Genotoxic effects of nicotine in human target cells of carcinogenesis

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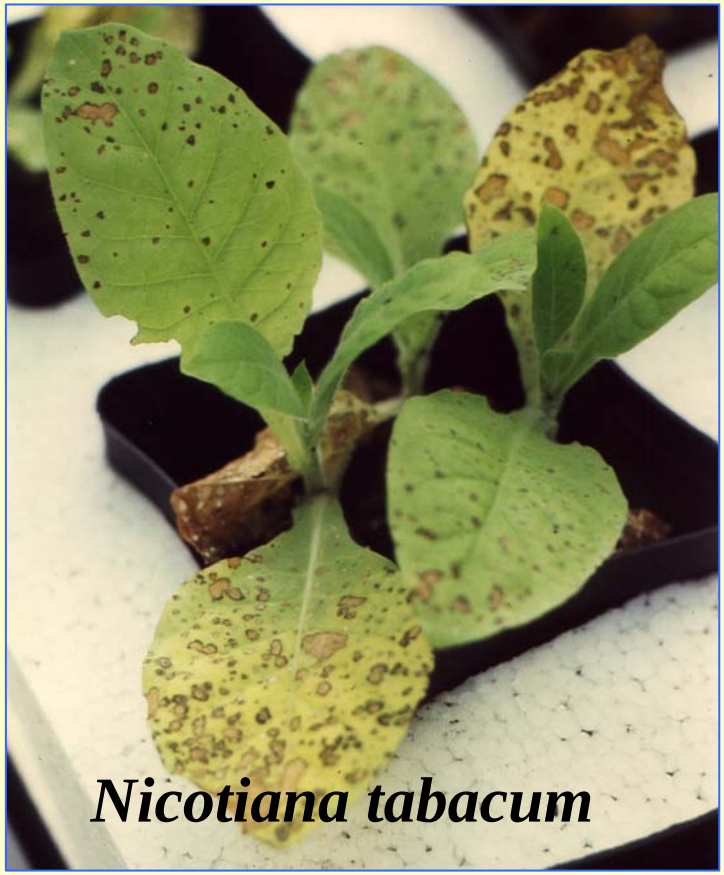
Tobacco smoke

- 4000 compounds
- Vapor phase and particle phase
- Side stream and main stream

Nicotine

Alkaloid of the tobacco plant



Columbus

Nicot

Effects of nicotine

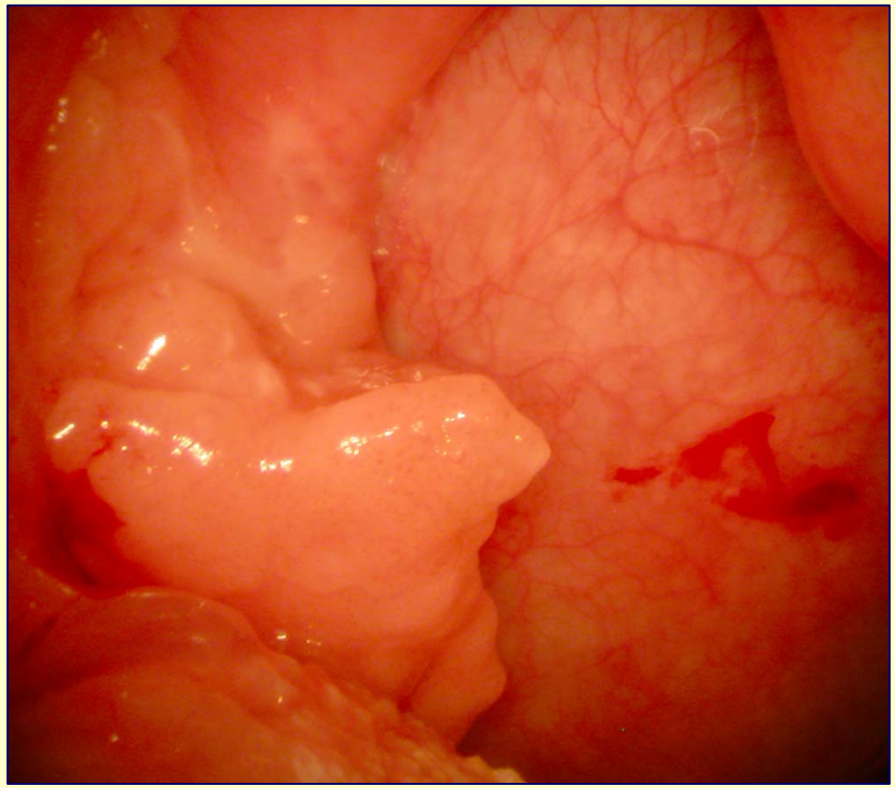
- Addiction
- Toxicity in high dosis
- Tumor promotion
- Tumor initiation ?

Genotoxic effects of nicotine ?

- DNA single strand breaks
  in human lymphatic tissue cells and lymphocytes
- Dependence of the DNA migration
  on the pH-value

M & M

Cell source I

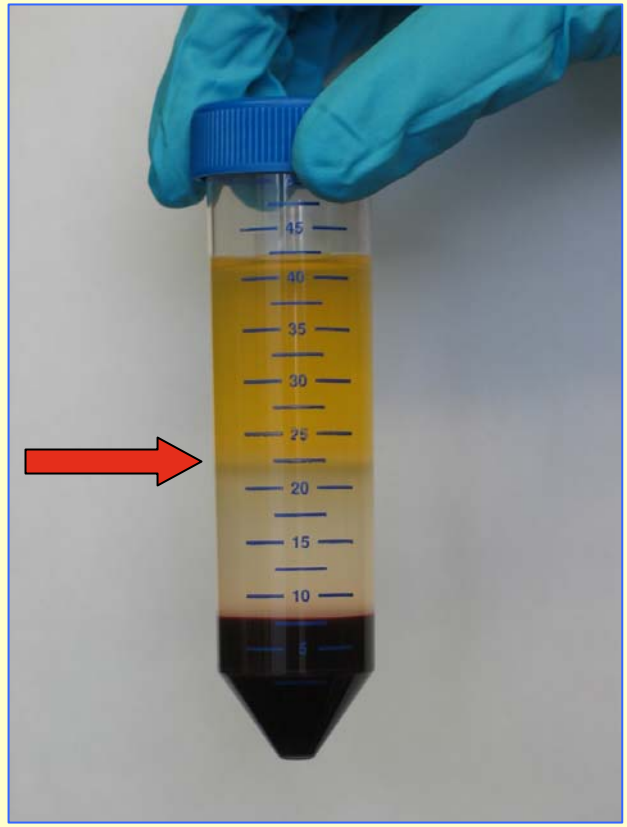


- Tonsilla palatina

Cell source II

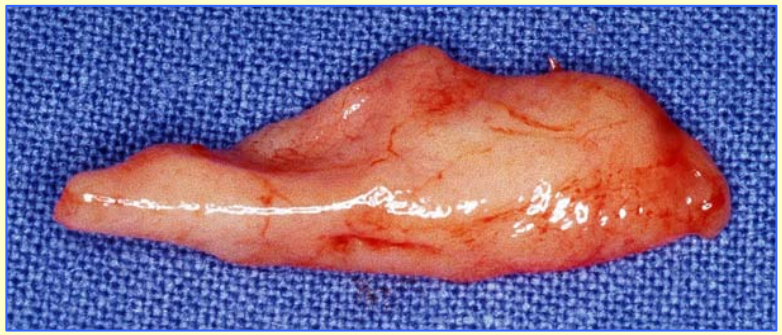
- Lymphocytes:

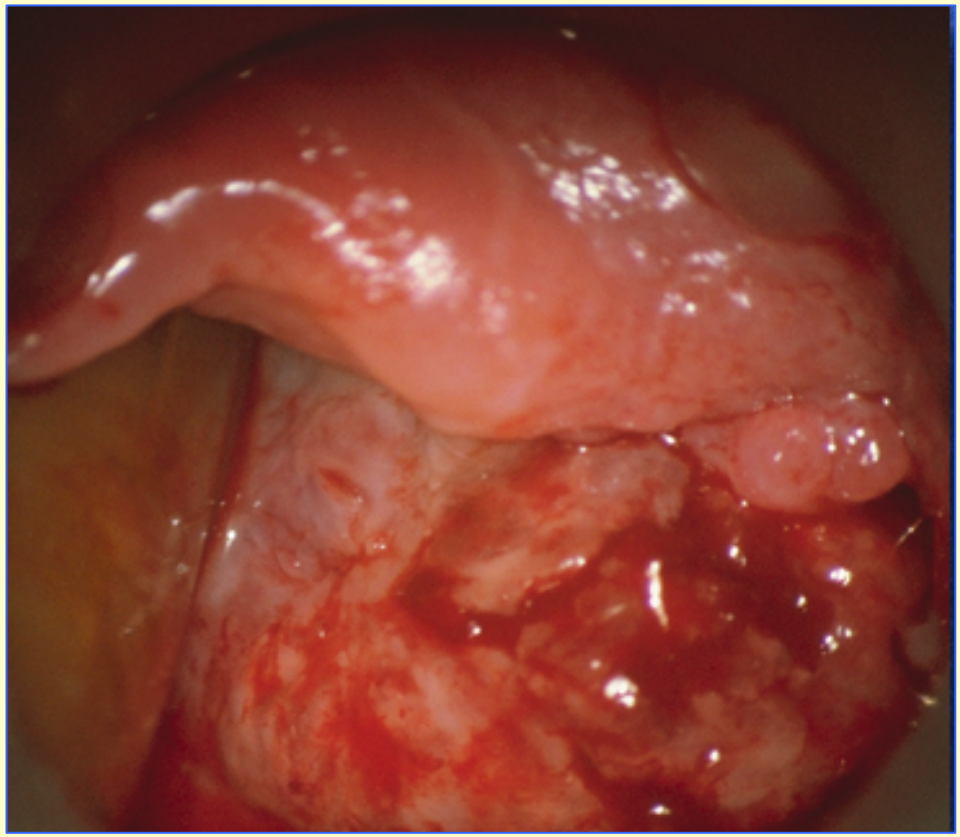
  isolated per Biocoll gradient



Cell source III

- Larynx
- Nasal turbinate





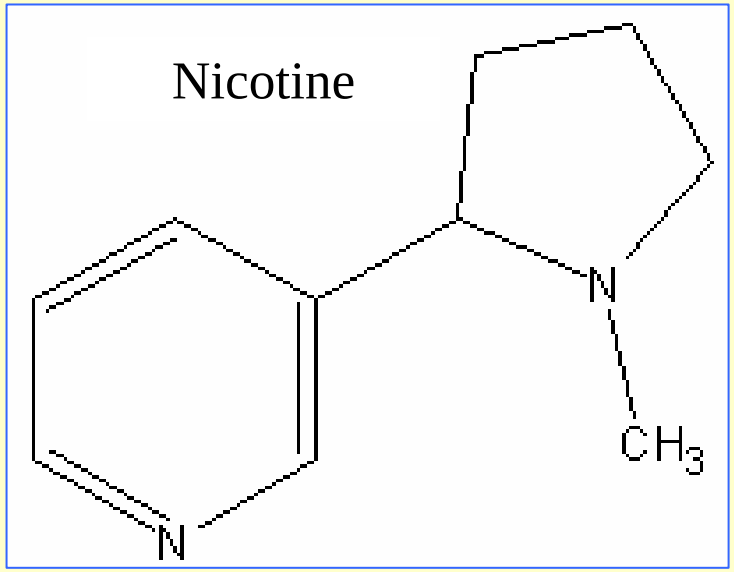
Exposure to Nicotine

- Exposure:

  0.125 - 4 mM / 1 h

- pH:

  neutral versus
  alkaline medium



Comet Assay I

- single cell microgel electrophoresis assay

- detection of - single strand breaks
  - alkali-instable sites
  - incomplete excision repair

Comet Assay II

Single cell suspension

⇩

Exposure to xenobiotic

⇩

Slide preparation

⇩

Alkaline lysis

⇩

Unwinding and electrophoresis

Comet Assay III

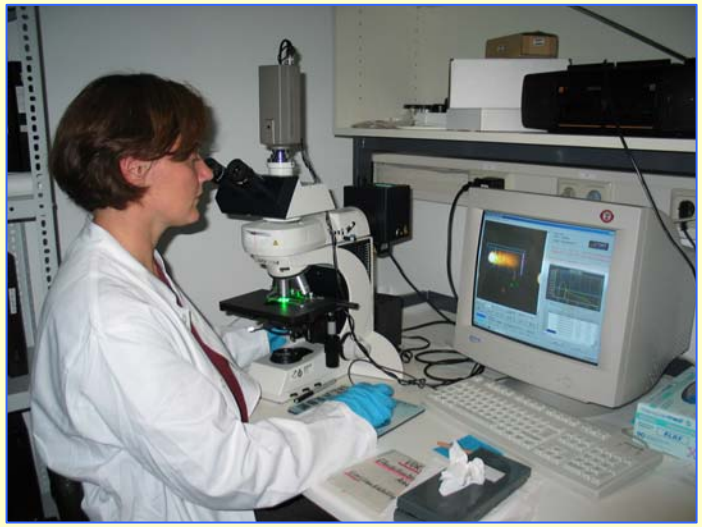
Neutralization

⇩

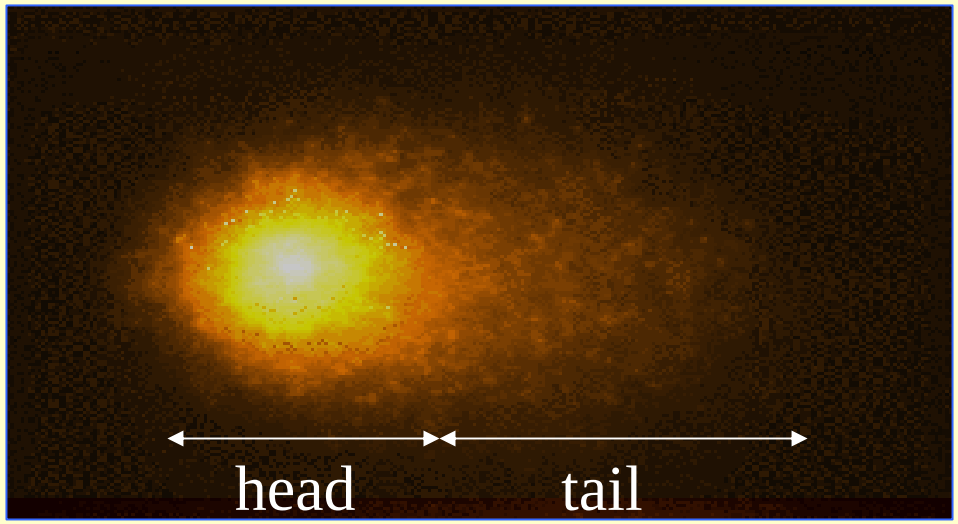
Staining with ethidium bromide

⇩

Image analysis



Comet Assay IV



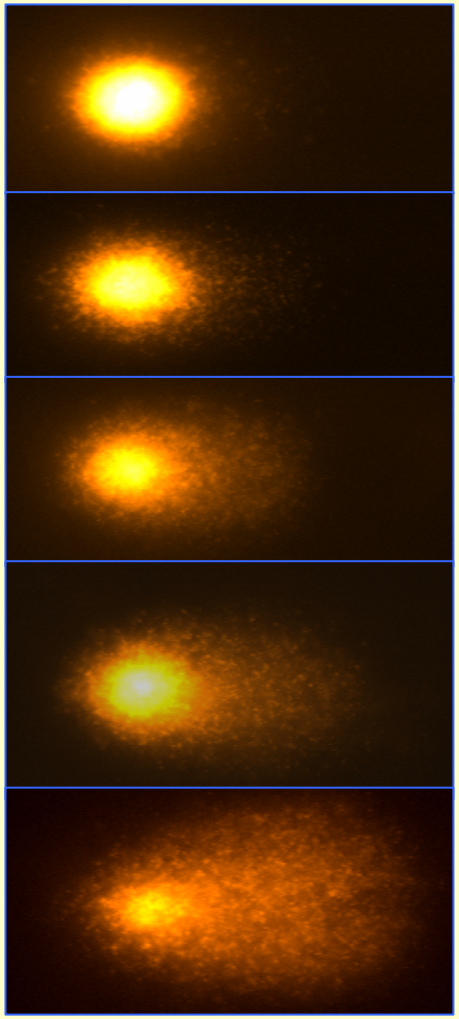
Olive tail moment (OTM):

percentage DNA in the tail

x

median migration distance

Comet Assay V



- Negative control:

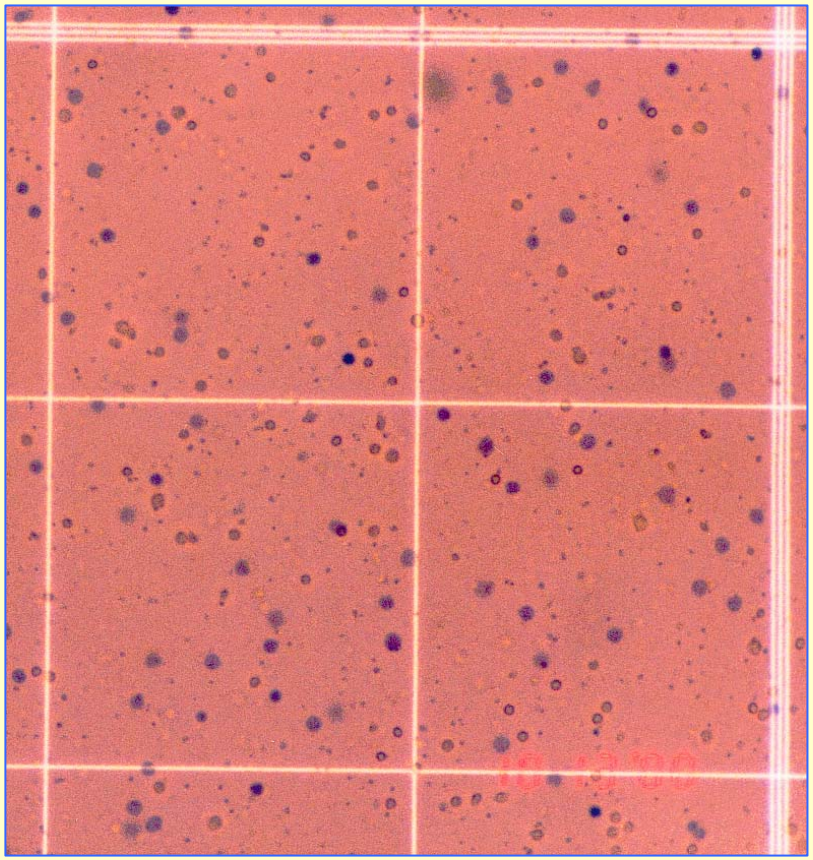
  phosphate buffered saline (PBS)

- Xenobiotic, e.g. nicotine

- Positive control:

  N-methyl-N´-nitro-N-nitrosoguanidine (MNNG)

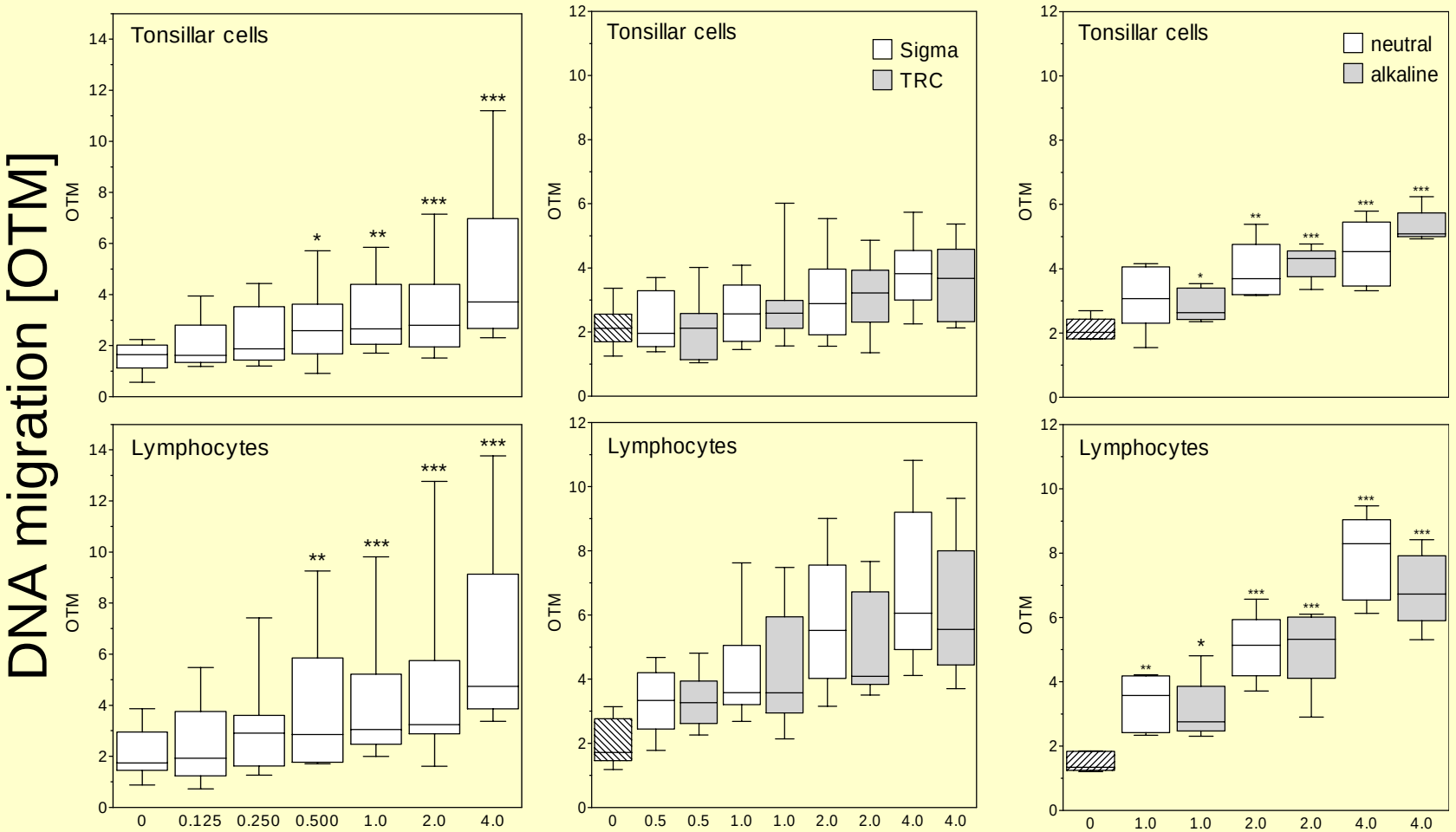
Trypanblue Exclusion Test



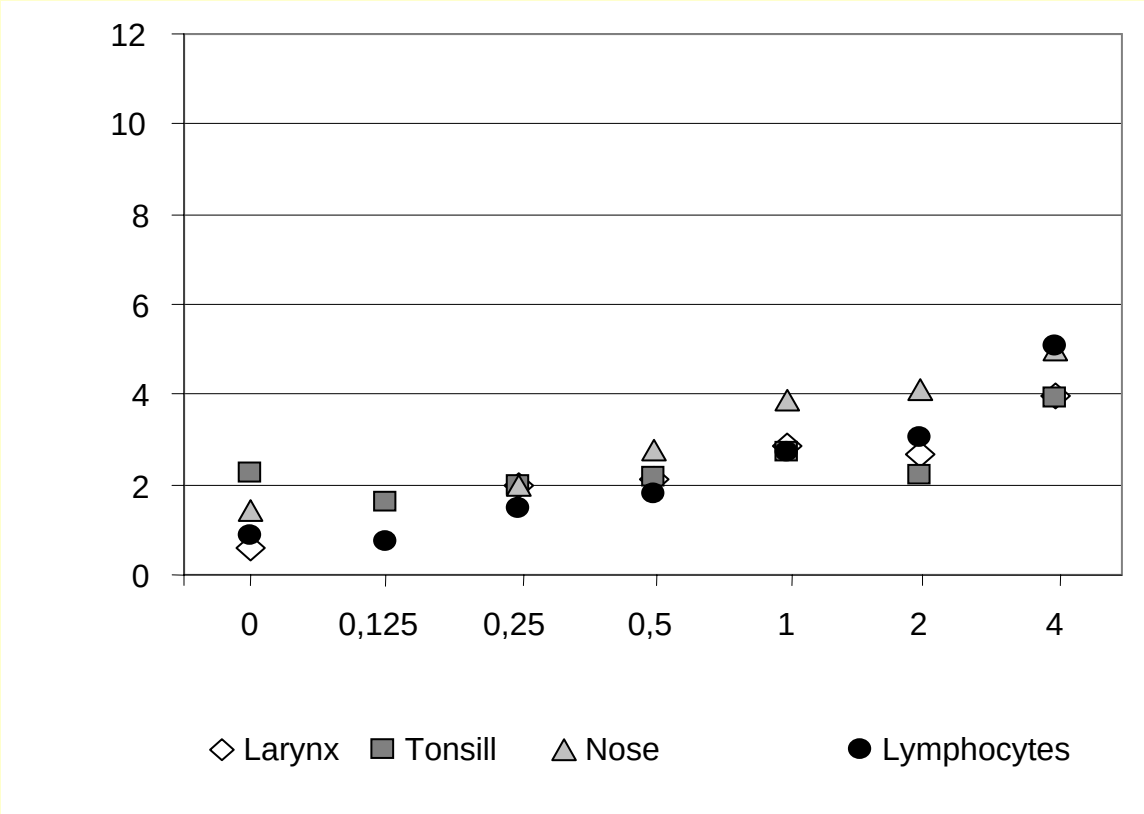
- determination of cytotoxicity

Results

Lymphatic Tissue & Lymphocytes



Mucosal cells - Nose



Summary

- Dose dependent genotoxic effects in human target cells of carcinogenesis

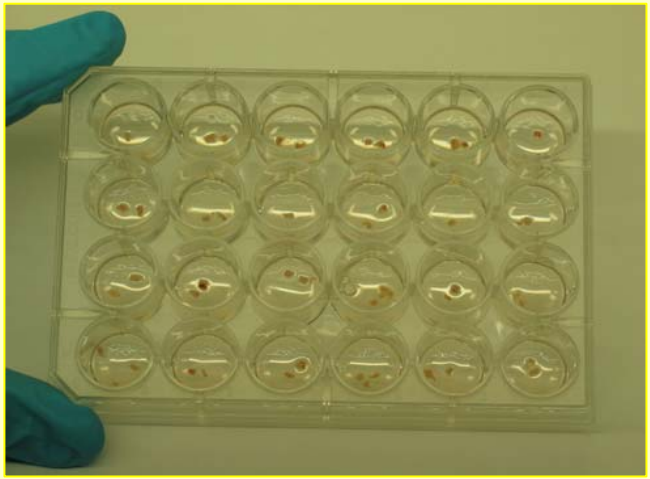
- pH-increase by alkaloid had no effect on the genotoxicity of nicotine

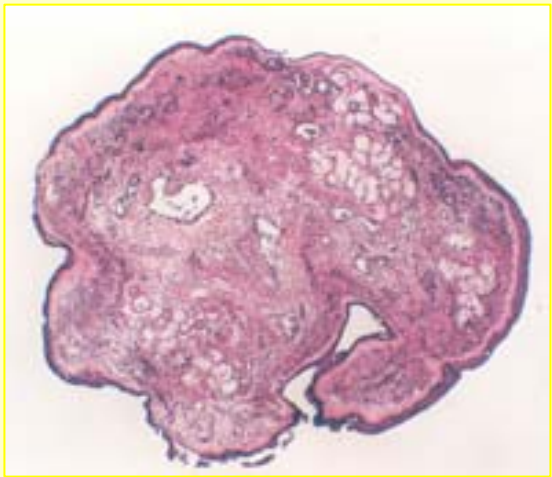
→ Nicotine adds to the mutagenicity of tobacco smoke

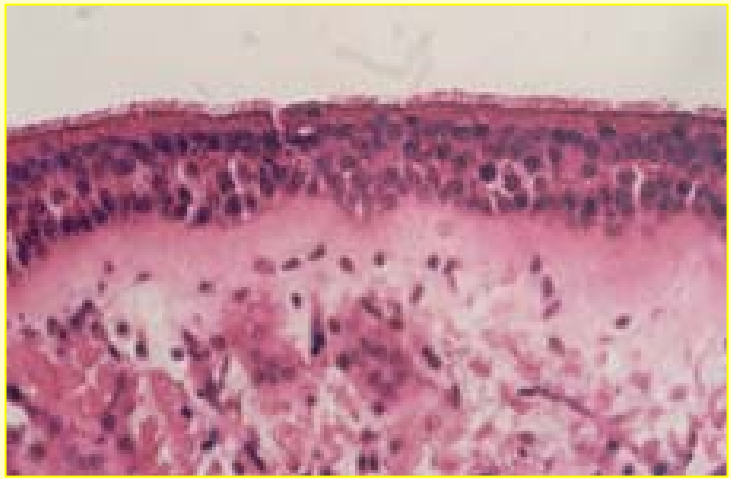
Future Studies

- Mini organ cultures
- Prolonged exposure
- Synergisms
- Apoptosis

Mini organ cultures







Regensburg