

Document Number: ANP041	INTRATRACHEAL INSTILLATION OF SUBSTANCES IN RODENTS <i>CONFIDENTIAL INFORMATION</i> MOLECULAR MEDICINE RESEARCH INSTITUTE	Effective Date: 4-10-07
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1.0 OBJECTIVE

- 1.1 The objective of this procedure is to describe the procedure for intra-tracheal instillation of substances in rats and mice.

2.0 SCOPE

- 2.1 This procedure applies to animals used for experimentation.

3.0 POLICY

- 3.1 It is the policy of MMRI to establish written and approved procedures to ensure that the health and well being of employees is protected, and that potentially hazardous procedures are performed in a safe manner.

4.0 RESPONSIBILITIES

- 4.1 It is the responsibility of Manager of Animal Research or designated alternate to implement this procedure and revise it when necessary.

5.0 REFERENCES

- 5.1 SOP# ANP016, Animal Anesthesia.
- 5.2 Experimental and Surgical Technique in the Rat. Academic Press, 1992
- 5.3 Intratracheal Instillation as an Exposure Technique for the Evaluation of Respiratory Tract Toxicity: Uses and Limitations, Kevin E. Driscoll et al. Toxicological Sciences 2000, **55**, 24-35 (2000)
- 5.4 Bioavailability and Metabolism of Hydroquinone After Intratracheal Instillation in Male Rats, Peter J. Deisinger and J. Caroline English. Drug Metabolism and Disposition 1999, Vol. 27, No. 4, 442-448

6.0 SAFETY PRECAUTIONS

- 6.1 It is the responsibility of all personnel to use good judgment and safe practices in the laboratories. Protective clothing (e.g., laboratory coats, coveralls, boots, face masks, aprons, rubber gloves and safety glasses) are provided by the company.
- 6.2 Sterile rubber gloves, facemasks, bouffants and safety glasses are worn when performing surgery.

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6.3 Used disposable scalpel blades, hypodermic needles and syringes are placed in a disposable sharps container located in the procedure room. When full, the container is disposed of safely.

6.4 All injury accidents are promptly reported to the appropriate Supervisor.

7.0 EQUIPMENT AND MATERIALS

Autoclaved surgical instruments

Anesthetic (Domitor® and Ketamine combination or Ketamine and Xylazine combination or isoflurane)

Animal clippers and blade #40

Nolvasan® or Betadine® surgical scrub and 70% Isopropyl Alcohol

Gauze Sponges

Syringes (1.0 mL)

Blunt needles (gauge 22 or 23)

Suture materials (3-0 to 5-0 black braided silk or Dexon) or staples.

Micro-Renathane, Type MRE 040 catheter or equivalent

Micro-Renathane, Type MRE 025 catheter or equivalent

0.9 % sterile saline

Lactated Ringers Solution

Heating Pads

Surgical Fiber-optic light

Magnifying loop or dissecting scope.

8.0 PROCEDURE

8.1 Instillation through the oropharynx

8.1.1 Anesthetize the animal as per SOP. Position animal against an angled restraining stand and secure via the upper incisors with a rubber band.

8.1.1 Pull tongue out and to one side and keep mouth open using thumb forceps.

8.1.2 Position the end of a fiber optic light at the surface of the skin of the neck by the pharyngoepiglottic region. The vocal cords can now be viewed as an inverted "V" that is opening and closing.

8.1.3 For rats, a catheter with a maximum gauge of 14 can be introduced between the vocal cords. For mice use a maximum of 20 gauge.

8.1.4 Instill a maximum volume of 1 mL/kg body weight.

8.2 Transtracheal instillation

8.2.1 Anesthetize the animal as per SOP.

8.2.2 Shave the ventral neck and prepare site with Nolvasan® or Betadine® surgical scrub alternating with 70% Isopropanol (2-3 times).

8.2.3 Make a midline skin incision on the ventral neck and expose the trachea by blunt dissection.

8.2.4 Insert a needle between two tracheal rings just below the larynx and inject a maximum volume of 1 mL/kg body weight into the lumen.

8.2.5 Close the ventral incision with 3-0 to 5-0 suture or staples.

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8.3 Instillation through a pre-placed indwelling endotracheal tube (rats only).

8.3.1 Anesthetize the animal as per SOP.

8.3.2 Shave the ventral neck and dorsal neck and prepare sites with Nolvasan® or Betadine® surgical scrub alternating with 70% Isopropanol (2-3 times).

8.3.3 Make a midline skin incision on the ventral neck and expose the trachea by blunt dissection.

8.3.4 Make a hole between 2 tracheal rings below the larynx using a 20 gauge needle and through this hole insert a polyurethane tube with a 0.025" O.D x 0.012" I.D. (Micro-Renathane MRE 025, Braintree Scientific, Inc., Braintree, MA). Secure the tube using a medical grade cyanoacrylate and suture to the sternohyoid muscles. Couple this tube to an MRE 040 tube (0.040" O.D. x 0.025" I.D.), tunnel subcutaneously and exteriorize below the nape.

8.3.5 Plug the tube with a stainless steel wire and staple or suture the incisions.

8.3.6 Let the rat recover from anesthesia on a heating pad

8.3.7 On the day of instillation, remove the plug and introduce a maximum volume of 1 mL/kg followed by 200 uL of air.

8.3.8 Plug tube again.