day 4             | Mean | 0.0069         | 0.0039         | 0.0070           | 0.0027          | 0.0382         |
|                          | SEM  | 0.0002         | 0.0002         | 0.0001           | 0.0001          | 0.0009         |
| Males, Day 15            | Mean | 0.0061         | 0.0037         | 0.0073           | 0.0022          | 0.0394         |
|                          | SEM  | 0.0002         | 0.0001         | 0.0002           | 0.0001          | 0.0005         |
| Females, Day 4           | Mean | 0.0083         | 0.0042         | 0.0066           | 0.0027          | 0.0352         |
|                          | SEM  | 0.0003         | 0.0001         | 0.0002           | 0.0002          | 0.0008         |
| Females, Day 15          | Mean | 0.0077         | 0.0039         | 0.0064           | 0.0024          | 0.0346         |
|                          | SEM  | 0.0002         | 0.0002         | 0.0003           | 0.0001          | 0.0005         |

Table 5. Terminal Organ Weight to Brain Weight Ratios

| Organ Weight:Brain Weight |      |                      |                        |                       |                      |
|---------------------------|------|----------------------|------------------------|-----------------------|----------------------|
| Group                     |      | Heart:Brain Wt Ratio | Kidneys:Brain Wt Ratio | Spleen:Brain Wt Ratio | Liver:Brain Wt Ratio |
| Males, Day 4              | Mean | 0.57                 | 1.01                   | 0.39                  | 5.54                 |
|                           | SEM  | 0.03                 | 0.03                   | 0.03                  | 0.25                 |
| Males, Day 15             | Mean | 0.61                 | 1.20                   | 0.36                  | 6.47                 |
|                           | SEM  | 0.01                 | 0.04                   | 0.01                  | 0.16                 |
| Females, Day 4            | Mean | 0.50                 | 0.80                   | 0.33                  | 4.25                 |
|                           | SEM  | 0.03                 | 0.03                   | 0.04                  | 0.23                 |
| Females, Day 15           | Mean | 0.51                 | 0.84                   | 0.31                  | 4.49                 |
|                           | SEM  | 0.04                 | 0.03                   | 0.02                  | 0.16                 |

### 3.5. Hematology

Total protein was increased in females at Day 15 compared with corresponding baseline values (Figure 3, Table 6 and 7). Remaining parameters were comparable within each sex at baseline, Day 4, and Day 15.

Figure 3.

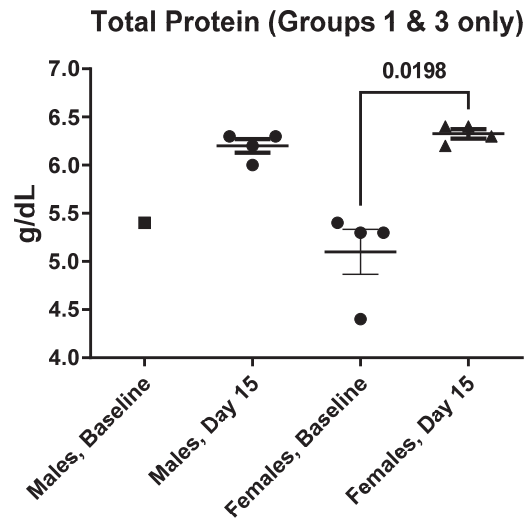


Table 6. Hematology – Baseline Group Means

| Group         |      | Total Protein<br>g/dL | RBC<br>x10.e6/uL | Hemoglobin<br>g/dL | Hematocrit<br>% | Hematocrit<br>spun % | MCV<br>fL | MCH<br>pg | MCHC<br>g/dL |
|---------------|------|-----------------------|------------------|--------------------|-----------------|----------------------|-----------|-----------|--------------|
| Males         | Mean | 5.7                   | 7.0              | 13.7               | 40.5            | 26.0                 | 58.0      | 19.5      | 34.0         |
| Day 4 cohort  | SEM  | 0.1                   | 0.1              | 0.3                | 0.5             | 12.0                 | 1.0       | 0.5       | 0.0          |
| Males         | Mean | 5.4                   | 6.4              | 12.5               | 39.0            | 36.0                 | 62.0      | 20.0      | 31.0         |
| Day 15 cohort | SEM  | 0.0                   | 0.0              | 0.0                | 0.0             | 0.0                  | 0.0       | 0.0       | 0.0          |
| Females       | Mean | 5.5                   | 7.1              | 13.0               | 37.5            | 36.5                 | 53.5      | 18.0      | 34.5         |
| Day 4 cohort  | SEM  | 0.7                   | 0.6              | 1.0                | 2.5             | 2.5                  | 0.5       | 0.0       | 0.5          |
| Females       | Mean | 5.1                   | 6.6              | 12.3               | 36.6            | 38.3                 | 56.2      | 19.0      | 33.2         |
| Day 15 cohort | SEM  | 0.2                   | 0.4              | 0.8                | 2.5             | 1.6                  | 1.6       | 0.3       | 0.6          |

| Group         |      | RDW<br>% | Platelets<br>x10.e3/uL | MPV<br>fL | WBC<br>x10.e3/uL | Segmented<br>Neutrophils<br>x10.e3/uL | Band<br>Neutrophils<br>x10.e3/uL | Lymphocytes<br>x10.e3/uL | Monocytes<br>x10.e3/uL |
|---------------|------|----------|------------------------|-----------|------------------|---------------------------------------|----------------------------------|--------------------------|------------------------|
| Males         | Mean | 12.0     | 977.0                  | 8.2       | 11.3             | 1.1                                   | 0.0                              | 49.7                     | 1.0                    |
| Day 4 cohort  | SEM  | 0.0      | 141.0                  | 0.2       | 1.5              | 0.3                                   | 0.0                              | 38.3                     | 1.0                    |
| Males         | Mean | 12.0     | 883.0                  | 8.4       | 12.7             | 0.5                                   | 0.0                              | 11.9                     | 0.3                    |
| Day 15 cohort | SEM  | 0.0      | 0.0                    | 0.0       | 0.0              | 0.0                                   | 0.0                              | 0.0                      | 0.0                    |
| Females       | Mean | 10.5     | 525.0                  | 8.3       | 7.6              | 0.9                                   | 0.0                              | 6.6                      | 0.1                    |
| Day 4 cohort  | SEM  | 0.5      | 133.0                  | 0.6       | 0.2              | 0.4                                   | 0.0                              | 0.5                      | 0.1                    |
| Females       | Mean | 10.6     | 803.8                  | 7.7       | 8.0              | 0.7                                   | 0.0                              | 7.0                      | 0.2                    |
| Day 15 cohort | SEM  | 0.4      | 84.8                   | 0.2       | 1.3              | 0.1                                   | 0.0                              | 1.3                      | 0.0                    |

| Group         |      | Eosinophils<br>x10.e3/uL | Basophils<br>x10.e3/uL | Segmented<br>Neutrophils<br>% | Band<br>Neutrophils<br>% | Lymphocytes<br>% | Monocytes<br>% | Eosinophils<br>% | Basophils<br>% |
|---------------|------|--------------------------|------------------------|-------------------------------|--------------------------|------------------|----------------|------------------|----------------|
| Males         | Mean | 1.0                      | 0.0                    | 9.5                           | 0.0                      | 88.5             | 1.0            | 1.0              | 0.0            |
| Day 4 cohort  | SEM  | 1.0                      | 0.0                    | 1.5                           | 0.0                      | 0.5              | 1.0            | 1.0              | 0.0            |
| Males         | Mean | 0.0                      | 0.0                    | 4.0                           | 0.0                      | 94.0             | 2.0            | 0.0              | 0.0            |
| Day 15 cohort | SEM  | 0.0                      | 0.0                    | 0.0                           | 0.0                      | 0.0              | 0.0            | 0.0              | 0.0            |
| Females       | Mean | 0.1                      | 0.0                    | 12.0                          | 0.0                      | 86.5             | 0.5            | 1.0              | 0.0            |
| Day 4 cohort  | SEM  | 0.0                      | 0.0                    | 5.0                           | 0.0                      | 4.5              | 0.5            | 0.0              | 0.0            |
| Females       | Mean | 0.1                      | 0.0                    | 4.0                           | 0.0                      | 86.5             | 1.7            | 1.3              | 0.0            |
| Day 15 cohort | SEM  | 0.0                      | 0.0                    | 0.0                           | 0.0                      | 2.2              | 0.2            | 0.4              | 0.0            |

Table 7. Hematology – Terminal Group Means

| Group           |      | Total Protein<br>g/dL | RBC<br>x10.e6/uL | Hemoglobin<br>g/dL | Hematocrit<br>% | Hematocrit<br>spun % | MCV<br>fL | MCH<br>pg | MCHC<br>g/dL |
|-----------------|------|-----------------------|------------------|--------------------|-----------------|----------------------|-----------|-----------|--------------|
| Males, Day 4    | Mean | 5.7                   | 6.5              | 12.3               | 38.2            | 38.2                 | 59.4      | 19.0      | 32.2         |
|                 | SEM  | 0.2                   | 0.1              | 0.2                | 0.5             | 0.7                  | 0.5       | 0.0       | 0.2          |
| Males, Day 15   | Mean | 6.2                   | 7.3              | 14.0               | 42.5            | 41.5                 | 58.3      | 19.0      | 32.8         |
|                 | SEM  | 0.1                   | 0.1              | 0.2                | 0.5             | 0.3                  | 0.3       | 0.0       | 0.3          |
| Females, Day 4  | Mean | 6.0                   | 6.8              | 12.6               | 38.0            | 37.7                 | 56.0      | 18.7      | 33.3         |
|                 | SEM  | 0.2                   | 0.1              | 0.2                | 0.6             | 1.2                  | 0.0       | 0.3       | 0.3          |
| Females, Day 15 | Mean | 6.3                   | 7.8              | 14.5               | 42.8            | 40.8                 | 55.0      | 18.8      | 30.3         |
|                 | SEM  | 0.0                   | 0.4              | 0.7                | 2.1             | 2.4                  | 1.1       | 0.3       | 3.8          |

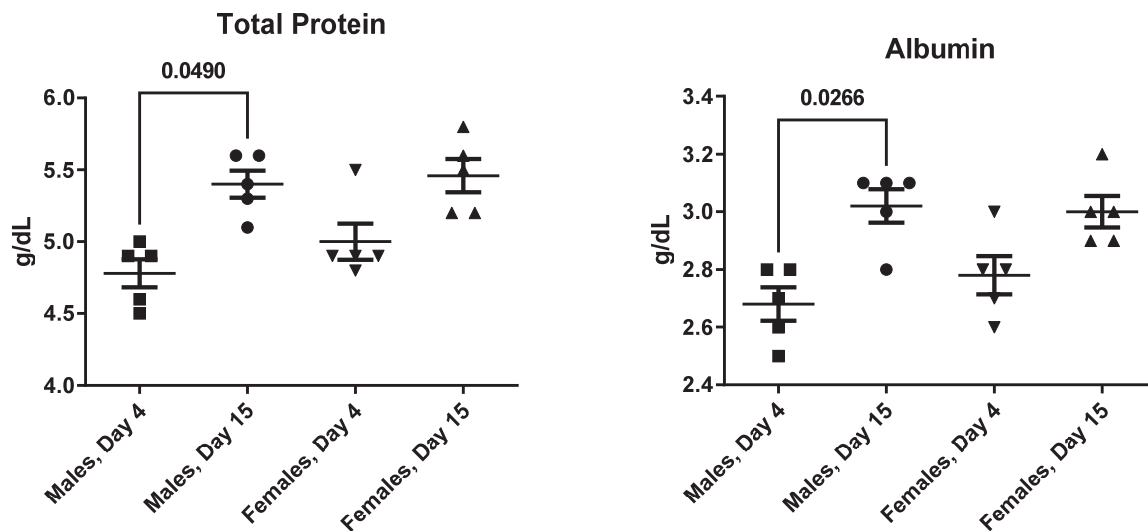
| Group           |      | RDW<br>% | Platelets<br>x10.e3/uL | MPV<br>fL | WBC<br>x10.e3/uL | Segmented<br>Neutrophils<br>x10.e3/uL | Band<br>Neutrophils<br>x10.e3/uL | Lymphocytes<br>x10.e3/uL | Monocytes<br>x10.e3/uL |
|-----------------|------|----------|------------------------|-----------|------------------|---------------------------------------|----------------------------------|--------------------------|------------------------|
| Males, Day 4    | Mean | 12.4     | 989.6                  | 8.0       | 9.2              | 0.9                                   | 0.0                              | 8.0                      | 0.3                    |
|                 | SEM  | 0.2      | 117.2                  | 0.1       | 1.1              | 0.2                                   | 0.0                              | 1.0                      | 0.1                    |
| Males, Day 15   | Mean | 12.0     | 998.0                  | 8.6       | 9.4              | 1.1                                   | 0.0                              | 8.0                      | 0.2                    |
|                 | SEM  | 0.0      | 60.2                   | 0.2       | 1.0              | 0.3                                   | 0.0                              | 1.1                      | 0.1                    |
| Females, Day 4  | Mean | 12.0     | 920.3                  | 8.0       | 8.5              | 0.7                                   | 0.0                              | 7.5                      | 0.2                    |
|                 | SEM  | 0.6      | 18.9                   | 0.2       | 2.7              | 0.3                                   | 0.0                              | 2.3                      | 0.1                    |
| Females, Day 15 | Mean | 10.8     | 845.8                  | 8.3       | 7.9              | 0.9                                   | 0.0                              | 6.7                      | 0.2                    |
|                 | SEM  | 0.3      | 60.0                   | 0.5       | 1.9              | 0.2                                   | 0.0                              | 1.8                      | 0.1                    |

| Group           |      | Eosinophils<br>x10.e3/uL | Basophils<br>x10.e3/uL | Segmented<br>Neutrophils<br>% | Band<br>Neutrophils<br>% | Lymphocytes<br>% | Monocytes<br>% | Eosinophils<br>% | Basophils<br>% |
|-----------------|------|--------------------------|------------------------|-------------------------------|--------------------------|------------------|----------------|------------------|----------------|
| Males, Day 4    | Mean | 0.0                      | 0.0                    | 9.6                           | 0.0                      | 86.6             | 3.2            | 0.6              | 0.0            |
|                 | SEM  | 0.0                      | 0.0                    | 2.2                           | 0.0                      | 2.3              | 0.5            | 0.2              | 0.0            |
| Males, Day 15   | Mean | 0.1                      | 0.0                    | 11.5                          | 0.0                      | 85.3             | 2.3            | 1.0              | 0.0            |
|                 | SEM  | 0.1                      | 0.0                    | 3.3                           | 0.0                      | 3.3              | 0.9            | 0.7              | 0.0            |
| Females, Day 4  | Mean | 0.1                      | 0.0                    | 9.0                           | 0.0                      | 87.3             | 2.7            | 1.0              | 0.0            |
|                 | SEM  | 0.1                      | 0.0                    | 2.0                           | 0.0                      | 1.3              | 0.3            | 0.6              | 0.0            |
| Females, Day 15 | Mean | 0.1                      | 0.0                    | 12.8                          | 0.0                      | 82.8             | 3.5            | 1.0              | 0.0            |
|                 | SEM  | 0.0                      | 0.0                    | 2.5                           | 0.0                      | 3.1              | 0.9            | 0.4              | 0.0            |

### 3.6. Clinical Biochemistry

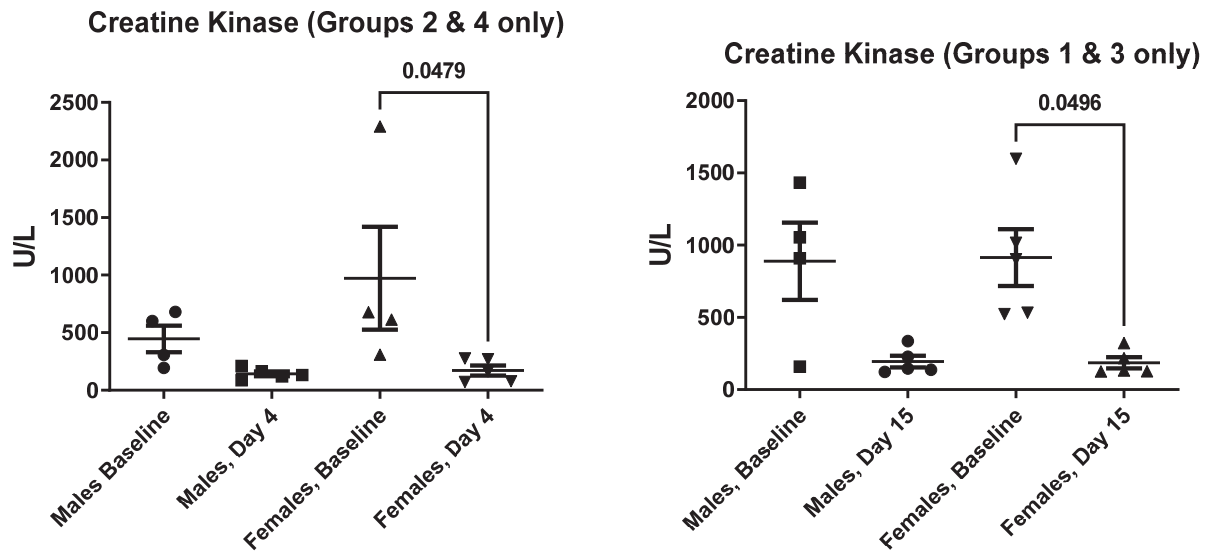
Total protein and albumin increased in males from Day 4 to Day 15 (Figure 4, Table 9).

Figure 4.



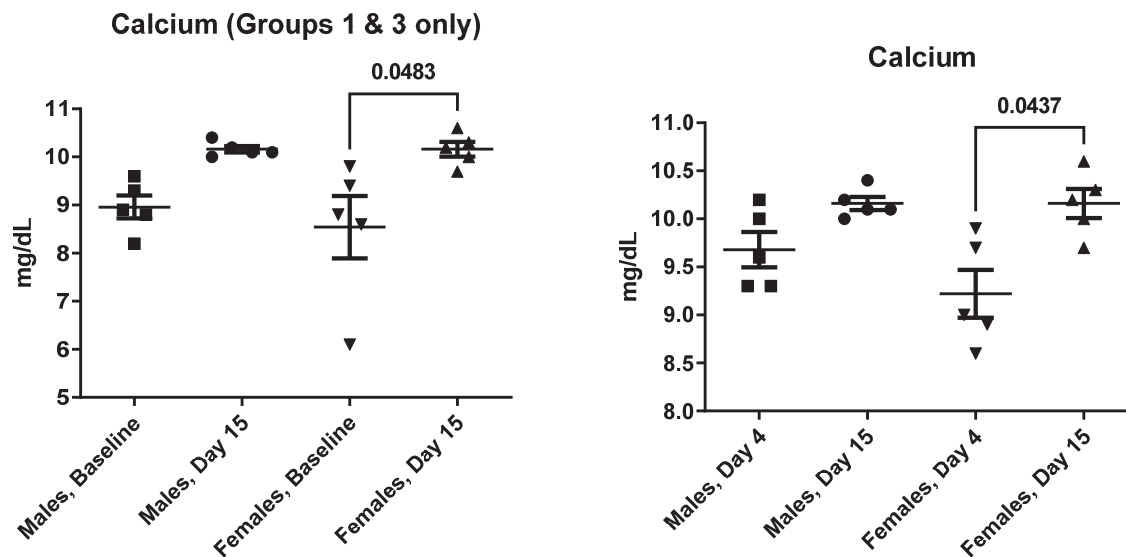
Creatine kinase was decreased in female rats on Days 4 and 15 compared with corresponding baseline levels (Figure 5, Table 8 and 9).

Figure 5.



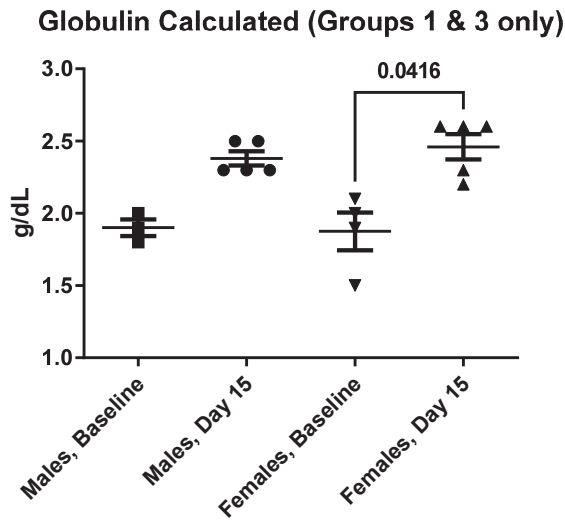
Calcium increased in females from baseline to Day 15 and from Day 4 to Day 15 (Figure 6, Table 8 and 9).

Figure 6.



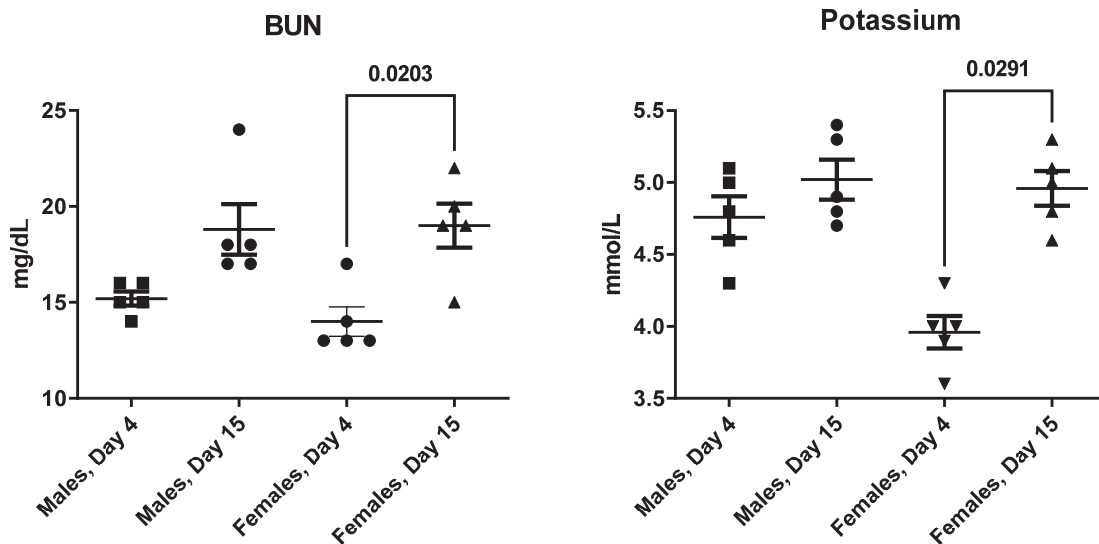
Globulin was increased at Day 15 in females compared with corresponding baseline levels (Figure 7, Table 8 and 9).

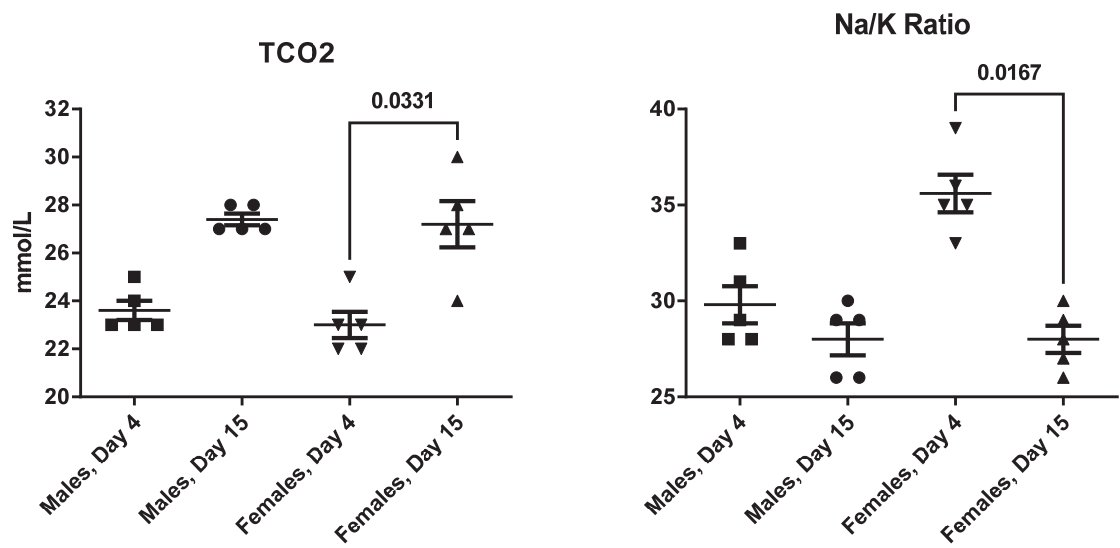
Figure 7.



BUN, potassium, and TCO2 increased in females from Day 4 to Day 15 while the Na/K ratio decreased from Day 4 to Day 15 in females (Figure 8, Table 9).

Figure 8.





All other biochemistry parameters remained unchanged in males and in females.

Table 8. Biochemistry Parameters – Baseline Group Means

| Group                 |      | Urea Nitrogen (mg/dL) | Creatinine (Jaffe) (mg/dL) | Sodium (mmol/L) | Potassium (mmol/L) | Chloride (mmol/L) | TCO2 (mmol/L) | Na/K Ratio |
|-----------------------|------|-----------------------|----------------------------|-----------------|--------------------|-------------------|---------------|------------|
| Males Day 4 cohort    | Mean | 16                    | 0.2                        | 139             | 5.3                | 102               | 25            | 26         |
|                       | SEM  | 1                     | 0.0                        | 1               | 0.2                | 2                 | 1             | 1          |
| Males Day 15 cohort   | Mean | 15                    | 0.2                        | 140             | 5.2                | 104               | 21            | 27         |
|                       | SEM  | 1                     | 0.0                        | 1               | 0.4                | 1                 | 1             | 2          |
| Females Day 4 cohort  | Mean | 13                    | 0.2                        | 140             | 4.1                | 109               | 20            | 35         |
|                       | SEM  | 1                     | 0.0                        | 1               | 0.2                | 3                 | 2             | 2          |
| Females Day 15 cohort | Mean | 14                    | 0.2                        | 140             | 4.2                | 107               | 21            | 34         |
|                       | SEM  | 1                     | 0.0                        | 1               | 0.4                | 3                 | 1             | 4          |

| Group                 |      | Anion Gap (mmol/L) | Osmolarity Calculated (mmol/L) | Calcium (mg/dL) | Phosphorus (mg/dL) | Magnesium (mg/dL) | Iron (ug/dL) | Total Protein (g/dL) |
|-----------------------|------|--------------------|--------------------------------|-----------------|--------------------|-------------------|--------------|----------------------|
| Males Day 4 cohort    | Mean | 18                 | 293                            | 9.5             | 7.1                | 1.7               | 194          | 4.9                  |
|                       | SEM  | 1                  | 1                              | 0.4             | 0.2                | 0.1               | 13           | 0.2                  |
| Males Day 15 cohort   | Mean | 20                 | 295                            | 9.0             | 8.0                | 1.7               | 225          | 4.5                  |
|                       | SEM  | 1                  | 1                              | 0.2             | 0.3                | 0.1               | 25           | 0.2                  |
| Females Day 4 cohort  | Mean | 16                 | 293                            | 8.5             | 5.3                | 1.5               | 324          | 5.0                  |
|                       | SEM  | 1                  | 1                              | 0.7             | 1.0                | 0.2               | 54           | 0.7                  |
| Females Day 15 cohort | Mean | 16                 | 294                            | 8.5             | 5.6                | 1.5               | 286          | 4.4                  |
|                       | SEM  | 1                  | 2                              | 0.6             | 0.6                | 0.1               | 9            | 0.3                  |

| Group                 |      | Albumin (g/dL) | Globulin Calc (g/dL) | Glucose (Hexokinase) (mg/dL) | Amylase (U/L) | Total Bilirubin (mg/dL) | Direct Bilirubin (mg/dL) | Indirect Bilirubin (mg/dL) |
|-----------------------|------|----------------|----------------------|------------------------------|---------------|-------------------------|--------------------------|----------------------------|
| Males Day 4 cohort    | Mean | 2.7            | 2.2                  | 185                          | 461           | 0.1                     | 0.0                      | 0.1                        |
|                       | SEM  | 0.1            | 0.1                  | 16                           | 18            | 0.0                     | 0.0                      | 0.0                        |
| Males Day 15 cohort   | Mean | 2.7            | 1.9                  | 190                          | 457           | 0.1                     | 0.0                      | 0.1                        |
|                       | SEM  | 0.1            | 0.1                  | 7                            | 41            | 0.0                     | 0.0                      | 0.0                        |
| Females Day 4 cohort  | Mean | 2.5            | 2.3                  | 154                          | 279           | 0.1                     | 0.0                      | 0.1                        |
|                       | SEM  | 0.1            | 0.4                  | 18                           | 11            | 0.0                     | 0.0                      | 0.0                        |
| Females Day 15 cohort | Mean | 2.6            | 1.9                  | 160                          | 274           | 0.1                     | 0.0                      | 0.1                        |
|                       | SEM  | 0.2            | 0.1                  | 14                           | 31            | 0.0                     | 0.0                      | 0.0                        |

| Group                 |      | Alkaline Phosphatase (U/L) | Alanine Amino-transferase (U/L) | Aspartate Amino-transferase (U/L) | Creatine Kinase (U/L) | Cholesterol (mg/dL) | Triglyceride (mg/dL) | Lipemia | Icterus | Hemolysis |
|-----------------------|------|----------------------------|---------------------------------|-----------------------------------|-----------------------|---------------------|----------------------|---------|---------|-----------|
| Males Day 4 cohort    | Mean | 385                        | 46                              | 61                                | 446                   | 63                  | 84                   | NA      | NA      | NA        |
|                       | SEM  | 14                         | 3                               | 3                                 | 116                   | 5                   | 15                   | NA      | NA      | NA        |
| Males Day 15 cohort   | Mean | 399                        | 40                              | 76                                | 889                   | 65                  | NA                   | NA      | NA      | NA        |
|                       | SEM  | 25                         | 3                               | 16                                | 267                   | 3                   | NA                   | NA      | NA      | NA        |
| Females Day 4 cohort  | Mean | 171                        | 40                              | 55                                | 973                   | 49                  | 59                   | NA      | NA      | NA        |
|                       | SEM  | 19                         | 3                               | 6                                 | 446                   | 3                   | 24                   | NA      | NA      | NA        |
| Females Day 15 cohort | Mean | 172                        | 40                              | 62                                | 914                   | 48                  | NA                   | NA      | NA      | NA        |
|                       | SEM  | 28                         | 6                               | 7                                 | 197                   | 4                   | NA                   | NA      | NA      | NA        |

Table 9. Biochemistry Parameters – Terminal Group Means

| Group           |      | Urea Nitrogen (mg/dL) | Creatinine (Jaffe) (mg/dL) | Sodium (mmol/L) | Potassium (mmol/L) | Chloride (mmol/L) | TCO2 (mmol/L) | Na/K Ratio |
|-----------------|------|-----------------------|----------------------------|-----------------|--------------------|-------------------|---------------|------------|
| Males, Day 4    | Mean | 15                    | 0.2                        | 141             | 4.8                | 103               | 24            | 30         |
|                 | SEM  | 0                     | 0.0                        | 0               | 0.1                | 1                 | 0             | 1          |
| Males, Day 15   | Mean | 19                    | 0.3                        | 141             | 5.0                | 101               | 27            | 28         |
|                 | SEM  | 1                     | 0.0                        | 0               | 0.1                | 0                 | 0             | 1          |
| Females, Day 4  | Mean | 14                    | 0.2                        | 141             | 4.0                | 106               | 23            | 36         |
|                 | SEM  | 1                     | 0.0                        | 1               | 0.1                | 1                 | 1             | 1          |
| Females, Day 15 | Mean | 19                    | 0.2                        | 139             | 5.0                | 103               | 27            | 28         |
|                 | SEM  | 1                     | 0.0                        | 0               | 0.1                | 1                 | 1             | 1          |

| Group           |      | Anion Gap (mmol/L) | Osmolarity Calculated (mmol/L) | Calcium (mg/dL) | Phosphorus (mg/dL) | Magnesium (mg/dL) | Iron (ug/dL) | Total Protein (g/dL) |
|-----------------|------|--------------------|--------------------------------|-----------------|--------------------|-------------------|--------------|----------------------|
| Males, Day 4    | Mean | 19                 | 297                            | 9.7             | 7.3                | 2.0               | 267          | 4.8                  |
|                 | SEM  | 1                  | 1                              | 0.2             | 0.3                | 0.1               | 34           | 0.1                  |
| Males, Day 15   | Mean | 17                 | 298                            | 10.2            | 7.6                | 2.2               | 220          | 5.4                  |
|                 | SEM  | 1                  | 1                              | 0.1             | 0.0                | 0.0               | 11           | 0.1                  |
| Females, Day 4  | Mean | 16                 | 296                            | 9.2             | 6.1                | 1.9               | 303          | 5.0                  |
|                 | SEM  | 0                  | 1                              | 0.2             | 0.4                | 0.1               | 20           | 0.1                  |
| Females, Day 15 | Mean | 14                 | 294                            | 10.2            | 6.9                | 2.0               | 304          | 5.5                  |
|                 | SEM  | 0                  | 0                              | 0.2             | 0.3                | 0.1               | 25           | 0.1                  |

| Group           |      | Albumin (g/dL) | Globulin Calc (g/dL) | Glucose (Hexokinase) (mg/dL) | Amylase (U/L) | Total Bilirubin (mg/dL) | Direct Bilirubin (mg/dL) | Indirect Bilirubin (mg/dL) |
|-----------------|------|----------------|----------------------|------------------------------|---------------|-------------------------|--------------------------|----------------------------|
| Males, Day 4    | Mean | 2.7            | 2.1                  | 157                          | 459           | 0.1                     | 0.0                      | 0.1                        |
|                 | SEM  | 0.1            | 0.0                  | 4                            | 15            | 0.0                     | 0.0                      | 0.0                        |
| Males, Day 15   | Mean | 3.0            | 2.4                  | 176                          | 543           | 0.1                     | 0.0                      | 0.1                        |
|                 | SEM  | 0.1            | 0.1                  | 9                            | 21            | 0.0                     | 0.0                      | 0.0                        |
| Females, Day 4  | Mean | 2.8            | 2.2                  | 163                          | 296           | 0.1                     | 0.0                      | 0.1                        |
|                 | SEM  | 0.1            | 0.1                  | 10                           | 10            | 0.0                     | 0.0                      | 0.0                        |
| Females, Day 15 | Mean | 3.0            | 2.5                  | 160                          | 332           | 0.1                     | 0.0                      | 0.1                        |
|                 | SEM  | 0.1            | 0.1                  | 4                            | 13            | 0.0                     | 0.0                      | 0.0                        |

| Group           |      | Alkaline Phosphatase (U/L) | Alanine Amino-transferase (U/L) | Aspartate Amino-transferase (U/L) | Creatine Kinase (U/L) | Cholesterol (mg/dL) | Triglyceride (mg/dL) | Lipemia | Icterus | Hemolysis |
|-----------------|------|----------------------------|---------------------------------|-----------------------------------|-----------------------|---------------------|----------------------|---------|---------|-----------|
| Males, Day 4    | Mean | 365                        | 46                              | 55                                | 143                   | 60                  | 75                   | NA      | NA      | NA        |
|                 | SEM  | 18                         | 3                               | 2                                 | 21                    | 3                   | 7                    | NA      | NA      | NA        |
| Males, Day 15   | Mean | 380                        | 46                              | 65                                | 194                   | 64                  | 81                   | NA      | NA      | NA        |
|                 | SEM  | 26                         | 2                               | 5                                 | 40                    | 2                   | 9                    | NA      | NA      | NA        |
| Females, Day 4  | Mean | 164                        | 39                              | 44                                | 172                   | 51                  | 46                   | NA      | NA      | NA        |
|                 | SEM  | 23                         | 4                               | 2                                 | 44                    | 2                   | 4                    | NA      | NA      | NA        |
| Females, Day 15 | Mean | 199                        | 50                              | 62                                | 185                   | 58                  | NA                   | NA      | NA      | NA        |
|                 | SEM  | 10                         | 3                               | 6                                 | 38                    | 2                   | NA                   | NA      | NA      | NA        |

### **3.7. Histopathology**

There was little to no variation between animals within or between treatment groups with the exception of the brain (Appendix V). Mild vacuolation of the white matter was observed throughout the brain at 4 days post dose. There were no indications of this lesion at Day 15, however. Epicardial hemorrhage was noted in one animal and minimal renal cortical mineralization noted in another; both of these latter lesions were considered to be background and not significant to the study.

## **4. CONCLUSION**

UM\_COU was clinically well-tolerated when administered as a single intravenous bolus to male and female adult Sprague-Dawley rats, evaluated through 15 days after dosing. There were no adverse effects on body weight or food consumption. Biochemistry changes were minor and limited to increases in total protein and albumin in males, decreased creatinine kinase levels and Na/K ratios in females, and increased calcium, globulin, BUN, potassium, and TCO<sub>2</sub> levels in females. Mild vacuolation of brain white matter was observed microscopically at 4 days post dose. However, there were no indications of this lesion by 15 days post dose.

**Appendix I. Individual Body Weight over Time**

| Group                            | Animal No. | Body Weight (g) |       |       |
|----------------------------------|------------|-----------------|-------|-------|
|                                  |            | Study Day       |       |       |
|                                  |            | 1               | 4     | 15    |
| 1<br>Males<br>Day 15<br>cohort   | 101        | 201.7           | NA    | 306.1 |
|                                  | 102        | 189.3           | NA    | 268.3 |
|                                  | 103        | 196.2           | NA    | 286.3 |
|                                  | 104        | 191.8           | NA    | 290.6 |
|                                  | 105        | 183.7           | NA    | 273.1 |
| 2<br>Males<br>Day 4<br>cohort    | 201        | 227.1           | 248.7 | NA    |
|                                  | 202        | 225.6           | 241.7 | NA    |
|                                  | 203        | 232.0           | 251.7 | NA    |
|                                  | 204        | 228.6           | 247.1 | NA    |
|                                  | 205        | 240.5           | 262.2 | NA    |
| 3<br>Females<br>Day 15<br>cohort | 301        | 204.1           | NA    | 227.5 |
|                                  | 302        | 207.5           | NA    | 232.0 |
|                                  | 303        | 229             | NA    | 260.4 |
|                                  | 304        | 202.6           | NA    | 229.6 |
|                                  | 305        | 199.8           | NA    | 216.8 |
| 4<br>Females<br>Day 4<br>cohort  | 401        | 217.8           | 218.4 | NA    |
|                                  | 402        | 224.5           | 221.2 | NA    |
|                                  | 403        | 224.6           | 227.6 | NA    |
|                                  | 404        | 218.8           | 222.5 | NA    |
|                                  | 405        | 215.3           | 218.3 | NA    |

## Appendix II. Individual Organ Weights

| Organ Weight (g) |            |       |       |        |        |                        |
|------------------|------------|-------|-------|--------|--------|------------------------|
| Group            | Animal No. | Brain | Heart | Liver  | Spleen | Kidneys (L&R combined) |
| Males, Day 4     | 201        | 1.669 | 0.902 | 9.953  | 0.675  | 1.629                  |
|                  | 202        | 1.651 | 1.026 | 8.694  | 0.522  | 1.751                  |
|                  | 203        | 1.653 | 0.873 | 10.054 | 0.791  | 1.759                  |
|                  | 204        | 1.881 | 0.949 | 8.816  | 0.676  | 1.750                  |
|                  | 205        | 1.813 | 1.165 | 10.333 | 0.740  | 1.871                  |
| Males, Day 15    | 101        | 1.771 | 1.121 | 12.284 | 0.568  | 2.228                  |
|                  | 102        | 1.735 | 1.027 | 10.592 | 0.654  | 1.814                  |
|                  | 103        | 1.775 | 1.025 | 11.613 | 0.651  | 2.147                  |
|                  | 104        | 1.670 | 1.069 | 11.127 | 0.633  | 2.094                  |
|                  | 105        | 1.736 | 1.054 | 10.622 | 0.637  | 2.163                  |
| Females, Day 4   | 401        | 1.606 | 1.034 | 8.084  | 0.734  | 1.363                  |
|                  | 402        | 1.818 | 0.885 | 7.497  | 0.598  | 1.479                  |
|                  | 403        | 1.934 | 0.897 | 8.455  | 0.649  | 1.475                  |
|                  | 404        | 2.012 | 0.927 | 7.269  | 0.571  | 1.436                  |
|                  | 405        | 1.875 | 0.887 | 7.703  | 0.467  | 1.600                  |
| Females, Day 15  | 301        | 1.825 | 0.814 | 8.003  | 0.652  | 1.395                  |
|                  | 302        | 1.713 | 0.904 | 8.272  | 0.538  | 1.590                  |
|                  | 303        | 1.848 | 1.259 | 8.991  | 0.615  | 1.590                  |
|                  | 304        | 1.732 | 0.782 | 7.522  | 0.495  | 1.310                  |
|                  | 305        | 1.829 | 0.842 | 7.337  | 0.460  | 1.597                  |

| Organ Weight:Body Weight |            |                |                |                 |                  |                |
|--------------------------|------------|----------------|----------------|-----------------|------------------|----------------|
| Group                    | Animal No. | Brain:BW Ratio | Heart:BW Ratio | Spleen:BW Ratio | Kidneys:BW Ratio | Liver:BW Ratio |
| Males, Day 4             | 201        | 0.0067         | 0.0036         | 0.0027          | 0.0065           | 0.0400         |
|                          | 202        | 0.0068         | 0.0042         | 0.0022          | 0.0072           | 0.0360         |
|                          | 203        | 0.0066         | 0.0035         | 0.0031          | 0.0070           | 0.0399         |
|                          | 204        | 0.0076         | 0.0038         | 0.0027          | 0.0071           | 0.0357         |
|                          | 205        | 0.0069         | 0.0044         | 0.0028          | 0.0071           | 0.0394         |
| Males, Day 15            | 101        | 0.0058         | 0.0037         | 0.0019          | 0.0073           | 0.0401         |
|                          | 102        | 0.0065         | 0.0038         | 0.0024          | 0.0068           | 0.0395         |
|                          | 103        | 0.0062         | 0.0036         | 0.0023          | 0.0075           | 0.0406         |
|                          | 104        | 0.0057         | 0.0037         | 0.0022          | 0.0072           | 0.0383         |
|                          | 105        | 0.0064         | 0.0039         | 0.0023          | 0.0079           | 0.0389         |
| Females, Day 4           | 401        | 0.0074         | 0.0047         | 0.0034          | 0.0062           | 0.0370         |
|                          | 402        | 0.0082         | 0.0040         | 0.0027          | 0.0067           | 0.0339         |
|                          | 403        | 0.0085         | 0.0039         | 0.0029          | 0.0065           | 0.0371         |
|                          | 404        | 0.0090         | 0.0042         | 0.0026          | 0.0065           | 0.0327         |
|                          | 405        | 0.0086         | 0.0041         | 0.0021          | 0.0073           | 0.0353         |
| Females, Day 15          | 301        | 0.0080         | 0.0036         | 0.0029          | 0.0061           | 0.0352         |
|                          | 302        | 0.0074         | 0.0039         | 0.0023          | 0.0069           | 0.0356         |
|                          | 303        | 0.0071         | 0.0048         | 0.0024          | 0.0061           | 0.0345         |
|                          | 304        | 0.0075         | 0.0034         | 0.0022          | 0.0057           | 0.0328         |
|                          | 305        | 0.0084         | 0.0039         | 0.0021          | 0.0074           | 0.0339         |

| Organ Weight:Brain Weight |            |                      |                       |                        |                      |
|---------------------------|------------|----------------------|-----------------------|------------------------|----------------------|
| Group                     | Animal No. | Heart:Brain Wt Ratio | Spleen:Brain Wt Ratio | Kidneys:Brain Wt Ratio | Liver:Brain Wt Ratio |
| Males, Day 4              | 201        | 0.54                 | 0.40                  | 0.98                   | 5.96                 |
|                           | 202        | 0.62                 | 0.32                  | 1.06                   | 5.27                 |
|                           | 203        | 0.53                 | 0.48                  | 1.06                   | 6.08                 |
|                           | 204        | 0.50                 | 0.36                  | 0.93                   | 4.69                 |
|                           | 205        | 0.64                 | 0.41                  | 1.03                   | 5.70                 |
| Males, Day 15             | 101        | 0.63                 | 0.32                  | 1.26                   | 6.94                 |
|                           | 102        | 0.59                 | 0.38                  | 1.05                   | 6.10                 |
|                           | 103        | 0.58                 | 0.37                  | 1.21                   | 6.54                 |
|                           | 104        | 0.64                 | 0.38                  | 1.25                   | 6.66                 |
|                           | 105        | 0.61                 | 0.37                  | 1.25                   | 6.12                 |
| Females, Day 4            | 401        | 0.64                 | 0.46                  | 0.85                   | 5.03                 |
|                           | 402        | 0.49                 | 0.33                  | 0.81                   | 4.12                 |
|                           | 403        | 0.46                 | 0.34                  | 0.76                   | 4.37                 |
|                           | 404        | 0.46                 | 0.28                  | 0.71                   | 3.61                 |
|                           | 405        | 0.47                 | 0.25                  | 0.85                   | 4.11                 |
| Females, Day 15           | 301        | 0.45                 | 0.36                  | 0.76                   | 4.39                 |
|                           | 302        | 0.53                 | 0.31                  | 0.93                   | 4.83                 |
|                           | 303        | 0.68                 | 0.33                  | 0.86                   | 4.87                 |
|                           | 304        | 0.45                 | 0.29                  | 0.76                   | 4.34                 |
|                           | 305        | 0.46                 | 0.25                  | 0.87                   | 4.01                 |

## Appendix III. Individual Hematology Values

## BASELINE VALUES

| Group                     | Animal No. | Total Protein g/dL | RBC x10.e6/uL | Hemoglobin g/dL | Hematocrit % | Hematocrit spun % | MCV fL | MCH pg | MCHC g/dL |
|---------------------------|------------|--------------------|---------------|-----------------|--------------|-------------------|--------|--------|-----------|
| 1<br>Male Day 15 cohort   | 101        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 102        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 103        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 104        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 105        | 5.4                | 6.4           | 12.5            | 39           | 36                | 62     | 20     | 31        |
| 2<br>Male Day 4 cohort    | 201        | 5.6                | 6.9           | 14              | 41           | 14                | 59     | 20     | 34        |
|                           | 202        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 203        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 204        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 205        | 5.7                | 7.0           | 13.4            | 40           | 38                | 57     | 19     | 34        |
| 3<br>Female Day 15 cohort | 301        | 5.3                | 7.3           | 13.7            | 40.0         | 41.0              | 55.0   | 19.0   | 34.0      |
|                           | 302        | 5.3                | 7.3           | 13.8            | 41.0         | 41.0              | 56.0   | 19.0   | 33.0      |
|                           | 303        | 5.4                | 6.4           | 12.5            | 39           | 36                | 62     | 20     | 31        |
|                           | 304        | QNS                | 4.9           | 9.3             | 27           | QNS               | 56     | 19     | 34        |
|                           | 305        | 4.4                | 6.9           | 12.4            | 36           | 35                | 52     | 18     | 34        |
| 4<br>Female Day 4 cohort  | 401        | 4.8                | 6.5           | 12              | 35           | 34                | 54     | 18     | 34        |
|                           | 402        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 403        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 404        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 405        | 6.1                | 7.6           | 14.0            | 40           | 39                | 53     | 18     | 35        |

| Group                     | Animal No. | RDW % | Platelets x10.e3/uL | MPV fL | WBC x10.e3/uL | Segmented Neutrophils x10.e3/uL | Band Neutrophils x10.e3/uL | Lymphocytes x10.e3/uL | Monocytes x10.e3/uL |
|---------------------------|------------|-------|---------------------|--------|---------------|---------------------------------|----------------------------|-----------------------|---------------------|
| 1<br>Male Day 15 cohort   | 101        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 102        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 103        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 104        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 105        | 12.0  | 883.0               | 8.4    | 13            | 1                               | 0                          | 12                    | 0                   |
| 2<br>Male Day 4 cohort    | 201        | 12    | 836                 | 8.3    | 9.8           | 0.8                             | 0.0                        | 88                    | 2                   |
|                           | 202        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 203        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 204        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 205        | 12.0  | 1118.0              | 8.0    | 13            | 1                               | 0                          | 11                    | 0                   |
| 3<br>Female Day 15 cohort | 301        | 11.0  | 1075.0              | 7.5    | 8.4           | 0.7                             | NA                         | 7.4                   | 0.2                 |
|                           | 302        | 10.0  | 752.0               | 7.5    | 6.6           | 0.8                             | NA                         | 5.6                   | 0.1                 |
|                           | 303        | 12.0  | 883.0               | 8.4    | 13            | 1                               | 0                          | 12                    | 0                   |
|                           | 304        | 10.0  | 563.0               | 7.6    | 7             | 1                               | NA                         | 6                     | 0                   |
|                           | 305        | 10.0  | 746.0               | 7.6    | 6             | 1                               | NA                         | 5                     | 0                   |
| 4<br>Female Day 4 cohort  | 401        | 10    | 658                 | 7.7    | 7.4           | 1.3                             | 0.0                        | 6.1                   | 0.0                 |
|                           | 402        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 403        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 404        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 405        | 11.0  | 392.0               | 8.8    | 8             | 1                               | 0                          | 7                     | 0                   |

**BASELINE VALUES, cont.**

| Group                        | Animal No. | Eosinophils<br>x10.e3/uL | Basophils<br>x10.e3/uL | Segmented<br>Neutrophils<br>% | Band<br>Neutrophils<br>% | Lymphocytes<br>% | Monocytes<br>% | Eosinophils<br>% | Basophils<br>% |
|------------------------------|------------|--------------------------|------------------------|-------------------------------|--------------------------|------------------|----------------|------------------|----------------|
| 1<br>Male Day 15<br>cohort   | 101        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 102        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 103        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 104        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 105        | 0.0                      | 0.0                    | 4.0                           | 0                        | 94               | 2              | 0                | 0              |
| 2<br>Male Day 4<br>cohort    | 201        | 2                        | 0.0                    | 8                             | 0                        | 88               | 2              | 2                | 0              |
|                              | 202        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 203        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 204        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 205        | 0.0                      | 0.0                    | 11.0                          | 0                        | 89               | 0              | 0                | 0              |
| 3<br>Female Day 15<br>cohort | 301        | 0.1                      | NA                     | NA                            | 7.8                      | 87.6             | 2.2            | 1.5              | NA             |
|                              | 302        | 0.1                      | NA                     | NA                            | 12.0                     | 84.3             | 1.4            | 1.8              | NA             |
|                              | 303        | 0.0                      | NA                     | 4.0                           | 0                        | 94               | 2              | 0                | 0              |
|                              | 304        | 0.1                      | NA                     | NA                            | 11                       | 86               | 1              | 1                | NA             |
|                              | 305        | 0.1                      | NA                     | NA                            | 15                       | 81               | 2              | 2                | NA             |
| 4<br>Female Day 4<br>cohort  | 401        | 0.1                      | 0.0                    | 17                            | 0                        | 82               | 0              | 1                | 0              |
|                              | 402        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 403        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 404        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 405        | 0.1                      | 0.0                    | 7.0                           | 0                        | 91               | 1              | 1                | 0              |

**Abbreviations:**

MCV = Mean Cell Volume

MCH = Mean Corpuscular Hemoglobin

MCHC = Mean Cell Hemoglobin Concentration

RDW = Red Cell Distribution Width

MPV = Mean Platelet Volume

WBC = White Blood Cells

## TERMINAL VALUES

| Group                     | Animal No. | Total Protein g/dL | RBC x10.e6/uL | Hemoglobin g/dL | Hematocrit % | Hematocrit spun % | MCV fL | MCH pg | MCHC g/dL |
|---------------------------|------------|--------------------|---------------|-----------------|--------------|-------------------|--------|--------|-----------|
| 1<br>Male Day 15 cohort   | 101        | 6.3                | 7.2           | 13.8            | 42.0         | 41.0              | 59.0   | 19.0   | 32.0      |
|                           | 102        | 6.2                | 7.6           | 14.6            | 44.0         | 42.0              | 58.0   | 19.0   | 33.0      |
|                           | 103        | 6.3                | 7.2           | 13.8            | 42           | 41                | 58     | 19     | 33        |
|                           | 104        | 6.0                | 7.3           | 13.8            | 42           | 42                | 58     | 19     | 33        |
|                           | 105        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
| 2<br>Male Day 4 cohort    | 201        | 5.8                | 6.5           | 12.4            | 39           | 40                | 60     | 19     | 32        |
|                           | 202        | 5.3                | 6.5           | 12.6            | 39           | 37                | 61     | 19     | 32        |
|                           | 203        | 6.2                | 6.4           | 12.0            | 37.0         | 40.0              | 58.0   | 19.0   | 32.0      |
|                           | 204        | 5.6                | 6.7           | 12.7            | 39           | 37                | 59     | 19     | 33        |
|                           | 205        | 5.5                | 6.3           | 11.8            | 37           | 37                | 59     | 19     | 32        |
| 3<br>Female Day 15 cohort | 301        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 302        | 6.4                | 6.6           | 12.4            | 37.0         | 34.0              | 56.0   | 19.0   | 34.0      |
|                           | 303        | 6.3                | 7.8           | 14.7            | 42           | 41                | 55     | 19     | 19        |
|                           | 304        | 6.4                | 8.1           | 15.3            | 46           | 44                | 57     | 19     | 34        |
|                           | 305        | 6.2                | 8.7           | 15.4            | 46           | 44                | 52     | 18     | 34        |
| 4<br>Female Day 4 cohort  | 401        | 6                  | 6.8           | 12.5            | 38           | 37                | 56     | 18     | 33        |
|                           | 402        | 6.3                | 6.9           | 12.9            | 39           | 40                | 56     | 19     | 33        |
|                           | 403        | 5.6                | 6.6           | 12.4            | 37.0         | 36.0              | 56.0   | 19.0   | 34.0      |
|                           | 404        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |
|                           | 405        | NA                 | NA            | NA              | NA           | NA                | NA     | NA     | NA        |

| Group                     | Animal No. | RDW % | Platelets x10.e3/uL | MPV fL | WBC x10.e3/uL | Segmented Neutrophils x10.e3/uL | Band Neutrophils x10.e3/uL | Lymphocytes x10.e3/uL | Monocytes x10.e3/uL |
|---------------------------|------------|-------|---------------------|--------|---------------|---------------------------------|----------------------------|-----------------------|---------------------|
| 1<br>Male Day 15 cohort   | 101        | 12.0  | 1169.0              | 8.3    | 8.0           | 0.9                             | 0.0                        | 6.8                   | 0.3                 |
|                           | 102        | 12.0  | 915.0               | 8.1    | 7.5           | 1.0                             | 0.0                        | 6.3                   | 0.2                 |
|                           | 103        | 12.0  | 996.0               | 9.1    | 12            | 0                               | 0                          | 11                    | 0                   |
|                           | 104        | 12.0  | 912.0               | 8.8    | 10            | 2                               | 0                          | 8                     | 0                   |
|                           | 105        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
| 2<br>Male Day 4 cohort    | 201        | 12    | 1087                | 7.6    | 10.8          | 0.6                             | 0                          | 9.8                   | 0.3                 |
|                           | 202        | 13.0  | 1077.0              | 8.0    | 7             | 1                               | 0                          | 6                     | 0                   |
|                           | 203        | 12.0  | 1032.0              | 8.0    | 8.4           | 1.4                             | 0.0                        | 6.6                   | 0.3                 |
|                           | 204        | 12.0  | 537.0               | 8.3    | 7             | 0                               | 0                          | 7                     | 0                   |
|                           | 205        | 13.0  | 1215.0              | 7.9    | 13            | 1                               | 0                          | 11                    | 1                   |
| 3<br>Female Day 15 cohort | 301        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 302        | 11.0  | 784.0               | 7.9    | 2.5           | 0.4                             | 0.0                        | 1.9                   | 0.1                 |
|                           | 303        | 11.0  | 726.0               | 9.9    | 9             | 1                               | 0                          | 7                     | 0                   |
|                           | 304        | 11.0  | 1002.0              | 8.0    | 12            | 1                               | 0                          | 10                    | 0                   |
|                           | 305        | 10.0  | 871.0               | 7.5    | 9             | 1                               | 0                          | 8                     | 0                   |
| 4<br>Female Day 4 cohort  | 401        | 13    | 890                 | 8.1    | 12.9          | 1.4                             | 0                          | 11.1                  | 0.3                 |
|                           | 402        | 12.0  | 916.0               | 7.7    | 4             | 0                               | 0                          | 3                     | 0                   |
|                           | 403        | 11.0  | 955.0               | 8.2    | 9.0           | 0.4                             | 0.0                        | 8.1                   | 0.3                 |
|                           | 404        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |
|                           | 405        | NA    | NA                  | NA     | NA            | NA                              | NA                         | NA                    | NA                  |

**TERMINAL VALUES, cont.**

| Group                        | Animal No. | Eosinophils<br>x10.e3/uL | Basophils<br>x10.e3/uL | Segmented<br>Neutrophils<br>% | Band<br>Neutrophils<br>% | Lymphocytes<br>% | Monocytes<br>% | Eosinophils<br>% | Basophils<br>% |
|------------------------------|------------|--------------------------|------------------------|-------------------------------|--------------------------|------------------|----------------|------------------|----------------|
| 1<br>Male Day 15<br>cohort   | 101        | 0.0                      | 0.0                    | 11.0                          | 0.0                      | 85.0             | 4.0            | 0.0              | 0.0            |
|                              | 102        | 0.1                      | 0.0                    | 13.0                          | 0.0                      | 84.0             | 2.0            | 1.0              | 0.0            |
|                              | 103        | 0.4                      | 0.0                    | 3.0                           | 0                        | 94               | 0              | 3                | 0              |
|                              | 104        | 0.0                      | 0.0                    | 19.0                          | 0                        | 78               | 3              | 0                | 0              |
|                              | 105        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
| 2<br>Male Day 4<br>cohort    | 201        | 0                        | 0                      | 6                             | 0                        | 91               | 3              | 0                | 0              |
|                              | 202        | 0.1                      | 0.0                    | 12.0                          | 0                        | 85               | 2              | 1                | 0              |
|                              | 203        | 0.0                      | 0.0                    | 17.0                          | 0.0                      | 79.0             | 3.0            | 1.0              | 0.0            |
|                              | 204        | 0.0                      | 0.0                    | 5.0                           | 0                        | 92               | 3              | 0                | 0              |
|                              | 205        | 0.1                      | 0.0                    | 8.0                           | 0                        | 86               | 5              | 1                | 0              |
| 3<br>Female Day 15<br>cohort | 301        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 302        | 0.0                      | 0.0                    | 18.0                          | 0.0                      | 77.0             | 5.0            | 0.0              | 0.0            |
|                              | 303        | 0.1                      | 0.0                    | 16.0                          | 0                        | 78               | 5              | 1                | 0              |
|                              | 304        | 0.1                      | 0.0                    | 8.0                           | 0                        | 89               | 2              | 1                | 0              |
|                              | 305        | 0.2                      | 0.0                    | 9.0                           | 0                        | 87               | 2              | 2                | 0              |
| 4<br>Female Day 4<br>cohort  | 401        | 0.1                      | 0                      | 11                            | 0                        | 86               | 2              | 1                | 0              |
|                              | 402        | 0.0                      | 0.0                    | 11.0                          | 0                        | 86               | 3              | 0                | 0              |
|                              | 403        | 0.2                      | 0.0                    | 5.0                           | 0.0                      | 90.0             | 3.0            | 2.0              | 0.0            |
|                              | 404        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |
|                              | 405        | NA                       | NA                     | NA                            | NA                       | NA               | NA             | NA               | NA             |

**Abbreviations:**

MCV = Mean Cell Volume

MCH = Mean Corpuscular Hemoglobin

MCHC = Mean Cell Hemoglobin Concentration

RDW = Red Cell Distribution Width

MPV = Mean Platelet Volume

WBC = White Blood Cells

Appendix IV. Individual Biochemistry Values

BASELINE VALUES

| Group                | Animal No. | Urea Nitrogen (mg/dL) | Creatinine (Jaffe) (mg/dL) | Sodium (mmol/L) | Potassium (mmol/L) | Chloride (mmol/L) | TCO2 (mmol/L) | Na/K Ratio |
|----------------------|------------|-----------------------|----------------------------|-----------------|--------------------|-------------------|---------------|------------|
| 1<br>(Male Day 15)   | 101        | 13                    | QNS                        | 139             | 5.8                | QNS               | QNS           | 24         |
|                      | 102        | 20                    | 0.2                        | 140             | 6.3                | 102               | 22            | 22         |
|                      | 103        | 14                    | 0.2                        | 141             | 4.4                | 104               | 22            | 32         |
|                      | 104        | 16                    | QNS                        | 138             | 5.3                | QNS               | QNS           | 26         |
|                      | 105        | 13                    | 0.2                        | 140             | 4.3                | 105               | 20            | 33         |
| 2<br>(Male Day 4)    | 201        | 19                    | 0.2                        | 137             | 6.0                | 99                | 24            | 23         |
|                      | 202        | 15                    | 0.2                        | 138             | 5.2                | 102               | QNS           | 27         |
|                      | 203        | 16                    | 0.2                        | 137             | 5.6                | 100               | 25            | 24         |
|                      | 204        | 17                    | 0.2                        | 139             | 5.1                | 101               | 26            | 27         |
|                      | 205        | 15                    | 0.2                        | 142             | 4.8                | 109               | 23            | 30         |
| 3<br>(Female Day 15) | 301        | 18                    | 0.2                        | 137             | 5.4                | 102               | 23            | 25         |
|                      | 302        | 11                    | 0.2                        | 138             | 4.7                | 102               | 24            | 29         |
|                      | 303        | 11                    | 0.2                        | 140             | 4.0                | 107               | 22            | 35         |
|                      | 304        | 14                    | <0.1                       | 145             | 2.9                | 119               | 16            | 50         |
|                      | 305        | 14                    | 0.2                        | 140             | 4.2                | 107               | 21            | 33         |
| 4<br>(Female Day 4)  | 401        | 15                    | 0.2                        | 140             | 3.7                | 112               | 19            | 38         |
|                      | 402        | 14                    | 0.2                        | 141             | 4.7                | 112               | 16            | 30         |
|                      | 403        | 13                    | 0.2                        | 139             | 3.9                | 109               | 18            | 36         |
|                      | 404        | 16                    | 0.2                        | 138             | 4.5                | 98                | 27            | 31         |
|                      | 405        | 9                     | QNS                        | 141             | 3.5                | 112               | QNS           | 40         |

## BASELINE VALUES, cont.

| Group                | Animal No. | Anion Gap<br>(mmol/L) | Osmolarity<br>Calculated<br>(mmol/L) | Calcium<br>(mg/dL) | Phosphorus<br>(mg/dL) | Magnesium<br>(mg/dL) | Iron<br>(ug/dL) | Total<br>Protein<br>(g/dL) |
|----------------------|------------|-----------------------|--------------------------------------|--------------------|-----------------------|----------------------|-----------------|----------------------------|
| 1<br>(Male Day 15)   | 101        | QNS                   | 292                                  | 9.3                | 8.4                   | 1.9                  | QNS             | QNS                        |
|                      | 102        | 22                    | 298                                  | 9.6                | 8.8                   | 1.8                  | 190             | 4.8                        |
|                      | 103        | 19                    | 298                                  | 8.8                | 7.5                   | 1.7                  | 213             | 4.5                        |
|                      | 104        | QNS                   | 293                                  | 8.9                | 8.2                   | 1.8                  | QNS             | QNS                        |
|                      | 105        | 19                    | 294                                  | 8.2                | 7.3                   | 1.5                  | 273             | 4.2                        |
| 2<br>(Male Day 4)    | 201        | 20                    | 290                                  | 10.6               | 7.5                   | 1.8                  | 229             | 5.3                        |
|                      | 202        | QNS                   | 294                                  | 8.2                | 7.0                   | 1.6                  | QNS             | QNS                        |
|                      | 203        | 18                    | 291                                  | 10.0               | 7.3                   | 1.7                  | 197             | 5.0                        |
|                      | 204        | 17                    | 295                                  | 10.0               | 7.4                   | 1.8                  | 169             | 4.9                        |
|                      | 205        | 15                    | 297                                  | 8.8                | 6.2                   | 1.5                  | 180             | 4.3                        |
| 3<br>(Female Day 15) | 301        | 17                    | 291                                  | 9.4                | 6.8                   | 1.8                  | 291             | 4.7                        |
|                      | 302        | 17                    | 290                                  | 9.8                | QNS                   | 1.5                  | QNS             | QNS                        |
|                      | 303        | 15                    | 293                                  | 8.6                | 5.8                   | 1.6                  | 306             | 4.5                        |
|                      | 304        | 13                    | 302                                  | 6.1                | 3.8                   | 1.1                  | 264             | 3.4                        |
|                      | 305        | 16                    | 292                                  | 8.8                | 6.0                   | 1.3                  | 281             | 4.8                        |
| 4<br>(Female Day 4)  | 401        | 13                    | 293                                  | 7.7                | 4.3                   | 1.5                  | 286             | 4.3                        |
|                      | 402        | 18                    | 294                                  | 7.4                | QNS                   | 1.2                  | 256             | QNS                        |
|                      | 403        | 16                    | 291                                  | 8.4                | QNS                   | QNS                  | QNS             | QNS                        |
|                      | 404        | 18                    | 293                                  | 10.5               | 6.3                   | 1.9                  | 430             | 5.6                        |
|                      | 405        | QNS                   | QNS                                  | QNS                | QNS                   | QNS                  | QNS             | QNS                        |

## BASELINE VALUES, cont.

| Group                | Animal No. | Albumin<br>(g/dL) | Globulin<br>Calc (g/dL) | Glucose<br>(Hexokinase)<br>(mg/dL) | Amylase<br>(U/L) | Total<br>Bilirubin<br>(mg/dL) | Direct<br>Bilirubin<br>(mg/dL) | Indirect<br>Bilirubin<br>(mg/dL) |
|----------------------|------------|-------------------|-------------------------|------------------------------------|------------------|-------------------------------|--------------------------------|----------------------------------|
| 1<br>(Male Day 15)   | 101        | 3.0               | QNS                     | 176                                | QNS              | 0.2                           | QNS                            | QNS                              |
|                      | 102        | 2.8               | 2.0                     | 187                                | 476              | 0.1                           | 0.0                            | 0.1                              |
|                      | 103        | 2.6               | 1.9                     | 200                                | 516              | 0.1                           | 0.0                            | 0.1                              |
|                      | 104        | QNS               | QNS                     | 212                                | QNS              | 0.2                           | QNS                            | QNS                              |
|                      | 105        | 2.4               | 1.8                     | 176                                | 378              | 0.1                           | 0.0                            | 0.1                              |
| 2<br>(Male Day 4)    | 201        | 2.8               | 2.5                     | 171                                | 509              | 0.1                           | 0.0                            | 0.1                              |
|                      | 202        | 2.6               | QNS                     | 222                                | 446              | 0.1                           | QNS                            | QNS                              |
|                      | 203        | 2.7               | 2.3                     | 211                                | 469              | 0.1                           | 0.0                            | 0.1                              |
|                      | 204        | 2.8               | 2.1                     | 188                                | 479              | 0.1                           | 0.0                            | 0.1                              |
|                      | 205        | 2.4               | 1.9                     | 132                                | 400              | 0.1                           | 0.0                            | 0.1                              |
| 3<br>(Female Day 15) | 301        | 2.6               | 2.1                     | 186                                | 362              | 0.1                           | 0.0                            | 0.1                              |
|                      | 302        | 2.9               | QNS                     | 190                                | 281              | QNS                           | 0.0                            | QNS                              |
|                      | 303        | 2.6               | 1.9                     | 172                                | 258              | 0.1                           | 0.0                            | 0.1                              |
|                      | 304        | 1.9               | 1.5                     | 120                                | 173              | 0.1                           | 0.0                            | 0.1                              |
|                      | 305        | 2.8               | 2.0                     | 132                                | 296              | 0.1                           | 0.0                            | 0.1                              |
| 4<br>(Female Day 4)  | 401        | 2.4               | 1.9                     | 146                                | 271              | 0.1                           | 0.0                            | 0.1                              |
|                      | 402        | 2.5               | QNS                     | 120                                | 261              | 0.1                           | 0.0                            | 0.1                              |
|                      | 403        | 2.4               | QNS                     | 146                                | 285              | 0.1                           | QNS                            | QNS                              |
|                      | 404        | 3.0               | 2.6                     | 204                                | 320              | 0.1                           | 0.0                            | 0.1                              |
|                      | 405        | 2.3               | QNS                     | QNS                                | 256              | QNS                           | QNS                            | QNS                              |

## BASELINE VALUES, cont.

| Group                | Animal No. | Alkaline Phosphatase (U/L) | Alanine Amino-transferase (U/L) | Aspartate Amino-transferase (U/L) | Creatine Kinase (U/L) | Cholesterol (mg/dL) | Triglyceride (mg/dL) | Lipemia | Icterus | Hemolysis |
|----------------------|------------|----------------------------|---------------------------------|-----------------------------------|-----------------------|---------------------|----------------------|---------|---------|-----------|
| 1<br>(Male Day 15)   | 101        | 439                        | 45                              | 70                                | 1054                  | 67                  | NA                   | Normal  | Normal  | Normal    |
|                      | 102        | 338                        | 35                              | 52                                | 159                   | 64                  | NA                   | Normal  | Normal  | Normal    |
|                      | 103        | 404                        | 34                              | 57                                | 909                   | 67                  | NA                   | Normal  | Normal  | Normal    |
|                      | 104        | QNS                        | 51                              | 139                               | QNS                   | 73                  | NA                   | Normal  | Normal  | Normal    |
|                      | 105        | 414                        | 37                              | 64                                | 1432                  | 55                  | NA                   | Normal  | Normal  | Normal    |
| 2<br>(Male Day 4)    | 201        | 354                        | 50                              | 67                                | 680                   | 82                  | 127                  | Normal  | Normal  | Normal    |
|                      | 202        | 408                        | 41                              | 60                                | QNS                   | 56                  | NA                   | Slight  | Normal  | Normal    |
|                      | 203        | 400                        | 47                              | 62                                | 602                   | 65                  | 88                   | Normal  | Normal  | Normal    |
|                      | 204        | 415                        | 52                              | 63                                | 308                   | 58                  | 50                   | Normal  | Normal  | Normal    |
|                      | 205        | 347                        | 39                              | 51                                | 194                   | 53                  | 69                   | Normal  | Normal  | Normal    |
| 3<br>(Female Day 15) | 301        | 231                        | 60                              | 80                                | 902                   | 50                  | NA                   | Slight  | Normal  | Normal    |
|                      | 302        | 227                        | 40                              | 78                                | 1597                  | 49                  | NA                   | Normal  | Normal  | Normal    |
|                      | 303        | 138                        | 35                              | 54                                | 1015                  | 57                  | NA                   | Slight  | Normal  | Normal    |
|                      | 304        | 84                         | 27                              | 46                                | 523                   | 34                  | NA                   | Slight  | Normal  | Normal    |
|                      | 305        | 181                        | 36                              | 51                                | 533                   | 50                  | NA                   | Normal  | Normal  | Normal    |
| 4<br>(Female Day 4)  | 401        | 216                        | 53                              | 76                                | 2290                  | 41                  | 35                   | Slight  | Normal  | Normal    |
|                      | 402        | 191                        | 36                              | 53                                | 678                   | 48                  | 21                   | Slight  | Normal  | Normal    |
|                      | 403        | 196                        | 36                              | 54                                | 613                   | 54                  | NA                   | Normal  | Normal  | Normal    |
|                      | 404        | 133                        | 39                              | 51                                | 310                   | 51                  | 122                  | Slight  | Normal  | Normal    |
|                      | 405        | 119                        | 37                              | 39                                | QNS                   | QNS                 | NA                   | Slight  | Normal  | Normal    |

TERMINAL VALUES

| Group                | Animal No. | Urea Nitrogen (mg/dL) | Creatinine (Jaffe) (mg/dL) | Sodium (mmol/L) | Potassium (mmol/L) | Chloride (mmol/L) | TCO2 (mmol/L) | Na/K Ratio |
|----------------------|------------|-----------------------|----------------------------|-----------------|--------------------|-------------------|---------------|------------|
| 1<br>(Male Day 15)   | 101        | 18                    | 0.3                        | 140             | 5.3                | 100               | 28            | 26         |
|                      | 102        | 24                    | 0.3                        | 141             | 5.4                | 101               | 27            | 26         |
|                      | 103        | 18                    | 0.2                        | 142             | 4.7                | 101               | 27            | 30         |
|                      | 104        | 17                    | 0.3                        | 140             | 4.8                | 101               | 28            | 29         |
| 2<br>(Male Day 4)    | 105        | 17                    | 0.2                        | 141             | 4.9                | 102               | 27            | 29         |
|                      | 201        | 16                    | 0.3                        | 140             | 4.8                | 101               | 24            | 29         |
|                      | 202        | 15                    | 0.2                        | 142             | 5.1                | 103               | 23            | 28         |
|                      | 203        | 14                    | 0.2                        | 142             | 4.3                | 105               | 23            | 33         |
|                      | 204        | 16                    | 0.2                        | 140             | 5.0                | 101               | 25            | 28         |
| 3<br>(Female Day 15) | 205        | 15                    | 0.2                        | 142             | 4.6                | 106               | 23            | 31         |
|                      | 301        | 22                    | 0.2                        | 139             | 5.3                | 102               | 27            | 26         |
|                      | 302        | 15                    | 0.2                        | 140             | 4.6                | 106               | 24            | 30         |
|                      | 303        | 19                    | 0.3                        | 139             | 5.1                | 100               | 30            | 27         |
|                      | 304        | 20                    | 0.3                        | 139             | 5.0                | 103               | 27            | 28         |
|                      | 305        | 19                    | 0.2                        | 139             | 4.8                | 102               | 28            | 29         |
| 4<br>(Female Day 4)  | 401        | 13                    | 0.2                        | 141             | 4.3                | 108               | 22            | 33         |
|                      | 402        | 13                    | 0.3                        | 141             | 4.0                | 104               | 25            | 35         |
|                      | 403        | 17                    | 0.2                        | 138             | 3.9                | 103               | 23            | 35         |
|                      | 404        | 14                    | 0.3                        | 142             | 3.6                | 108               | 22            | 39         |
|                      | 405        | 13                    | 0.2                        | 142             | 4.0                | 107               | 23            | 36         |

## TERMINAL VALUES, cont.

| Group                | Animal No. | Anion Gap<br>(mmol/L) | Osmolarity<br>Calculated<br>(mmol/L) | Calcium<br>(mg/dL) | Phosphorus<br>(mg/dL) | Magnesium<br>(mg/dL) | Iron<br>(ug/dL) | Total<br>Protein<br>(g/dL) |
|----------------------|------------|-----------------------|--------------------------------------|--------------------|-----------------------|----------------------|-----------------|----------------------------|
| 1<br>(Male Day 15)   | 101        | 17                    | 297                                  | 10.4               | 7.5                   | 2.3                  | 226             | 5.6                        |
|                      | 102        | 18                    | 300                                  | 10.2               | 7.7                   | 2.1                  | 197             | 5.4                        |
|                      | 103        | 19                    | 300                                  | 10.1               | 7.7                   | 2.3                  | 200             | 5.6                        |
|                      | 104        | 16                    | 298                                  | 10.0               | 7.6                   | 2.1                  | 231             | 5.1                        |
| 2<br>(Male Day 4)    | 105        | 17                    | 297                                  | 10.1               | 7.6                   | 2.1                  | 247             | 5.3                        |
|                      | 201        | 20                    | 294                                  | 10.2               | 7.9                   | 2.1                  | 306             | 4.9                        |
|                      | 202        | 21                    | 298                                  | 9.6                | 7.9                   | 1.9                  | 384             | 5.0                        |
|                      | 203        | 18                    | 298                                  | 9.3                | 6.5                   | 1.8                  | 211             | 4.6                        |
|                      | 204        | 19                    | 295                                  | 10.0               | 7.1                   | 1.9                  | 226             | 4.9                        |
| 3<br>(Female Day 15) | 205        | 18                    | 298                                  | 9.3                | 7.3                   | 2.2                  | 209             | 4.5                        |
|                      | 301        | 15                    | 294                                  | 10.0               | 7.5                   | 2.0                  | 289             | 5.5                        |
|                      | 302        | 15                    | 294                                  | 9.7                | 5.7                   | 1.8                  | 320             | 5.2                        |
|                      | 303        | 14                    | 293                                  | 10.6               | 7.2                   | 2.2                  | 393             | 5.8                        |
|                      | 304        | 14                    | 295                                  | 10.2               | 7.0                   | 2.1                  | 255             | 5.6                        |
| 4<br>(Female Day 4)  | 305        | 14                    | 294                                  | 10.3               | 7.1                   | 1.9                  | 264             | 5.2                        |
|                      | 401        | 15                    | 295                                  | 9.0                | 5.5                   | 1.7                  | 277             | 4.9                        |
|                      | 402        | 16                    | 296                                  | 9.7                | 5.7                   | 2.2                  | 241             | 5.5                        |
|                      | 403        | 16                    | 293                                  | 9.9                | 7.4                   | 2.0                  | 332             | 4.9                        |
|                      | 404        | 16                    | 297                                  | 8.6                | 5.6                   | 1.8                  | 306             | 4.9                        |
|                      | 405        | 16                    | 298                                  | 8.9                | 6.2                   | 1.7                  | 358             | 4.8                        |

TERMINAL VALUES, cont.

| Group                | Animal No. | Albumin<br>(g/dL) | Globulin<br>Calc (g/dL) | Glucose<br>(Hexokinase)<br>(mg/dL) | Amylase<br>(U/L) | Total<br>Bilirubin<br>(mg/dL) | Direct<br>Bilirubin<br>(mg/dL) | Indirect<br>Bilirubin<br>(mg/dL) |
|----------------------|------------|-------------------|-------------------------|------------------------------------|------------------|-------------------------------|--------------------------------|----------------------------------|
| 1<br>(Male Day 15)   | 101        | 3.1               | 2.5                     | 182                                | 584              | 0.1                           | 0.0                            | 0.1                              |
|                      | 102        | 3.1               | 2.3                     | 162                                | 515              | 0.1                           | 0.0                            | 0.1                              |
|                      | 103        | 3.1               | 2.5                     | 172                                | 603              | 0.1                           | 0.0                            | 0.1                              |
|                      | 104        | 2.8               | 2.3                     | 207                                | 491              | 0.1                           | 0.0                            | 0.1                              |
|                      | 105        | 3.0               | 2.3                     | 155                                | 522              | 0.1                           | 0.0                            | 0.1                              |
| 2<br>(Male Day 4)    | 201        | 2.7               | 2.2                     | 153                                | 472              | 0.1                           | 0.0                            | 0.1                              |
|                      | 202        | 2.8               | 2.2                     | 151                                | 476              | 0.1                           | 0.0                            | 0.1                              |
|                      | 203        | 2.6               | 2.0                     | 157                                | 449              | 0.1                           | 0.0                            | 0.1                              |
|                      | 204        | 2.8               | 2.1                     | 174                                | 493              | 0.1                           | 0.0                            | 0.1                              |
|                      | 205        | 2.5               | 2.0                     | 150                                | 407              | 0.1                           | 0.0                            | 0.1                              |
| 3<br>(Female Day 15) | 301        | 2.9               | 2.6                     | 153                                | 357              | 0.1                           | 0.0                            | 0.1                              |
|                      | 302        | 2.9               | 2.3                     | 161                                | 292              | 0.1                           | 0.0                            | 0.1                              |
|                      | 303        | 3.2               | 2.6                     | 149                                | 362              | 0.2                           | 0.0                            | 0.2                              |
|                      | 304        | 3.0               | 2.6                     | 175                                | 322              | 0.1                           | 0.0                            | 0.1                              |
|                      | 305        | 3.0               | 2.2                     | 160                                | 325              | 0.1                           | 0.0                            | 0.1                              |
| 4<br>(Female Day 4)  | 401        | 2.8               | 2.1                     | 144                                | 284              | 0.1                           | 0.0                            | 0.1                              |
|                      | 402        | 3.0               | 2.5                     | 174                                | 300              | 0.1                           | 0.0                            | 0.1                              |
|                      | 403        | 2.7               | 2.2                     | 197                                | 312              | 0.1                           | 0.0                            | 0.1                              |
|                      | 404        | 2.8               | 2.1                     | 140                                | 263              | 0.1                           | 0.0                            | 0.1                              |
|                      | 405        | 2.6               | 2.2                     | 161                                | 322              | 0.1                           | 0.0                            | 0.1                              |

## TERMINAL VALUES, cont.

| Group                | Animal No. | Alkaline Phosphatase (U/L) | Alanine Amino-transferase (U/L) | Aspartate Amino-transferase (U/L) | Creatine Kinase (U/L) | Cholesterol (mg/dL) | Triglyceride (mg/dL) | Lipemia | Icterus | Hemolysis |
|----------------------|------------|----------------------------|---------------------------------|-----------------------------------|-----------------------|---------------------|----------------------|---------|---------|-----------|
| 1<br>(Male Day 15)   | 101        | 383                        | 52                              | 66                                | 228                   | 62                  | 103                  | Normal  | Normal  | Normal    |
|                      | 102        | 322                        | 43                              | 81                                | 336                   | 61                  | 97                   | Normal  | Normal  | Normal    |
|                      | 103        | 400                        | 42                              | 63                                | 123                   | 64                  | 63                   | Normal  | Normal  | Normal    |
|                      | 104        | 332                        | 43                              | 53                                | 145                   | 71                  | 86                   | Normal  | Normal  | Normal    |
|                      | 105        | 464                        | 51                              | 61                                | 138                   | 63                  | 57                   | Normal  | Normal  | Normal    |
| 2<br>(Male Day 4)    | 201        | 353                        | 51                              | 60                                | 166                   | 72                  | 70                   | Normal  | Normal  | Normal    |
|                      | 202        | 393                        | 43                              | 56                                | 133                   | 59                  | 100                  | Normal  | Normal  | Normal    |
|                      | 203        | 313                        | 42                              | 48                                | 119                   | 56                  | 74                   | Normal  | Normal  | Normal    |
|                      | 204        | 417                        | 54                              | 59                                | 211                   | 56                  | 54                   | Normal  | Normal  | Normal    |
|                      | 205        | 347                        | 38                              | 51                                | 87                    | 56                  | 76                   | Normal  | Normal  | Normal    |
| 3<br>(Female Day 15) | 301        | 189                        | 57                              | 80                                | 217                   | 54                  | NA                   | Normal  | Normal  | Normal    |
|                      | 302        | 205                        | 49                              | 51                                | 128                   | 56                  | NA                   | Normal  | Normal  | Normal    |
|                      | 303        | 233                        | 54                              | 68                                | 323                   | 63                  | NA                   | Normal  | Normal  | Normal    |
|                      | 304        | 171                        | 49                              | 60                                | 125                   | 63                  | NA                   | Normal  | Normal  | Normal    |
|                      | 305        | 198                        | 42                              | 50                                | 133                   | 52                  | NA                   | Normal  | Normal  | Normal    |
| 4<br>(Female Day 4)  | 401        | 170                        | 50                              | 48                                | 78                    | 49                  | 45                   | Normal  | Normal  | Normal    |
|                      | 402        | 226                        | 43                              | 42                                | 71                    | 59                  | 29                   | Normal  | Normal  | Normal    |
|                      | 403        | 193                        | 40                              | 50                                | 276                   | 53                  | 51                   | Normal  | Normal  | Normal    |
|                      | 404        | 87                         | 28                              | 41                                | 163                   | 44                  | 48                   | Slight  | Normal  | Normal    |
|                      | 405        | 145                        | 33                              | 41                                | 270                   | 50                  | 55                   | Normal  | Normal  | Normal    |

Appendix V. Individual Microscopic Findings

| Animal ID | Heart | Liver | Kidney | Spleen | Brain | Other comments                                |
|-----------|-------|-------|--------|--------|-------|-----------------------------------------------|
| 101       | 0     | 0     | 0      | 0      | 0     |                                               |
| 102       | 0     | 0     | 0      | 0      | 0     |                                               |
| 103       | 0     | 0     | 0      | 0      | 0     |                                               |
| 104       | 0     | 0     | 0      | 0      | 0     |                                               |
| 105       | 0     | 0     | 0      | 0      | 0     |                                               |
| 201       | 1     | 0     | 0      | 0      | 0     | Epicardial chronic inflammation (AV junction) |
| 202       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 203       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 204       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 205       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 301       | 0     | 0     | 0      | 0      | 0     |                                               |
| 302       | 0     | 0     | 0      | 0      | 0     |                                               |
| 303       | 0     | 0     | 0      | 0      | 0     | focal mineral in kidney                       |
| 304       | 0     | 0     | 0      | 0      | 0     |                                               |
| 305       | 0     | 0     | 0      | 0      | 0     |                                               |
| 401       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 402       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 403       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 404       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |
| 405       | 0     | 0     | 0      | 0      | 1     | White matter vacuolation                      |

0 = no lesion  
1 = mild  
2 = moderate  
3 = severe



**Evaluation of Brain Following a Single Intravenous Dose of UM\_COU in Rats**

**Study Number: 2022109**

**Michigan State University In Vivo Facility  
East Lansing, Michigan 48824**

**Sponsor: Peter J.H. Scott, PhD.  
Division of Nuclear Medicine/Department of Radiology  
University of Michigan Medical School**

**Sponsor Contact: Peter J.H. Scott, PhD.**

**APPROVAL SIGNATURES**

The following individuals confirm that this report accurately represents their interpretation of the data:



Date: 2022.12.21  
16:41:00 -05'00'

12/21/2022

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Teresa Krieger-Burke, PhD, DVM

Date

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## Evaluation of Brain Following a Single Intravenous Dose of UM\_COU in Rats

### 1. OBJECTIVE

The objective of this study was to investigate potential adverse effects on brain histology following a single intravenous dose of UM\_COU in male and female rats.

### 2. MATERIALS AND METHODS

#### 2.1. Test Article

|                                             |                                                        |
|---------------------------------------------|--------------------------------------------------------|
| Compound:                                   | UM_COU                                                 |
| Lot Number:                                 | TRK50                                                  |
| Active Moiety (used for dose calculations): | 100% assumed                                           |
| Vehicle:                                    | 5% USP Ethanol, 95% 0.9% USP Saline, 10mM NAOAc , pH 5 |
| Dose:                                       | 86 µg/kg                                               |
| Dose Volume:                                | 5 mL/kg                                                |
| Dose Route of Administration:               | Intravenous                                            |
| Storage Conditions:                         | Refrigerated, protected from light                     |

#### 2.2. Dose Preparation

Formulations and vehicle were provided by Sponsor ready-to-dose. All formulations were stored under refrigerated conditions, protected from light, until time of use. Formulation were used within 7 days of receipt.

#### 2.3. Animal Information

Eight (8) male and eight (8) female Sprague-Dawley rats were obtained from Charles River Laboratories and weighed 212-225 (males) and 199-215 (females) at the time of dosing initiation. Animals were pair housed in solid bottom cages with aspen bedding for the duration of the study. All animals had free access to standard rodent chow and fresh water throughout the study. Animals were maintained on an automated 12/12 hour light/dark cycle with 7:00am as the start of the light phase.

This study was conducted in accordance with the current guidelines for animal welfare (Guide for the Care and Use of Laboratory Animals, 8th Ed., 2011). The procedures used in this study have been reviewed and approved by the Institutional Animal Care and Use Committee.

## 2.4. Experimental Schedule

Animals were assigned to an experimental schedule according to Table 1 below.

Table 1. Treatment Schedule

| Group | No. of Animals | Animal Numbers | Sex | Treatment       | Scheduled Necropsy Day |
|-------|----------------|----------------|-----|-----------------|------------------------|
| 1     | 2              | 101-102        | M   | vehicle         | Day 2                  |
| 2     | 2              | 201-202        | F   | vehicle         | Day 2                  |
| 3     | 2              | 301-302        | M   | UM_COU 86 µg/kg | Day 2                  |
| 4     | 2              | 401-402        | F   | UM_COU 86 µg/kg | Day 2                  |
| 5     | 2              | 501-502        | M   | vehicle         | Day 15                 |
| 6     | 2              | 601-602        | F   | vehicle         | Day 15                 |
| 7     | 2              | 701-702        | M   | UM_COU 86 µg/kg | Day 15                 |
| 8     | 2              | 801-802        | F   | UM_COU 86 µg/kg | Day 15                 |

Test Subject Arrival Date: October 11, 2022

In-Life Start Date (Study Day 1): October 18, 2022

End Date (Completion of In-Life Phase): November 1, 2022

Data Collection Rooms: B-23 and B-346, Life Sciences building, MSU

Clinical Observations: pre-dose and daily thereafter

Body Weights: prior to dosing and at time of necropsy

Organ/Tissue Collection: Brain at time of necropsy

Disposition of Animals at Study Completion: Euthanized and carcasses incinerated

## 2.5. Description of Experimental Procedure

Eight (8) male and eight (8) female Sprague-Dawley rats were used on study. Animals were received from the supplier and were housed in room B-23 in the vivarium of the Life Sciences building at Michigan State University. Animals were pair housed in plastic solid bottom cages containing aspen bedding, with free access to standard rodent chow and fresh water throughout the study. Animals were maintained on an automated 12/12 hour light/dark cycle with 7:00am as the start of the light phase.

Animals acclimated for 1 week. All animals were dosed on October 17, 2022, considered Study Day 1. Animals were lightly anesthetized using isoflurane in oxygen and received a single intravenous bolus in a dose volume of 5 mg/kg of vehicle or UM\_COU at 86 µg/kg, according to group assignment. Animals were recovered from anesthesia immediately following dosing.

Clinical observations were recorded daily.

Animals were euthanized by thoracotomy under isoflurane anesthesia on Study Day 2 (Groups 1 through 4) and Study Day 15 (Groups 5 through 8). Terminal body weights were recorded. Brain was collected, weighed, and immediately fixed in 10% neutral-buffered formalin. Brain specimens were delivered on the day of collection to the MSU Investigative Histopathology Laboratory for processing and slide

preparation. Slides were stained with Hematoxylin & Eosin, and reviewed by a board-certified veterinary pathologist.

## 2.6. Statistical Analyses

Statistical comparisons were not conducted due to the limited number of animals in each treatment group.

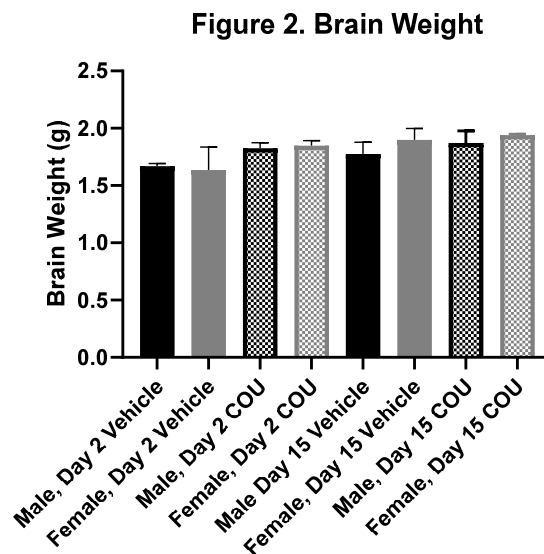
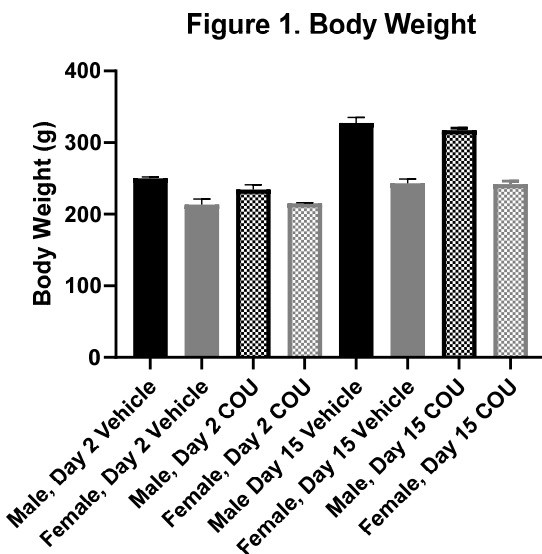
## 3. RESULTS

### 3.1. Clinical Observations

There were no drug-related clinical findings. All animals remained within normal limits throughout the study.

### 3.2. Body and Brain Weights

Body weight gain over time in males and females treated with UM\_COU was similar to corresponding vehicle controls (Figure 1, Table 2).



Brain weights and brain-to-body weight ratios of UM\_COU-treated males and females were similar to corresponding vehicle controls (Figure 2, Table 2).

Table 2. Brain Weights and Terminal Body Weights

| Group                           | Animal No. | Body Weight (g) | Brain Weight (g) | Brain:Body Weight Ratio |
|---------------------------------|------------|-----------------|------------------|-------------------------|
| 1<br>Males, Vehicle<br>Day 2    | 101        | 252             | 1.65             | 0.007                   |
|                                 | 102        | 248             | 1.69             | 0.007                   |
|                                 | Mean       | <b>250</b>      | <b>1.67</b>      | <b>0.007</b>            |
| 2<br>Females, Vehicle<br>Day 2  | 201        | 221             | 1.44             | 0.006                   |
|                                 | 202        | 206             | 1.84             | 0.009                   |
|                                 | Mean       | <b>214</b>      | <b>1.64</b>      | <b>0.008</b>            |
| 3<br>Males, UM_COU<br>Day 2     | 301        | 241             | 1.87             | 0.008                   |
|                                 | 302        | 228             | 1.78             | 0.008                   |
|                                 | Mean       | <b>235</b>      | <b>1.83</b>      | <b>0.008</b>            |
| 4<br>Females, UM_COU<br>Day 2   | 401        | 216             | 1.89             | 0.009                   |
|                                 | 402        | 214             | 1.80             | 0.008                   |
|                                 | Mean       | <b>215</b>      | <b>1.85</b>      | <b>0.009</b>            |
| 5<br>Males, Vehicle<br>Day 15   | 501        | 320             | 1.67             | 0.005                   |
|                                 | 502        | 335             | 1.88             | 0.006                   |
|                                 | Mean       | <b>328</b>      | <b>1.78</b>      | <b>0.005</b>            |
| 6<br>Females, Vehicle<br>Day 15 | 601        | 237             | 1.80             | 0.008                   |
|                                 | 602        | 249             | 2.00             | 0.008                   |
|                                 | Mean       | <b>243</b>      | <b>1.90</b>      | <b>0.008</b>            |
| 7<br>Males, UM_COU<br>Day 15    | 701        | 315             | 1.98             | 0.006                   |
|                                 | 702        | 320             | 1.76             | 0.006                   |
|                                 | Mean       | <b>318</b>      | <b>1.87</b>      | <b>0.006</b>            |
| 8<br>Females, UM_COU<br>Day 15  | 801        | 238             | 1.95             | 0.008                   |
|                                 | 802        | 246             | 1.93             | 0.008                   |
|                                 | Mean       | <b>242</b>      | <b>1.94</b>      | <b>0.008</b>            |

### 3.3. Brain Histopathology

Minimal to mild vacuolation of white matter tracts was noted in all animals. White matter from the diencephalon (corpus collosum and thalamic regions) was particularly affected. The vacuoles varied in size, but were generally small (5-10  $\mu\text{m}$ ), crisp, and had a clear center, devoid of debris or membranous material. Rare perineuronal and perivascular regions of vacuolation (edema) were also present across all treatment and control groups. Representative photomicrographs are shown below (Figures 3 and 4).

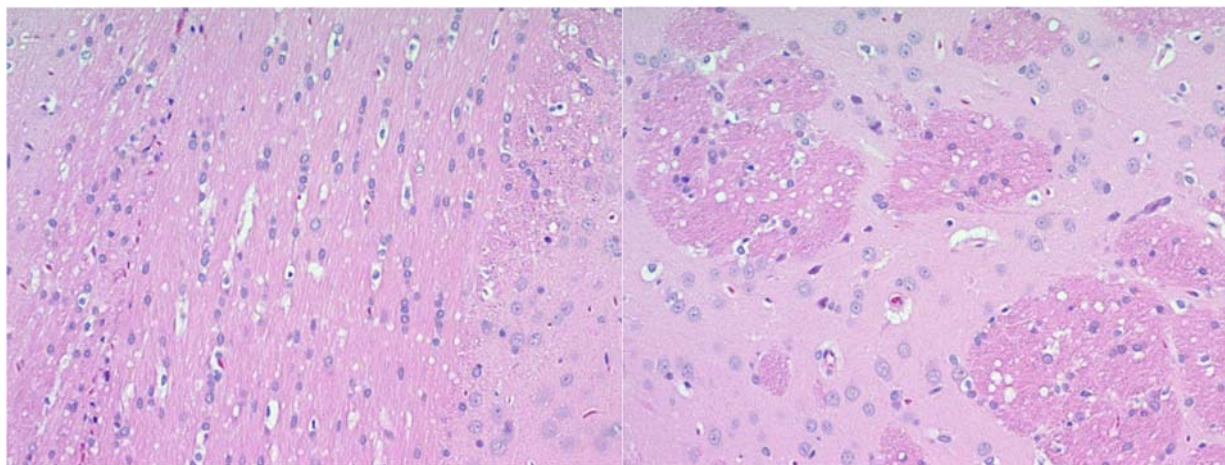


Figure 3. Representative white matter vacuolation in vehicle-treated brain (Rat 102)

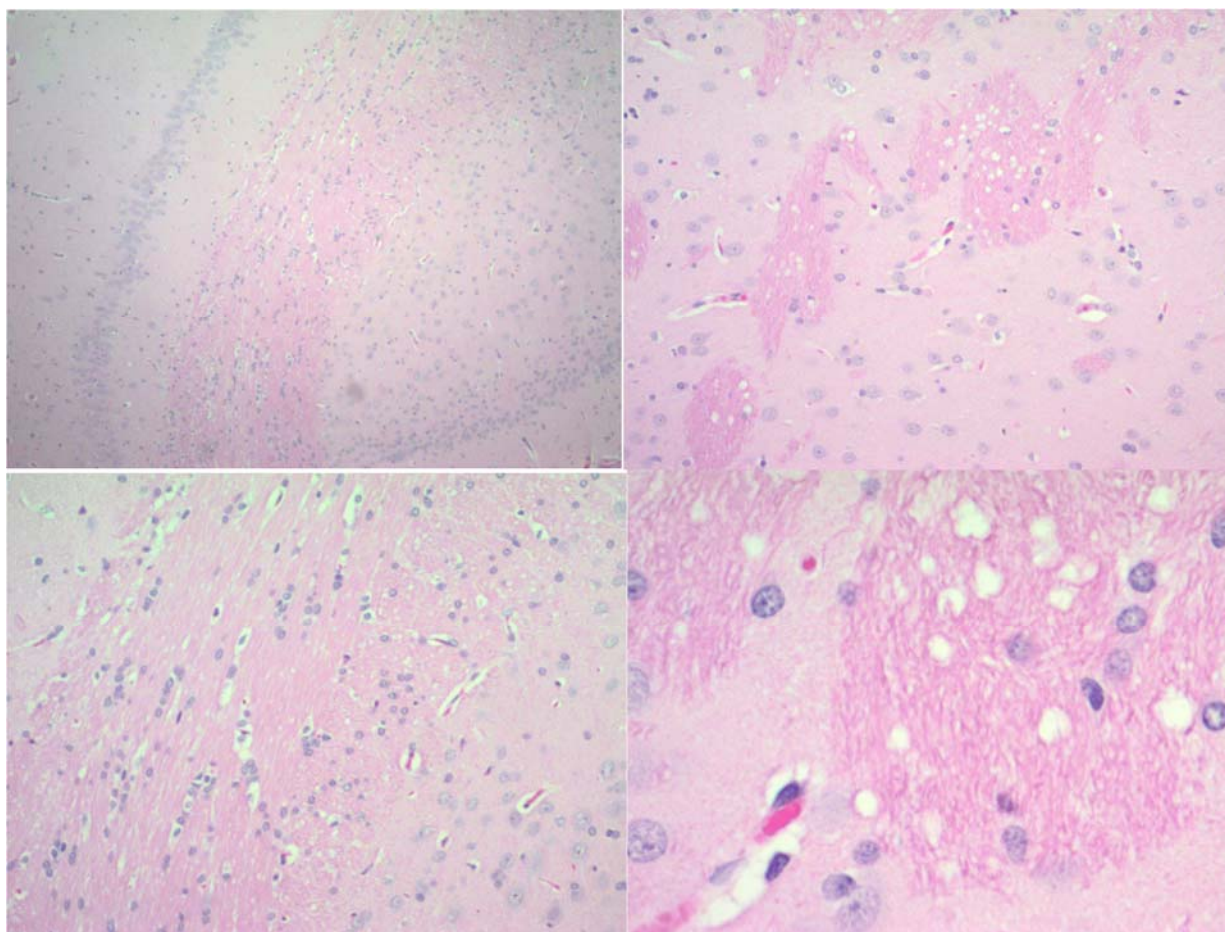


Figure 4. Representative white matter vacuolation in UM\_COU-treated brain (Rat 802)

#### **4. DISCUSSION**

A screening study of UM\_COU in rats (study 2022101) was conducted at the same facility prior to the current study. White matter vacuolation was observed in brain following a single intravenous bolus of UM\_COU at 86 µg/kg in the former study. However, only compound-treated rats were evaluated due to the screening nature of the study. The current study investigated whether vacuolation in brain was an adverse effect of the compound by comparing compound-treated brain specimens to vehicle-treated specimens. Because vehicle-treated animals had brain lesions similar to compound-treated, it was determined that these lesions not directly compound related. Instead, the vacuolation in brain appears to be vehicle-related or to be due to specimen processing. Similarities in central nervous system lesions associated with toxicity versus processing and fixation artifact have been described (Kaufmann W, Bolon B, Bradley A, Butt M, Czasch S, Garman RH, George C, Gröters S, Krinke G, Little P, McKay J, Narama I, Rao D, Shibutani M, Sills R. Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicol Pathol. 2012 Jun;40(4 Suppl):87S-157S). Moreover, white matter vacuolation is a very common postmortem artifact associated with fixation, if there is excessive exposure to alcohol (<https://ntp.niehs.nih.gov/nnl/nervous/brain/index.htm>). The fact that formulated COU and vehicle both contained 5% ethanol USP (v/v), offers a possible explanation.

#### **5. CONCLUSION**

UM\_COU was clinically well-tolerated when administered as a single intravenous bolus at 86 µg/kg to male and female adult Sprague-Dawley rats, evaluated through 15 days after dosing. There were no adverse effects on body weight gain or on brain weight. Minimal to mild vacuolation of brain white matter was observed in all animals, with no distinction between compound-treated and vehicle-treated animals. Taken together with results from study 2022101, where male and female Sprague-Dawley rats were treated with UM\_COU in a similar manner, 86 µg/kg is now considered a no-observed-adverse-effect dose of UM\_COU when administered as a single intravenous bolus to rats.