

Title of Dataset

Potent Lung Tumor Promotion by Inhaled MWCNT

Introduction

In the lung, carcinogenesis is a multi-stage process that includes initiation by a genotoxic agent, promotion that expands the population of cells with damaged DNA to form a tumor, and progression from benign to malignant neoplasms. We have previously shown that Mitsui-7, a long and rigid multi-walled carbon nanotube (MWCNT), promotes pulmonary carcinogenesis in a mouse model. To investigate the potential exposure threshold and dose-response for tumor promotion by this MWCNT, 3-methylcholanthrene (MC) initiated (10 µg/g, i.p., once) or vehicle (corn oil) treated B6C3F1 mice were exposed by inhalation to filtered air or MWCNT (5 mg/m³) for 5 hours/day for 0, 2, 5, or 10 days and were followed for 17 months post-exposure for evidence of lung tumors.

Methods Collection

MWCNT

- MWCNT used in this study (MWNT-7, lot #061220-31) were obtained from Mitsui & Company (Tokyo, Japan).

MWCNT Inhalation Exposure

- The MWCNT aerosol was generated using an acoustical-based computer controlled whole body inhalation system.
- The target concentration of the mouse exposure was 5 mg/m³ for a duration of 5 hours/day.

Initiation Promotion Protocol

- Six-week-old male B6C3F1 mice (Jackson Laboratories, Bar Harbor, ME) were used.
- All animals used in this study were housed in an AAALAC-accredited, specific pathogen-free, environmentally controlled facility.
- All procedures involving animals were approved by the NIOSH Institutional Animal Care and Use Committee.
- Mice were treated following a two-stage (initiation-promotion) protocol. This two-stage initiation-promotion protocol involves the administration of a DNA

damaging agent, methylcholanthrene (MC), followed by administration of a suspected carcinogen, MWCNT.

- All mice received a single dose of either MC (10 µg/g bw, i.p.) or vehicle (corn oil). One week after receiving MC or vehicle, mice were exposed to MWCNT by whole body inhalation (5 mg/m³, 5 hours/day) or filtered air (control) for 2, 5 or 10 days.
- Mice were euthanized 17 months after exposure to allow time for tumor development.

MWCNT Lung Burden

- MWCNT lung burden determinations were made using a UV/visible spectrophotometer-based method.

Necropsy, Histopathology and Lung Tumor Counts

- At 17 months (512-521 days) post-exposure, mice were euthanized by intraperitoneal barbiturate overdose with ≥100 mg/kg bw pentobarbital i.p. followed by exsanguination.
- The lungs were fixed by intratracheal perfusion with 1 ml of 10% neutral buffered formalin (NBF). The mice were then necropsied following standard techniques.
- Slides were examined by a board-certified veterinary pathologist using light microscopy.
- Polarized light microscopy was occasionally used to confirm the presence or absence of foreign material (presumptive test material).
- The severity of non-neoplastic lesions was graded on a 5-point scale of minimal (1), mild (2), moderate (3), marked (4), or severe (5) to be consistent with toxicologic pathology guidelines.
- Peer review of the study was conducted by two additional veterinary pathologists. The peer review included a review of all bronchioloalveolar adenocarcinomas and all lung slides from 5% of total cases.
- The reported histopathology findings represent the consensus of all three veterinary pathologists following the peer review.

Citations

Dale W. Porter, Marlene S. Orandle, Ann Hubbs, Lauren M. Staska, David Lowry, Michael Kashon, Michael G. Wolfarth, Walter McKinney & Linda M. Sargent. Potent lung tumor promotion by inhaled MWCNT. Potent lung tumor promotion by inhaled MWCNT. *Nanotoxicology* 2024, VOL. 18, NO. 1, 69–86. <https://doi.org/10.1080/17435390.2024.2314473>

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