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Vaporized Delta-9-tetrahydrocannabinol Inhalation in Female Sprague Dawley Rats: A Pharmacokinetic and Behavioral Assessment

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Abstract

Background: Delta-9-tetrahydrocannabinol (THC) is the main psychoactive component of cannabis. Historically, rodent studies examining the effects of THC have used intraperitoneal injection as the route of administration, heavily focusing on male subjects. However, human cannabis use is often through inhalation rather than injection.

Objective: We sought to characterize the pharmacokinetic and phenotypic profile of acutely inhaled THC in female rats, compared to intraperitoneal injection, to identify any differences in exposure of THC between routes of administration.

Methods: Adult female rats were administered THC via inhalation or intraperitoneal injection. Serum samples from multiple timepoints were analyzed for THC and metabolites 11-

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Research Involving Animals: All experiments were conducted in compliance with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals and approved by the University at Buffalo Institutional Animal Care and Use Coimnittee.

Availability of Data and Materials:
The data that support the findings of this study are available from the corresponding author, PT, on special request.

hydroxy-delta-9-tetrahydrocannabinol and 11-nor-9-carboxy-delta-9-tetrahydrocannabinol using ultra-performance liquid chromatography-tandem mass spectrometry. Rats were similarly treated for locomotor activity analysis.

Results: Rats treated with 2 mg/kg THC intraperitoneally reached a maximum serum THC concentration of 107.7±21.9 ng/mL. Multiple THC inhalation doses were also examined (0.25 mL of 40 or 160 mg/mL THC), achieving maximum concentrations of 43.3±7.2 and 71.6±22.5 ng/mL THC in serum, respectively. Significantly reduced vertical locomotor activity was observed in the lower inhaled dose of THC and the intraperitoneal injected THC dose compared to vehicle treatment.

Conclusion: This study established a simple rodent model of inhaled THC, demonstrating the pharmacokinetic and locomotor profile of acute THC inhalation, compared to an i.p. injected THC dose, in female subjects. These results will help provide support for future inhalation THC rat research which is especially important when researching behavior and neurochemical effects of inhaled THC as a model of human cannabis use.

Keywords

Delta-9-tetrahydrocannabinol (THC); cannabis; liquid-chromatography/mass-spectrometry; rodent; serum; locomotor activity

1. Introduction

Cannabis (*Cannabis sativa* L.) is the second most used psychoactive drug in the United States (US), with nearly 12 million young adults reporting cannabis use in 2018 (1). As legalization of cannabis for both medical and recreational use becomes more common, there is increased interest in understanding both the short- and long-term effects of cannabis use. According to a 2020 study, cannabis use among both adolescents and adults in the US has continuously increased since 2006, positively correlating with increasing medical marijuana legalization (2). Delta-9-tetrahydrocannabinol (THC), which is responsible for the psychoactive effects of cannabis, exerts its effects via interaction with the endocannabinoid system as a cannabinoid receptor 1 (CB_1) agonist (3, 4). The endocannabinoid system is largely comprised of two G-protein coupled receptors, CB_1 and cannabinoid receptor 2 (CB_2), as well as endogenous ligands including anandamide (AEA) and 2-arachidonylglycerol (2-AG), and various other associated proteins and enzymes, responsible for synthesis, breakdown, and transportation of these ligands (5, 6). Cannabis use induces a host of physiological and cognitive changes in humans, both in acute exposure as well as after chronic use, resulting in altered physiology, executive function, nociception, and appetite, among others (7-12). These results have been observed in preclinical rodent models as well (13-15).

Route of administration is an important factor to consider in both clinical and preclinical investigations of cannabis use and its effects. Human cannabis use is primarily through inhalation, and previous research has demonstrated that the route of administration alters the pharmacokinetics of THC in humans (16, 17). A multitude of clinical studies investigating the pharmacokinetics of acute THC inhalation has been published, with diverse results

(16, 18-22). Due to differences in and lack of standardization across studies, maximum concentration (C_{max}) values of THC can differ drastically across studies, with mean C_{max} values ranging from 4 ng/mL (in whole blood) (20) to 163 ng/mL (in plasma) (18). Preclinical studies offer opportunity to control for many factors that cannot be controlled easily in clinical studies (*e.g.* environment, diet, previous drug exposure). However, the majority of rodent THC studies (both pharmacokinetic and phenotypic) use intraperitoneal (i.p.) injected THC rather than inhalation administration. Just as in clinical models, this can alter the pharmacokinetic profile of the compound, as well as its phenotypic effects. Recent years have seen increased investigation of inhaled THC in preclinical models (see (23-26) among others) but there is limited knowledge of how pharmacokinetic and pharmacodynamic properties may differ depending on sex, age, strain, and equipment.

Route of administration must be considered when looking at behavioral responses to THC. I.p. injected THC is subject to first-pass metabolism while inhaled THC is not, so the time course the psychoactive drug and its metabolites take through the body are altered depending on the route of administration. This could potentially effect when phenotypic effects are observed, and the magnitude of such effects. Altered locomotion is a common phenotype in many drugs of abuse, including THC. High doses of THC have been demonstrated to produce suppressed locomotion in preclinical literature of both i.p. injected and inhaled THC (23, 27-30), while there is some evidence of low doses of THC resulting in increased locomotor activity (31). The cannabinoid tetrad test, an established paradigm used in preclinical work to test if compounds have CB_1 agonist activity, includes locomotion as one of the four assessments (32, 33). Observing the effects of inhaled and i.p. injected THC on locomotion alongside the pharmacokinetic profiles offers a more substantial understanding of how different routes of administration may or may not lead to the same outcome.

The goal of the present study was to develop a simple THC inhalation model in rodents that produces similar levels of THC [C_{max}/Dose] to what is observed after human use of cannabis (23, 24, 34, 35).

2. Material and Methods

2.1 Animals:

Adult female Sprague Dawley (Taconic, Rensselaer, NY) rats 9-12 weeks old, were used in this study. All animals were single housed in a temperature-controlled environment on a 12-hour reverse light cycle (lights off 0900-1800). Food and water were provided *ad libitum* except during drug exposure. All experiments were conducted in compliance with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals and approved by the University at Buffalo Institutional Animal Care and Use Committee.

2.2 Drug:

THC was obtained from the NIDA Drug Supply Program. THC for inhalation was prepared in a 95% ethanol vehicle from a stock solution of 200 mg/mL. THC for i.p. injections was prepared in a vehicle solution of 1:1:18 ethanol:Kolliphor®:sterile saline, for a final concentration of 1 mg/mL THC, and i.p. injected at a volume of 2 μL/g.

2.3 Treatment:

2.3.1 Delta-9-tetrahydrocannabinol Inhalation: Rats were placed in a sealed inhalation chamber (2.5 gallon; 16.5x11.3x5.5 inches) for a 5-minute habituation period. THC solution was vaporized using a Volcano vaporizer (Storz and Bickel, Germany). THC solution (0.25 mL) was dispensed onto steel pads for final amounts of 10 or 40 mg. After allowing ethanol to fully evaporate, the steel pad was placed into the vaporizer, and the drug was vaporized (at heat setting 9, approximately 226 °C). Vaporized THC was collected in an 8 L plastic balloon. THC vapor was administered into each airtight chamber through a fitted adaptor which was then sealed, and a 10-minute exposure period followed. Exposures were conducted under a fume hood to eliminate any risk of exposure to the investigators. Animals were continuously monitored throughout inhalation. All animals were returned to their home cages before the start of testing.

2.3.2 Delta-9-tetrahydrocannabinol Intraperitoneal Injections: Animals were administered an i.p. injection of 2 mg/kg after body weight was taken and returned to their home cages before the start of testing.

2.4 Blood Collection:

Blood was collected at pre-determined timepoints of 5, 10, 20, 80, 160, 320, and 480 minutes. This time was either from the time of injection, or from the time the animals were removed from the 10-minute inhalation administration. Animals were rapidly anesthetized with 2% isoflurane, and blood was collected via tail vein. Each animal had blood collected at 1-2 timepoints, with no more than 1% of the animal's body weight being collected in total (total n=36). Collected blood was placed into a 1.5 mL microcentrifuge tube and allowed to clot at room temperature for 30-45 minutes. The sample was then centrifuged for 15 minutes at 4°C and 604 x g. Serum was aliquoted and placed into a −80°C freezer for storage until analysis.

2.5 Analysis of test samples using UPLC-MS/MS

2.5.1 Materials and Reagents: Commercially available standards (purity > 98%) for delta-9-tetrahydrocannabinol (THC), 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH), and 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC) were obtained from Cerilliant (Round Rock, TX, USA). Additionally, deuterated internal standard (purity >98%) (IS) delta-9-tetrahydrocannabinol-d3 (THC-d3) was also obtained from Cerilliant (Round Rock, TX, USA). LC-MS grade water, acetonitrile, isopropanol, methanol, and formic acid were sourced from Fisher Scientific (Fair Lawn, NJ, USA). Blank rat serum was obtained from Innovative Research, Inc. (Novi, MI, USA).

2.5.2 Instrumentation and Analytical Conditions: The ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) bioanalytical method was developed based on a previously validated method for use of detection of cannabinoids in low THC varieties of cannabis (36). A Waters Acquity Class-I UPLC coupled with a Xevo TQ-S Micro triple quadrupole mass spectrometer was used for separation and detection of cannabinoids and their metabolites. The system was controlled by MassLynx 4.2 with data processed using TargetLynx XS™ (Waters, Milford, MA, USA). Chromatographic

separation was achieved as previously described (36), with some modifications; namely, the gradient elution started at 18% A which was linearly decreased to 0% over 4.5 minutes before a steep return to the initial conditions for re-equilibration of the column. The sample injection volume was 4 μL.

2.5.3 Preparation of calibration and quality control samples: Two mix stock solutions (10 and 1 μg/mL) were prepared by combining the appropriate volume of 100 μg/mL primary stock of each compound to get the final concentrations, per compound. These mix stocks were then used to make eight working stock solutions (25, 50, 100, 250, 500, 1000, 1500, and 2500 ng/mL). Each calibration standard was prepared by spiking 18 μL of blank serum with 2 μL of the associated working stock to yield serum concentrations in the range of 2.5-250 ng/mL. The quality control samples were prepared from a different primary stock using the same method at the four following concentrations: 2.5 (lower limit of quantification (LLOQ)), 6 (low quality control (LQC)), 80 (medium quality control (MQC)), and 180 ng/mL (high quality control (HQC)).

2.5.4 Sample Preparation: Samples were thawed to room temperature prior to analysis. Each sample was mixed by vortex and 20 μL was subjected to a simple and fast protein precipitation method for the removal of endogenous substances and extraction. Test samples, blank, calibration, and quality control standards were quenched with 100 μL of methanol containing 0.05% formic acid and 10 ng/mL IS. The samples were vortex mixed for 5 minutes at 650 rpm before being transferred to a 96-well Millipore (Burlington, MA, USA) multiscreen Solvinert 0.45 μm filter plates. The samples were filtered by centrifugation at 850×g for 2 minutes at 4°C. The filtrate was then subjected to UPLC-MS/MS analysis.

2.5.5 Bioanalytical Method Validation: The Food and Drug Administration (FDA) guidelines for validation of bioanalytical methods were used to assess sensitivity, selectivity, linearity, carryover, accuracy, precision, recovery, and stability (37). The sensitivity of the method was determined by measuring the limit of detection (LOD) and the LLOQ. Selectivity was assessed by analyzing six different samples of blank rat serum to ensure the absence of interference at retention times of the analytes and IS. Calibration curves were created by plotting the analyte to internal standard peak area ratio against the nominal concentration in serum. The linear range of this method was 2.5-250 ng/mL and the linear fit was determined by 1/X weighing method for all sample runs. Carryover was assessed by running blank serum samples immediately following HQCs and comparing the peak analyte area in the blank to that of the LLOQ. Inter- and intra-day accuracy and precision were evaluated on three different days using QC samples at all four concentrations (n=6, each). Accuracy was evaluated by comparing the spiked concentration of the QC to the curve and precision was evaluated by comparing the observed values to one another. Recovery was assessed by comparing the analyte area in serum spiked with the compound before protein precipitation (pre-spike) or after protein precipitation (post-spike). The recovery was calculated as the ratio of the analyte area under the curve in the pre- versus post-spike samples. The possible matrix effect of serum was assessed by spiking the quencher solution (methanol containing 0.05% formic acid and 10 ng/mL IS) with the working stock solutions

used to generate the calibration curve and adding either 20 μL of water or blank serum. The ratio of the mean analyte area under the curve of the calibration standards was compared to determine the percent matrix effect. Stability was assessed for conditions that were most likely to occur during sample collection, storage, and analysis. This included benchtop, autosampler, freeze-thaw, and stock stability.

2.6 Locomotor Activity:

A separate cohort of adult female Sprague Dawley rats (n=15) were tested for locomotor activity in an OpenField (OF) arena (Coulbourn Instruments, Holliston, MA). All animals underwent a habituation session, followed by five THC (or vehicle) administration days, each separated by a 3-day abstinence period. OF testing and analysis was conducted as previously described (38). On experimental days, animals first underwent either the i.p. injection of vehicle or THC, or 10 minutes of vehicle or THC exposure. Immediately following vehicle or drug administration, animals were placed into the OF chamber for 45 minutes.

2.7 Data Analysis:

All graphs and statistical analyses were generated using GraphPad Prism 8 (San Diego CA). A p value <0.05 was deemed significant.

2.7.1 Pharmacokinetic Analyses: For the calculations of pharmacokinetic parameters, serum concentration-time data was subjected to non-compartmental analysis using Phoenix Version 8.3 (Certara, Princeton, NJ, USA). Maximum serum concentration (C_{max}) and time to reach C_{max} (T_{max}) were identified directly from concentration-time data for THC and its metabolites. A linear trapezoidal method was used the calculation of area under the serum concentration-time profile (AUC), and clearance (CL/F) was calculated as Dose/AUC. Serum concentrations found below the LLOQ were excluded from the pharmacokinetic analysis (39).

2.7.2 Locomotor Analyses: Two-way RM Analyses of the Variance (ANOVAs) were performed in GraphPad Prism 8 to analyze the open field data over time. One-way RM ANOVAs and paired t-tests were performed to analyze the totaled locomotor data. Post-hoc analyses were done with Tukey's multiple comparisons or Sidak's multiple comparisons tests if applicable.

3. Results

3.1 UPLC-MS/MS method validation:

The bioanalytical method was successfully validated for sensitivity, selectivity, linearity, carryover, accuracy, precision, recovery, matrix effect, and stability in serum. A representative chromatogram is shown in Figure 1.

3.2 Sensitivity and selectivity:

The LOD was found to be 1 ng/mL for each analyte as the response at this concentration was consistently a signal to noise ratio > 3, while the LLOQ was selected to be 2.5 ng/mL

for each analyte as it showed a signal to noise ratio > 10 with accuracy and precision within 20 %. When six different blank rat serum samples were analyzed, no endogenous substances were eluted at the retention time of the analytes or IS.

3.3 Linearity and carryover:

Calibration curves for the range of 2.5-250 ng/mL were found to be linear for all runs with the coefficient of determination value greater than 0.99 for all runs, indicating an adequate linear fit. Carryover analysis of a blank sample immediately following an HQC of 180 ng/mL produced an analyte peak area <20% of the LLOQ for the analytes and <5% of IS, demonstrating negligible carryover.

3.4 Accuracy and precision:

Table 1 shows the results of intra- and inter-day accuracy and precision evaluations carried out on three different days using n=6 at each QC level. All values were within the acceptable limits of 15% of the target concentration, or 20% for the LLOQ.

3.5 Recovery and matrix effect:

The mean recovery from serum was 95.6 ± 6.2, 91.1 ± 8.4, and 90.4 ± 12.0% for THC, THC-COOH, and 11-OH-THC, respectively. The percent recovery was consistent across QCs of different concentrations and always within 15%. The mean analyte response in serum across the various concentrations was 100.4 ± 8.8, 98.6 ± 10.8, and 94.4 ± 11.6% of the response in water for THC, THC-COOH, and 11-OH-THC, respectively, indicating the absence of matrix effects in serum.

3.6 Stability:

The stability of the analytes in serum was assessed in conditions most likely to occur during sample preparation and storage. The benchtop, autosampler, freeze-thaw, and long-term stock stability were assessed. The analytes were stable in serum for up to 4 hr at room temperature. Extracted samples were stable in the autosampler for 48 hr at 10° C. The compounds were stable in serum for up to two freeze-thaw cycles (thawed to room temperature after storage at −80° C). Stocks were stable after two months storage in −20°C.

3.7 Dose Calculation:

Dose was estimated according to the equation (34):

$$\text{Dose} = C \times T \times RMV \times (DF/BW)$$

where C is the concentration of drug in chamber (mg/L), T is the time of exposure (min), RMV is the respiratory minute volume (L/min), DF is the deposition factor, and BW is the body weight (kg). The RMV for female Sprague Dawley rats weighing approximately 0.225 kg is 0.075 L/min (40). The deposition factor for rodents is 0.10. The average body weight of the female rats was 0.225 kg. To calculate the concentration of drug in the chamber the following equation was used:

$$\frac{\text{concentration of solution (mg/ml)} \times \text{volume of solution vaporized (mL)} \times \%\ \text{recovery after vaporization at } 226^{\circ}\text{C}}{\text{volume of the chamber (L)}}$$

The % recovery after vaporization was selected from (41). For the 40 mg/mL solution this value would be 0.6 mg/L. For the 160 mg/mL solution the calculated concentration value would be 2.5 mg/L. Once the concentration of drug in the chamber was calculated the estim0.75ated dose delivered was determined to be 0.19 mg/kg for the 40 mg/mL solution and 0.76 mg/kg for the 160 mg/mL solution.

To determine the human equivalent dose (HED) the following equation was used (42):

$$\text{HED (mg/kg)} = \text{Animal Dose (mg/kg)} \times (\text{weight animal (kg)} / \text{weight human(kg)})^{0.33}$$

The value used for animal weight was 0.225 kg and for human weight was 75 kg. The HED for 0.19 mg/kg dose would be 0.028 mg/kg and for the 0.76 mg/kg dose would be 0.11 mg/kg.

3.8 Pharmacokinetics:

Concentration-time profiles for THC, 11-OH-THC, and THC-COOH after inhalation or i.p. administration of THC are shown in Figure 2. The 2 mg/kg i.p. dose of THC resulted in an average C_{max} of 107.7±21.9 ng/mL after 10 minutes post-injection, while 0.19 mg/kg inhaled THC resulted in an average C_{max} of 43.3±7.2 ng/mL at 10 minutes after the conclusion of inhalation, and 0.76 mg/kg inhaled THC resulted in an average C_{max} of 71.6±22.5 ng/mL 5 minutes after inhalation was completed. Increasing the inhaled THC dose from 0.19 mg/kg to 0.76 mg/kg resulted in a 1.6 fold-change of C_{max} values. For each timepoint on the THC curves, n=3-4 (Figure 2A).

The results of the serum analysis were utilized to estimate the pharmacokinetic parameters for each of the three THC doses administered, with results presented in Table 2. The 2 mg/kg i.p. THC dose had a greater C_{max} than either the 0.19 mg/kg or 0.76 mg/kg vaporized THC dose, as well as greater total drug exposure. While both the 2 mg/kg i.p. and 0.19 mg/kg vaporized doses had peak concentrations 10 minutes post administration, the 0.76 mg/kg vaporized dose observed peak concentrations at 5 minutes after exposure. Following the i.p. dose, a slightly lower elimination rate was observed than the vaporized doses, and a longer half-life as well. While the apparent volume of distribution with the 2 mg/kg i.p. dose was higher than the 0.19 mg/kg vaporized dose, it was lower than the 0.76 mg/kg vaporized dose. Similarly, the volume of distribution at steady state was much higher in the 0.76 mg/kg vaporized dose than the 0.19 mg/kg vaporized dose. Again, clearance of the 2 mg/kg i.p. dose was greater than in the 0.19 mg/kg vaporized dose, but lower than the 0.76 mg/kg vaporized dose.

The primary metabolite, 11-OH-THC, had an average C_{max} of 84.2 ± 19.3 ng/mL 20 minutes after a 2 mg/kg i.p. injection, while 0.19 mg/kg inhaled THC resulted in an average C_{max} of 14.4±1.4 ng/mL 5 minutes after the conclusion of inhalation, and 0.76

mg/kg inhaled THC resulted in an average C_{max} of 35.6±7.4 ng/mL at 5 minutes after inhalation was completed. For each time point on the 11-OH-THC curves, n=1-3; 11-OH-THC in multiple test samples fell below the LLOQ, and were therefore excluded from the pharmacokinetic data analysis (Figure 2B).

Another phase I metabolite, THC-COOH had an average C_{max} of 87.0 ± 21.8 ng/mL 160 minutes after the 2 mg/kg i.p. injection, while 0.19 mg/kg inhaled THC resulted in an average C_{max} of 20.9 ± 4.1 ng/mL 10 minutes after inhalation, and 0.76 mg/kg inhaled THC resulted in an average C_{max} of 19.9 ng/mL 20 minutes after inhalation was completed. For each time point on the THC-COOH curves, n=0-4; plasma concentrations fell below LLOQ and were excluded from the data analysis (Figure 2C). C_{max} values for all compounds analyzed are summarized in Table 3.

3.9 Locomotor Activity:

The effect of i.p. injected and inhaled THC on locomotor activity was tested in an OpenField arena. Four parameters were measured: floor plane distance, margin distance, center distance, and vertical plane entries (rearing events). For each parameter, a Two-Way RM ANOVA was performed, with Time and Treatment as factors, and the appropriate post-hoc analysis (Sidak's multiple comparison for the i.p. test, and Tukey 's multiple comparison for the inhalation test). Total distances/entries were analyzed with One-way RM ANOVAs or paired T-tests.

For i.p. injected THC, there was a significant effect of Time on floor plane distance [$F(3.978,71.61)=33.76$, $p<0.0001$], margin distance [$F(3.688,66.38)=12.00$, $p<0.0001$], and center distance [$F(4.672,84.09)=18.88$, $p<0.0001$; Figure 3A, C-D]. For vertical plane entries, there was a significant effect of Time [$F(4.782,86.07)=27.43$, $p<0.0001$] and Treatment [$F(1,18)=4.898$, $p=0.0400$; Figure 3B]. Analyzing total entries, there was a significant reduction in vertical plane entries after THC administration when compared to vehicle [$t(9)=2.700$, $p=0.0244$; Figure 3B].

For the vaporized THC, there was a significant effect of Time [$F(5.280, 169.0)=89.89$, $p<0.0001$] and an interaction of Time x Treatment [$F(16, 256)=3.059$, $p<0.0001$] on floor plane distance. Post-hoc comparisons showed that at 20 minutes, the 0.76 mg/kg THC dose subjects had significantly higher floor plane distance compared to the vehicle group ($p=0.0161$; Figure 4A). For vertical plane entries, there was a significant effect of Time [$F(3.662,113.5)=50.10$, $p<0.0001$; Figure 4B]. There was a significant effect of Time [$F(5.159,165.1)=20.32$, $p<0.0001$] and a significant interaction of Time x Treatment [$F(16,256)=2.430$, $p=0.0020$] for margin distance (Figure 4C). There were no significant post-hoc comparisons. For center distance there was a significant effect of Time [$F(5.325,170.4)=43.16$, $p<0.0001$] and a significant interaction of Time x Treatment [$F(16,256)=2.148$, $p=0.0071$]. Post hoc analysis found that at 20 minutes, the 0.76 mg/kg vaporized THC group had significantly higher center distance travelled compared to the vehicle group ($p=0.0459$; Figure 4D). Analyzing total entries, there was a significant effect of treatment on vertical plane entries [$F(1.177,16.48)=4.648$, $p=0.0410$]. Tukey's multiple comparisons test showed that 0.19 mg/kg INH resulted in significantly decreased vertical plane entries compared to vehicle ($p=0.0003$).

4. Discussion

Our results demonstrate a model for THC inhalation in female rats at two different doses, in comparison to an i.p. injected dose within the well-established range used in preclinical THC research (24, 27, 43-45). The pharmacokinetic results obtained for our i.p. study are comparable to previous i.p. THC pharmacokinetic studies in rodents (46, 47). However, few comprehensive time-course studies of i.p. injected THC in rats exist in the literature, which further emphasizes the need for understanding the pharmacokinetic properties of THC in preclinical models, regardless of route of administration. Compared to our i.p. reference dose of THC, we found that both inhaled doses of THC resulted in more rapid absorption and metabolism of THC, as well as lower C_{max} values. This is supported by our dose calculations, which found that the low dose of inhaled THC is approximate to a 0.19 mg/kg dose of THC, while the higher dose of inhaled THC is approximate to a 0.76 mg/kg dose of THC. While the two different doses of inhaled THC resulted in a 1.6-fold change of C_{max} values, the total THC exposure was largely equivalent L (87.0 hr*ng/mL vs. 94.4 hr*ng/mL). This may reflect a limitation to controlling the amount of drug delivered in this route of administration compared to i.p. injection in a rodent model, as body weight and respiratory rate can vary in individual animals. As this paradigm delivers vapor to the exposure chamber rather than directly to the rat, this may be a small limitation but avoids the stress of restraining a subject to directly infuse the vapor in the nose/mouth. This approach therefore provides important data on the pharmacokinetic and pharmacodynamic response to vapor THC in female rats without the confound of restraint.

In addition to quantifying THC levels in serum, we also quantified two metabolites, 11-OH-THC and THC-COOH. The primary metabolite of THC, 11-OH-THC, has similar psychoactive properties as THC, and is therefore important to consider when investigating the behavioral effects of THC in preclinical models. Another metabolite THC-COOH, while inactive, and therefore having no psychotropic activity to consider in behavior analysis, is often used in cannabis drug testing in clinical settings. THC-COOH remains detectable in the bloodstream for longer time periods in humans, often 7 days or more after administration (18, 48). Metabolism of THC is mediated by the Cytochrome P450 (CYP) family of enzymes in both humans and rats (49); differences in CYP-mediated metabolism have been observed across species (50, 51), which must be considered when comparing preclinical THC inhalation models with the available clinical publications. Our inhaled THC serum concentration-time curves show rapid absorption of THC into the systemic circulation with a rapid decline in both THC and 11-OH-THC levels, while THC-COOH concentrations remain steady throughout (Figure 2). Previous THC inhalation models in rats have seen similar results, with peak THC concentrations immediately following vapor exposure with rapid elimination (23, 26, 34, 52). Our results for the 0.76 mg/kg vaporized THC specifically produced very similar C_{max} and T_{max} values as Baglot et al., one of the only other studies looking at THC inhalation in female Sprague Dawley rats; similarities in time course and T_{max} continued when comparing results for 11-OH-THC levels as well (26). Differences may therefore be attributed to difference in inhalation paradigm. Additionally, our results are similar to those seen in a 2018 clinical pharmacokinetic study, which also observed in their

acute inhalation analysis that the primary metabolite, 11-OH-THC, was up to 30% of THC, and there was a greater exposure to the THC-COOH metabolite than to THC (22).

To accompany the pharmacokinetic data collected, we additionally tested the effect of each THC dose on locomotor activity. Locomotion is a commonly tested parameter to measure the phenotypic effect of THC, and is grouped into the classic “tetrad” test, which is commonly used to determine if compounds have CB_1 agonist activity (32, 33). We observed minimal effect of the 0.76 mg/kg inhaled THC on horizontal distance traveled or altered thigmotaxis over a 45-minute period, but we did see reduced rearing activity for both the i.p. injected THC dose (Figure 3), as well as the lower inhaled THC dose (Figure 4). Previous studies looking at comparable doses of i.p. injected THC have seen similar results: horizontal locomotion is often not effected until doses at or exceeding 15 mg/kg (27-29). Other studies looking at acute inhaled THC have described inconsistent results in horizontal locomotion (23-25, 30). Suppressed locomotion was observed in studies with higher blood concentration levels of THC (30), or in paradigms that did not also determine blood concentration values of THC (23), but additional studies with varying doses and blood concentration levels of THC have observed little to no effect of inhaled THC on locomotor activity (24, 25).

There is evidence of THC having a biphasic effect in rodents, including on locomotor measures, where low doses can actually increase locomotor activity, while high doses will create the hypolocomotor effect more commonly observed (31). This may explain the slightly heightened locomotion seen 20 minutes into the open field session for the 0.76 mg/kg cohort, in comparison to vehicle (Figure 4A). This effect could reflect max THC and/or 11-OH-THC levels in the brain; Hložek et al. saw peak THC brain levels 15 minutes after THC inhalation in males (35), while Baglot et al. saw peak THC brain levels at 30 minutes (26). The study from Katsidoni et al. also saw increased rearing activity at the low dose of THC (31). Conversely, Bruijnzeel and colleagues found that after inhaled cannabis, rearing activity was decreased in rats, along with decreased horizontal locomotion and higher blood plasma levels of THC than the present study (30). Thus, the doses used in our study showed decreased rearing activity, and minimal effects on floor plane activity. Our results suggest that rearing activity is more sensitive to acute vaporized THC administration than horizontal locomotion (Figures 3 and 4).

Additionally, THC administration has been shown to produce anxiolysis at low doses in rodents (30, 53-55). Altered thigmotaxic activity, the tendency for rodents to avoid unsafe spaces (i.e., the center of the open field), can indicate anxiolytic effects of administered drugs (56). Our inhaled 0.76 mg/kg THC dose did see increased center distance travelled at 20 minutes into the session compared to the vehicle group, which could indicate a small anxiolytic effect corresponding to this dose (Figure 4D). Our i.p. injected THC dose of 2 mg/kg may fall into a window that does not affect anxiety, or it could speak to a difference due to route of administration.

In 2014, Manwell and colleagues utilized a similar approach as used here, with a vaporizer and THC solution diluted in ethanol, albeit at lower doses and looking at male rats rather than females (24). Information on female pharmacokinetics is important for future prenatal

THC inhalation research. This study collected blood samples at two timepoints, therefore not covering the entire absorption, distribution, metabolism, and elimination process but rather capturing a small window of data, and each timepoint only has data from a single subject. Their data suggests that there may be some sex differences in how the inhaled THC is metabolized, but without more data it is difficult to make an exact comparison. Another study from 2017 used the vaporizer as well, adapting it to expel directly into the animal chamber rather than using the balloons that are used with the vaporizer in humans (35). This alters the amount of vapor being expelled into the chamber compared with our results and Manwell et al.'s, but is otherwise comparable from an apparatus viewpoint. This modification seems to have allowed for greater C_{max} of THC in serum, but the pharmacokinetic profile is comparable. Other studies have begun to use e-cigarette vaporizers (23, 26, 57, 58), which may be advantageous given the rise in consuming THC with "vapes," but are more complex and require significantly more variables to be tested. These custom apparatuses often have multiple factors to consider when comparing across studies, including the duration of each puff, the temperature at which the e-cigarette vaporizes the THC, and the effect the vehicle solution may have on phenotypic response. Overall, the pharmacokinetic data from an e-cigarette mode of delivery shows similar trends to that seen from our results and others (23, 26, 34, 52, 57, 58). One notably different study from Nguyen and colleagues achieved mean C_{max} levels of 303 ng/mL THC in female Sprague Dawley rats at 5 minutes post-30 minute inhalation of 200 mg/mL THC (59). This may be attributed to the high concentration of THC solution coupled with the extended period of exposure compared to our model and other models. Here, we present an alternative inhalation paradigm that is advantageous to modified e-cigarette models which require more complex design (multiple components and custom software are often necessary to ran these apparatuses) and/or more costly equipment, while still capturing the full plasma concentration-time profile of THC in serum out to 320 minutes post-inhalation at multiple doses, as well as representative phenotypic data, in female rats. To our knowledge, this is the first study examining the use of the Volcano Vaporizer for a THC inhalation paradigm in female rats.

The most common form of cannabis consumption in human users is inhalation, both for recreational and medicinal purposes (60, 61). Multiple clinical studies have looked at the pharmacokinetics of acute THC use in humans, both administered as cannabis or as an isolated compound (16, 18-22). These studies tend to vary regarding important demographic factors such as subject age, sex, and cannabis use history, as well as drug formulation (smoking vs vaping), dose of THC administered, and duration of exposure. This results in a wide variety of THC C_{max} values in blood, plasma, and/or serum, from 4 ng/mL (20) to 162 ng/mL (18) just in these studies mentioned. While this may reflect the differences in smoking activity in the general population, it can make it difficult to make any solid conclusions from a clinical research standpoint. In addition to different smoking habits, cannabis in the United States, as well as other countries, has seen increased potency in recent years, which can further complicate research focused on specific doses of THC, both preclinical and clinical (62, 63). Finally, individual subjects can have vastly different pharmacokinetic results within the same study conditions; one study found that 6 subjects all administered the same dose of THC under the same regime had plasma C_{max} values ranging

from 70-280 ng/mL, further emphasizing the large variability between not only studies, but individuals (18). The authors concluded that this variation may have been due to individual differences in inhalation (i.e., inhalation volume, duration, and frequency). A clinical study performed at a similar dose to those used here showed plasma concentration C_{max} ranging from 73.81 ± 63.09 ng/mL comparable to the C_{max} values achieved in this study (64).

Use of preclinical rodent models allows for control of some factors that may complicate clinical research, such as route of administration. As previously mentioned, a large majority of current THC preclinical research is focused on i.p. injection of THC, which is not representative of the common clinical route of administration. Use of inhaled THC can instead control for the effect that route of administration can have on the pharmacokinetics of and phenotypic response to the drug. Drugs i.p. injected are subject to first-pass metabolism via the liver (65, 66), whereas inhaled drugs bypass this step. It therefore is advantageous to match the route of administration as best as possible in preclinical models of cannabis use to the prevalent mode of use in humans, inhalation. While oral consumption is another common route of administration, it was not the focus of this study. It has recently been demonstrated in a preclinical model that the route of administration can indeed influence the phenotypic response to the drug. A 2021 publication (67) found that administering THC in multiple different routes of administration had an effect on the potency of drug and the onset and duration of effect in a drug discrimination paradigm, further supporting the notion that preclinical THC research would benefit from matching the inhalation route of administration. Our serum THC concentration results clearly demonstrate the effect route of administration has on the pharmacokinetic properties of THC; both inhaled doses of THC saw increased rates of absorption and quicker clearance from the bloodstream as compared to the i.p. injected THC (Figure 2A).

As with all research studies, there are some limitations in the work presented here. This paper primarily focuses on female rats, and it has been previously established that the pharmacological properties of THC in rodents may be dependent on both sex and strain (68-70). While our focus for this study was examining female rats as a first step for additional work, investigating the same paradigm in male rats may be valuable in the future. Additionally, it may be useful to investigate the effects of chronic THC administration prior to collecting test blood samples, to elucidate the consequences chronic use may have on the pharmacokinetic properties of THC in rats. There has been clinical research demonstrating that chronic THC consumption can result in detection of THC and/or metabolites for a significantly longer time period than seen in infrequent users (48, 71, 72), even though prolonged psychological effects may not be seen. As previously discussed, clinical research investigating inhaled THC has used multiple formulations (vaped as an isolated compound or smoked within the *cannabis* plant); there is evidence to suggest the pharmacokinetics of these two methods of inhalation are comparable (73), but more attention to possible differences is still needed. As more preclinical work with inhaled THC is reported, distinguishing differences will become clearer. The present study included locomotor testing for 45-minute periods after THC administration; it may be beneficial to look at time periods extending out multiple hours after exposure, as there may be longer term effects of THC and metabolites as they remain in the bloodstream for multiple hours. Finally, this rodent model of passive inhalation cannot be directly compared with active inhalation models in clinical

research, as each animal will have unique breathing rates within the inhalation chamber. A similar limitation does exist in human models, however, where each subject may have different inhalation volumes and hold times in even the most regulated testing regimes.

5. Conclusion

Here we present a validated UPLC-MS/MS method for the quantification of THC, 11-OH-THC, and THC-COOH in rat serum, as well as a preclinical inhalation model for further THC research. This model is a simple and safe method for the administration of inhaled THC which could be implemented in both acute and chronic preclinical THC research going forward. The serum C_{max} values for both our low and high dose of inhaled THC (43.3 ng/mL and 71.6 ng/mL, respectively) were found to be within a range observed in clinical studies investigating smoked or vaped cannabis (mean C_{max} ranges from 4-162 ng/mL) at similar doses (16, 18-21), and could be utilized in future phenotypic and neurological studies investigating the effects of THC consumption in rats.

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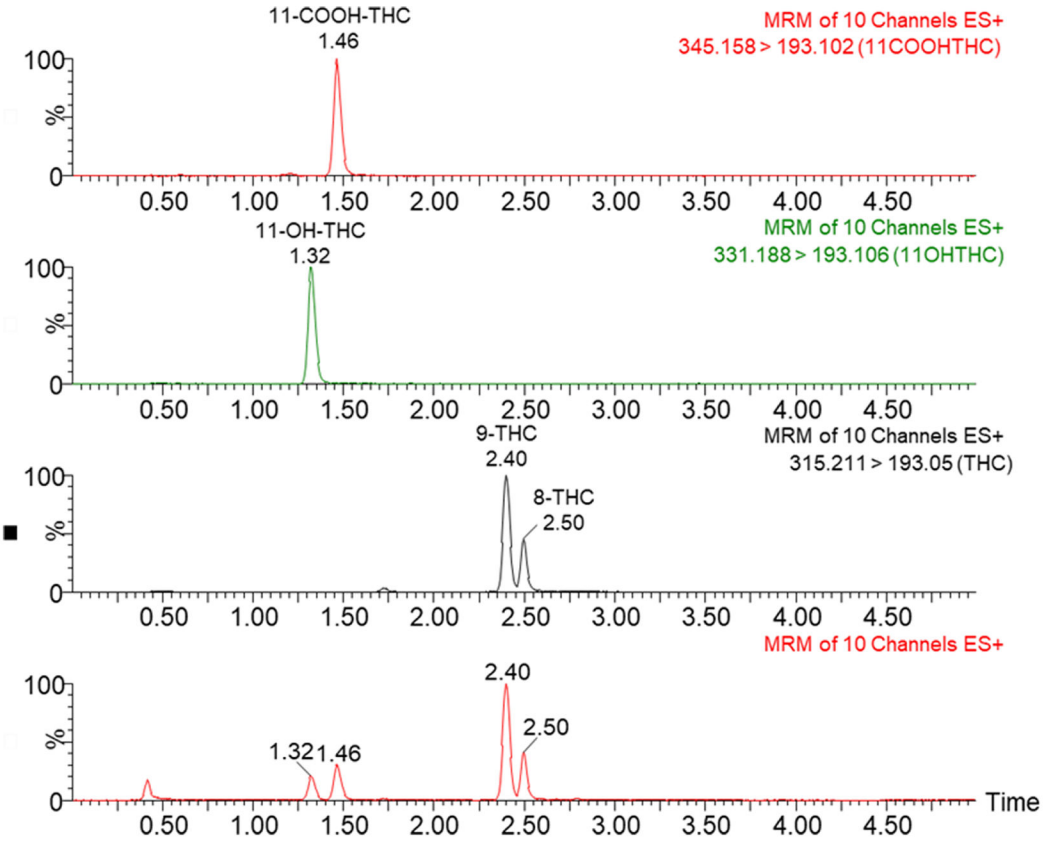

Figure 1:

Representative chromatograms for THC and its metabolites in positive ionization mode. Retention times (in minutes) are labeled above each peak.

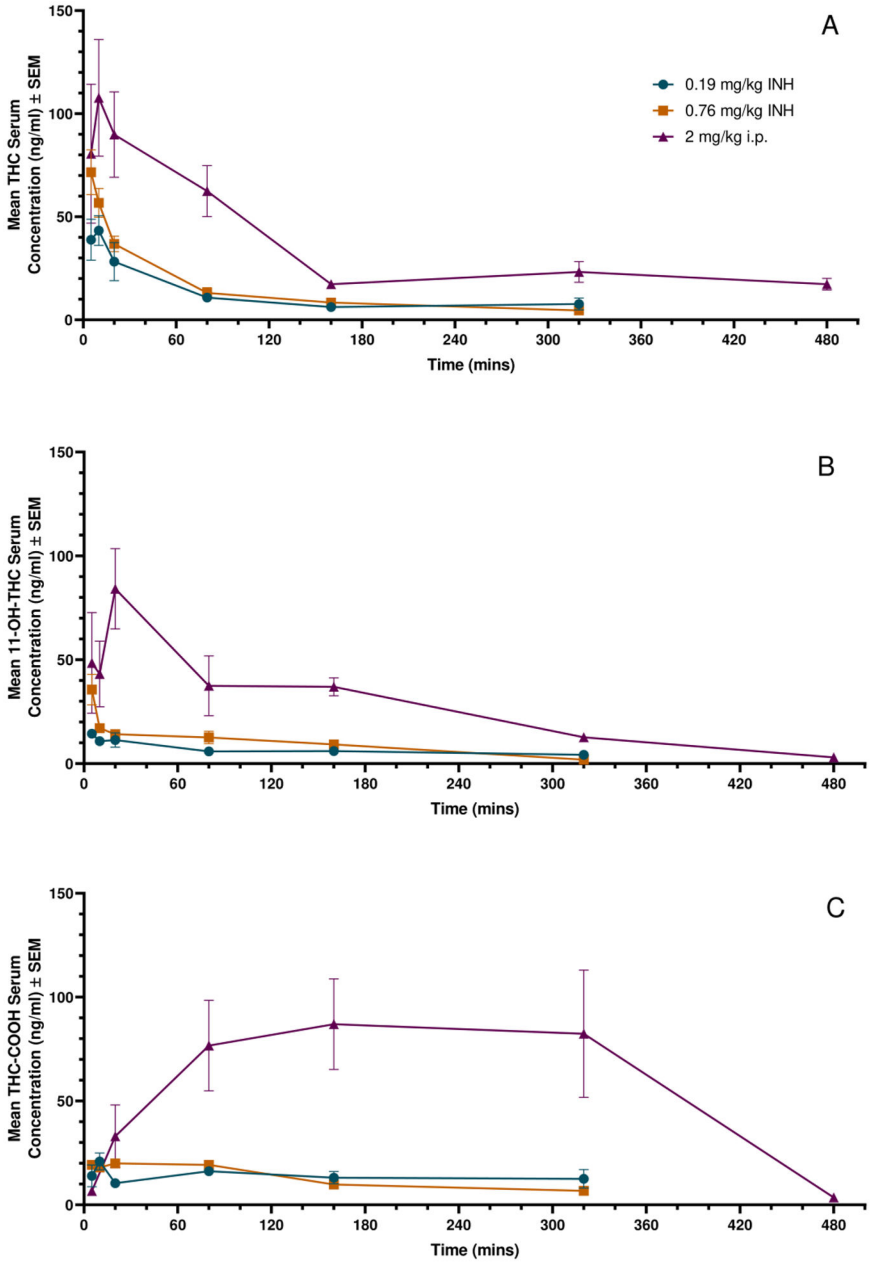

Figure 2: Mean serum concentration-time profiles of THC and its metabolites after inhalation (INH) and i.p. administration of THC in rats.

Data represented as Mean ± SEM. A: THC in serum after 0.2 or 0.76 mg/kg inhaled THC or 2 mg/kg i.p. injected THC, n=3-4 at each time point. B: 11-OH-THC in serum after 0.2 or 0.76 mg/kg inhaled THC or 2 mg/kg i.p. injected THC, n=1-4 at each time point. C: THC-COOH after 0.2 or 0.76 mg/kg inhaled THC or 2 mg/kg i.p. injected THC, n=1-4 at each time point.

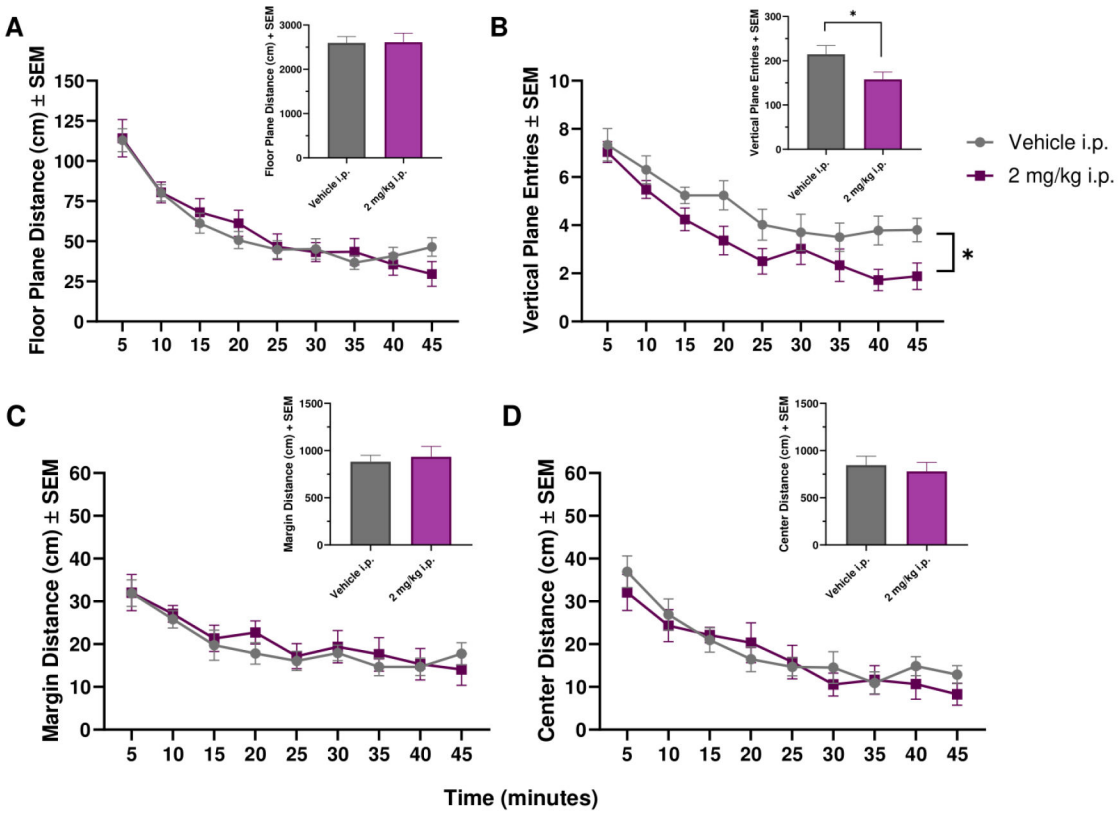

Figure 3: Mean locomotor activity of THC after i.p. administration in female Sprague Dawley rats.

Mean floor plane distance, vertical plane entries, margin distance, and center distance after administration of i.p. vehicle or 2 mg/kg i.p. THC over a 45 minute period. n=10 for each group. * indicates $p<0.05$.

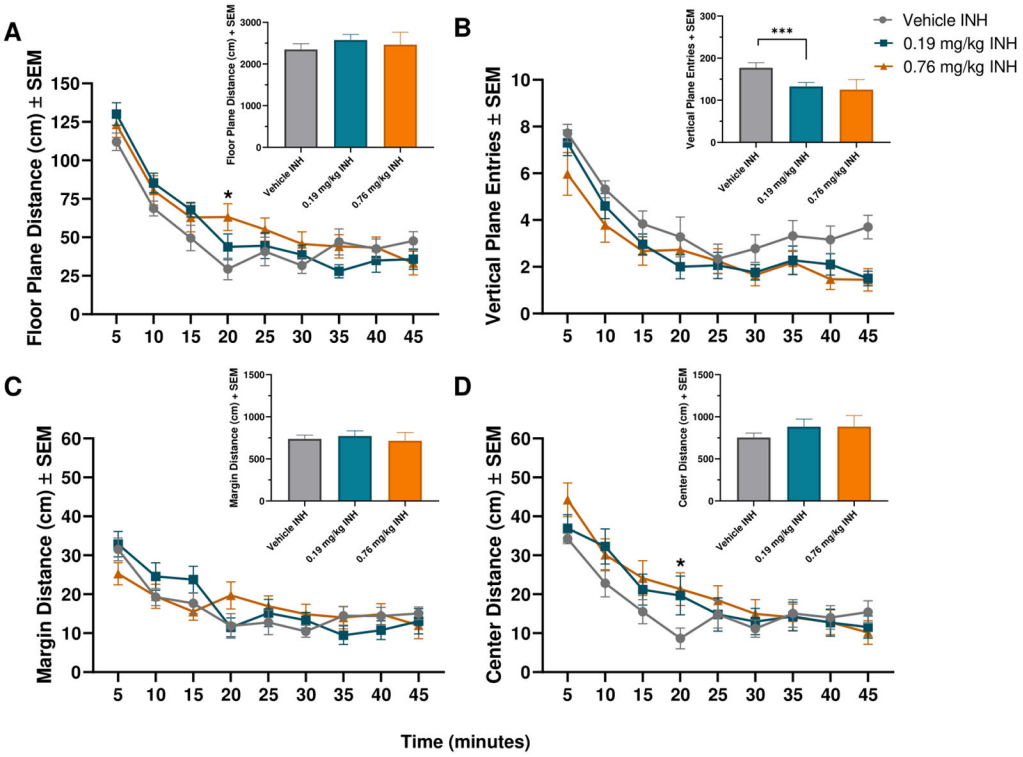

Figure 4: Mean locomotor activity after inhaled THC in female Sprague Dawley rats.

Mean floor plane distance, vertical plane entries, margin distance, and center distance after administration of inhaled vehicle, 0.19 mg/kg, or 0.76 mg/kg inhaled THC. n=10-15 for each group. * indicates $p<0.05$, *** indicates $p<0.001$. Panels A and D, * indicates significant difference between vehicle and 0.76 mg/kg THC groups.

Table 1.

Accuracy and precision of the assay in rat serum. n=6 at each concentration

Rat Serum						
THC						
Concentration (ng/mL)	**Intra-day**			**Inter-day**		
	Measured Concentration (mean ± SD; ng/mL)	**Precision (% RSD)**	**Accuracy (% bias)**	**Measured Concentration (mean ± SD; ng/mL)**	**Precision (% RSD)**	**Accuracy (% bias)**
2.5	3.0 ± 0.5	15.3	19.4	2.8 ± 0.4	12.7	13.4
6	5.2 ± 0.6	11.5	−14.2	5.6 ± 0.6	11.3	−7.3
80	84.4 ± 5.6	6.6	5.5	82.3 ± 8.1	9.8	2.9
180	161.6 ± 10.5	6.5	−10.2	159.3 ± 14.8	9.3	−11.5
THC-COOH						
Concentration (ng/mL)	**Intra-day**			**Inter-day**		
	Measured Concentration (mean ± SD; ng/mL)	**Precision (% RSD)**	**Accuracy (% bias)**	**Measured Concentration (mean ± SD; ng/mL)**	**Precision (% RSD)**	**Accuracy (% bias)**
2.5	2.9 ± 0.3	8.6	17.3	2.7 ± 0.4	13.0	9.1
6	5.3 ± 0.5	9.7	−11.6	5.4 ± 0.5	8.9	−10.4
80	85.6 ± 4.3	5.0	7.0	81.5 ± 4.1	5.1	1.8
180	164.3 ± 8.7	5.3	−8.8	164.2 ± 10.9	6.7	−8.8
11-OH-THC						
Concentration (ng/mL)	**Intra-day**			**Inter-day**		
	Measured Concentration (mean ± SD; ng/mL)	**Precision (% RSD)**	**Accuracy (% bias)**	**Measured Concentration (mean ± SD; ng/mL)**	**Precision (% RSD)**	**Accuracy (% bias)**
2.5	2.2 ± 0.3	12.3	−10.4	2.1 ± 0.2	7.8	−14.7
6	5.3 ± 0.5	8.6	−11.7	5.5 ± 0.5	9.3	−8.6
80	79.5 ± 10.7	13.5	−0.7	82.7 ± 10.2	12.4	3.4
180	153.5 ± 7.7	5.0	−14.7	154.6 ± 14.3	9.3	−14.1

Table 2:

Pharmacokinetics parameters of THC in female Sprague Dawley rats. Abbreviations: C_{max}, maximum serum concentration; AUC_{inf}, area under the curve from 5 minutes post-exposure to infinity; k_e, elimination rate constant; $t_{1/2}$, terminal half-life; V_d/F, volume of distribution; V_{ss}, volume of distribution at steady state; CL or CL/F, clearance; **, indicating apparent volume of distribution or clearance.

Parameter	2 mg/kg i.p.	0.19 mg/kg vaporized	0.76 mg/kg vaporized
C_{max} (ng/mL)	107.7	43.3	71.6
T_{max} (min)	10	10	5
AUC_{inf} (hr*ng/mL)	364.4	87.0	94.4
k_e (1/hr)	0.2	0.3	0.3
$t_{1/2}$ (hr)	3.4	2.2	2.7
V_d/F (L/kg)**	26.9	7.4	33.0
V_{ss} (L/kg)	-	8.6	24.4
CL or CL/F (L/hr/kg)**	5.5**	2.3	8.5

Table 3:

Mean peak serum concentrations (C_{max}) of THC and its metabolites after inhalation (INH) and i.p. administration of THC in rats (n=3 for each value, mean ± SEM).

THC Dose and route of administration	C_{max} (ng/mL)		
	THC	11-OH-THC	THC-COOH
2 mg/kg (i.p.)	107.7 ± 21.9	84.2 ± 19.3	87.0 ± 21.8
0.19 mg/kg (INH)	43.3 ± 7.2	14.4 ± 1.4	20.9 ± 4.1
0.76 mg/kg (INH)	71.6 ± 10.9	35.6 ± 7.4	19.9 ± 2.2