Review of Histopathological Changes in the Respiratory Tract of Rat Induced by Inhalation of the Mainstream Smoke of Standard Reference Cigarette 1R4F
Outline

• Goal
• Anatomy and Histology
• Histotechnical Work
• Results
  – INBIFO
  – Literature
Goal

• Overview of the methods and procedures regarding the histotechnical work and histopathological evaluation as well as an overview of the histopathological changes following subchronic exposure to cigarette smoke
Rationales

• Inhalation studies, special emphasis on the histopathology of the target organs, i.e., respiratory tract organs
• Regulatory guidelines (OECD, EPA)
• Human relevance - association of tobacco smoke inhalation with laryngeal and bronchial cancers in humans
Rat Respiratory Tract
Nose

• Anatomy
  – vestibule
  – cartilagenous septum that divides the nasal cavity into 2 halves
  – dorsal, middle, and ventral meatuses
  – turbinates or conchae

• Histology
  – 3 basic epithelial types
    – squamous epithelium
    – respiratory epithelium
    – olfactory epithelium
Anatomy

• Larynx consists of 5 cartilages
  – epiglottis
  – thyroid
  – cricoid
  – arytenoid
  – u-shaped
Histology

• 4 types of epithelium
  – squamous
  – respiratory
  – pseudostratified cuboidal
  – squamoid
Trachea

• Anatomy
  – connects the larynx with the lungs
  – 15 incomplete cartilaginous rings dorsally interconnected by ligaments
  – tracheal bifurcation at the level of 6th rib into a smaller left and larger right extrapulmonary main bronchus

• Histology
  – pseudostratified respiratory epithelium
    – ciliated cells
    – serous cells
    – mucous (goblet) cells
    – basal cells
## Lung

<table>
<thead>
<tr>
<th></th>
<th>Man</th>
<th>Rat</th>
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<tbody>
<tr>
<td></td>
<td>Left lung</td>
<td></td>
</tr>
<tr>
<td>•</td>
<td>2 lobes</td>
<td>1 lobe</td>
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<tr>
<td></td>
<td>10 segmental bronchi</td>
<td>12 to 13 segmental bronchi</td>
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<tr>
<td>•</td>
<td>Right lung</td>
<td></td>
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<tr>
<td>•</td>
<td>3 lobes</td>
<td>4 lobes</td>
</tr>
<tr>
<td>•</td>
<td>10 segmental bronchi</td>
<td>14 to 15 segmental bronchi</td>
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<tr>
<td></td>
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<td>few subsegmental bronchi</td>
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</tbody>
</table>
Cells of the Pulmonary Parenchyma

- Alveolar type I epithelial cells
- Alveolar type II epithelial cells
- Pulmonary endothelial cells
- Interstitial cells
- Macrophages
Tissue Sectioning Method

• Sections at pre-established levels
  – nose: Young (1981)
  – larynx: Lewis (1980)
  – trachea: INBIFO
  – lung: Lamb and Reid (1969)

• Purpose
  – adequate morphological evaluation
  – uniform histological examination
  – ensure correct comparison within and among studies
Histopathological Evaluation

- Recording of findings in a consistent manner
- Use of standardized dictionary of criteria
- Consistent scoring system
- Evaluation in a blinded fashion, i.e., without the knowledge of treatment groups
Results INBIFO

• Study reference
  – subchronic cigarette smoke inhalation studies

• Experimental condition
  – “nose only” mode
  – TPM concentration range 40 to 200 µg TPM/l
  – exposure duration: 6 h/d, 7 d/week for 21 or 90 days
Histopathology

• Nose
  – respiratory epithelium (levels 1 and 2)
    – reserve cell hyperplasia
    – squamous metaplasia
    – goblet cell hypertrophy/hyperplasia
  – olfactory epithelium (levels 2, 3, and 4)
    – ulceration
    – atrophy
    – squamous metaplasia
Nasal Septum, Level 1, Normal
Nasal Septum, Level 1, Goblet Cell Hypertrophy/Hyperplasia
Nasal Septum, Level 1, Goblet Cell Hypertrophy/Hyperplasia, AB-PAS Stain
Dorsolateral Wall, Nose Level 1, Normal
Dorsolateral Wall, Nose Level 1, Squamous Metaplasia
Maxilloturbinate Tip, Nose Level 1, Normal
Maxilloturbinate Tip, Nose Level 1, Hyperplasia
Dorsomedial Region, Nose Level 2, Normal Olfactory Epithelium (low power)
Dorsomedial Region, Nose Level 2, Normal Olfactory Epithelium (high power)
Dorsomedial Region, Nose Level 2, Olfactory Epithelium, Atrophy