**FINAL SCRIPT: APPROVED FOR FILMING**

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# Title: Development of a Preclinical Inhalation Model to Test Vaporized Cannabis Distillates


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# Author Questionnaire

**1. Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

**2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

**3. Filming location:** Will the filming need to take place in multiple locations? **No**

**Current Protocol Length**
Number of Steps: 18
Number of Shots: 41

# Introduction

***Videographer: Obtain headshots for all authors available at the filming location.***

1.1. **Roham Gorgani:** We explore how inhaled cannabis vape products impact lung physiology by combining traditional molecular biology techniques and emerging computational techniques to gain insights on how vaping changes lung function.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1*

What are the most recent developments in your field of research?

1.2. **Roham Gorgani:** We have shown that cannabis vapor elicits transcriptional changes in lung epithelial cells similar to cannabis smoke, suggestive of damage. Also, mechanistic insights into vaping-related lung injury are emerging, particularly with THC products containing vitamin E acetate.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

1.3. **Roham Gorgani:** Most studies on THC-containing products rely on non-inhalation methods such as oral gavage and injections, which offer valuable insights but differ from typical human use. Our goal is to model cannabis exposure in a more physiologically relevant manner with inhalation.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.9*

What are the current experimental challenges?

1.4. **Roham Gorgani:** The potency of new cannabis products provides challenges in dosing. We want to mimic human use patterns and achieve physiologically relevant doses without adverse effects in the mice.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.7*

What significant findings have you established in your field?

1.5. **Roham Gorgani:** We establish a standardized exposure dose that delivers physiologically relevant doses of THC to the mice without adverse behavioral effects.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.5*

***Videographer: Obtain headshots for all authors available at the filming location.***

**Testimonial Questions :**

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Roham Gorgani:** With frequent new lab members, this JoVe publication will streamline training and improve productivity by standardizing our exposure protocol. We also hope it fosters collaborations by enabling others to study additional organs of interest, and serves as a citable resource in future publication from our lab and the inhalation toxicology community.

   1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

*Videographer: Please film the testimonial*

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at McGill University

# Protocol

**2. System Setup, Calibration, and Animal Acclimation for Controlled E-Cigarette Exposure in Mice**

**Demonstrator:** Roham Gorgani

2.1. To begin, assemble all components of the system **[1]**. Insert a closed tube into the buffer chamber of the puffing pump to prevent leakage **[2]**.

2.1.1. WIDE: Talent assembling the components of the puffing system according to the schematic in the reference figure.

2.1.2. Shot of a closed tube being inserted into the buffer chamber of the puffing pump.

2.2. Turn on the system and launch the software **[1]**. Select the **Experimentation Sessions** module **[2]**.

2.2.1. Talent switching on the power to the puffing system and launching the associated software.

2.2.2. SCREEN: 2025-04-11-15-52-04.mkv 00:09-00:16

2.3. Select **New Study** to begin a new experimental session **[1]**. Define the experimental groups and subjects to be studied **[2]**. Then choose the experimental template that matches the desired exposure regimen **[3]**.

2.3.1. SCREEN: 2025-04-11-15-52-04.mkv 00:17-00:22

2.3.2. SCREEN: 2025-04-11-15-52-04.mkv 01:54-02:26

*Video Editor: please speed up the video*

2.3.3. SCREEN: 2025-04-11-15-52-04.mkv 02:40-02:50

2.4. In the Session Properties window, complete the Operator section to document the equipment usage **[1]**.

2.4.1. SCREEN: 2025-04-11-15-52-04.mkv 02:52-03:00

2.5. For calibration, select the **Desired Channel** in the software **[1]**. Follow the steps outlined in the operating software **[2]**.

2.5.1. SCREEN: 2025-04-11-15-52-04.mkv 03:12-03:20

2.5.2. SCREEN: 2025-04-11-15-52-04.mkv 03:20-03:36

2.6. Next, select the **Desired Pump** in the software **[1]**. Perform the system flow test by following the operating software instructions **[2]**. Confirm that flow is directed toward the rotameter **[3-TXT]**. Cancel any prompts to start data recording if the experiment is not ready to begin **[4]**.

2.6.1. SCREEN: 2025-04-11-15-52-04.mkv 03:38-03:45

2.6.2. SCREEN: 2025-04-11-15-52-04.mkv 03:45-03:57
2.6.3. Shot showing the rotameter and flow direction indicator. **TXT: If the system fails test, clean all components and tubing and repeat test**
2.6.4. SCREEN: 2025-04-11-15-52-04.mkv 03:58-04:02

2.7. ~~For the creation of puff profiles, first create a bias flow profile set at 2 liters per minute to provide adequate aeration throughout the experiment **[1-TXT]**. Create puff profiles for e-cigarette exposure. Modify the puff to deliver a volume of 78 milliliters over a 2.4-second duration at 1, 2, or 4 puffs per minute **[2]**.~~
**NOTE: This is shown in other steps. Step deleted to prevent redundancy**
~~2.7.1. SCREEN: The bias flow profile is being set to 2 liters per minute using the profile configuration tab. **TXT: Refer Technote 037 from manufacturer for instructions**~~
~~2.7.2. SCREEN: Profile editor is being used to input puff volume, duration, and frequency parameters.~~

2.8. For animal preparation, acclimate male and female C57BL/6 *(C-Fifty-Seven-B-L-Bar-Six)* ~~mice aged 10 to 12 weeks and weighing approximately 20 to 30 grams **[1]**. Acclimate the animals~~ gradually over 3 days to the exposure system **[2]**.
**NOTE:** VO edited to accommodate the deleted shot
~~2.8.1. Shot of labeled cages housing male and female C57BL/6 mice.~~
**AUTHOR'S NOTE: Shot not filmed**
2.8.2. Talent placing mice into the exposure system.

2.9. To begin acclimation, place each mouse in a soft restraint **[1]**. Initiate a 2 liters per minute bias airflow with room air for a duration equivalent to their experimental exposure of 10, 20, or 30 minutes **[2]**.
2.9.1. Talent inserting a mouse into the soft restraint chamber.
2.9.2. SCREEN: 2025-04-11-15-52-04.mkv 04:03-04:32

2.10. Fully retract the mesh component of the restraint **[1]**. Then hold the entire restraint in front of the mouse and allow the mouse to enter the plunger component on its own **[2]**.
2.10.1. Talent retracting the mesh part of the restraint.
2.10.2. Talent positioning the restraint and mouse entering the plunger chamber voluntarily.

2.11. Verify that the mouse's nose is visible in the plunger section **[1]**. Secure a binder clip just behind the mouse to prevent backward movement **[2]**. When handling mice of different sexes or genotypes, use color-coded binder clips to identify and distinguish them **[3]**.
2.11.1. Shot of mouse's nose through the plunger section.
2.11.2. Talent attaching a binder clip gently behind the mouse.

2.11.3. Talent selecting and applying color-coded binder clips to different mice based on sex or genotype.

**3. Controlled E-Cigarette Aerosol Exposure and Recovery Procedures in Mice**

3.1. Place six restrained mice into the nose-only exposure tower of the system **[1]**. Initiate the bias flow at 2 liters per minute with room air to ensure sufficient airflow through the chamber **[2]**.

3.1.1. Talent inserting six restrained animals into the slots of the nose-only exposure tower.

3.1.2. SCREEN: 2025-04-11-15-52-04.mkv 04:33-04:50

3.2. In the task docker ~~on the right-hand side of the screen~~, right-click on the **E-cigarette** *(E-Cigarette)* **profile created** and select **Task Properties** ~~from the menu~~ **[1]**.

3.2.1. SCREEN: 2025-04-11-15-52-04.mkv 04:54-04:57

3.3. Under Puff Frequency, enter the desired puff interval such as 30 seconds for a 2 puffs per minute regime **[1]**. Click **OK** when prompted to confirm the changes **[2]**. Follow the prompts at the end of the session to save the puff regime as a template for future use **[3]**.

3.3.1. SCREEN: 2025-04-11-15-52-04.mkv 04:59-05:04 Puff interval is being input.

3.3.2. SCREEN: 2025-04-11-15-52-04.mkv 05:04-05:09 OK is being clicked, then Yes is being selected sin the pop-up dialog.

3.3.3. SCREEN: 2025-04-11-15-52-04.mkv 09:03-09:23

3.4. When ready to begin the exposure, double-click on the modified **E-cigarette profile** to start it **[1]**. Start a timer simultaneously to track the exposure duration **[2]**.

3.4.1. SCREEN: 2025-04-11-15-52-04.mkv 05:10-05:28

3.4.2. Talent pressing start on a stopwatch or digital timer.

3.5. To evaluate dose across exposure durations, perform exposures lasting 10 minutes, 20 minutes, and 30 minutes at a rate of 1 puff per minute **[1-TXT]**. ~~Then, test varying intensity by maintaining a 10-minute exposure and increasing puff frequency to 1, 2, and 4 puffs per minute **[2]**.~~

3.5.1. SCREEN: 2025-04-11-15-52-04.mkv 05:57-06:09

**TXT: Test varying intensity by maintain 10 min exposure and increasing puff frequency**

3.5.2. SCREEN: Exposure time is being maintained at 10 minutes and increment puff frequency to 1, 2, and 4 puffs per minute.

**NOTE: Converted to on-screen text**

3.6. After the exposure ends, restart the bias flow with room air **[1]**. ~~Carefully return the animals from the tower back to their respective cages **[2]**.~~

3.6.1. SCREEN: 2025-04-11-15-52-04.mkv 05:53-05:57

3.6.2. ~~Talent transferring animals from restraints into labeled home cages~~. **AUTHOR'S NOTE: Shot not filmed**

3.7. To release animals from the restraints, remove the binder clip first **[1]**. Fully retract the mesh toward the plunger and allow the animal to exit the restraint on its own **[2]**. If needed, gently tug the tail to encourage backward movement **[3]**.

3.7.1. Talent removing the binder clip from behind the mouse.

3.7.2. Talent retracting the mesh and letting the mouse exit naturally.

3.7.3. Talent gently pulling the mouse's tail to prompt it to exit the restraint.

# Results

**4. Representative Results**

4.1. Serum THC-COOH *(T-H-C-C-O-O-H)* concentrations significantly increased in a time-dependent manner with 10, 20, and 30 minutes of exposure to THC vapor at 1 puff per minute **[1]**.

4.1.1. LAB MEDIA: Figure 2A. *Video editor: Please sequentially highlight the columns corresponding to 10, 20 and 30 min*

4.2. Increasing puff frequency during a 10-minute exposure resulted in a dose-dependent rise in serum THC-COOH levels for 1, 2, and 4 puffs per minute **[1]**.

4.2.1. LAB MEDIA: Figure 2B. *Video editor: Please sequentially highlight the columns corresponding to 1, 2 and 4*

4.3. Mice exposed to THC vapor for 10 minutes at 2 puffs per minute and 4 puffs per minute exhibited significant hypo-locomotion, with distance travelled reduced to 0.29 and 0.05 meters respectively **[1]**.

4.3.1. LAB MEDIA: Figure 3. *Video editor: Please sequentially highlight the columns corresponding to 2 puffs/min and 4 puffs/min*

**Pronunciation Guide:**

Pharmacology

- **Pronunciation Link**: https://www.merriam-webster.com/dictionary/pharmacology
- **IPA**: /ˌfɑːrməˈkɑːlədʒi/
- **Phonetic Spelling**: far-muh-KOL-uh-jeeMerriam-WebsterMerriam-Webster+1Merriam-Webster+1

3. Therapeutics

- **Pronunciation Link**: https://www.merriam-webster.com/dictionary/therapeutics
- **IPA**: /ˌθɛrəˈpjuːtɪks/
- **Phonetic Spelling**: ther-uh-PYOO-tiksMerriam-Webster+4Merriam-Webster+4Merriam-Webster+4Merriam-Webster

4. Tetrahydrocannabinol (THC)

- **Pronunciation Link**: https://www.merriam-webster.com/dictionary/tetrahydrocannabinol
- **IPA**: /ˌtɛtrəˌhaɪdroʊˈkænəbɪnɔːl/
- **Phonetic Spelling**: tet-ruh-HY-droh-KAN-uh-bin-awlMerriam-Webster+1Merriam-Webster+1

5. Cannabinoid

- **Pronunciation Link**: https://www.merriam-webster.com/dictionary/cannabinoid
- **IPA**: /ˈkænəbɪnɔɪd/
- **Phonetic Spelling**: KAN-uh-bin-oydMerriam-Webster+3Merriam-Webster+3Merriam-Webster+3

6. C57BL/6

- **Pronunciation Link**: No confirmed link found
- **IPA**: /si ˈfɪfti ˈsɛvən bi ɛl sɪks/
- **Phonetic Spelling**: see FIFTY-SEVEN B-L SIX

7. THC-COOH

- **Pronunciation Link**: No confirmed link found
- **IPA**: /ti eɪtʃ si si oʊ oʊ eɪtʃ/
- **Phonetic Spelling**: tee-AYCH-SEE-SEE-OH-OH-AYCH