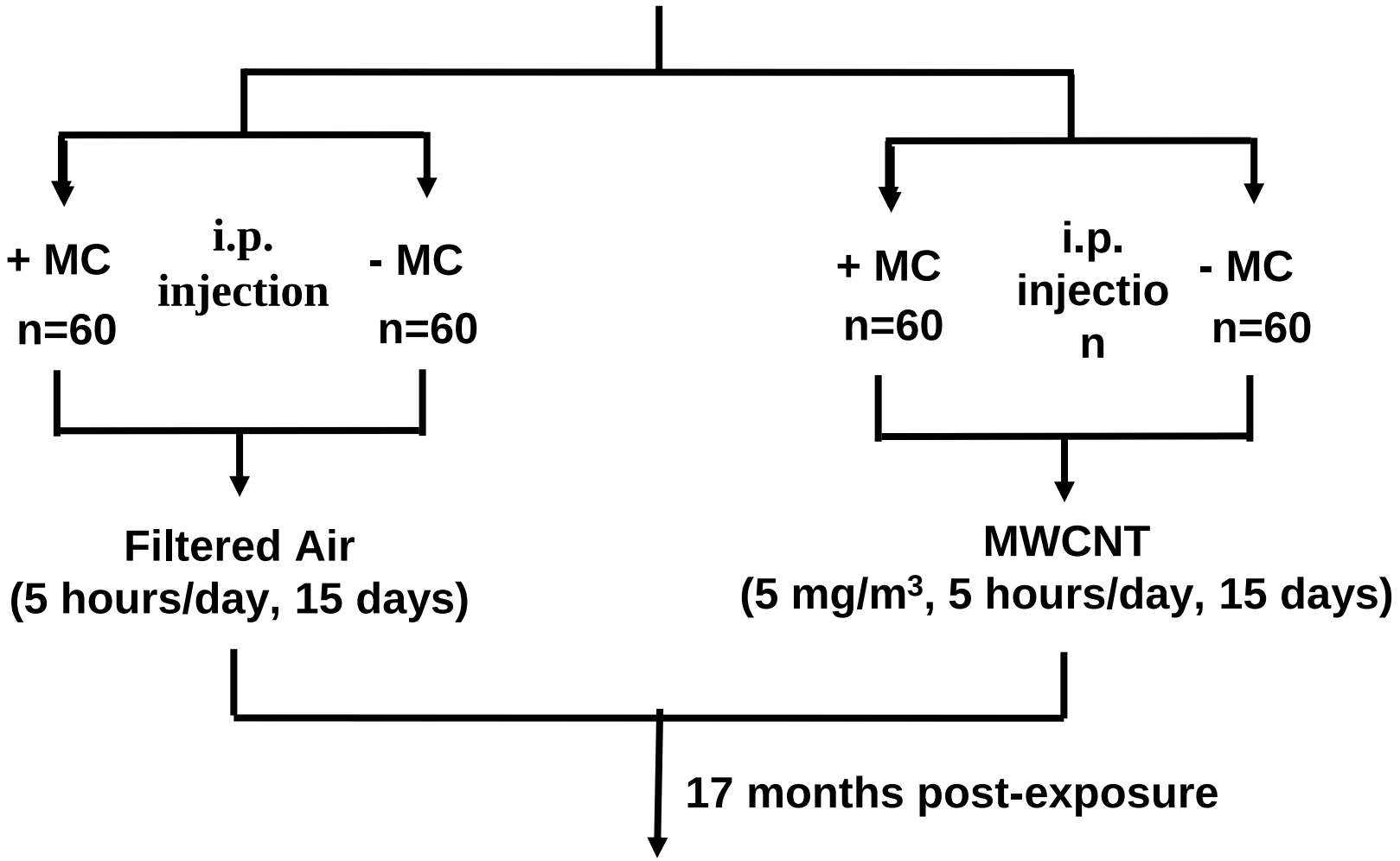
# Initiation, promotion model using the B6C3F1 mouse

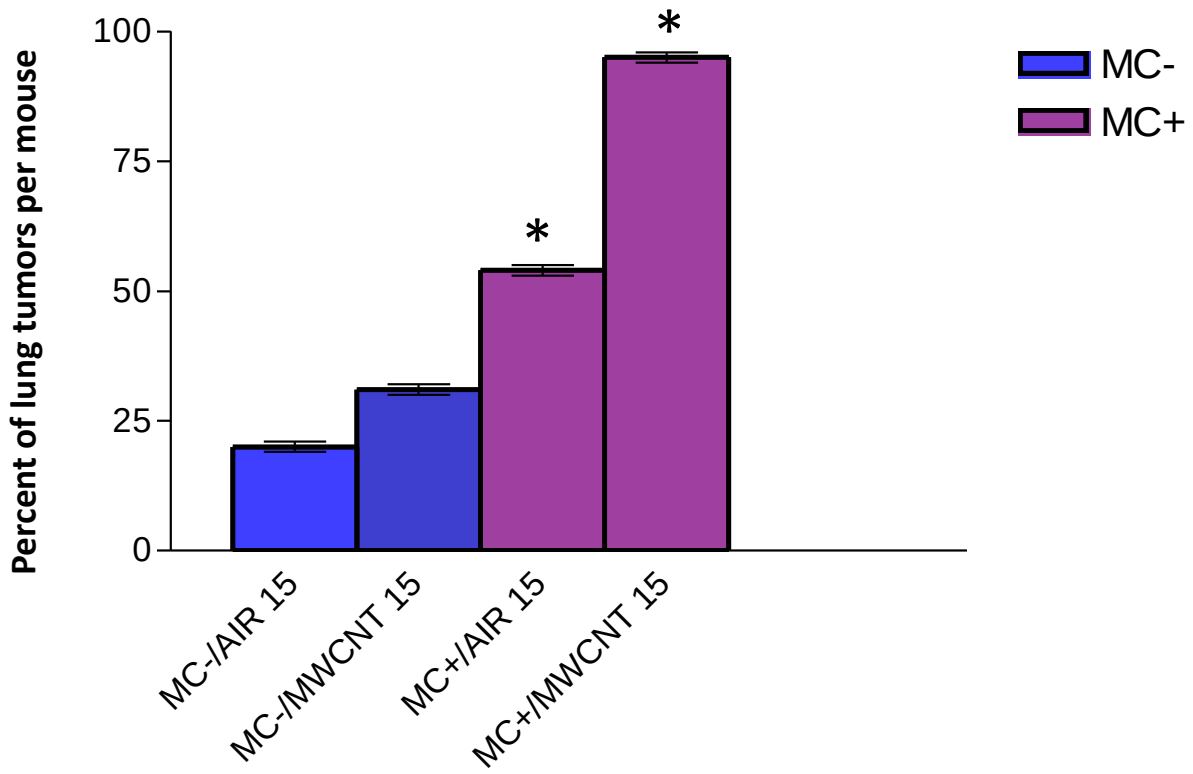
- B6C3F1 mouse strain used by the National Toxicology Program to estimate  potential carcinogenesis of test compounds.
- Animals given a low dose of a DNA damaging agent (initiation).
- Initiation followed by an agent that may encourage the growth of DNA damaged cells (promotion).

*“The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.”*

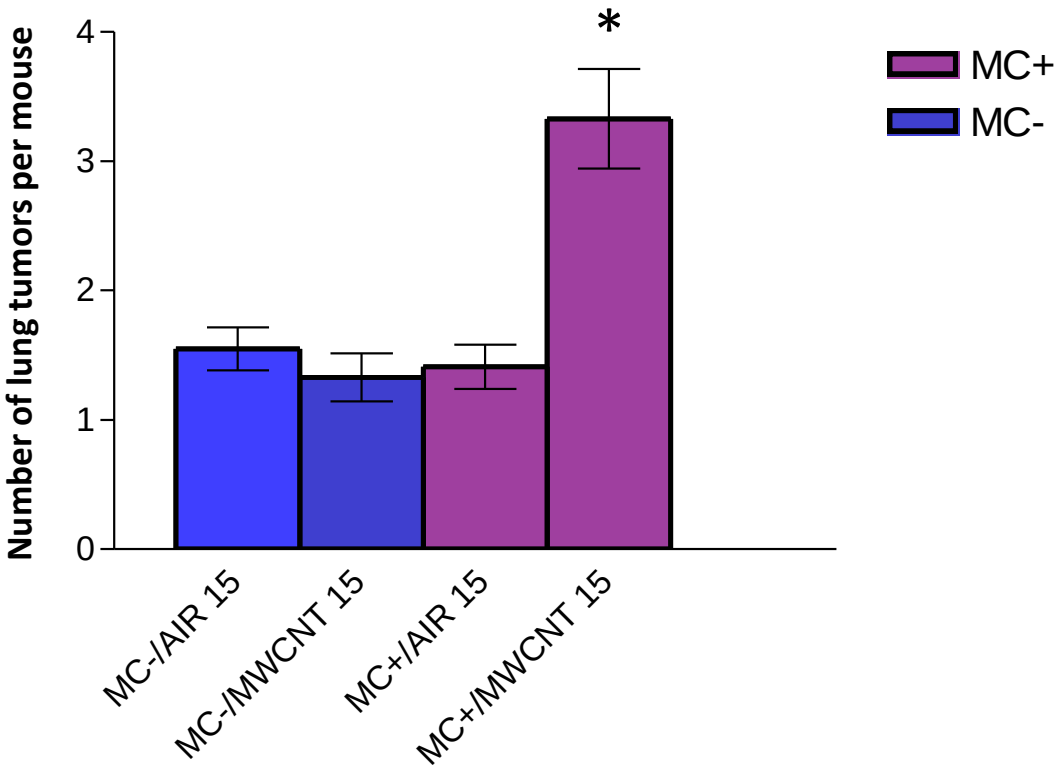



**Quantitative determination of lung tumors using histopathological techniques and expression of tumor specific proteins. Deposition 31.2 μg/mouse, *Sargent et al, 2014*.**

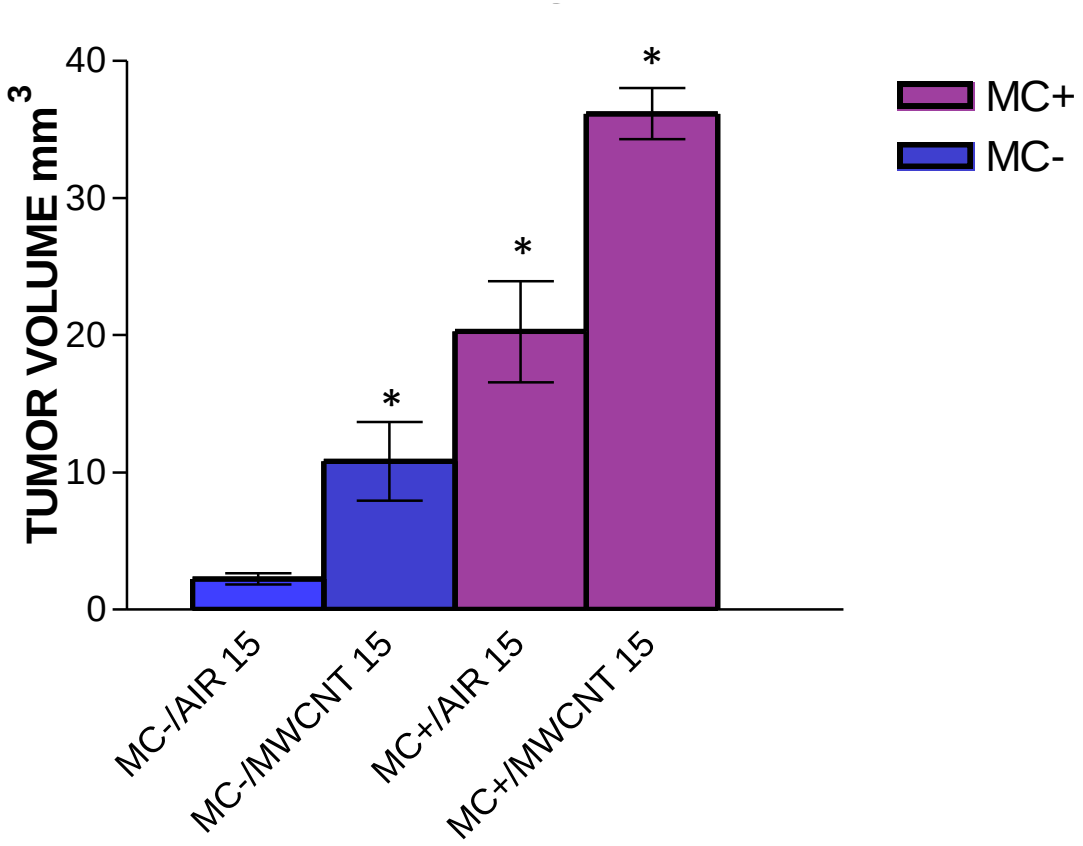
# Incidence of lung carcinomas in B6C3F1 mice 17 months following exposure to 15 days of inhaled Mitsui MWCNT



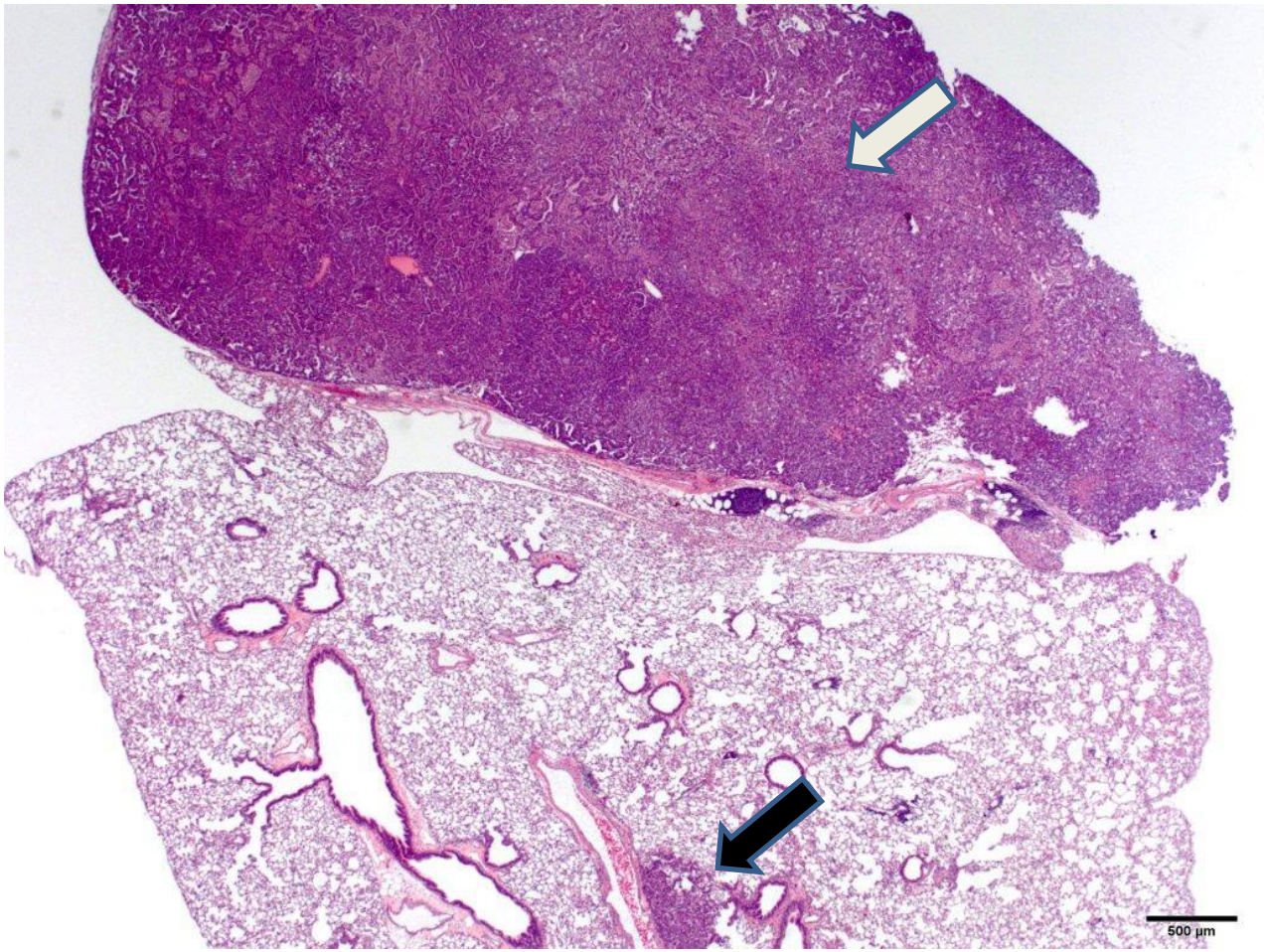
# Mean Number of lung tumors in B6C3F1 mice 17 months following exposure to 15 days of inhaled Mitsui MWCNT



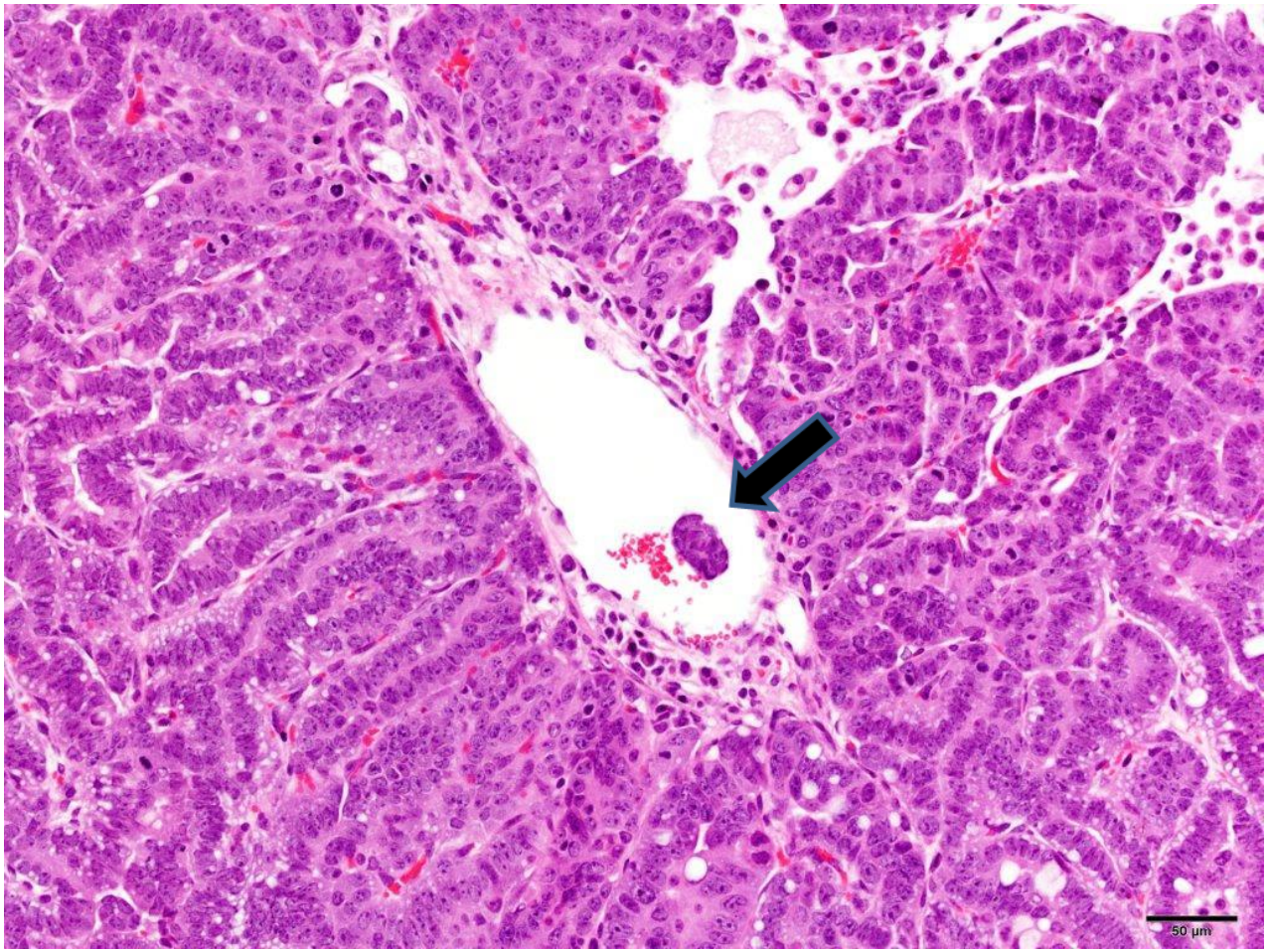
# Mean volume of lung tumors in B6C3F1 mice 17 months following exposure to 15 days of inhaled Mitsui MWCNT



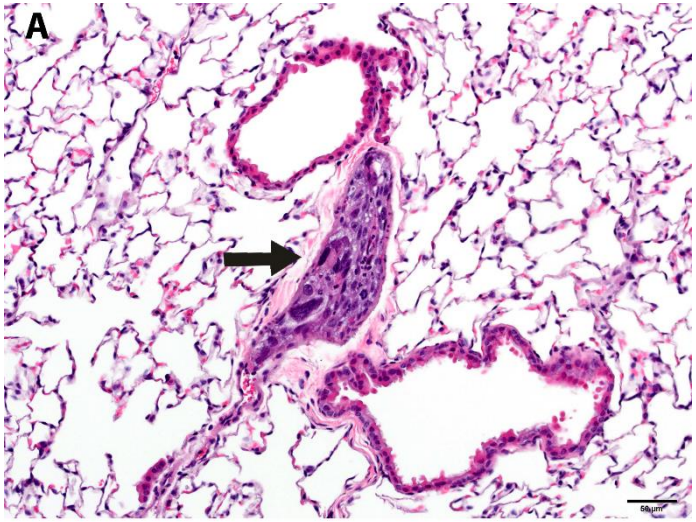
# Lung MC+/MWCNT. Alveolar/bronchiolar carcinoma (white arrow) and smaller mass (black arrow) considered an adenoma (2x)

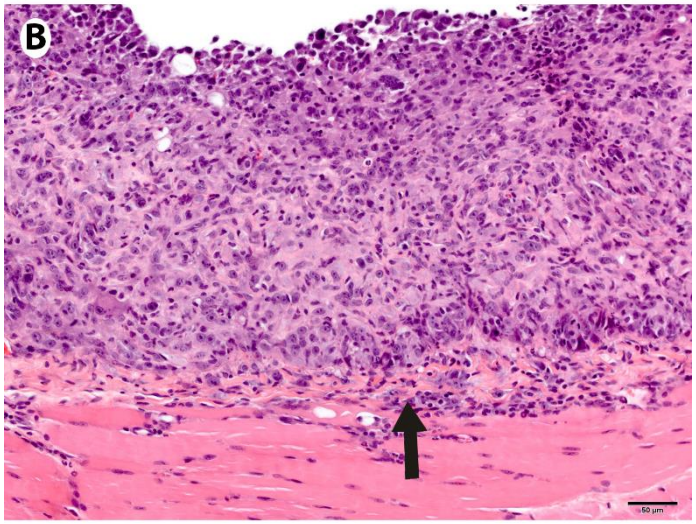


# Metastasis of an alveolar bronchiolar carcinoma( 40X)

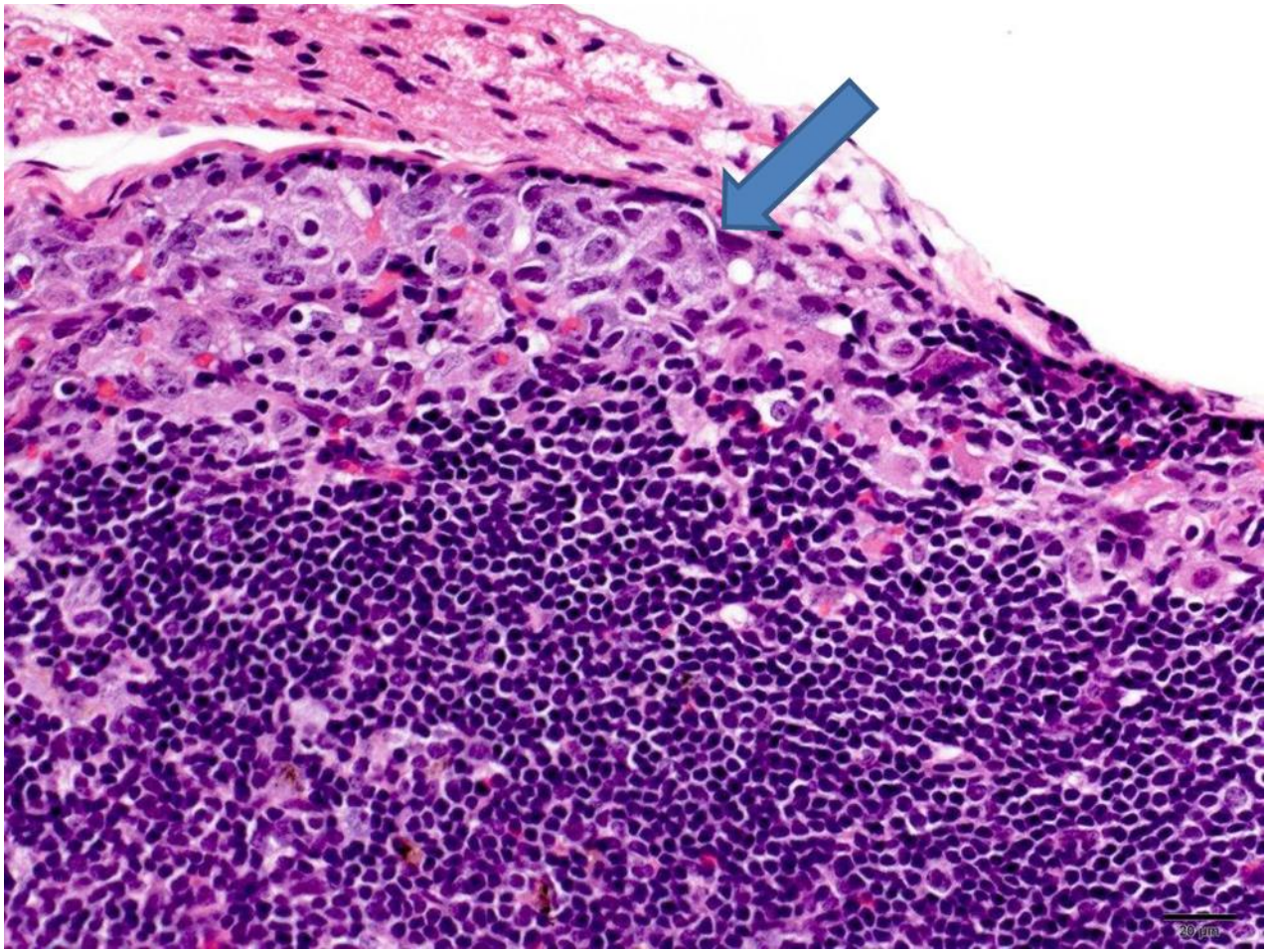


**Nine % of the MC+MWCNT 15 day-treated mice had Malignant serosal lesion in the in the diaphragm and the lung.** Molecular markers compared to human mesothelioma in progress

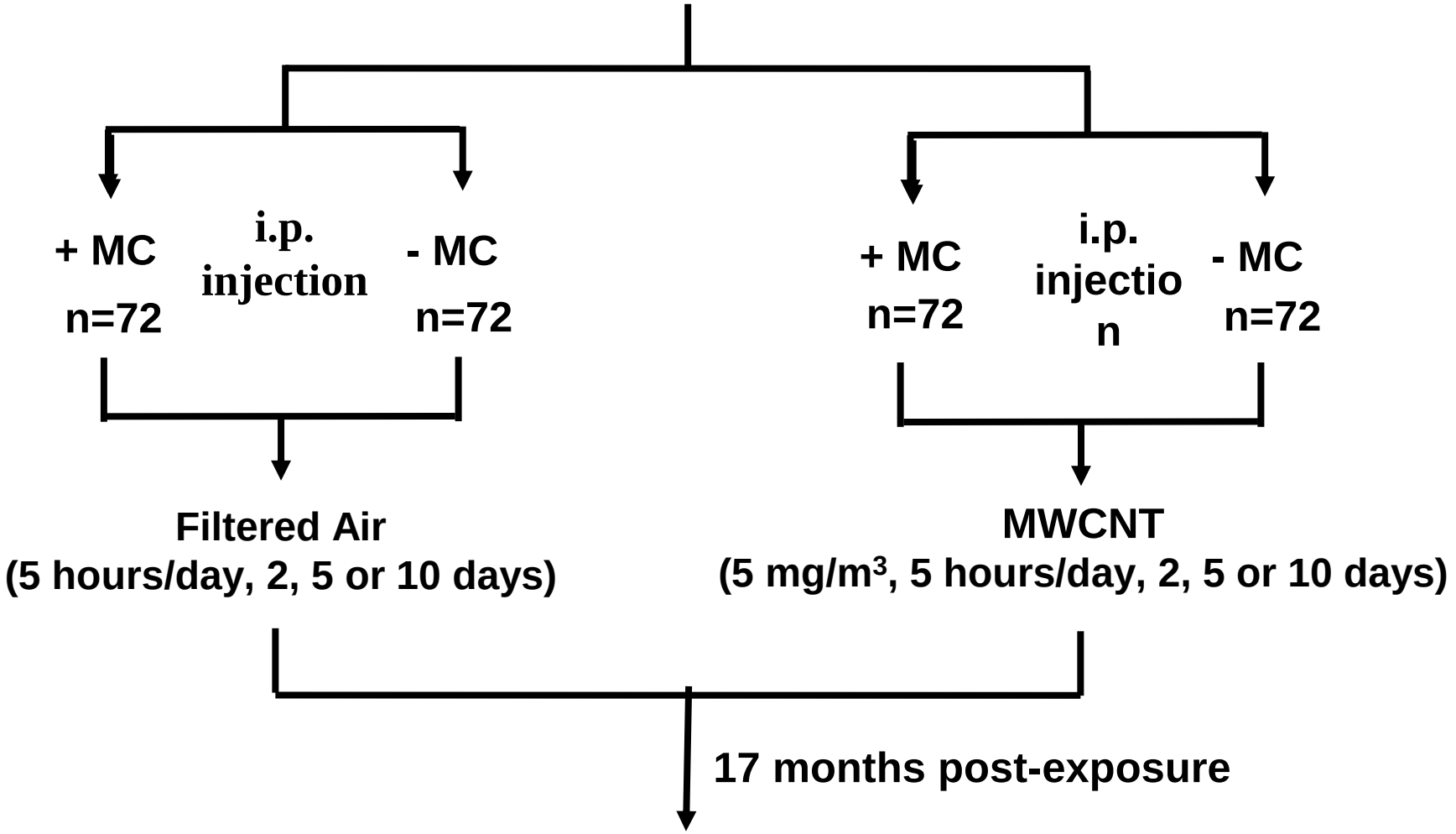


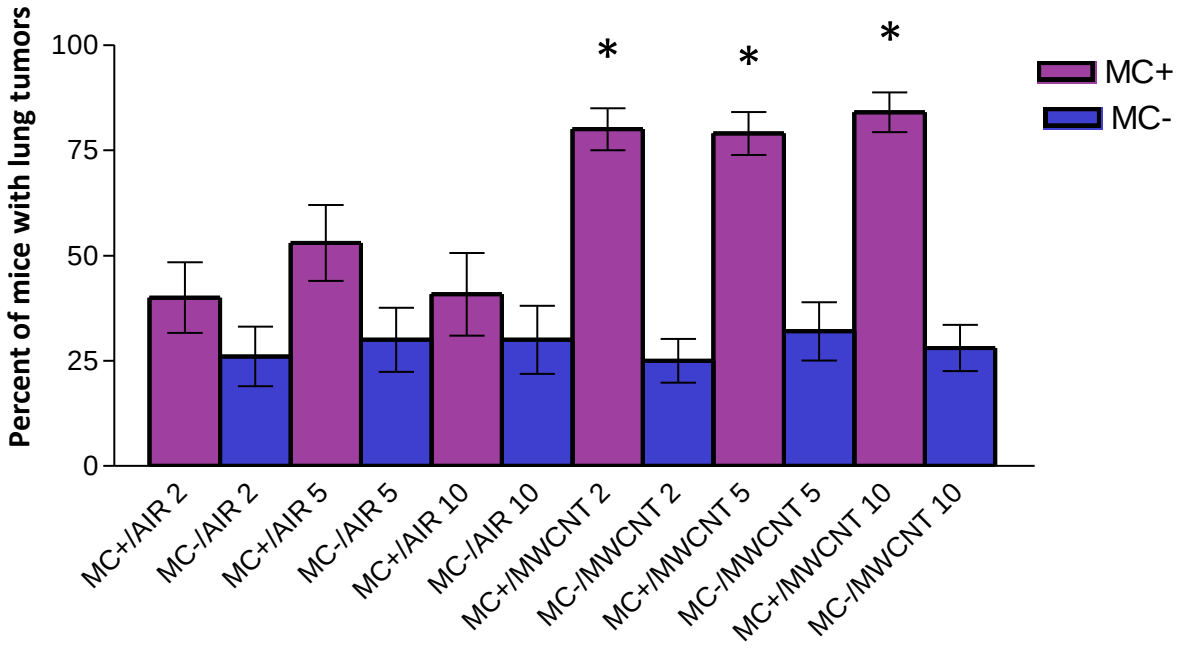
**Tracheobronchial lymph node of mouse 301 MC+/MWCNT.**
**Metastasis of presumptive mesotheliomia to the subcapsular sinus (blue arrow).**
**Presumptive test article (MWCNT) in macrophages (white arrows).**



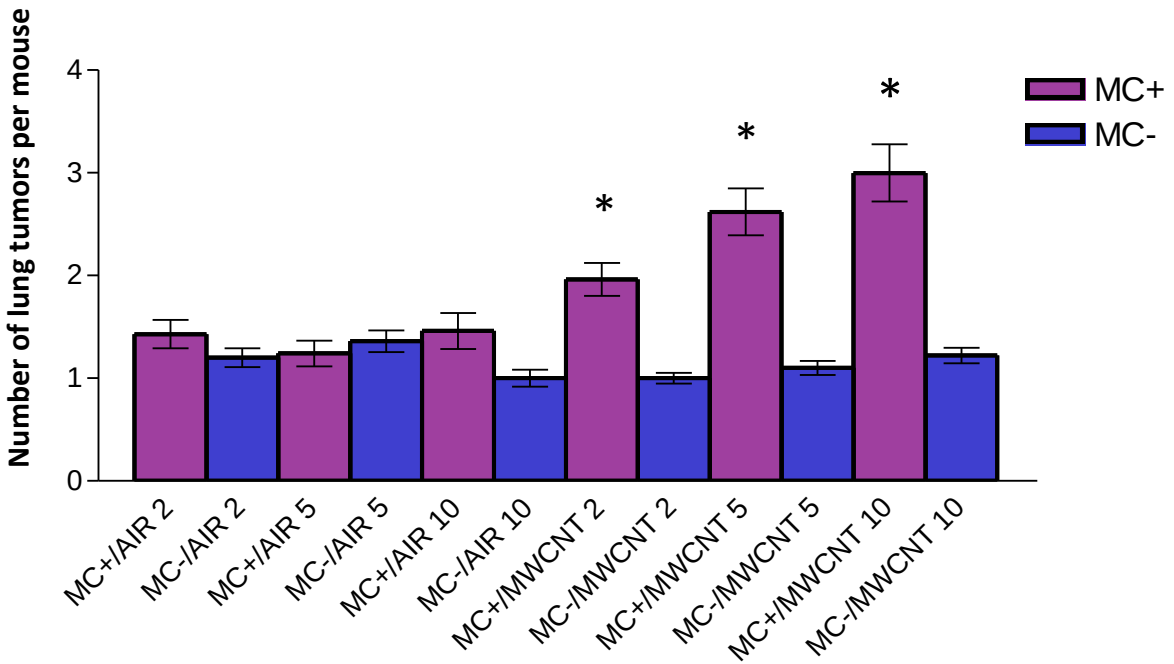
# Investigation of dose related tumor promotion.


Study II:Mice (male, B6C3F1, 6 weeks old)
+ MC
n=72
i.p.
injection
- MC
n=72
+ MC
n=72
i.p.
injectio
n
- MC
n=72
Filtered Air
(5 hours/day, 2, 5 or 10 days)
MWCNT
(5 mg/m3, 5 hours/day, 2, 5 or 10 days)
17 months post-exposure
Quantitative determination of lung tumors using histopathological techniques and expression of tumor specific proteins.
Deposition 4, 10 or 20 μg/mouse.

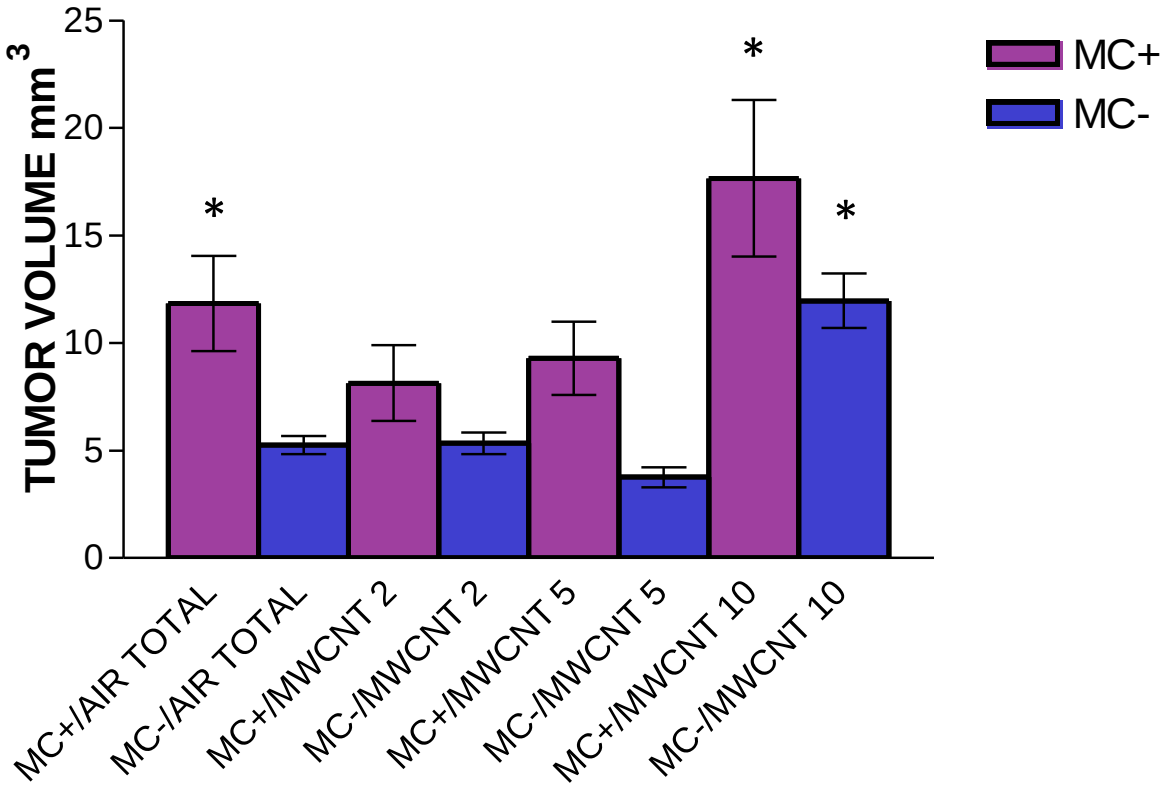
# Mean percent of mice with tumors after exposure to either corn oil or Methylcholanthrene followed by either air or 2, 5, or 10 days of inhaled MWCNT



# Mean number of tumor per mouse after exposure to either corn oil or Methylcholanthrene followed by either air or 2, 5, or 10 days of inhaled MWCNT



# Mean volume of tumor per mouse after exposure to either corn oil or Methylcholanthrene followed by either air or 2, 5, or 10 days of inhaled MWCNT



# Conclusions

- The data demonstrates that MWCNTs are a carcinogen that promotes the growth of spontaneously and chemically initiated lung cells, resulting in the development of lung adenocarcinoma.
- The threshold for tumor promotion was not found.
- MWCNTs are a strong tumor promoter.
- The MWCNT lung burden of the mice in this study approximates feasible human occupational exposures.
- The results of this study indicate that caution should be used to limit human inhalation exposures to MWCNTs.

# Collaborators

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# Incidence of lung carcinomas in B6C3F1 mice 17 months following exposure to 15 days of inhaled Mitsui MWCNT

| TOTAL SUMMARY | Total # of mice | Mice with Lung tumors | Total # Lung tumors | Number of Lung tumors per mouse | Percent of mice with lung tumors | Volume of lung tumors |
|---|---|---|---|---|---|---|
| **MC+/AIR** | 54 | 29 | 41 | 1.41±0.017 | *53.70±0.9 | 20.26±3.68 |
| **MC-/AIR** | 56 | 14 | 16 | 1.33±0.18 | 21.43±0.85 | 36.17±1.87 |
| **MC+/MWCNT 15 day** | 42 | 41 | *135 | *3.29±0.037 | *97.62±0.95 | 2.23±0.39 |
| **MC-/MWCNT 15 day** | 55 | 18 | 20 | 1.33±0.019 | 30.61±0.86 | 10.79±2.87 |

| Number of Lung Tumors | | | | |
|---|---|---|---|---|
| | Air MC- | Air MC+ | MWCNT MC- | MWCNT MC+ |
| Number of Animals Examined | 60 | 60 | 55 | 55 |
| Bronchiolo-alveolar Adenoma | | | | |
| | 6 | 30 | 11 | 80 |
| | | | | |
| Bronchiolo-alveolar Adenocarcinoma | | | | |
| | 8 | 16 | 8 | 61 |

Incidence of lung carcinomas in B6C3F1 mice 17 months following exposure to Mitsui MWCNT for 2, 5 or 10 days

| **TOTAL SUMMARY** | **Total # of mice** | **Total number Lung tumors** | **Number of Lung tumors per mouse** | **Percent of mice with lung tumors** | **Volume of lung tumors** |
|---|---|---|---|---|---|
| **MC+/AIR** | 94 | 60 | 1.36±0.083 | 44.0±0.052 | *11.84±2.21 |
| **MC-/AIR** | 109 | 37 | 1.19±0.054 | 28.43±0.85 | 5.25±0.42 |
| **MC-/2 days MWCNT** | 71 | 18 | 1.00 ±0.052 | 25.61±0.052 | 5.33±0.50 |
| **MC-/5 days MWCNT** | 66 | 23 | 1.10±0.069 | 32.0±0.069 | 3.75±0.47 |
| **MC-/10 days MWCNT** | 65 | 22 | 1.22±0.076 | 28.0±0.055 | *11.96±1.26 |
| **MC+/2 days MWCNT** | 65 | **102** | ***1.96±0.160** | *80±0.050 | *8.13±1.05 |
| **MC+/5 days MWCNT** | 63 | **131** | ***2.62±0.229** | *79±0.051 | *9.29±1.77 |
| **MC+/10 days MWCNT** | 62 | **156** | ***3.00±0.278** | *84±0.047 | *17.66±3.63 |

| Incidence of Select Group 2 Nonneoplastic Lesions | | | | |
|---|---|---|---|---|
| | **Air MC-** | **Air MC+** | **MWCNT MC-** | **MWCNT MC+** |
| **Lung No. Examined** | **60** | **60** | **55** | **55** |
| Hyperplasia, Alveolar, Focal | 7 | 8 | 14 | 30 |
| Marked | 1 | 1 | 3 | 15 |
| Hyperplasia, Bronchio-alveolar | 1 | 9 | 45 | 37 |
| Foreign Material (presumptive MWCNT) | 0 | 0 | 55 | 55 |
| Infiltration, macrophage | 3 | 9 | 32 | 39 |
| Inflammation, lymphohistiocytic | 10 | 11 | 17 | 21 |