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METABOLISM AND DISPOSITION OF [^{14}C]- METHYCYCLOSILOXANES IN RATS

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METABOLISM AND DISPOSITION OF ^{14}C-OCTAMETHYLCYCLOTETRASILOXANE ([^{14}C]D_4) OR ^{14}C-DECAMETHYLCYCLOPENTASILOXANE ([^{14}C]D_5) FOLLOWING SINGLE ORAL BOLUS GAVAGE ADMINISTRATION TO FISCHER 344 RATS

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Highlights

- Comparison of the low vs. high dose oral gavage administration of D_4 and D_5 demonstrated dose-dependent kinetic behavior.
- Data and modeling results suggest differences in metabolism between low and high dose administration indicating high dose administration results in or approaches non-linear saturated metabolism.
- These low dose data sets were used to refine the D_4/D_5 multi-route harmonized PBPK model to allow for a better description of the disposition and toxicokinetics of D_4/D_5 following oral exposure.
- With a refined oral uptake description, the model could be used in risk assessment to better define the internal dose of D_4 and D_5 following exposure to D_4 and D_5 via multiple routes.

ABSTRACT

Octamethylcyclotetrasiloxane (D_4) and decamethylcyclopentasiloxane (D_5) are low molecular weight cyclic volatile methyl siloxanes (cVMSs) primarily used as intermediates or monomers in the production of high molecular weight silicone polymers. The use of D_4 as a direct ingredient in personal care products has declined significantly over the past 10 years, although it may be present as a residual impurity in a variety of consumer products. D_5 is still used as an intentional ingredient in cosmetics, consumer products and in dry cleaning. Persons who may be exposed include occupational exposure for workers, and potential inhalation or dermal exposure for consumers and the general public. Because of the diverse use, especially of D_5, and the potential for human exposure, a comprehensive program was undertaken to understand the kinetics, metabolism, enzyme induction and toxicity of D_4 and D_5 in rats following relevant routes of exposure. Physiologically based pharmacokinetic (PBPK) models utilizing these studies have been reported for D_4 and D_5 in the rat and human following dermal and inhalation exposures, with the oral uptake component of the model being limited in its description. Data from high dose oral studies in corn oil and simethicone vehicles and neat were used in the D_4/D_5 harmonized PBPK model development. It was uncertain if the inability to adequately describe the oral uptake was due to unrealistic high doses or unique aspects of the

chemistry of D_4/D_5. Low dose studies were used to provide data to refine the description of oral uptake in the model by exploring the dose dependency and the impact of a more realistic food-like vehicle. Absorption, distribution, metabolism and elimination (ADME) of D_4 and D_5 was determined following a single low oral gavage dose of ^{14}C-D_4 and ^{14}C-D_5 at 30 and 100 mg/kg bw, respectively, in a rodent liquid diet.

Comparison of the low vs. high dose oral gavage administration of D_4 and D_5 demonstrated dose-dependent kinetic behavior. Data and modeling results suggest differences in metabolism between low and high dose administration indicating high dose administration results in or approaches non-linear saturated metabolism. These low dose data sets were used to refine the D_4/D_5 multi-route harmonized PBPK model to allow for a better description of the disposition and toxicokinetics of D_4/D_5 following oral exposure. With a refined oral uptake description, the model could be used in risk assessment to better define the internal dose of D_4 and D_5 following exposure to D_4 and D_5 via multiple routes.

Keywords – Absorption, Disposition, Metabolism, Elimination, Rat, Octamethylcyclotetrasiloxane, Decamethylcyclopentasiloxane.

INTRODUCTION

The cyclic volatile methyl siloxanes (cVMS) are a class of silicone compounds that have an unusual combination of physicochemical properties that results in their attractiveness for use in the manufacture of silicone polymers and in consumer products. The use of D_4 as a direct ingredient in personal care products has declined significantly over the past 10 years although it may be present as a residual impurity in a variety of products. D_5 is still used as an intentional ingredient in cosmetics, consumer products and in dry cleaning. These cyclic siloxanes have been thoroughly evaluated with regard to understanding their safety profile. The hazard profile of these substances has recently been reviewed [1, 2](Dekant and Klaunig*,* 2016, Franzen *et al.*, 2017). In addition, global human health risk assessments and detailed evaluations of biological relevance of any toxicological findings have been recently published [2-10]

Persons who may be exposed include occupational exposure for workers, and potential inhalation or dermal exposure for consumers and the general public [11, 12]. D_4 and D_5 are low molecular weight silicon based materials with octanol-water partitioning coefficients (i.e. log K_{ow}, a surrogate measure of lipophilicity) of 6.98 and 8.07 [13, 14], respectively, and water solubilities of 56 and 17 µg/L [15], respectively.

Physiologically based pharmacokinetic (PBPK) models have been published [16-21] describing the biological and physicochemical processes regulating the kinetic disposition of either D_4 or D_5 in rats and humans following different routes of exposure. Inhalation and dermal exposures in rat and human have been described by models of Reddy and Dobrev [18-21]. Recently an integrated D_4/D_5 multi-route model [22], describing dermal and inhalation exposures based on previous models [16, 17] has been developed, and kinetics following inhalation and dermal exposures have been found to be similar. Previous models have shown

different kinetics following high oral doses of D_4 and D_5 administered neat and in various vehicles (i.e., corn oil, simethicone) compared to inhalation and dermal exposures.

An initial multi-route PBPK model by Sarangapani [17], based on a previously published inhalation PBPK model by Andersen for D_4 [16] failed to describe the pharmacokinetics of D_4, following oral exposure. A refined model [17] describing delivery of D_4 from the gastrointestinal (GI) tract to the deep blood compartment as D_4 within the triglyceride core in chylomicrons via the lymphatic system, and transport of D_4 from the deep blood compartment to the two fat compartments provided the best fits to observed plasma D_4, exhaled D_4, and D_4 metabolites excreted in the urine following oral exposure. This model demonstrated that the pharmacokinetics of D_4 following oral exposure were sensitive to the mode of entry into the blood compartment as well as the vehicle used (corn oil, simethicone) or neat.

Currently a multi-route harmonized PBPK model is under development for use in risk assessment to define the internal dose of D_4 and D_5. The model will be able to accurately describe internal dose of D_4 and D_5 following aggregate dermal and inhalation exposure and also the oral route of exposure, particularly low dose exposure that would occur through incorporation of D_4 and D_5 into food matrices, if migration of low level impurities of D_4 and D_5 from siloxane polymers used in food contact applications occurs. Although the oral route is a minor route of exposure for D_4 and D_5, the incorporation of this route will allow accurate estimates of total exposure for use in an aggregate risk assessment.

In model development, it was recognized that for oral exposure, as compared to dermal and inhalation exposures, D_4 and D_5 uptake does not result in a free form that is present following dermal and inhalation exposure, where free D_4 and D_5 are available for transport, metabolism and elimination.

Oral toxicity studies with D4 and D5 commonly use a corn oil vehicle, therefore initial oral kinetic studies also utilized corn oil [17, 23]. Difference were seen in liver enlargement using different vehicles that prompted oral kinetic studies to be carried out delivering D_4 and D_5 in a corn oil vehicle at high doses, 300 and 1000 mg/kg bw, respectively, but also as neat chemical or in a simethicone vehicle, at the same high doses [1, 17]. The significant differences seen in liver enlargement with D_4 and D_5 following oral dosing neat or with various vehicles, raised the question of what is the most appropriate vehicle that reflects exposure in humans.

To explore the impact of a more realistic food vehicle, studies were conducted to determine the absorption, distribution, metabolism and elimination of D_4 and D_5 following a single oral gavage in rodent liquid diet at lower doses of ^{14}C -D_4 and ^{14}C-D_5 at 30 and 100 mg/kg bw, respectively. These low dose studies were used to provide data to refine the description of oral uptake in the model and examine the behaviour of D_4 and D_5 following administration under more relevant oral exposure conditions (a lower dose incorporated into a food vehicle, a rodent liquid diet) vs. the high doses in other vehicles studied previously.

METHODS

Test substances

The ^{14}C-octamethylcyclotetrasiloxane ([^{14}C]D_4) and ^{14}C-decamethylcyclopentasiloxane ([^{14}C]D_5) used in the studies at 30 and 100 mg/kg bw, respectively were prepared by the Dow Corning Corporation, Auburn, MI. Chemical identity and radiochemical purity of the labeled test articles were determined using Gas Chromatography with Mass Spectrometric detection (GC/MS) and High Performance Liquid Chromatography (HPLC) with a radioactivity flow-through detector (RAD). More than one batch of radiolabeled material was used. Specific activities (mCi/g) of

126 [^{14}C]D_4 were 9.717 and 10.242 mCi/g. Specific activities (mCi/g) of [^{14}C]D_5 were
127 29.081 mCi/g and 29.453 mCi/g. Radiochemical purities were: D_4, 98.6 and 99.8% and
128 D_5, 98.4 and 99.8. Non-radiolabeled **D_4 and D_5** were supplied by the Dow Corning
129 Corporation, Auburn, MI with purities of D_4, 99.8% and D_5, 99.0%.

130 *Animals*

131 CDF®(Fischer 344)/CrlBR rats, ~9 weeks of age minimum at dosing, were purchased from
132 Charles River Laboratories, Inc. The mean body weights of female and male rats from the
133 various groups ranged from 150-160 g and 200-231 g, respectively at the time of dosing.
134 In-dwelling jugular vein cannulae, implanted by the animal supplier, were used to facilitate
135 collection the multiple blood samples collected from each animal (~0.2 mL per time point).

136 The studies were approved by the Laboratory Animal Care and Use Committee of Dow
137 Corning Corporation, fully accredited by the Association for the Assessment and Accreditation of
138 Laboratory Animal Care International. Animals were acclimated to the laboratory environment
139 (12-h light/dark cycle at 18–26°C and 24–70% relative humidity) and found in good health.
140 Animals were housed individually in suspended wire-mesh cages except for the mass balance
141 portion of the study where they were housed in glass Roth-style glass metabolism cages (air
142 flow 500 to 1000 ml/min, 24-48 h acclimation). Animals were provided LabDiet® Certified
143 Rodent Diet #5002 (PMI Nutrition International, St. Louis, MO) and municipal water *ad libitum.*

144 *Preparation of Dosing Solutions*

145 [^{14}C]D_4 and [^{14}C]D_5 were administered in dosing solutions prepared with a
146 Rodent Liquid Diet (RLD) product# F6112SP (Bio-Serv, Frenchtown, NJ). The dosing
147 solutions were prepared in RLD to deliver approximately 0.29 mCi/kg bw, with a
148 nominal dose of 30 mg D_4/kg bw and 100 mg D_5/kg bw in 5 ml dosing solution/kg bw.

Dosing Solution Analysis

Samples of each dosing solution were analytically verified for concentration and homogeneity. The specific activity of the dosing solution was measured by liquid scintillation analysis on the day of preparation as well as the day of administration prior to initiation of dosing. Parent concentration, stability and homogeneity of ^{14}C-D_4 and ^{14}C-D_5 in the rodent liquid diet vehicle were evaluated by extracting in hexane (two phase extraction) and analyzing by GC/MS and direct analysis by liquid scintillation counting (LSC), against solvent standards of the test articles.

Prepared dose solutions were homogenous and ranged from 90 to 103% of targeted parent and radioactivity concentrations. The mean radioactivity in the dose solutions prepared for all the groups ranged from 0.0424-0.0571 mCi/g of dose solution. The mean parent concentration in dose solutions ranged from 5.17 – 5.51 mg D_4/g of dose solution and 18.61 – 19.86 mg D_5/g of dose solution.

Test substance administration

Test article was administered as a single dose by oral gavage in a RLD vehicle. RLD vehicle was chosen since a food matrix was desired to evaluate uptake kinetics especially at lower doses. The corn oil vehicle was chosen for one subset of female rats in the D_5 low dose study (blood kinetics) to compare to a previous D_5 high dose study where a corn oil vehicle was used.

Vehicle only and test article solutions were administered by oral gavage with a 15 gauge, 100 mm plastic oral feeding tube and syringe at 5 mL/kg bw. Volume was adjusted based upon the most recent individual body weights. The weight of the dose solution administered was determined gravimetrically.

The actual mean dose administered (D4 low) ranged from 27.41 – 30.92 mg/kg in female rats and 26.67– 30.04 mg/kg in male rats. Females received a mean range of 41.39 – 51.38 μCi/animal and males received a mean range of 56.70 – 69.54 μCi/animal.

The actual mean dose administered (D5 low) ranged from 91.48 – 112.94 mg/kg in female rats and 92.20 – 99.15 mg/kg in male rats. Females received a mean range of 35.06 – 42.45 μCi/animal and males received a mean range of 46.24 – 54.76 μCi/animal.

No clinical signs of toxicity were observed in the animals following oral gavage administration.

Overview of study design

Studies were conducted with a single oral gavage administration of 30 mg ^{14}C-D_4/kg bw in a rodent liquid diet vehicle (D_4 low) and 100 mg ^{14}C-D_5/kg bw in a RLD vehicle (D_5 low). In addition, a group of females in the D_5 low study for blood kinetics was administered 100 mg ^{14}C-D_5/kg bw in a corn oil vehicle to measure blood kinetics for comparison of vehicles. Study groups consisted of female and male Fischer 344 rats designated for determining blood kinetics, mass balance (absorption, distribution, metabolism and excretion) and tissue kinetics. Blood (~0,2 mL) was collected at 15, 30, 60 min, and 2, 4, 8, 24, 48, 72, 96, 120, 144 and 168 h post-dosing via a jugular vein cannula. ^{14}C-Activity and parent were measured in blood and kinetic parameters determined.

Collection intervals for urine, CO_2 and feces were 24 h through 168 h (in addition, urine was collected at 0-12 and 12-24 h) in the mass balance groups. Collection intervals for expired volatiles were: 0-1, 1-2, 2-4, 4-6, 6-12, 12-24, and 24 h intervals through 168 h. Selected tissues and remaining carcass (including pelt) were collected at 168 h post-dosing. Radioactivity (^{14}C) and parent were measured in collected samples and urine was profiled.

Tissues were collected at 2, 6, 12, 24, 48, 72, 120 and 168 h post-dosing in the groups to determine tissue kinetics. Tissues collected at these times were adrenals, digestive tract (without contents), perirenal fat, brown fat (D_5 study only), liver, lung, ovaries, spleen, testes and uterus. Radioactivity (^{14}C-activity) and parent were measured in extracted samples.

Analytical chemistry

Blood and tissues samples were collected and extracted in tetrahydrofuran (THF) based on the method of Varaprath [24]. A stable-isotope (^{13}C) isomer of the test material served as an internal standard for the low dose studies. Tetrakis(trimethylsiloxy)silane (M4Q) served as an internal standard for the high dose studies. Extraction solvent/ISTD was pre-aliquoted in a volume targeting a minimum 2:1 v/w ratio of solvent to tissue mass. Tissue samples were subjected to physical mincing after being introduced to extraction solvent. All tissues were vortex-mixed for five minutes, sonicated for 5 min (except blood), centrifuged, and extract removed to a new vial.

A separate aliquot of each extract was dried using magnesium sulfate and analyzed by GC/MS. Analysis was performed in electron ionization (EI) mode on a Hewlett Packard 6890GC/5973N MSD. The analytical column used was a Hewlett Packard HP-5MS (30 m x 0.25 mm x 0.25 µm). Parent D_4, M4Q, [^{13}C]D_4, parent D_5, and [^{13}C]D_5 were quantitated from the ion fragments m/z 281, 281, 285, 355, and 360, respectively.

Radioactivity was quantified in the THF extracts of blood and tissues by direct analysis using a Packard Tri-Carb 3100TR liquid scintillation analyzer. In addition, the remaining pellet after THF extraction was solubilized using 35% tetraethyl ammonium hydroxide (TEAH). Aliquots of the solubilized pellet were processed for liquid scintillation analysis to determine the amount of radioactivity remaining after extraction. Remaining carcass and fecal homogenate

218 samples were also solubilized in TEAH and analyzed for radioactivity. Total radioactivity was
219 calculated by summing radioactivity from extracts and solubilized pellets.

220 Selected urine samples and fecal samples were analyzed by HPLC/RAD to obtain a
221 qualitative metabolite profile.

222 The concentration of parent D_4 or D_5 in blood and tissues was reported as μg D_4 or D_5/g
223 sample. The radioactivity concentrations were reported as μg equivalents (μg eq) D_4 or D_5 per g
224 sample. The calculation of equivalents was based upon the specific activity D_4 or D_5 in the dose
225 solution administered.

226 Expired volatiles were collected onto charcoal sorbent tubes and expired CO_2 was
227 collected in KOH traps. Charcoal tubes were desorbed with toluene and parent and/or ^{14}C-
228 activity were determined. Excretion rates were reported as μg or μg equivalents (μg eq) of D_4 or
229 D_5/h. Excretion rates of CO_2 were reported as μg eq/h.

230 *Data analysis and quality assurance*

231 Calculations of mean and standard deviation (or standard error of the mean) of sample
232 concentrations were performed using Microsoft Excel™ v5, v7, v9 and 2007. Calculations of μg
233 eq were performed using Provantis™ Version 8.2 (D_4 and D_5 low dose studies). Blood Area-
234 Under-the–Curves (AUC)s for parent and radioactivity including statistical analyses were
235 calculated using SAS/STAT software, v8.2 and v9.3.

236 These studies were conducted in accordance with EPA Toxic Substance Control Act (EPA-
237 TSCA, 1984) Good Laboratory Practice Standards.

238 **Statistical Analysis**

Statistical comparisons between parent and ^{14}C-activity Area under the Curves (AUCs) were conducted using the Bailer method, Nedelman et al., [25] and the Satterthwaite approximation method, Nedelman and Jia [26]. Terminal half-lives of concentration of parent and ^{14}C-activity vs time were determined in blood, tissues, urine, feces and expired volatiles.

RESULTS

Mass Balance: Dose Recovered and Disposition

Mass balance studies are key to understanding the absorption, distribution, metabolism and elimination of D4 and D4 following single low dose oral administration.

D_4

The mean percentage of the administered dose recovered was 87.01% and 85.86% in female and males rats, respectively.

The key elimination pathways were excretion in urine and expiration of volatiles. Urine contained 32.08% and 40.02% of the recovered dose in females and males, respectively (**Table 1**). Expired volatiles contained 29.90 and 18.41% of the recovered dose (D_4 low) in females and males, respectively. The percentage of the recovered dose in feces accounted for 22.56 and 27.21% of the recovered dose in females and males, respectively. At 168 h, tissues and remaining carcass (including pelt) accounted for 10.73% and 8.68% of the recovered radioactivity (D_4 low) in females and males, respectively.

Absorption of radioactivity, expressed as percent-recovered radioactivity is the summation of radioactivity found in urine, tissues and carcass, expired volatiles, and expired CO_2 (**Table 1**) while the percent found in feces represents unabsorbed dose

(assuming no enterohepatic circulation). The percentage absorbed was 77.2% and 72.5% in females and males, respectively following administration of ^{14}C-D_4 at 30 mg/kg bw in a RLD vehicle.

D_5

The mean percentage of the administered dose recovered ranged from 95.5% and 103.24% in females and males.

The predominate route of elimination was in feces as the radioactivity recovered accounted for 82.52 and 82.99% of the recovered dose (D_5 low) in females and males, respectively (**Table 1**). Urine contained 8.15% and 8.99% of the recovered dose) in females and males, respectively. Expired volatiles contained 2.01 and 1.26% of the recovered dose (D_5 low) in females and males, respectively. The percentages of the dose recovered in the urine, feces and expired $^{14}CO_2$ of female and male rats were similar. At 168 h, the remaining carcass (including pelt) accounted for 5.07% and 4.72% of the recovered radioactivity (D_5 low) in females and males, respectively. Following administration of ^{14}C-D_5 at 100 mg/kg bw in a RLD vehicle, the percentage absorbed was 17.4% and 16.9% in females and males, respectively.

Blood Kinetics

D_4

Parent D_4 and total ^{14}C-activity were measured in blood following a single oral gavage administration of ^{14}C-D_4 at 30 mg/kg bw to female and male Fischer 344 rats, Blood kinetic parameters for parent D_4 and ^{14}C-activity in blood are presented in **Table 2 and Figure 1A** (female data, high dose data shown for comparison). For D_4 at 30 mg/kg bw in a rodent diet vehicle, parent concentrations were measurable through 72 h and 48 h for females

and males (data not shown), respectively. Radioactivity concentrations were measurable through 168 h for both females and males (data not shown). The peak blood concentration, C_{max}, occurred at 2 h for both D_4 and total ^{14}C-activity in females; the peak blood concentrations were at 2 h for D_4 and 4 h for total ^{14}C-activity in male animals (data not shown). C_{max} concentrations were similar between females and males. Noteworthy is that for the high dose female data shown for comparison, T_{max} is delayed for both parent and radioactivity as compared to the low dose female data which denotes a delay in gastric emptying.

Calculated AUCs from the blood time course data (D_4 low) indicated that ^{14}C-activity was absorbed; AUCs in μg ^{14}C-equivalents D_4 x hr/g of blood were similar between females and males. There was a statistically significant difference between AUCs for parent D_4 and ^{14}C-activity for females and males. This indicates that metabolites contribute to a portion of the AUC for ^{14}C-activity. A statistically significant difference was observed for parent AUCs between females and males. Terminal half-lives of elimination for either parent D_4 or ^{14}C-activity were similar between females and males; however, the elimination of ^{14}C-activity was slower than that for parent.

D_5

Blood kinetic parameters for parent D_5 and radioactivity in blood following a single oral gavage administration of ^{14}C-D_5 at 100 mg/kg bw to jugular vein cannulated female and male Fischer 344 rats are presented in **Table 3** for both females and males with D_5 and female data only in **Figure 1B** (female high dose data shown for comparison).

Parent concentrations for D_5 at 100 mg/kg bw in a rodent diet vehicle were measurable through 168 h and 72 h for females and males (data not shown), respectively. Radioactivity concentrations were measurable through 168 h for both females and males

(data not shown). In females and males, the peak blood concentration, C_{max}, occurred at 4 h for both D_5 and total radioactivity and the concentrations were similar.

As part of the study where ^{14}C-D_5 at 100 mg/kg bw was administered in a rodent liquid diet vehicle (**Table 3 and Figure 1B**), a group of females was administered the same dose in a corn oil vehicle. Parent and total radioactivity concentrations were measurable through 168 hr. In this group of females, the peak blood concentration occurred at 4 h for both D_5 and total radioactivity. In females dosed with ^{14}C-D_5 in a corn oil vehicle, C_{max} were higher than those observed in the females that were dosed with ^{14}C-D_5 in a RLD vehicle (**Table 3**). With the high dose female data shown for comparison, T_{max} is delayed for both parent and radioactivity as compared to the low dose female data which denotes a delay in gastric emptying.

Calculated AUCs from the blood time-course data (D_5 low) indicated that ^{14}C-activity was absorbed (AUC in μg ^{14}C-equivalents D_5 x hr/g of blood) and were similar, no statistically significant difference, between females and males; AUCs for parent D_5 were also similar between sexes. There was a statistically significant difference between blood AUCs for parent D_5 and ^{14}C-activity for females, males and females (corn oil). There was not a statistically significant difference in ^{14}C-activity blood AUCs between females dosed with D_5 (low) in the RLD vs. the corn oil vehicle; however, the blood parent AUCs were significantly different. Terminal ^{14}C-activity half-lives of elimination were similar for females and males. Parent D_5 terminal half-lives were different between females and males with the blood elimination half-life in females slower by ~4 fold.

Tissue Distribution and Kinetics: Parent (D_4 and D_5) and ^{14}C-Activity in Blood and Tissues

Key tissues described in PBPK models are blood, liver, fat and lung and those are highlighted in the results.

Parent D_4

A summary of pharmacokinetic parameters for tissues (C_{max}, T_{max}, $t_{1/2}$ terminal) are found in **Table 4.**

Parent D_4 was detected in all tissues with the C_{max} in most tissues at 2 h post-dosing, except in fat where the highest concentration was observed at 12 and 24 h post-dosing for males and females, respectively. Parent D_4 was measurable in tissues through 168 h post-dosing in all animals. The C_{max} of parent D_4 in blood was lower than C_{max} levels in tissues. Examples of tissue concentration of parent time-courses, liver and perirenal fat, for female animals are depicted in **Figures 2A and 3A**.

Terminal half-lives of elimination ($t_{1/2}$) (**Table 4**) of parent D_4 were fastest in blood; 20 and 18.7 h for females and males, respectively. Slower $t_{1/2}$s of elimination in fat were: 233.6 and 166.8 h for females and males, respectively.

^{14}C-Activity D_4

^{14}C-Activity **(Table 4)** was detected in all tissues with the highest concentrations in all tissues at 2 h post-dosing, except in fat where the highest concentration was observed at 12 h post-dosing. ^{14}C-Activity was measurable in tissues through 168 h post-dosing in all animals. In blood the C_{max} of ^{14}C-activity was lower than C_{max} concentrations in tissues. Examples of tissue concentration time-courses of ^{14}C-activity, liver and perirenal fat, in female animals are depicted in **Figures 2A and 3A**.

The half-lives of elimination (terminal phase) of radioactivity (**Table 4**) in blood were; 104.5 and 80.6 h for females and males, respectively. The slowest $t_{1/2}$s of

eliminations were in perirenal fat; 225.2 and 217.9 h for females and males, respectively and in lungs, 311.9 and 212 h for females and males, respectively.

Parent D_5

Pharmacokinetic parameters for tissues for parent D_5 and ^{14}C-activity are found in **Table 5.** Parent D_5 was detected in all tissues with the C_{max} in tissues typically observed at 2, 4 or 6 h post-dosing, except in brown fat and perirenal fat where the highest concentrations were observed at 12 and 48 h post-dosing. Parent D_5 was measurable in tissues through 168 h post-dosing in all animals. In blood C_{max} parent D_5 was lower than C_{max} levels in tissues. Examples of tissue parent concentration time-courses, liver and perirenal fat, in female animals is depicted in **Figures 2B and 3B**.

Half-lives of elimination (terminal phase) of parent D_5 (**Table 5)** were fastest in liver (17.62 h) and blood (18.90 h); for females and males, respectively. Slower $t_{1/2}$s of elimination were in the digestive tract: 441.20 and 538.00 h for females and males, respectively. The half-life of parent in blood for the female corn oil group was longer than for females administered D_5 in a rodent diet vehicle; 76 and 72 h, respectively.

^{14}C-Activity D_5

Radioactivity **(Table 5)** was detected in key model tissues with the highest concentrations at 2, 4, or 6 h post-dosing, except in brown fat and perirenal fat where the highest concentrations were observed at 12 and 48 h post-dosing. Total radioactivity was measurable in tissues through 168 h post-dosing in all animals. The Cmax for total radioactivity in blood was at lower levels than C_{max} levels in tissues. Examples of tissue 14C-acitivity time-courses, liver and perirenal fat, in female animals is depicted in **Figures 2B and 3B**.

The half-live of elimination (terminal phase) of total radioactivity (**Table 5**) was fastest in liver and lung. The slower $t_{1/2}$s of elimination were in the digestive tract (386.17 h) and perirenal fat (341.32 h) for females and males, respectively.

Tissue Kinetics and Metabolism

Total radioactivity concentration, ^{14}C-activity, is composed of both parent D_4 (or D_5) and metabolites of D_4 (or D_5). The difference between total radioactivity and parent D_4 concentrations, denotes the contribution of ^{14}C-radioactivity attributed to metabolites.

D_4

Calculated AUCs for ^{14}C-activity and parent D_4 in blood and tissues following a single oral gavage administration are presented in **Table 6.** Comparisons of calculated ^{14}C-activity AUCs (μg eq*h/g) and parent AUCs (μg*h/g) in female and male tissues revealed the following order from greatest to least: perirenal fat > digestive tract > lung or liver > spleen > blood.

The ratio of the AUCs for D_4 in tissues to blood was greater than 1 for all tissues. The highest tissue-to-blood AUC ratios were observed in perirenal fat; 240.7 and 189.1, for females and males, respectively. Liver to blood partitioning was 10.4 and 7.6 for females and males, respectively.

The percentage of the total radioactivity attributed to metabolites in tissues and blood from females ranged from 9.51% to 83.29% with blood (83.29%) and liver (61.53%) with the greatest percentage of metabolites and the fat (9.51%) with the least percentage of metabolites. Similar percentages of radioactivity attributed to metabolites were observed for males.

D_5

Blood and tissues had measurable concentrations of both parent D_5 and metabolites. Calculation of the Area-Under-the-Curves (AUC) for total radioactivity as well as parent D_5 in blood and tissues following a single oral gavage administration are presented in **Table 7.**

Comparisons of calculated total radioactivity AUCs (μg eq*h/g) and parent AUCs in female and male tissues revealed the following order, greatest to least: brown fat > digestive tract > perirenal fat > liver > lung > spleen > blood.

The ratio of the AUCs for D_5 in tissues to blood was greater than 1 for all tissues. The highest tissue-to-blood AUC ratios were observed in brown fat, 100.9 and 72.9, for females and males, respectively.

The percentage of the total radioactivity attributed to metabolites in tissues and blood from females ranged from 7.74% to 56.09% with liver (56.09%) and blood (47.81%) having the highest percentages. The percentage of the total radioactivity attributed to metabolites in tissues and blood from males ranged from 5.12% to 71.38% with liver (71.38%) and blood (46.24%) having the highest percentages.

The percentage of total radioactivity in the blood attributed to metabolites following administration of ^{14}C-D_5 (low) in a corn oil vehicle to a subset of females was 27.95% which was a lower percentage of metabolites than observed in the female group where administration of D_5 was in a rodent diet vehicle, 47.81%. There was a statistically significant difference in mean blood parent AUCs between females that were dosed with D_4 in RLD *vs.* dosed with D_4 in the corn oil vehicle.

Elimination

Radioactivity was measurable through 168 h in feces, expired volatiles, urine, and as $^{14}CO_2$ following single oral gavage administration of D_4 or D_5.

D_4

Graphical presentations of ^{14}C-activity and parent D_4 in expired volatiles, feces and of radioactivity in urine are presented in **Figures 4A – 6A (**female animals only, data from a high dose study are shown for comparison**)**.

A total of 29.9 and 18.4% of the recovered dose was accounted for in expired volatiles in females and males, respectively. The highest concentrations (^{14}C-activity) measured in expired volatiles was during the 1-2 and 2-4 h collection intervals for females (168 μg eq/h) and males (189 μg eq/h, data not shown), respectively.

A total of 32.1 and 40.0% of the recovered dose was accounted for in the urine in females and males, respectively. The highest concentration (^{14}C-activity) in urine was measured at the 12-24 h collection interval which was 150 and 253 μg eq/g in female and male rats (data not shown), respectively.

A total of 22.6 and 27.2% of the recovered dose were accounted for in feces in females and males, respectively. The highest concentration (^{14}C-activity) in feces was measured at the 0-24 h collection interval which was 152 and 173 μg eq/g in female and male rats (data not shown), respectively.

Concentrations of ***parent*** D_4 were measurable through 48 h in feces and through 168 h in expired volatiles following single oral gavage administration of ^{14}C-D_4. The highest concentration of parent in feces was measured at the 0-24 h collection interval and was 98.61 and 75.89 μg/g in female and male rats, respectively. The highest

amounts measured in expired volatiles were during the 1-2 and 2-4 h collection intervals for females (119.28 μg /h) and males (112.60 μg eq/h) respectively.

Calculation of AUCs for ^{14}C-activity, parent D_4 and terminal half-lives of elimination are presented for expired volatiles, urine, and feces in **Table 8.**

Terminal half-lives of elimination for radioactivity were similar for female and male animals for expired volatiles, urine and feces. Half-lives ranged from 50.1 to 63.9 h in females and 35.7 to 55.2 h in males.

The percentage of the total radioactivity attributed to metabolites in excreta from *females* following an oral administration of a low dose of D_4 ranged from 30.93% to 100.00%: feces (48.26%), expired volatiles (30.93%), and urine (100.00%), with similar results for males.

D_5

Graphical presentations of ^{14}C-activity and parent D_5 concentrations in expired volatiles, feces and of radioactivity in urine are presented in **Figures 4B – 6B** (female animals only, data from a high dose study are shown for comparison).

A total of 2.0 and 1.3% of the recovered dose was accounted for in expired volatiles in females and males, respectively. The highest amounts of radioactivity measured in expired volatiles was during the 4-6 h collection interval for females (17.1 μg eq/h) and males (15.8 μg eq/h, data not shown), respectively. The highest amounts of parent D_5 measured in expired volatiles was during the 4-6 h collection intervals for females (15.2 μg/h) and males (15.7 μg/h, data not shown), respectively.

A total of 8.2 and 9.0% of the recovered dose was accounted for in the urine in females and males, respectively. The highest concentration in urine was measured at the 12-24 h collection interval, 84 and 144 μg eq/g in female and male rats (data not shown), respectively.

A total of 83% of recovered radioactivity was found in feces. The largest percentage of elimination of radioactivity excreted in feces occurred during the first collection interval, 0-24 h. The highest concentration in feces was measured at the 0-24 h collection interval, 1618 and 1890 μg eq/g in female and male rats (data not shown), respectively. Concentrations of parent D_5 were measurable through 96 h in feces and through 168 h in expired volatiles following single oral gavage administration of ^{14}C-D_5. The highest concentration of parent in feces was measured in the 0-24 h collection interval with 1786 and 1513 μg/g in female and male rats (data not shown), respectively.

Calculation of the AUCs for total radioactivity as well as parent D_5 in feces, urine, expired volatiles and CO_2 following a single oral gavage administration are presented in **Table 9.**

Terminal half-lives of elimination for radioactivity were shorter in male animals for expired volatiles, urine, feces, and CO_2 **(Table 11)**. Half-lives ranged from 28.36 to 69.77 h in females and 25.72 to 49.39 h in males.

The AUC for total radioactivity is composed of both parent D_5 and labeled metabolites. Comparison to the AUC derived from parent D_5 demonstrates the percentage of the ^{14}C-pool that is composed of metabolites. Only feces (males) contained both parent and metabolites. No parent D_5 was found in urine samples; only metabolites

were present. The percentage of the total radioactivity attributed to metabolites in excreta from females following administration was: urine (100.00%) and in males it was: feces (19.40%) and urine (100.00%),.

Urine Analysis: Metabolite Profile

The radioactivity eliminated in the urine consisted entirely of polar metabolites of D_4 and D_5. The metabolite peak assignments are based on retention time comparison to urinary metabolite profiles performed in a separate study [27]. No confirmation of identity was conducted within these studies.

D_4

The mean percentage of radioactivity that can be attributed to individual metabolites from urine at 0-12, 12-24, and 24-48 h collection intervals following oral administration is presented in **Table 10**. Dimethylsilanediol represented the greatest percentage of total urinary radioactivity. The percentages for the three collection intervals ranged from 51 to 59% in female animals and 55 to 65% in male animals. The percentages for methylsilanetriol for the three collection intervals ranged from 20-25% in female rats and 16-27% in male rats. Dimethyldisiloxane-1, 3, 3, 3-tetrol as a percentage of urinary activity for the three collection intervals ranged from 9.5-13.1% in females and 9-13% in male animals. The average sum of de-methylated peak percentages (oxidative metabolism) ranged from 30 to 39% for females and males.

No gender differences in identified metabolites were noted, except that hexamethyltrisiloxane-1, 5-diol was only present in female animals during the 0-12 and 12-24 h collection intervals.

D_5

The mean percentage of radioactivity that was attributed to individual metabolites from urine at 0-12 and 12-24, hours following oral administration D_5 in the low dose study is presented in **Table 11**. Dimethylsilanediol represented the greatest percentage of total urinary radioactivity followed by methylsilanetriol. The percentages for dimethylsilanediol for the two collection intervals ranged from 53 to 58% in female animals and 50 to 53% in male animals and the percentages for methylsilanetriol for the three collection intervals ranged from 35 to 36% in female rats and 38 to 42% in male rats. Dimethyldisiloxane-1, 3, 3, 3-tetrol as a percentage of urinary activity for the two collection intervals ranged from 1 to 2% in females and from 2% in male animals. The average sum of de-methylated peak percentages ranged from 39 to 41% for females and 44 to 47% for males.

Statistical analysis indicates a difference between gender averaged sums at the 0-12, 12-24 and 48-72 (data not shown) h collection intervals for demethylated metabolites, $p < 0.05$ using a two-tailed t-test assuming equal variance.

Fecal Analysis: Metabolite Profile

D_4

Metabolite peak assignments are based on retention time comparison to fecal metabolite profiles performed in a separate study [27]. Parent D_4 represented the major percentage of radioactivity in the fecal samples analyzed from the 0-24 h collection interval (**Table 12**). Seventy-one percent of fecal radioactivity was identified as D_4 in female rats and 51% in male rats for the 0-24 h collection interval. At the same collection interval, 13% and 26% of the radioactivity was attributed to methylsilanetriol in females and males, respectively. In the 24-48 h collection interval, less D_4 was observed, 41 and 24% in females and males, respectively. However, the percentage of

methylsilanetriol was greater in the 24-48 h collection interval, 55 and 53% of the total fecal radioactivity in female and male animals, respectively.

No gender differences in identified metabolites were noted except dimethyldisiloxane-1, 3, 3, 3-tetrol was not present at the 0-24 h collection interval in females.

D_5

Parent D_5 represented the major percentage of radioactivity in the fecal samples analyzed from the 0-24 h collection interval for both sexes (**Table 13**). Ninety-three and 91% percent of fecal radioactivity was D_5 in female and male rats, respectively, for the 0-24 h collection interval.

An attempt was made to identify unknown peaks in the HPLC/RAD chromatogram by collecting fractions from 0-24 h fecal extracts. Hydroxylated D_5 metabolite (D_4D'OH) was observed in extracts by GC/MS; however, peak assignment in the radiochemical profile was inconclusive (data not shown).

DISCUSSION

An integrated multi-species and multi-dose route PBPK model for volatile cyclic methyl siloxanes describes the pharmacokinetic behavior of D_4 and D_5 by the inhalation and dermal routes of exposure [22]. Data from oral studies with a high dose (300 mg/kg bw) of D_4 were also described in earlier models [17, 21] although the oral uptake was not well described. Additional attempts were made to model the kinetic dispositions of D_4 and D_5 following high oral doses in various lipophilic vehicles or as neat material [28].

554 The additional low dose kinetic studies reported here were conducted to
555 determine if the different behavior of D_4 and D_5 following oral exposure compared to
556 inhalation and dermal exposures was a result of a high bolus dose, which may have
557 impacted the physiology of uptake. In addition, a dosing vehicle was chosen to be more
558 representative of a food like matrix to better understand absorption from food matrices.
559 Initial comparisons were conducted to assess dose dependent differences in kinetic
560 profiles for both D_4 and D_5 high dose and low dose data sets and reported previously [29,
561 30]. More detailed comparisons of these data sets are provided in the supplemental
562 materials.

563 Comparison of the low and high dose (Appendix A, **Tables A.4, A.6 in Supplementary**
564 **material**) oral studies with D_4 showed that there was evidence of a dose dependency in the
565 pharmacokinetics. Comparison of the data sets from the low (RLD) and high dose (corn oil) oral
566 gavage of ^{14}C-D_4 in female rats showed that a greater percentage of the administered dose is
567 absorbed following low dose oral gavage administration (77%, low dose compared to 55%, high
568 dose). Following low dose administration; more of the recovered dose was found in expired
569 volatiles (30% (low) vs. 16% (high)) and was excreted in urine as metabolites. In addition, a
570 greater percentage of the recovered low dose was eliminated as metabolites in feces (48%);
571 with the high dose, there was no statistical difference between parent and ^{14}C-activity. Mean
572 blood AUCs (Appendix A, **Table A.2 in Supplementary material**) for both parent (29 μg x h/g
573 blood (low) vs. 161 μg x h/g blood (high)) and ^{14}C-activity (175 μg x h/g blood (low) vs. 933 μg x
574 h/g blood (high)) were not proportional to dose since a 10-fold difference in oral dose resulted
575 in approximately a 5-fold difference in mean blood AUCs.

Data suggest that dose-dependency is related to saturation of metabolism at the high dose of D_4, as an increased percentage of recovered dose was found in the urine and an increase in metabolites was found in feces following low dose administration compared to high dose administration. Based on this data, the refined PBPK model [28, 31], estimates an increased metabolism in the liver with low dose vs. high dose administration (~47% metabolized at the low dose compared to only ~20% metabolized at the high dose) [29].

Dose dependency in D_4 oral uptake was also evaluated by Dobrev et al., [21]. They demonstrated dose-dependent absorption of D_4 following oral gavage of D_4 in corn oil from 10 to 300 mg/kg bw. Kinetic determinants were assessed for metabolism and oral uptake of D_4 following gavage in corn oil by utilization of closed-chamber disappearance curves. PBPK models analyses of the results, demonstrate that uptake from the gut and release from blood into the chamber air for oral doses from 10 to 300 mg/kg were consistent with prolonged and slow uptake of D_4 from the GI tract and reduced absorption at higher doses. This same dose dependent absorption was observed when comparing the D_4 low (reported here) and high dose studies previously described [17, 29].

Comparison of the low and high dose oral studies with D_5 also showed that there was evidence of a dose dependency in the pharmacokinetics [30]. Comparison of data sets from the low and high dose oral gavage of ^{14}C-D_5 in female rats indicates a similar percentage of the recovered dose is absorbed following low and high dose oral gavage administrations; 17 and 22%, respectively (Appendix A, **Table A.1 in Supplementary material**). However, there was a greater percentage of the recovered dose in the urine with the low dose (8.15%) study vs the high dose (4.39%) study suggesting that the process of metabolism maybe saturating at the higher dose level. Consequently, a greater

599 percentage of the recovered dose was found in expired volatiles with the high dose (11%)
600 study compared to the low dose (2%) study.

601 Mean blood AUCs (Appendix A, **Table A.3**) for both parent (165 μg x h/g blood (low) vs.
602 889 μg x h/g blood (high)) and ^{14}C-activity (315 μg x h/g blood (low) vs. 1060 μg x h/g blood
603 (high)) were not proportional to dose since a 10-fold difference in oral dose resulted in only a 3-
604 5-fold difference in mean blood AUCs.

605 Non-linearity in responses was observed between low and high dose (Appendix A,
606 **Tables A.5, A.7, A.8 in Supplementary material**) D_5 studies in females as demonstrated by: 1) a
607 greater percentage of the recovered dose was found in urine as metabolites following low dose
608 administration, 2) a greater percentage of the recovered dose was found in expired volatiles
609 following high dose administration, 3) mean blood AUCs for ^{14}C and parent were not
610 proportional to dose and 4) expired volatile mean AUCs were not proportional to dose. Similar
611 observations were made between the low and high dose D_5 data for male animals (not reported
612 here).

613 In the D_5 study reported here at the lower dose, in addition to a group of animals to
614 investigate blood kinetics when administered in a RLD vehicle, a group of female animals was
615 administered the low dose in corn oil to directly compare vehicles at the same dose level. The
616 blood AUCs for ^{14}C-activity and parent were similar between the corn oil group vs the RLD group
617 (^{14}C: 315 and 320 μg x h/g blood, respectively and Parent: 164 and 230 μg x h/g blood
618 respectively) indicating that the amount absorbed at this lower dose was most likely not
619 impacted by vehicle, at least for D5 for these two vehicles.

620 Due to the highly lipophilic nature of D_4 and D_5, these materials are likely primarily
621 absorbed along with lipids from both the corn oil and rodent liquid diet vehicles and absorbed in

622 a sequestered form, possibly directly into plasma lipid or via chylomicrons [31]. Sequestered
623 dietary lipid in the plasma is primarily distributed to the deep liver compartments and diffuse fat
624 [31]. The corn oil vehicle appears to be similar to the RLD vehicle when comparing kinetics for
625 D_5 at the lower dose; but it is uncertain if similar finding would be found for D_4 as the same
626 assessment was not done for D_4. Campbell et al., [31] reports differences in the kinetic behavior
627 of D_4 and D_5 by the oral route. Initially, the D_4 oral absorption parameterization was used to
628 simulate D_5 oral absorption; however, the use of the refined oral uptake description for D_4 gave
629 a similar over-prediction of the peak rate of exhaled D_5. This indicated that D_5 was even more
630 sequestered from exhalation so the model was adjusted with D_5 so that the only pathway of
631 absorption was via sequestration. It is possible for D_4, that the vehicle may have a greater
632 impact on absorption since D_4 appears to be absorbed by both absorption of free D_4 and
633 sequestered D_4 but it is difficult to say with certainty without a direct comparison.

634 In the high dose studies [1, 17] different vehicles were suggested to impact absorption.
635 D_4 was administered at 300 mg/kg bw as a neat material and also in corn oil and simethicone
636 vehicles [17]. Oral absorption differed when compared for D_4 administered neat, in corn oil and
637 simethicone vehicles in female rats; 28, 52 and 12% of the administered dose absorbed,
638 respectively. In a similar study, a high dose oral (1000 mg/kg bw) study with D_5 in female rats,
639 absorption was also compared when D_5 was administered as a neat material, and also in corn oil
640 and simethicone vehicles and the amount absorbed was 10, 20, and 26% of the administered
641 dose, respectively [1]. Although this pattern differed from the D4 study, evaluations based on
642 blood area under the curve (AUC) supported that absorption increased after administration of
643 D5 in corn oil and decreased after administration in simethicone fluid compared to neat
644 administration, similar to what was reported in the D_4 study[1]. The authors of the D_5 high dose
645 oral study [23] reported that "the discrepancy between absorption assessed by blood curve

analysis and mass balance analysis may be caused by evaporation of parent D_5 from the excreted fecal matter that remained in the body of the cage. Animals dosed with corn oil and especially simethicone had a higher occurrence of loose fecal matter that adhered to the side of the cages". This could falsely elevate the percent absorbed when including the expired volatiles that may have been contaminated with volatilized D_5. This suggests the blood AUC analysis may be more reliable for assessing absorption differences in this study. Overall, these high dose oral studies evaluating absorption for D_4 and D_5 in the different vehicles assessed in these studies suggest a vehicle impact on absorption for both D_4 and D_5 that needs to be considered when evaluating oral dosing studies.

SUMMARY

Comparison of the low vs. high dose oral gavage administration of D_4 and D_5 demonstrated dose-dependent kinetic behavior. Data presented in this paper and the refined PBPK model [31] suggest differences in metabolism between the low and high dose administration indicating high dose administration results in or approaches non-linear saturated metabolism. In addition, the data reported here and previously reported [7] suggest the pharmacokinetics of D_4 (and D_5), following oral dosing is different from D_4 and D_5 delivered by the inhalation or dermal routes and require a refined model, describing delivery of D_4 and D_5 from the GI tract to the nonexchangeable/deep blood compartment. In addition, studies with high doses [1, 17] suggests vehicle my influence absorption. These low dose data sets were used to refine the D_4/D_5 multi-route harmonized PBPK model to allow for a better description of the disposition and pharmacokinetics of D_4/D_5 following administration of D_4/D_5 under more realistic oral exposure conditions (low dose incorporated into a food like vehicle). The model with a refined oral uptake description is described in Campbell et al., [31] and can be used in risk

669 assessment to better define the internal dose of D_4/D_5 following exposure to D_4/D_5 via multiple

670 routes.

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FIGURE LEGENDS

Figure 1. Blood time-course of parent and radioactivity in female rats through 168 h following single oral gavage dosing with A) [^{14}C]D_4 at 30 or 1000 mg of test article/kg body weight in rodent liquid diet and corn oil vehicle, respectively or B) [^{14}C]D_5 at 100 or 1000 mg of test article/kg body weight in a corn oil vehicle. For comparison, data for D_4 at 1000 mg/kg bw (high dose), [17] and for D_5 at 300 mg/kg bw (high dose) are included.

Figure 2. Liver tissue time-course of parent and radioactivity in female rats through 168 h following single oral gavage dosing with A) [^{14}C] D_4 at 30 mg/kg body weight in rodent liquid diet or B) [^{14}C]D_5 at 100 mg/kg body weight in a rodent diet vehicle.

Figure 3. Perirenal fat tissue time-course of parent and radioactivity in female rats through 168 h following single oral gavage dosing with A) [^{14}C]D_4 at 30 mg/kg body weight in rodent liquid diet or B) [^{14}C]D_5 at 100 mg of test article/kg body weight in a rodent diet vehicle.

Figure 4. Time-course of parent and radioactivity in expired volatiles in female rats through 168 h following single oral gavage dosing with A) [^{14}C]D_4 at 30 or 1000 mg/kg body weight in rodent liquid diet and corn oil vehicle, respectively or B) [^{14}C]D_5 at 100 or1000 mg/kg body weight in rodent liquid diet and corn oil vehicle, respectively. For comparison, data for D4 at 1000 mg/kg bw (high dose), [17] and for D5 at 300 mg/kg bw (high dose) are included.

Figure 5. Time-course of radioactivity in urine in female rats through 168 h following single oral gavage dosing with A) [^{14}C]D_4 at 30 or 1000 mg/kg body weight in rodent liquid diet and corn oil vehicle, respectively or B) [^{14}C]D_5 at 100 or1000 mg/kg body weight in rodent liquid diet and corn oil vehicle, respectively. For comparison, data for D4 at 1000 mg/kg bw (high dose). [17] and for D5 at 300 mg/kg bw (high dose) are included.

Figure 6. Time-course of parent and radioactivity in feces in female rats through 168 h following single oral gavage dosing with A) [^{14}C]D_4 at 30 or 1000 mg/kg body weight in rodent liquid diet and corn oil vehicle, respectively or B) [^{14}C]D_5 at 100 or1000 mg/kg body weight in rodent liquid diet and corn oil vehicle, respectively. For comparison, data for D4 at 1000 mg/kg bw (high dose), [17] and for D5 at 300 mg/kg bw (high dose) are included.

SUPPLEMENTAL DATA

Appendix A. Supplementary material Tables A.1-A.8: D_4 and D_5 high dose disposition and kinetic data

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Figure Caption

Figr-1

Figure 1A.

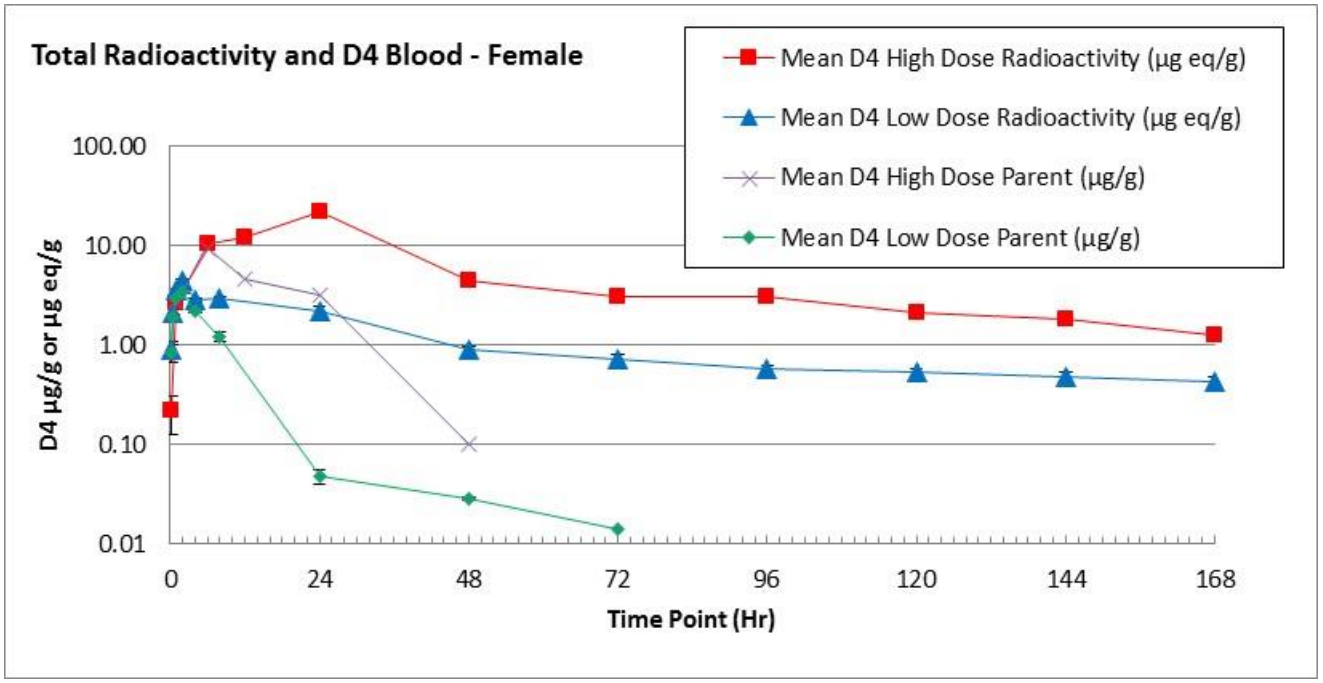
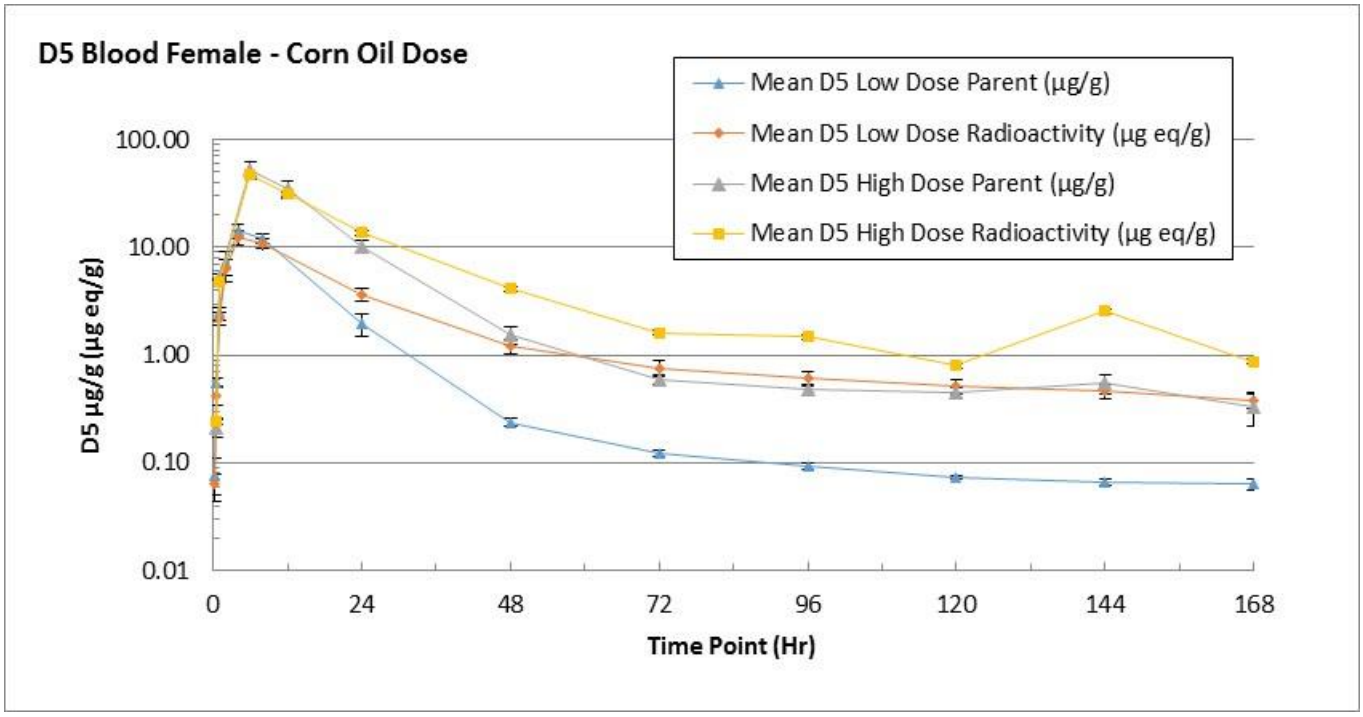
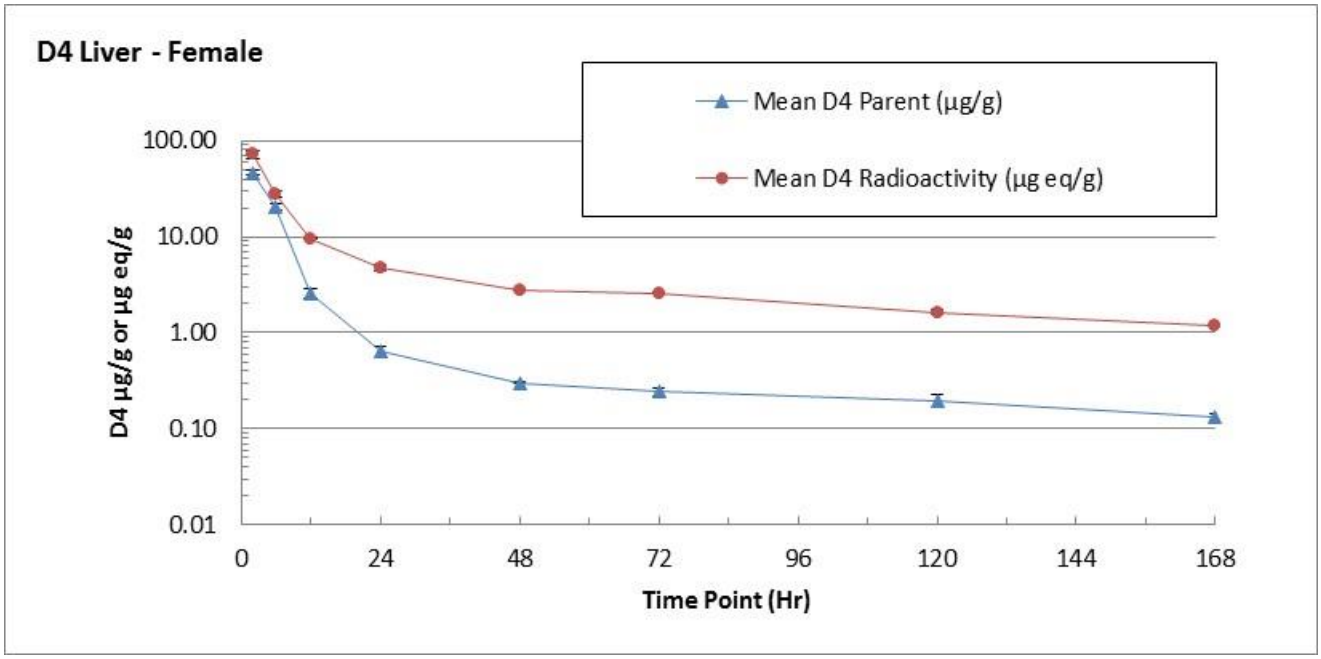


Figure 1B.

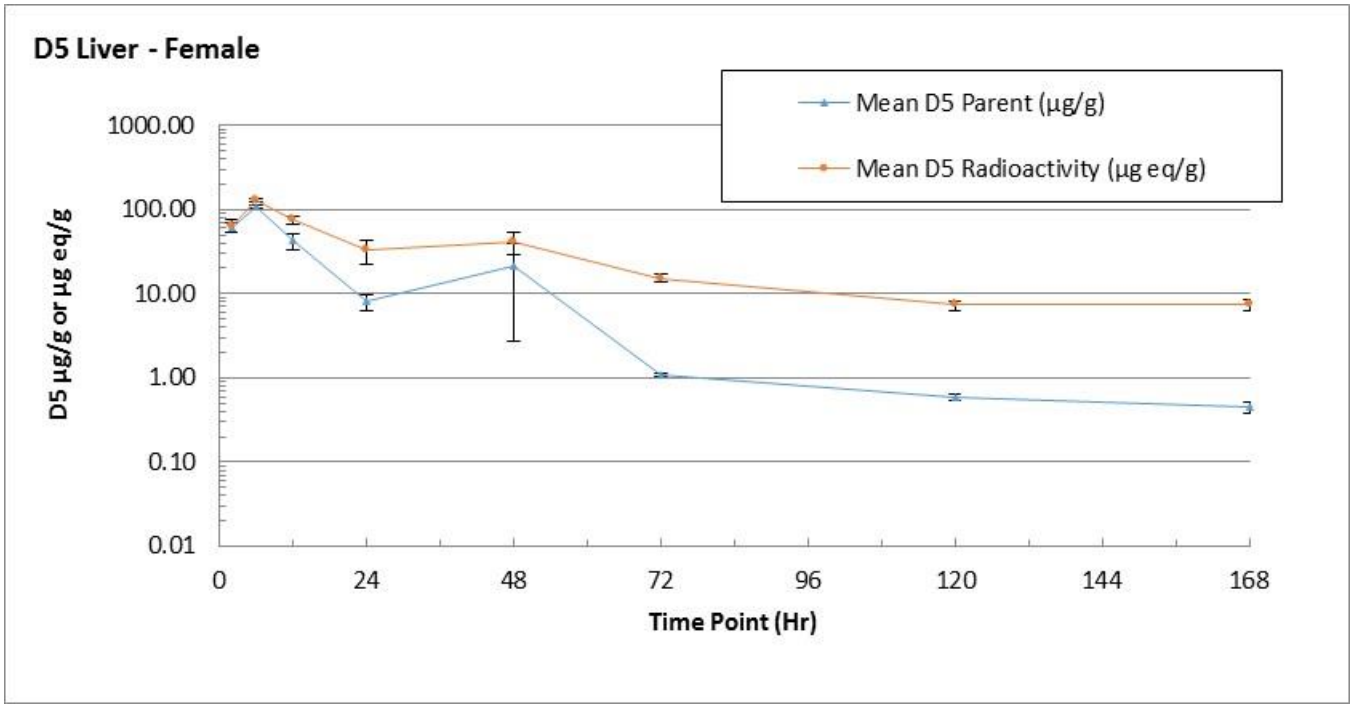


Figures 2A-2B

A

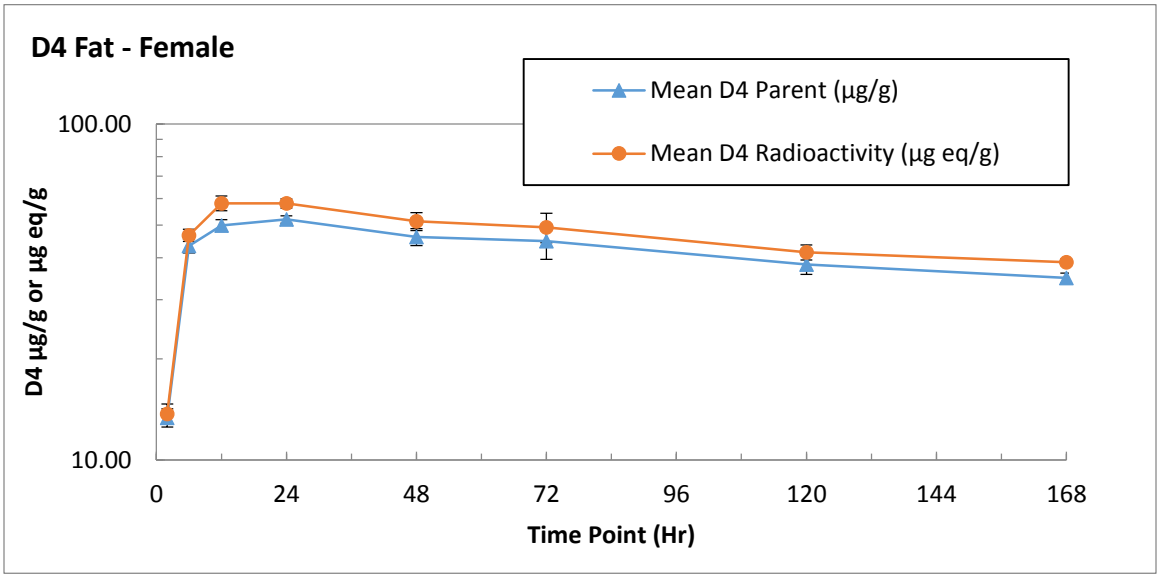


B



Figures 3A-3B

A



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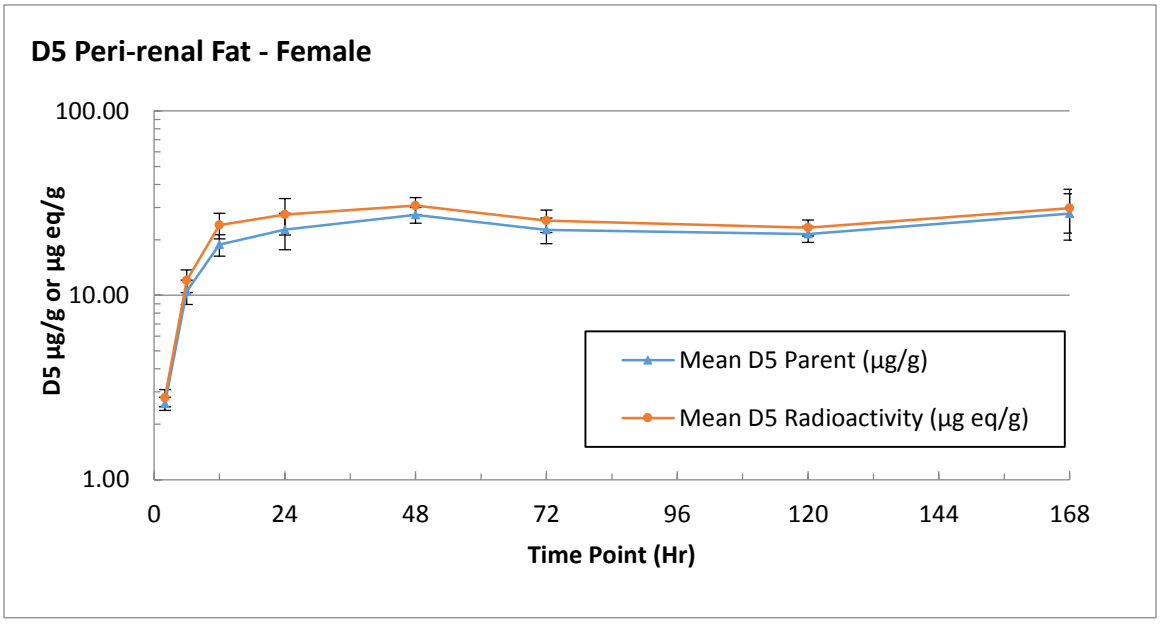
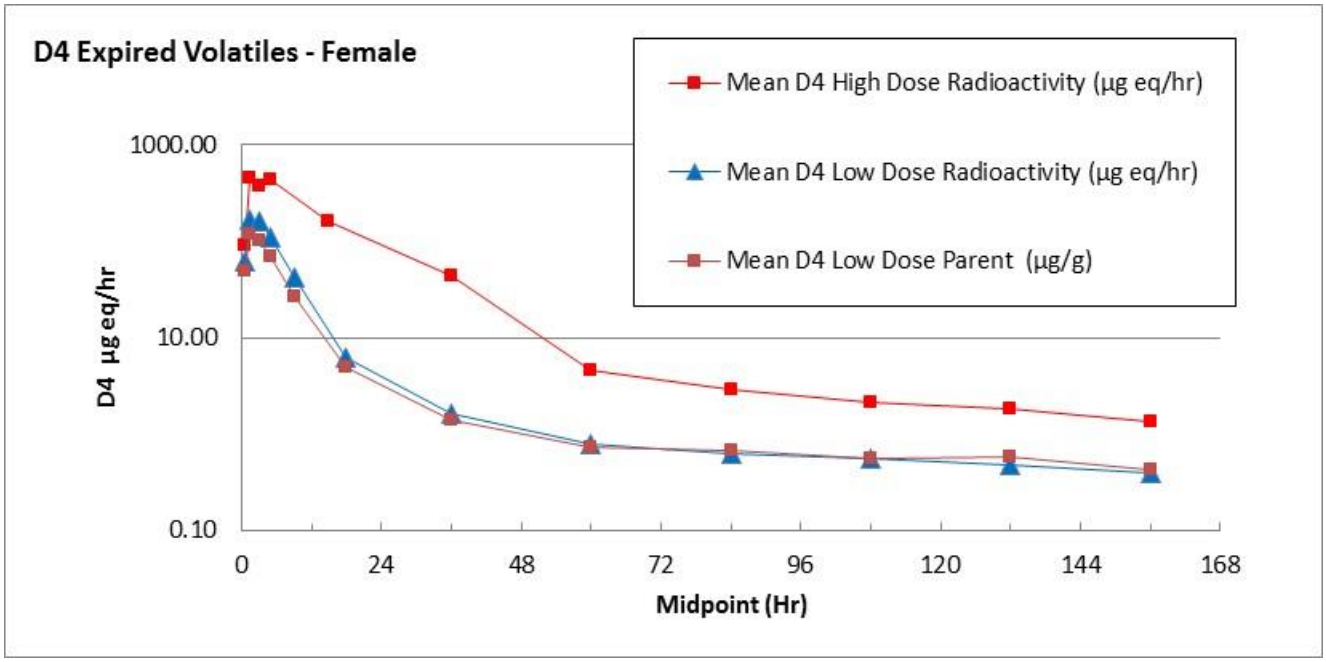


Figure 4A-4B

A



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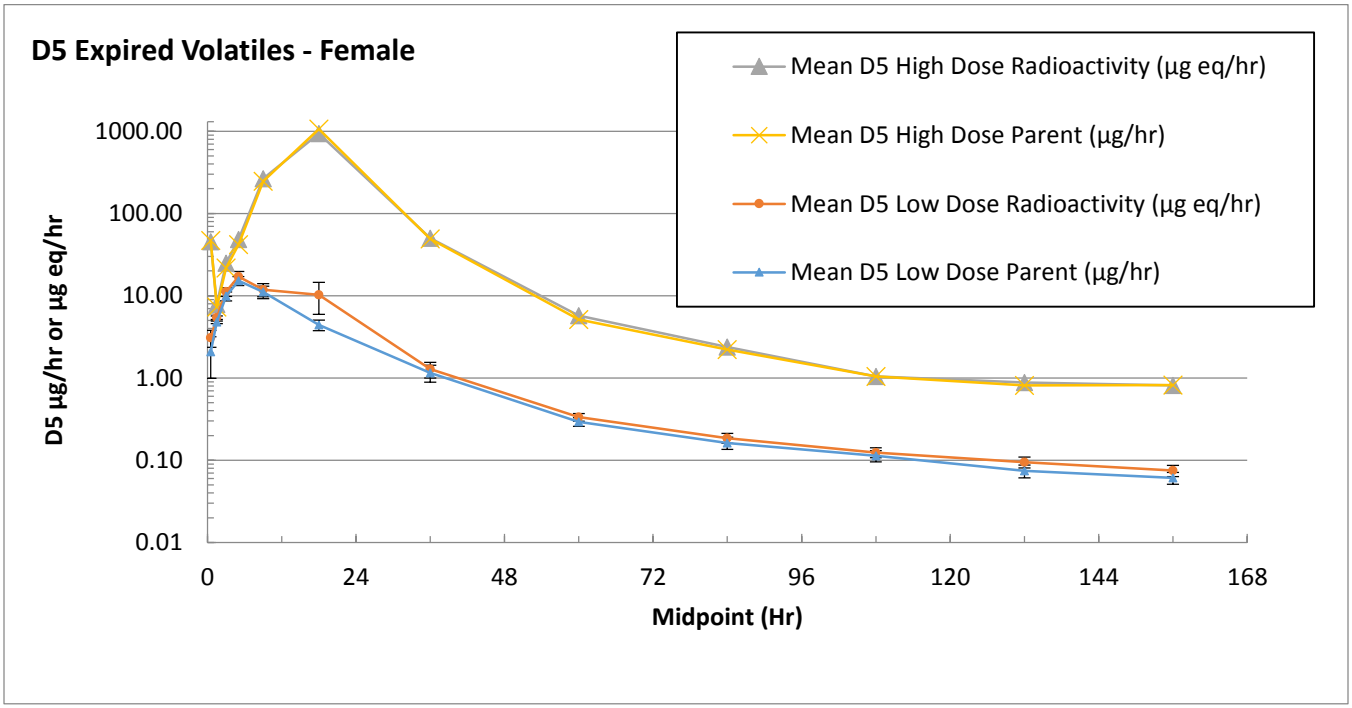
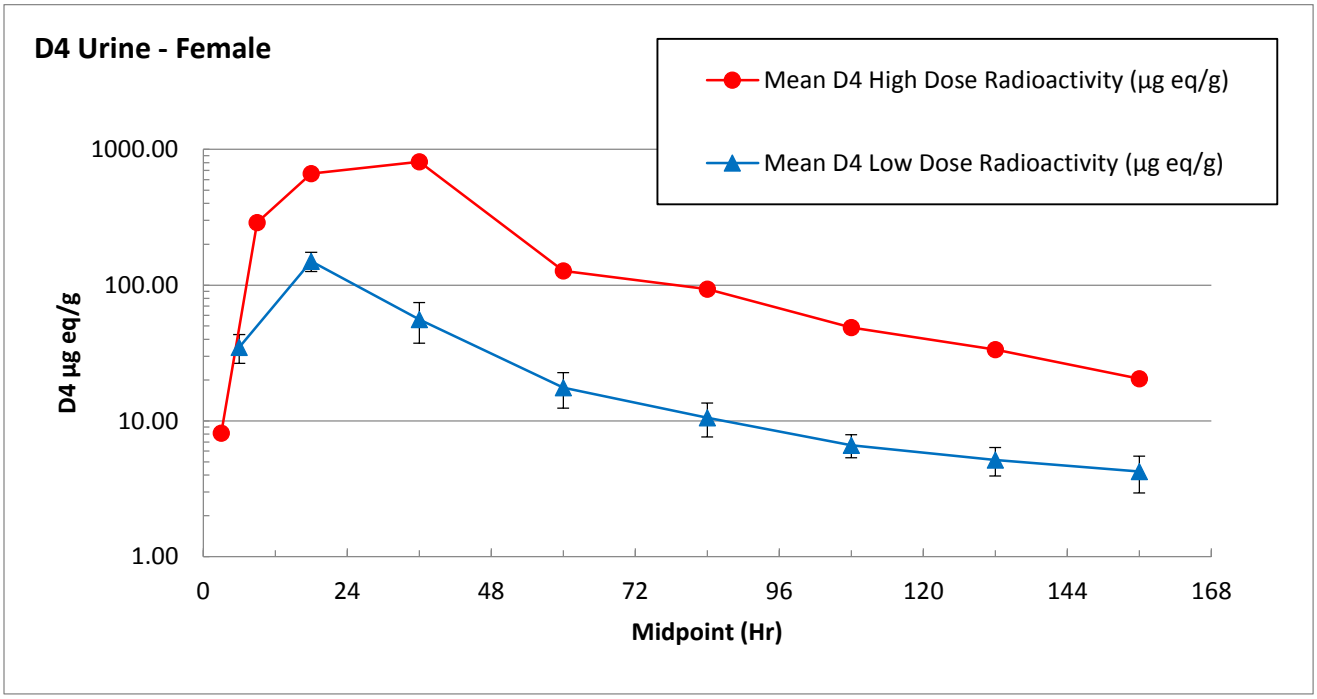


Figure 5A-5B

A



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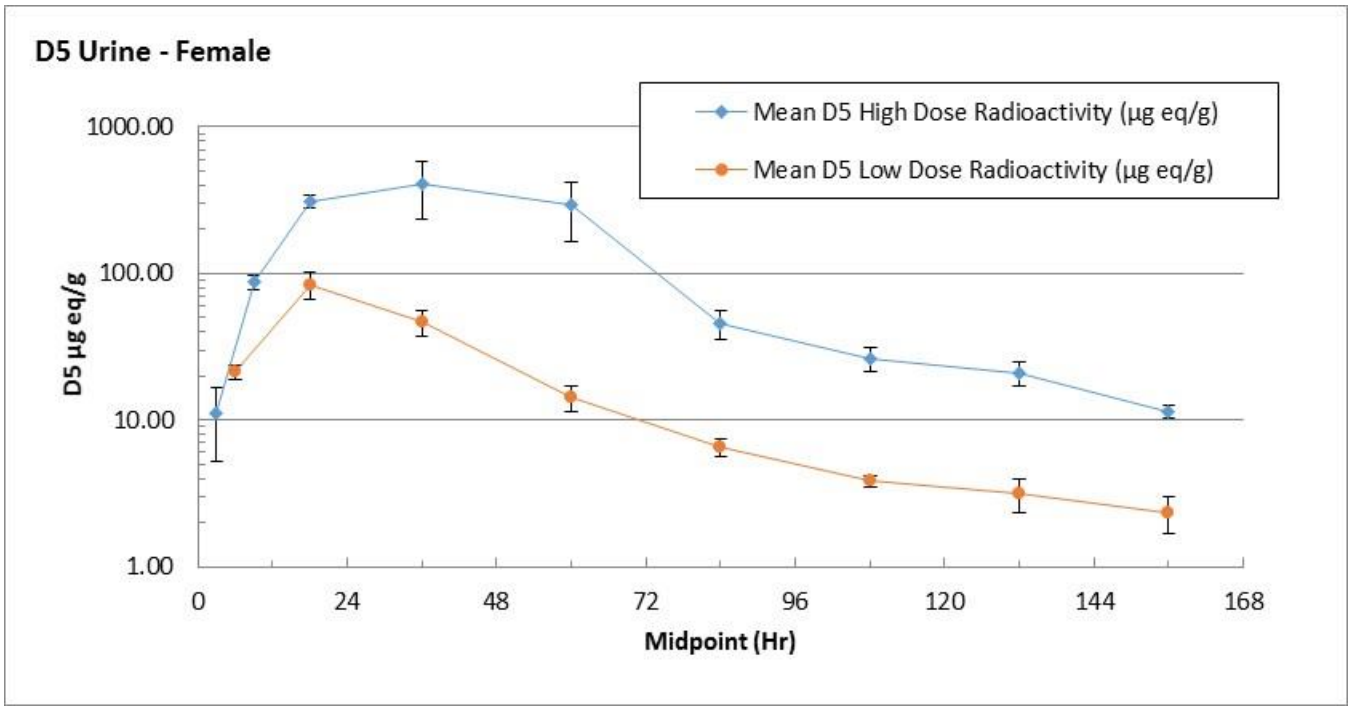
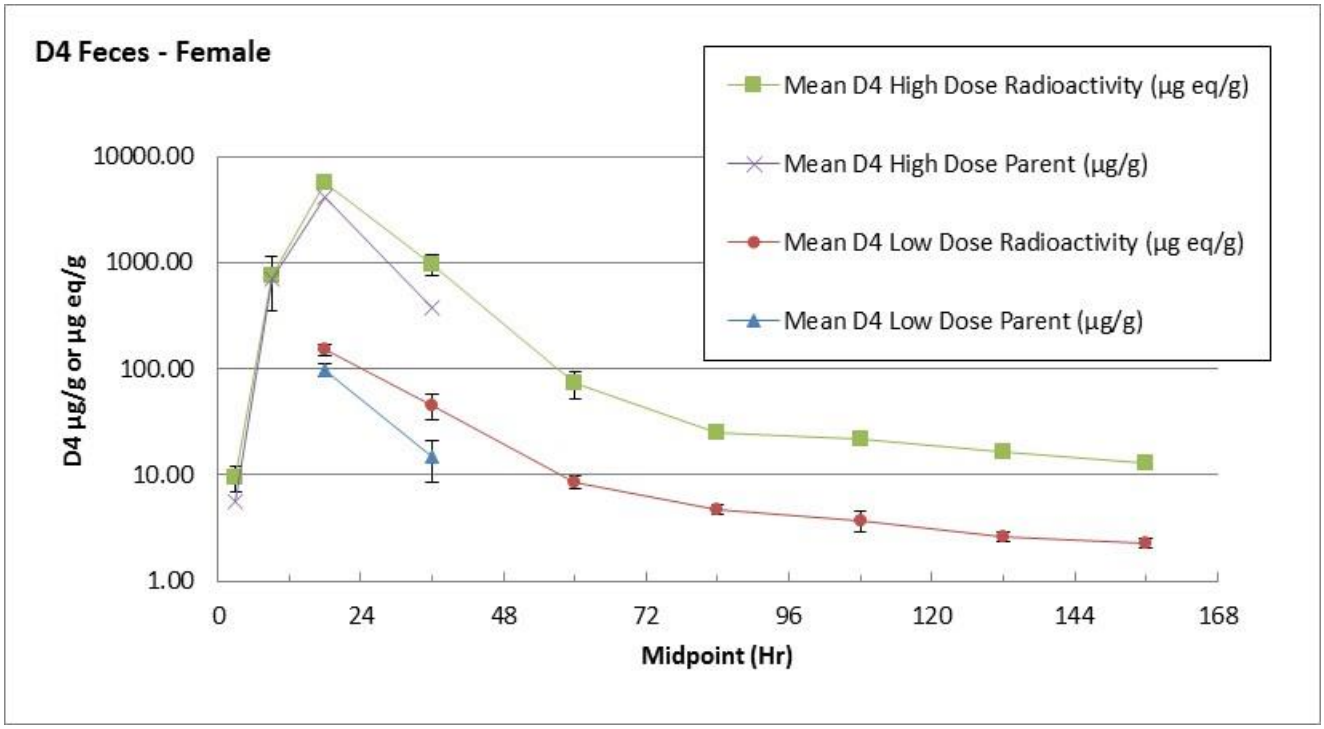


Figure 6A-6B

A



B