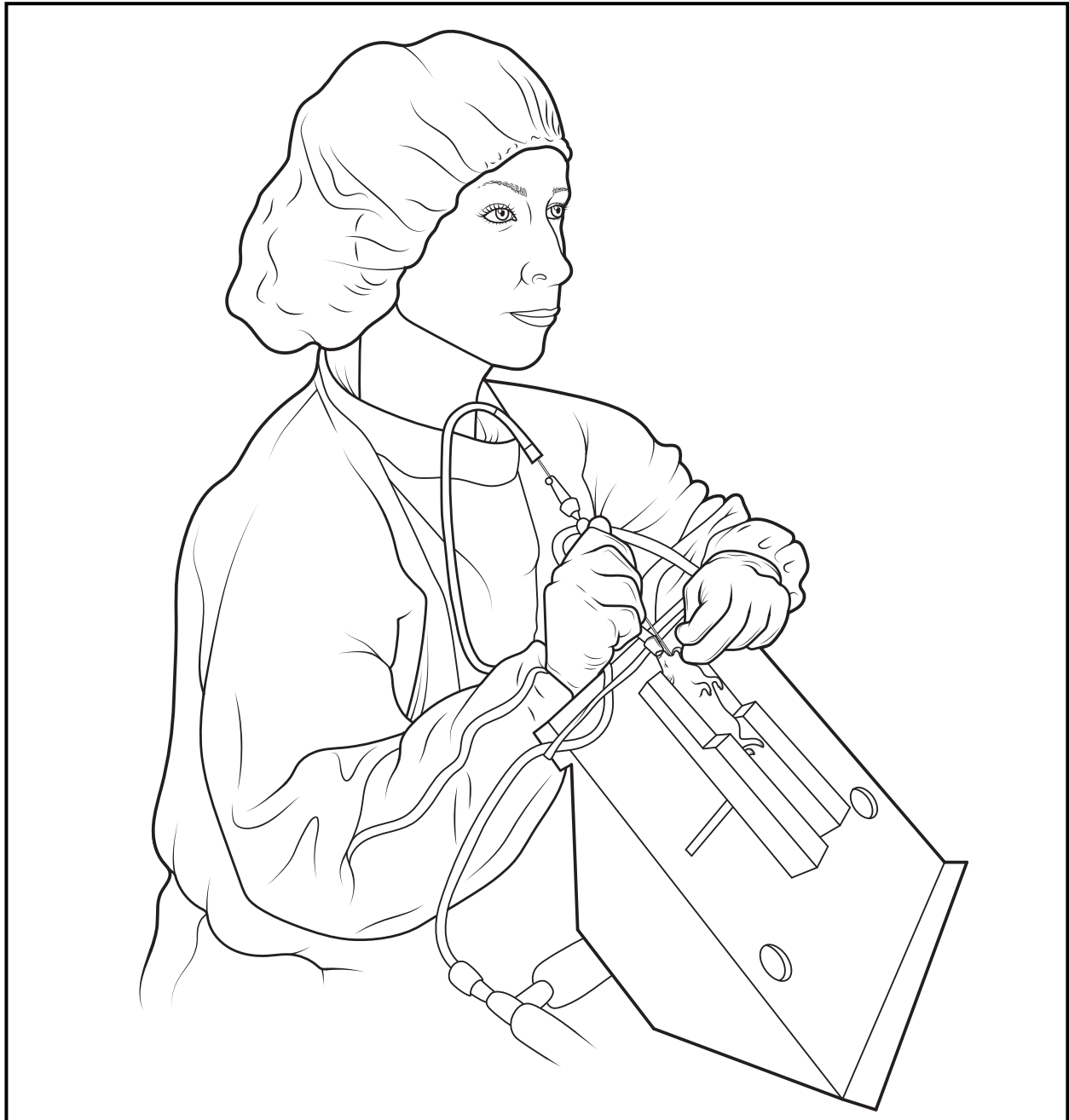


How to Complete a Bronchoscopic Procedure on Mice

An Illustrated Lab Manual

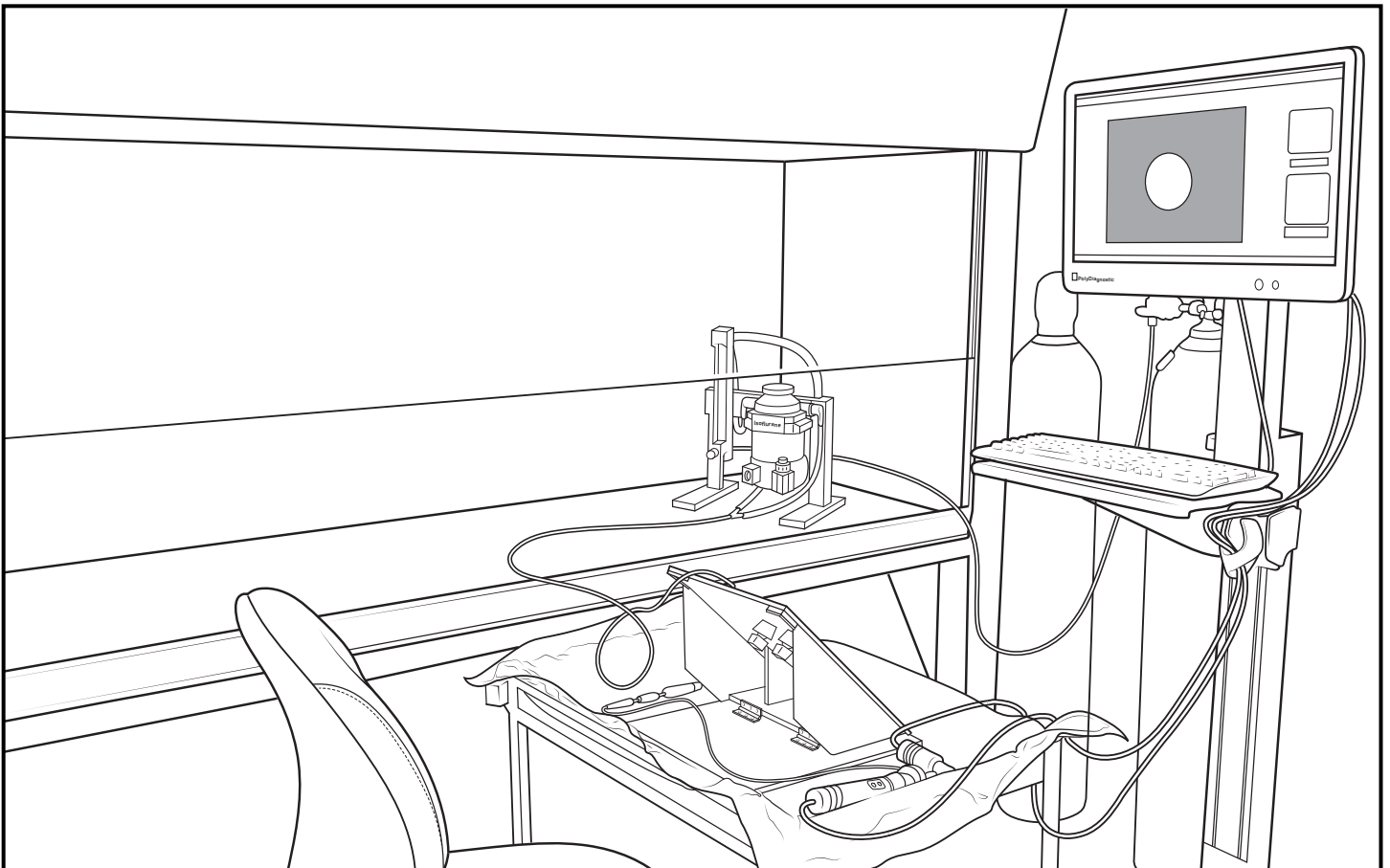


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Laboratory Set Up



For pulmonary research, it is important that the anatomy of the lungs in the animal model used, resembles the human lungs – both morphologically and physiologically. The mouse lungs, though smaller in size, closely resemble those of a human. The human lungs are divided into five lobes; two left lobes and three right lobes (**Figure C**). The left lobes are smaller to compensate for the space the heart takes up in the thoracic region. The mice lungs are also divided into five lobes, but are distributed differently; one left lobe and four right lobes (**Figure A and B**). **Figure A** depicts mouse lung anatomy, with the heart and trachea removed. **Figure B** depicts mouse lung anatomy with the heart, trachea, and some vasculature. **Figure C** depicts human thorax anatomy, including the lungs, heart (in pericardial sac), trachea, and some vasculature. **Figure D** depicts the internal airway anatomy of human lungs. On page 3, **Figure E** depicts the internal mouse airway anatomy.

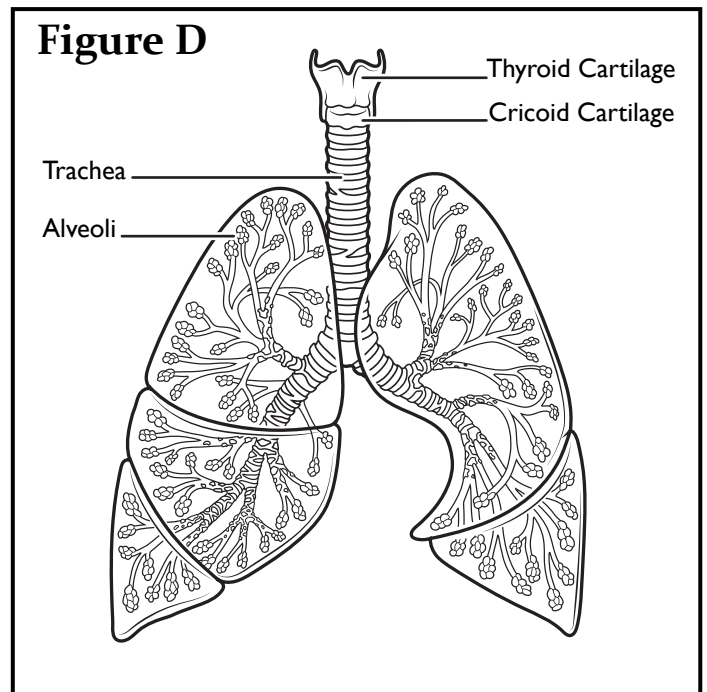
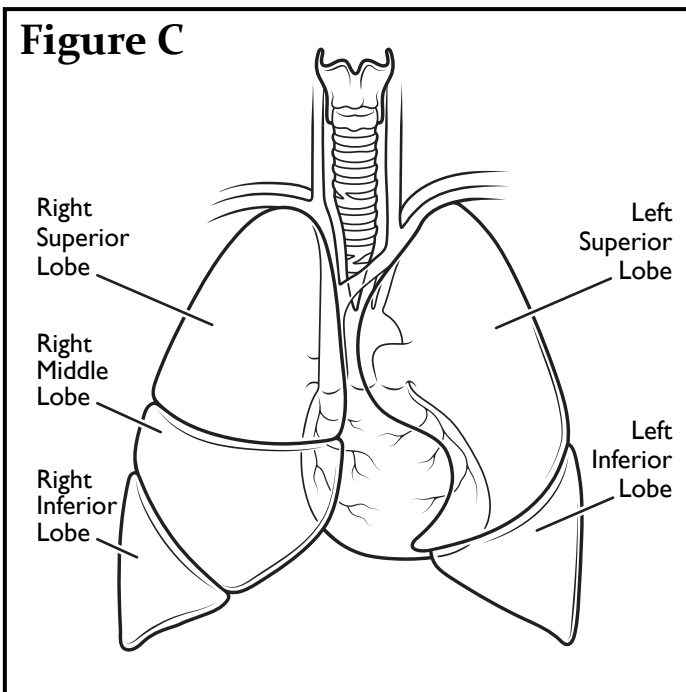
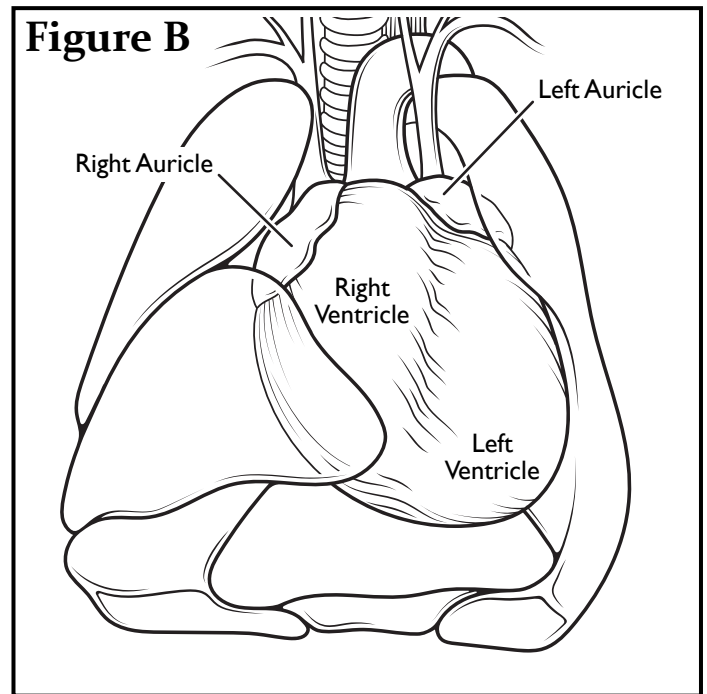
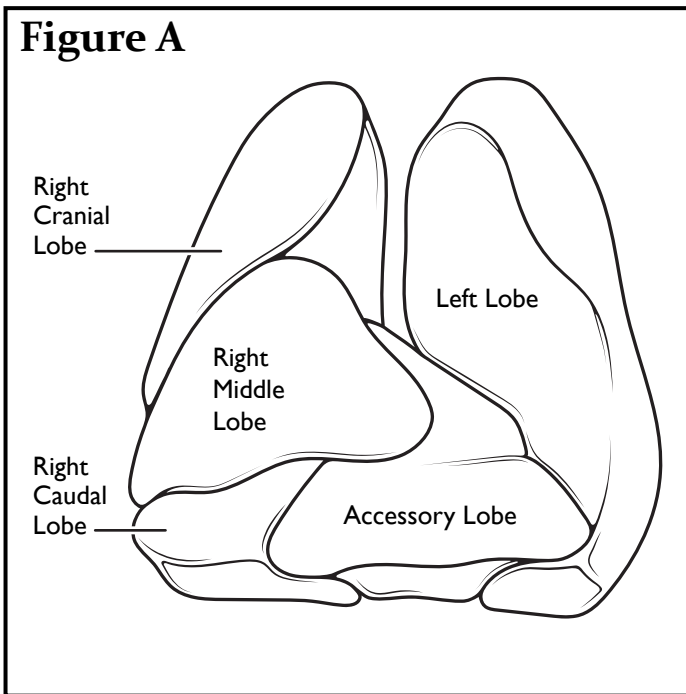


Figure E

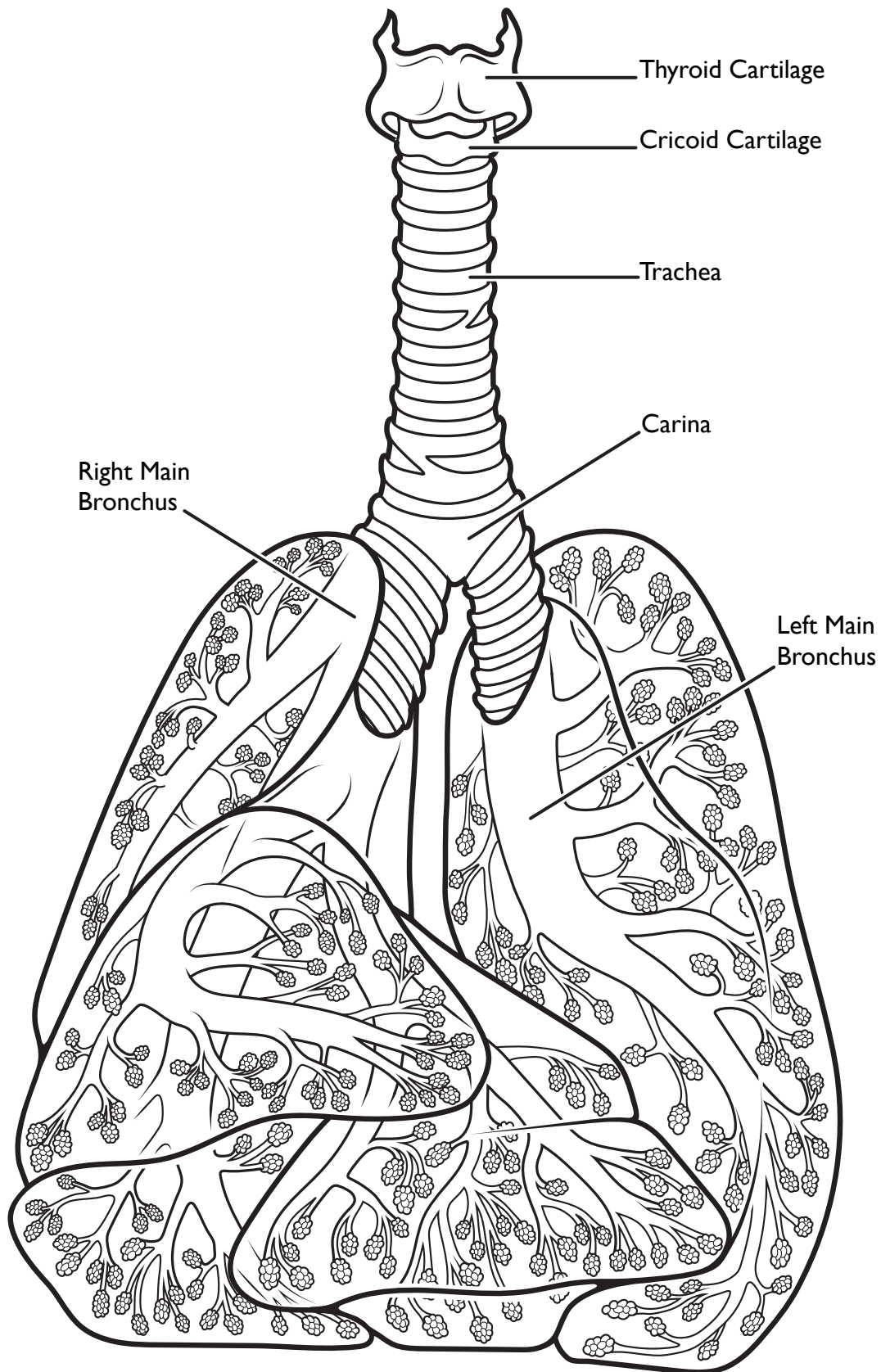
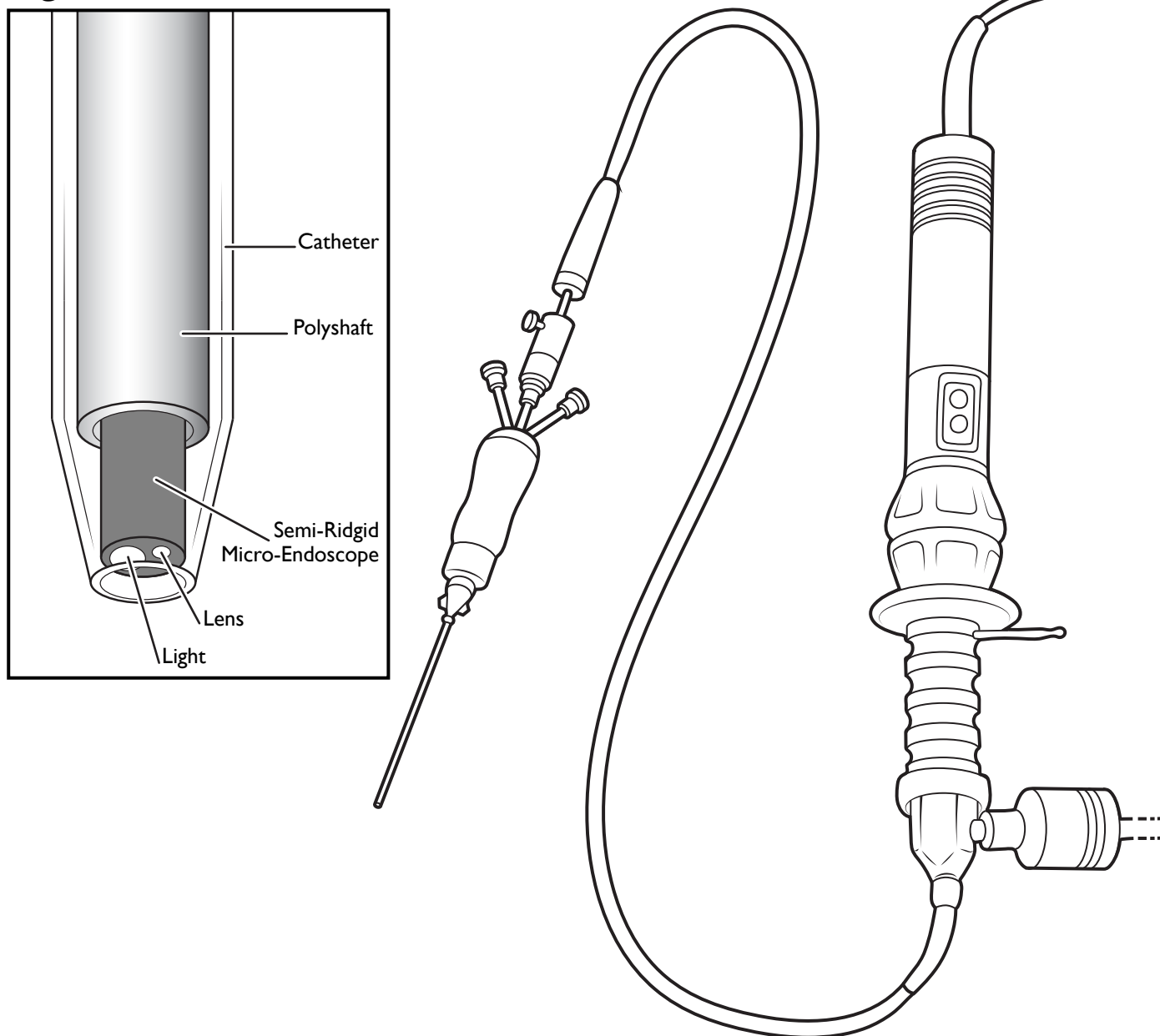


Figure F

The image above shows the semi-rigid micro-endoscope used for instillation of compounds in mouse lungs. The bronchoscope is a medical guidance device that contains a fiber-optic LED light cable and a 3000 pixel image system. This human micro-endoscope works well as a mouse bronchoscope because of its small size (0.45mm in diameter). **Figure F** depicts the setup for instillation of a compound using the bronchoscope as guidance for placement of a catheter. The bronchoscope is sheathed by a thin polyshaft canula within a sterile catheter (illustrated in the insert). When the procedure is being conducted, it is important to note that the bronchoscope, polyshaft, and catheter are all at the same level, so the catheter is the only part that comes in contact with the mouse airway to avoid scratching the mucosal surface of the airways. Focus and zoom are adjusted via the handheld piece that also allows for still image acquisition.

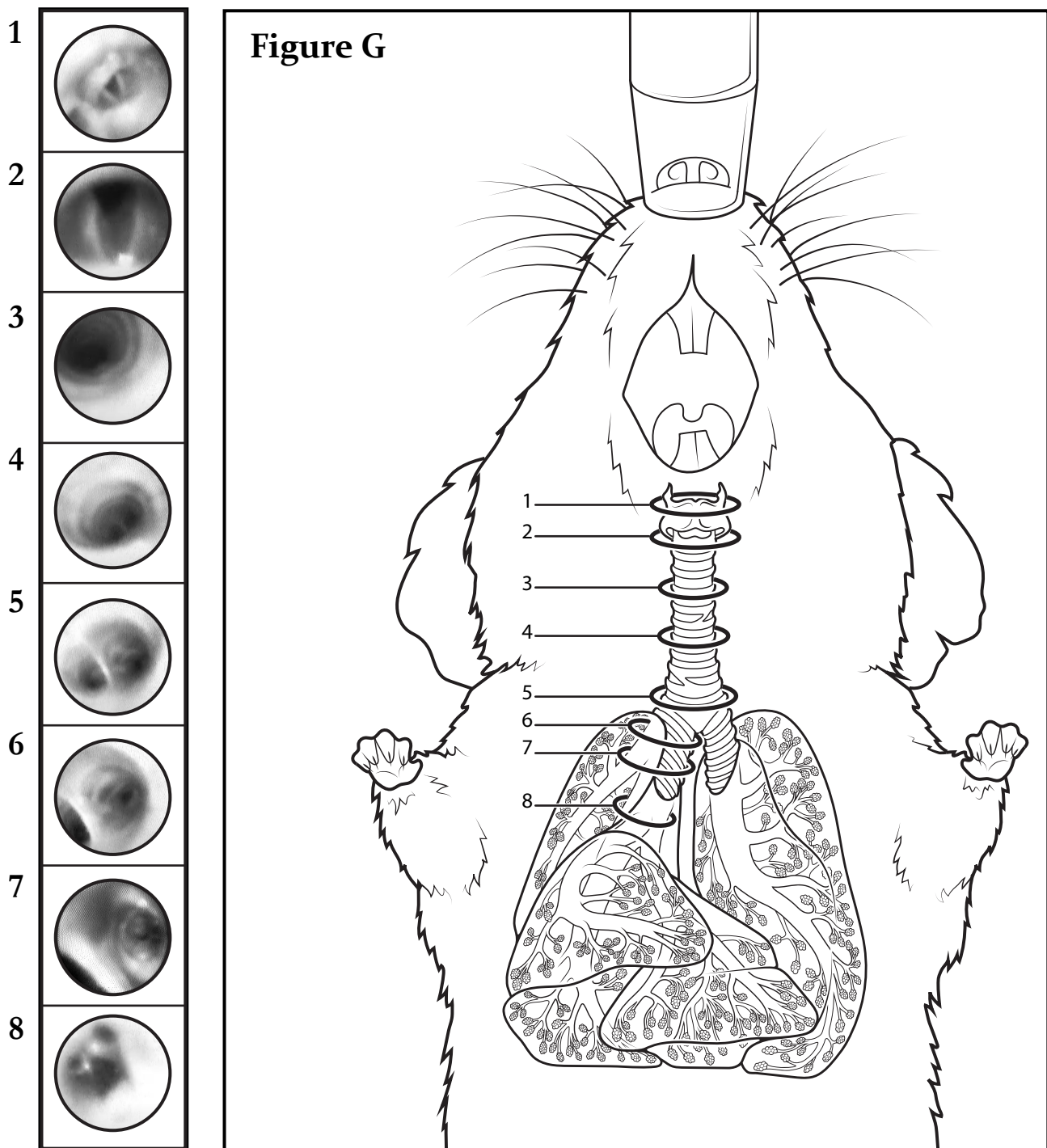
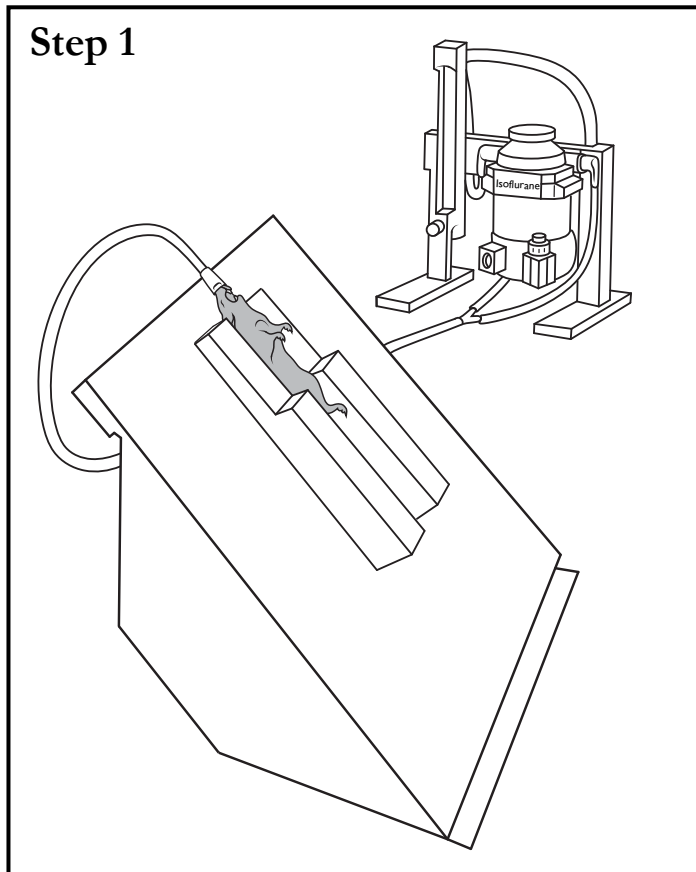
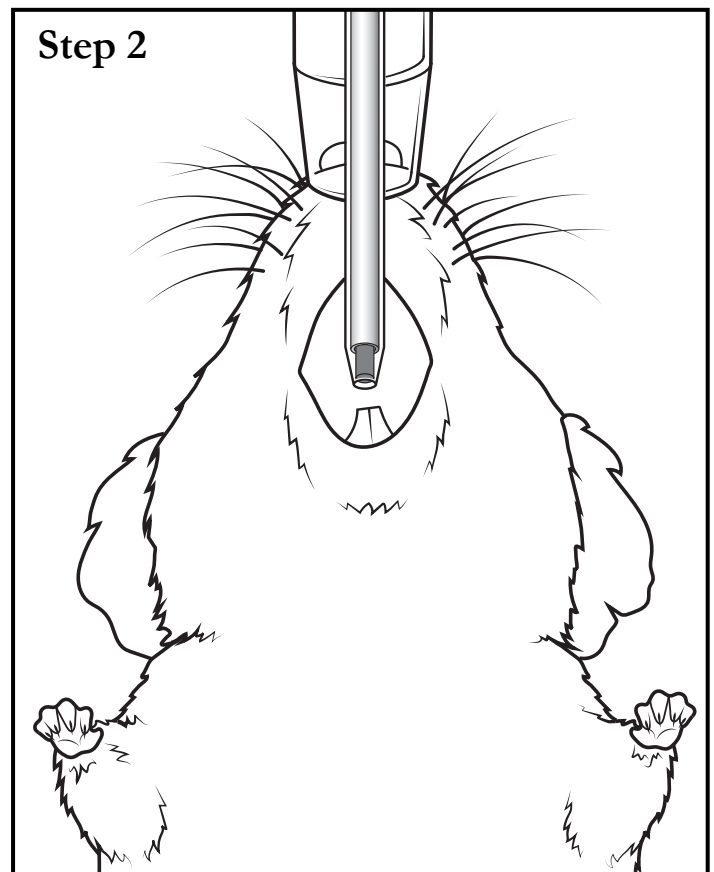


Figure G shows the murine lung structure and the insets (1-8) are still images from mouth cavity towards the secondary bronchi. Upon entering the airways of the mouse, one must go through the pharynx and past the epiglottis (1) that functions as a valve towards the larynx and then pass through the vocal cords (2) to enter the trachea (3, 4). The trachea, with its cartilage rings, leads the way to the first branch point, the carina of trachea (5), where the airway divides into right and left primary (main) bronchi. In the mouse, the right primary bronchus leads toward the second branch point (6) where the secondary bronchus toward the right superior lobe goes up (7) and the secondary bronchus toward the right middle lobe, right caudal lobe and the accessory lobe goes downward (8).

Before the procedure begins, the mouse is anaesthetised with isoflurane, a gas anaesthesia. After the mouse is unconscious, it is placed in a supine position, at a 45 degree angle. This position eases the use of the bronchoscope into the mouse lungs.

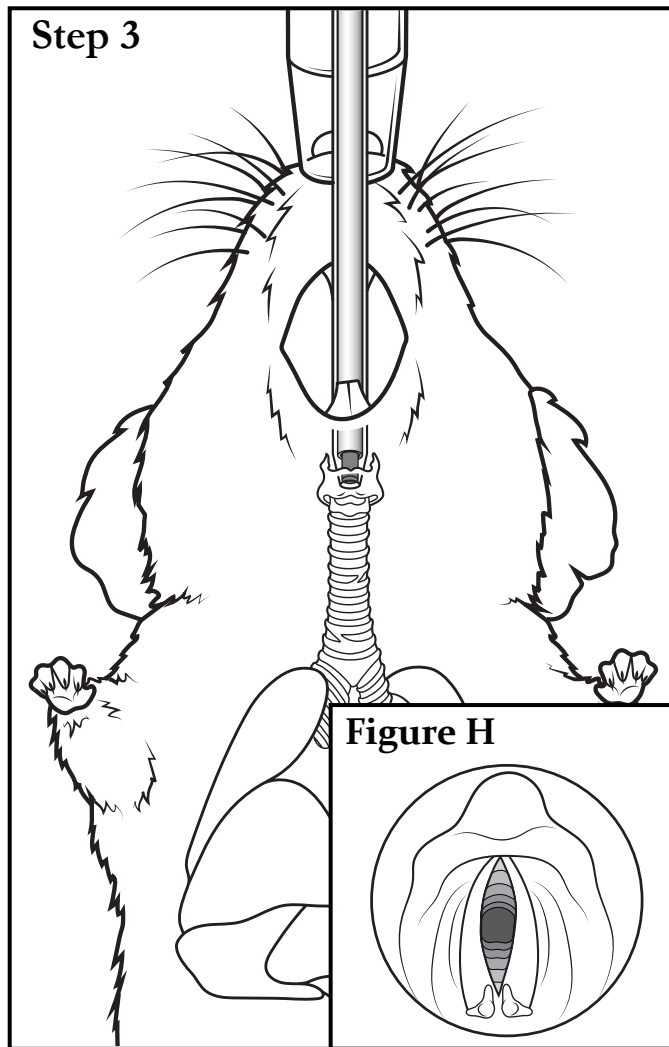


Step 1: The mouse is rendered unconscious using isoflurane, and is placed at a 45-degree angle on its back.



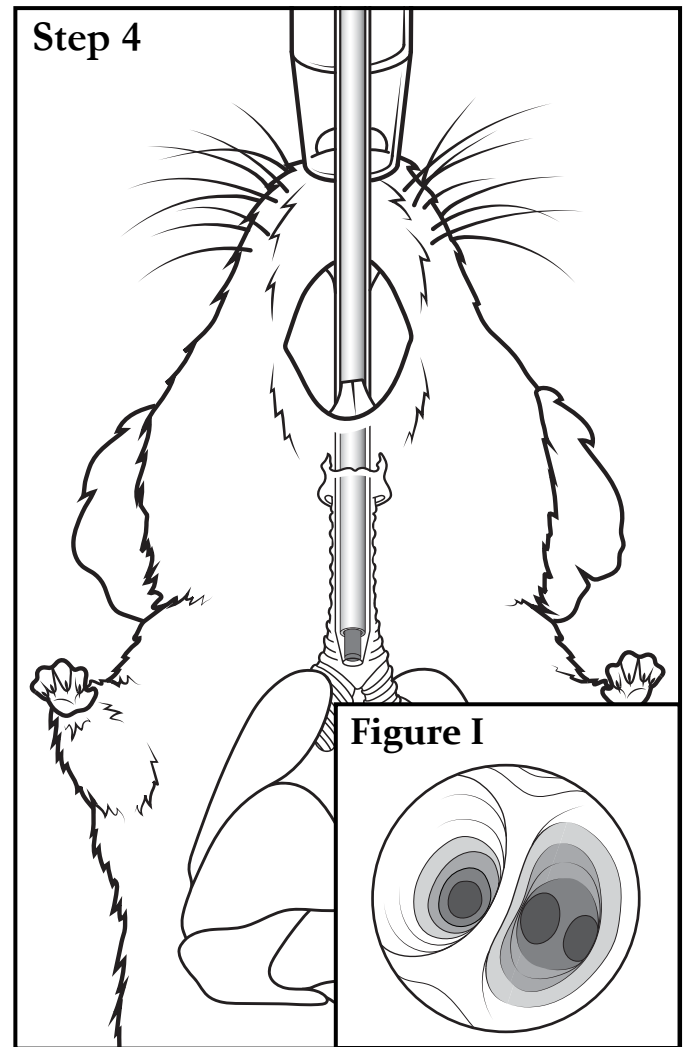
Step 2: The bronchoscope is inserted in the mouth of the mouse. The mouse is continuously administered isoflurane anesthesia during the procedure and the bronchoscopist sits behind the mouse to be able to insert the bronchoscope directly straight into the trachea and lungs.

After inserting the bronchoscope in the mouth, the epiglottis and vocal cords will be visible at the far end of the mouth (pharynx). With the mouse lying on its back, the bronchoscopist should aim at going under the epiglottis to be able to enter the larynx through the vocal cords. The epiglottis may behave "slippery" and the bronchoscope often ends in the esophagus instead of the trachea.



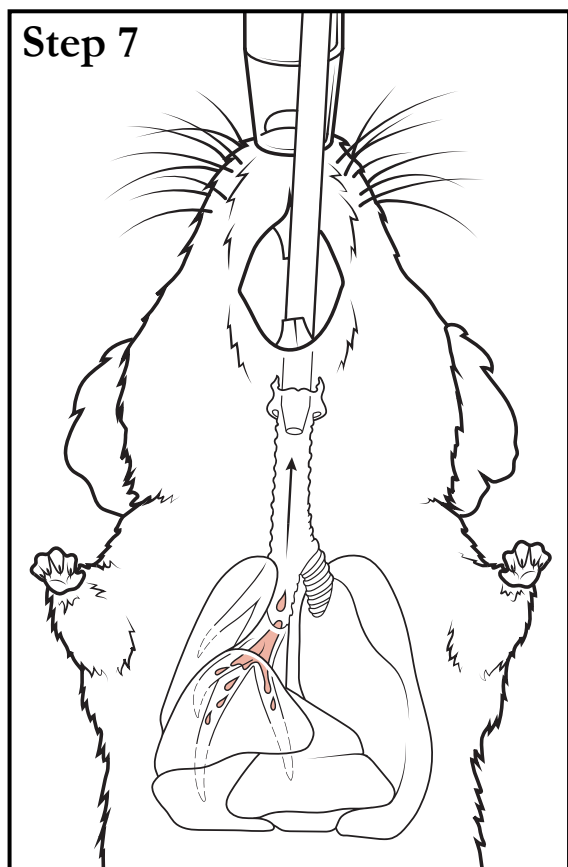
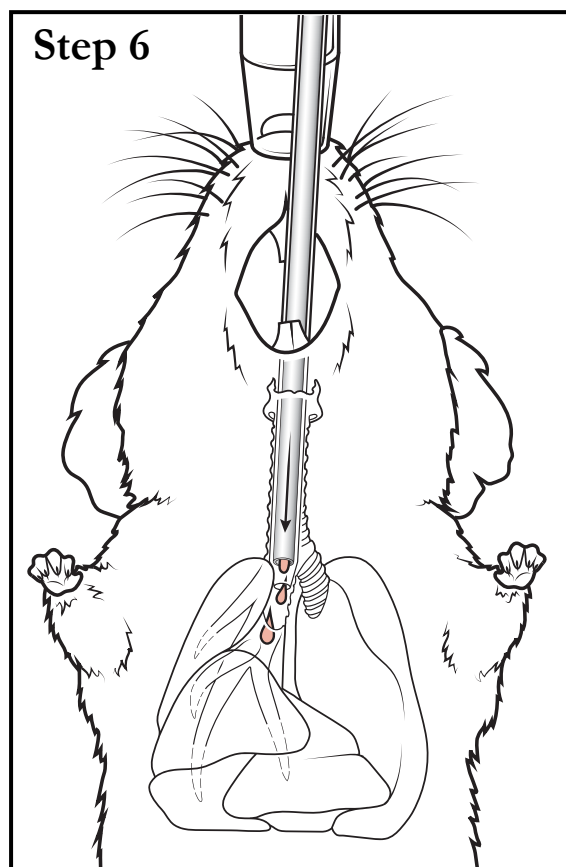
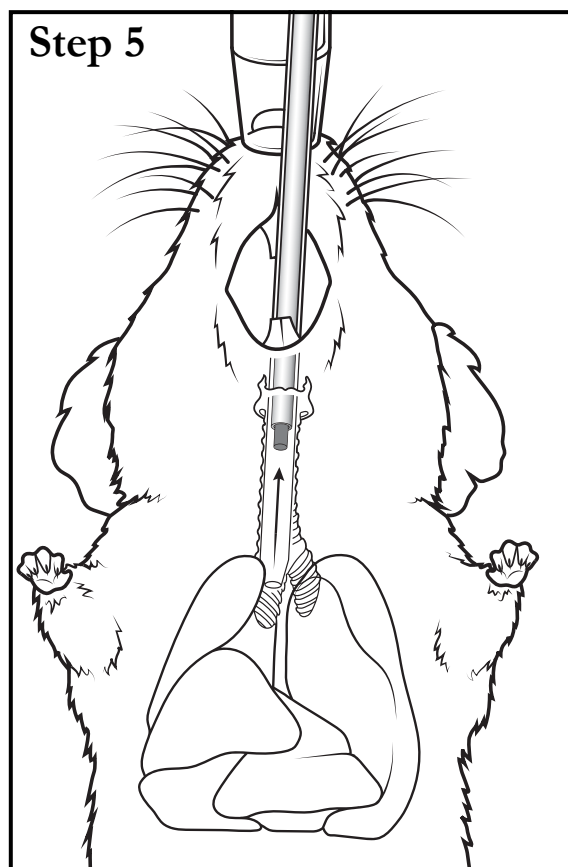
Step 3: The bronchoscope must pass through the curved vocal cords to enter the trachea, which is often difficult. The mild gas anesthesia allows the mouse to continue breathing, which causes the vocal chords to open and close with each breath. The bronchoscopist must be skilled in their timing to gently and successfully maneuver through the vocal cords and enter the trachea.

Figure H: A view of the vocal cords as it is seen through the bronchoscope. The vocal cords are seen here as a double crescent shape. Underneath the mucosa, The vocal ligament attaches anteriorly to the thyroid cartilage, and is opened posteriorly by the arytenoid cartilage. The arytenoid cartilage sits on top of the cricoid cartilage.



Step 4: Once inside the trachea, the cricoid cartilage rings keep the trachea open and make it easy to travel further into the airway with the bronchoscope. The division into the two main bronchi ends the journey through the trachea, and the carina of trachea marks the point where the bronchoscopist should choose to go either to the left lung or the right lung. With the small diameter of the bronchi in the mouse, the bronchoscope with the catheter can go no further than to the left main (primary) bronchus or to the right main (primary) bronchus looking into the two right secondary bronchi.

Figure I: A view of the division of the trachea, at the level of the carina. Seen here is the right and left main bronchus, the right main bronchus being the larger of the two.

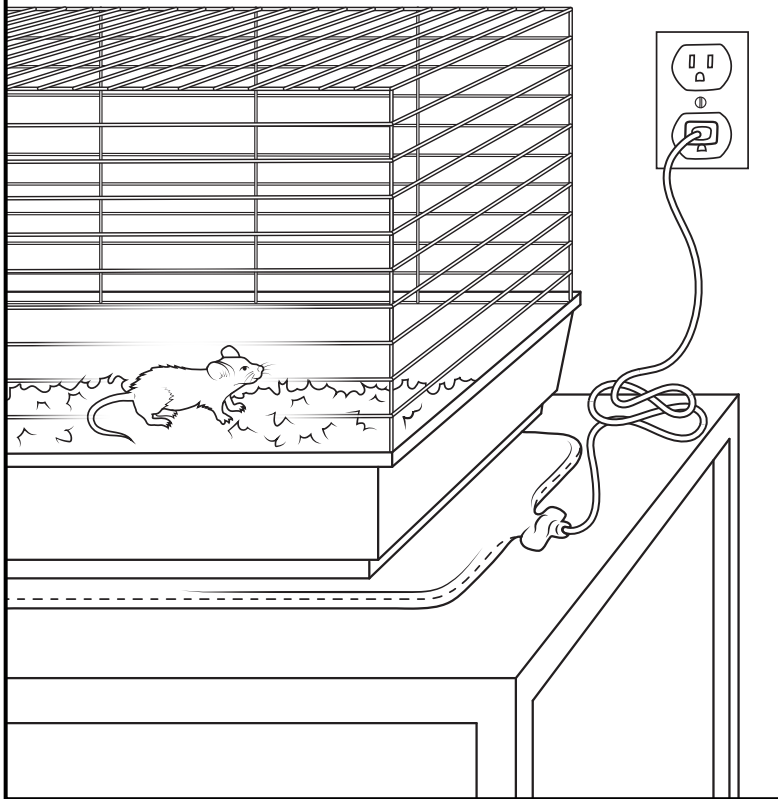


Step 5: After placement of the catheter in the right main bronchus, the bronchoscope with the polyshaft are removed, and the catheter is left inside the right lung.

Step 6: The compound is instilled into the right lung of the mouse, via a syringe with a polyshaft attached (identical to the one surrounding the bronchoscope in early steps) that is gently inserted through the catheter.

Step 7: After successful injection, the catheter and syringe are removed.

Step 8



Step 8: After the procedure is complete, the mouse is taken off the stand, and the isoflurane gas mask tube is removed. The mouse is then placed in its cage, on a heating pad to keep warm until it regains consciousness.

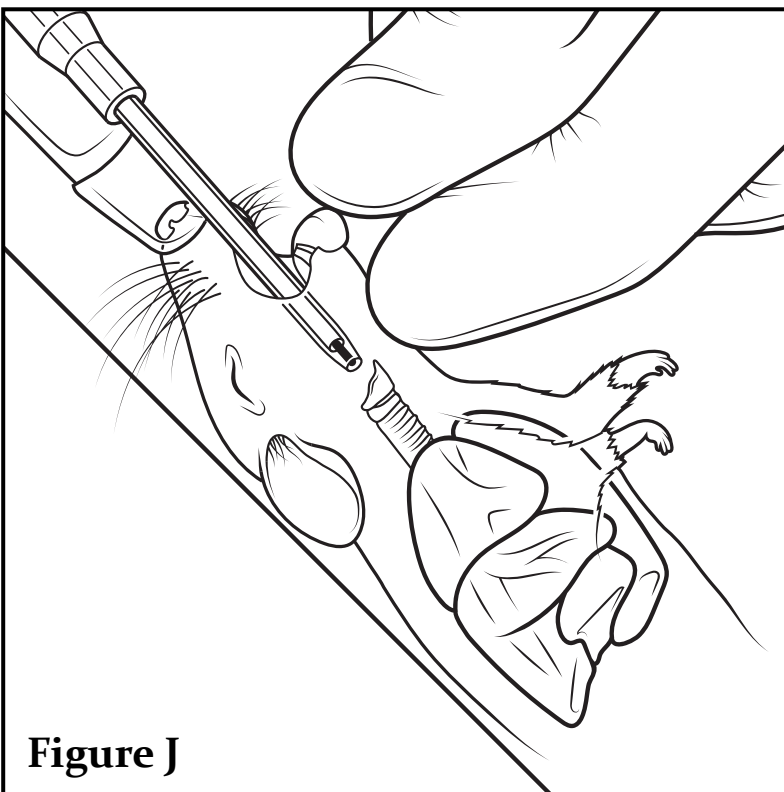


Figure J: The procedure from a lateral view, depicting the bronchoscope before entering the vocal chords.

Figure J