【S-6】

Pulmonary Toxicity of Single Walled Carbon Nanotubes

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Single walled carbon nanotubes (SWCNT) are, in their purest form, a single layer of carbon atoms in a cylindrical arrangement. Individual SWCNT are 1.5 nm in diameter and up to a millimeter or more in length. SWCNT are of commercial interest because of their great tensile strength, high conductivity, high surface area, unique electronic properties and high adsorption capacity. However, little is known concerning the potential adverse effects of SWCNT in the lung. In the present study, SWCNT, produced by the HiPco process and purified to remove contaminating iron, were evaluated. Mice were exposed to 10, 20, or 40 μg of SWCNT by pharyngeal aspiration. Saline was used as the vehicle control. Pulmonary responses were evaluated 1-60 days post-exposure. Aspiration of SWCNT was mimiced by nebulization of this material. Nebulized SWCNT exhibited two distinct morphologies, i.e., aggregates and dispersed SWCNT, as determined by transmission electron microscopy. Aspiration of SWCNT resulted in dose dependent oxidant stress (depletion of GSH), pulmonary damage (protein and LDH in bronchoalveolar lavage fluid), and inflammation (neutrophils, IL-1β, TNF-α, TNF-α and TGF-β1 in bronchoalveolar lavage samples), which peaked 1-7 days post-exposure before declining toward baseline. Sites of deposition of aggregates of SWCNT developed granulomatous lesions within 7 days post-exposure with the development of fibrosis, which progressed through 60 days post-exposure. Alveolar regions distal to SWCNT aggregates developed interstitial fibrosis, which progressed from 7-60 days post-exposure. Gold labeling of SWCNT indicated that this interstitial fibrotic reaction was associated with the deposition of nanotubes or nanoropes rather than aggregates of SWCNT. These data indicate that acute pulmonary exposure of rats to SWCNT results in a dose-dependent fibrotic response, which develops rapidly and progresses with time post-exposure. Therefore, inhalation of SWCNT may be a concern.

Pulmonary Toxicity of Single Walled Carbon Nanotubes

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Background

Single Walled Carbon Nanotubes (SWCNT)

A. Long tube-like configuration of carbon molecules
B. Single layer of carbon atoms in a cylindrical arrangement
C. Nanotube = 1.5 nm in diameter, up to 1 mm in length
D. High tensile strength, high surface area, unique electronic properties, high adsorption capacity
E Used in electronics, structural materials, etc

Issue

A. Potentially wide commercial applications
B Little information is available concerning the potential adverse effects of inhalation of SWCNT

Objective

A. Characterize the pulmonary responses to SWCNT
B. Determine the dose-dependence of the responses
C Determine the duration of responses post-exposure

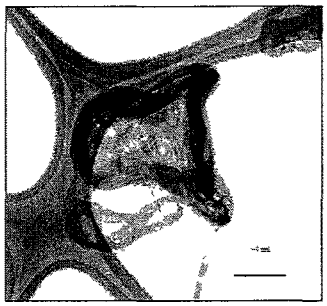
Test Material

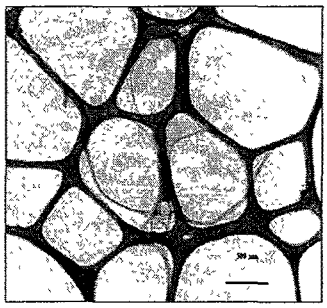
A. SWCNT were produced by the high-pressure carbon monoxide process (HiPCO)
B. Unpurified SWCNT – contain 30% metal catalyst (ultrafine Fe, or Fe/Ni)
C. Purified SWCNT – acid treated; < 0.2 wt% metals
D. Suspended material supplied by NASA

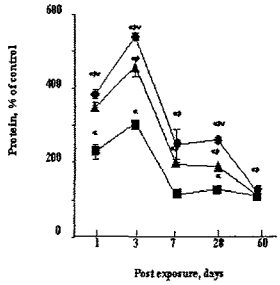
Methods

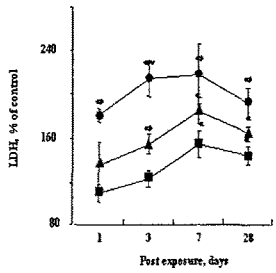
A. Exposure
   1) Pharyngeal aspiration of mice
   2) PBS or SWCNT (10, 20, or 40 μg/mouse)
   3) Sacrifice 1 – 60 days post-exposure

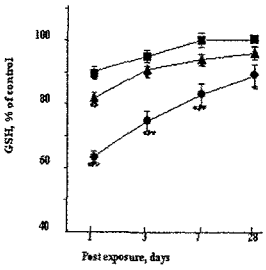
B. Endpoints
   1) Damage – BAL protein and LDH
   2) Inflammation – BAL cells and cytokines
   3) Oxidant stress – lung GSH
   4) Histology – particle deposition, inflammation, granulomas, and fibrosis

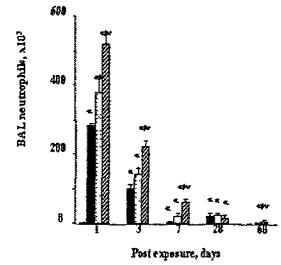


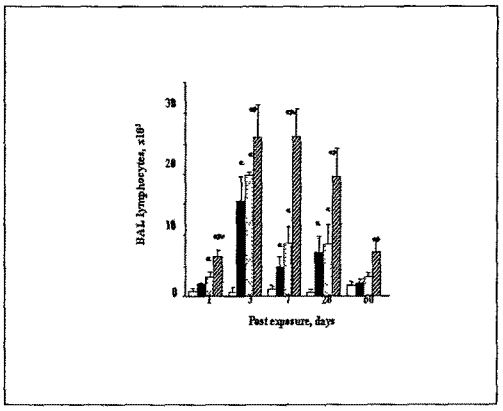


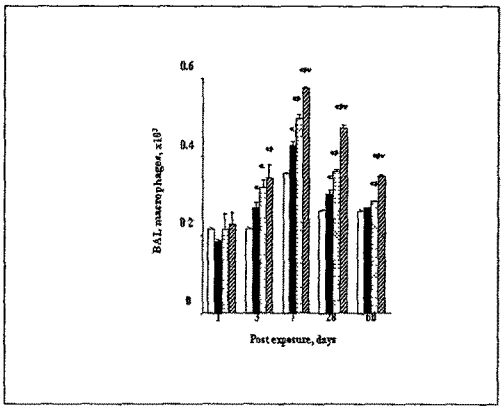

Protein, % of control
600
400
200
0
1
3
7
28
60
Post exposure, days

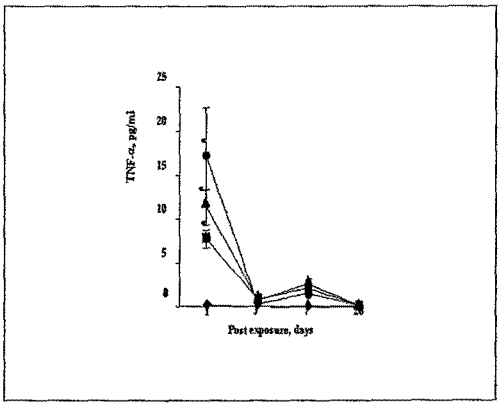

LDH, % of control
240
160
80
1
3
7
28
Post exposure, days

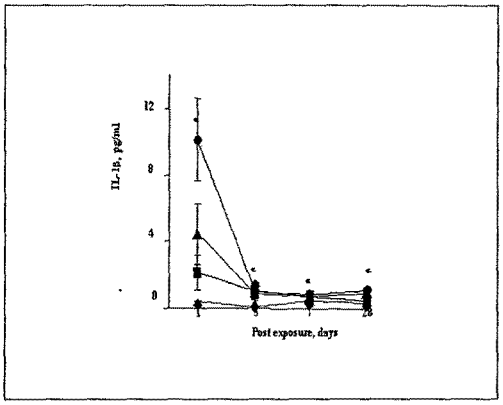

GSH, % of control
100
80
60
40
1
3
7
28
Post exposure, days

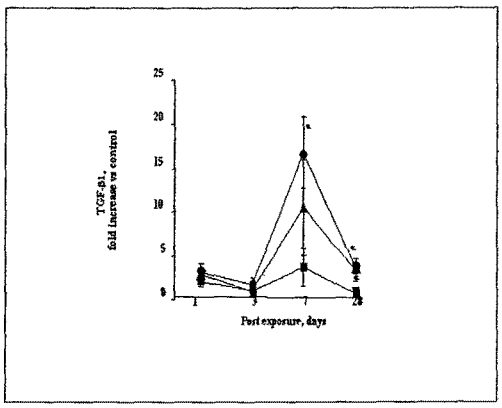

BAL neutrophils, x10³
600
400
200
0
1
3
7
28
60
Post exposure, days

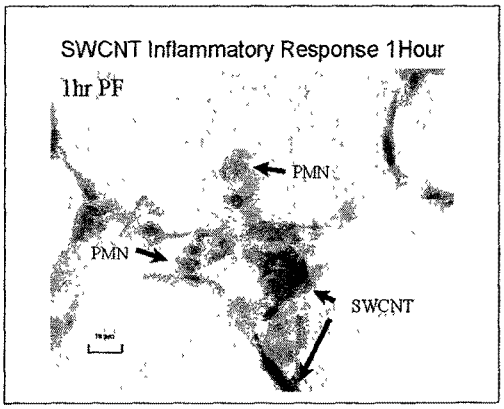

BAL lymphocytes, x10⁵
Post exposure, days

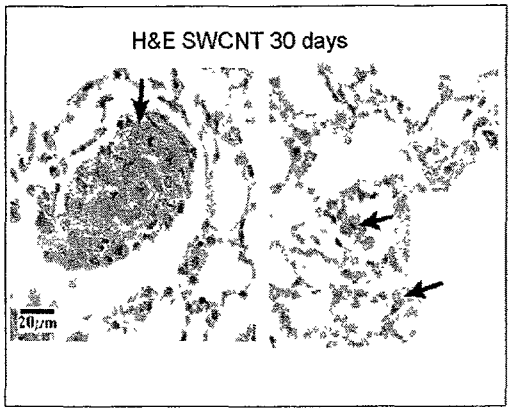

BAL macrophages, x10⁵
Post exposure, days

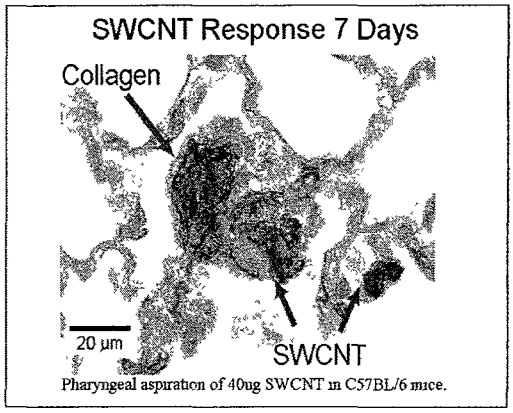

TNF-α, pg/ml
Post exposure, days

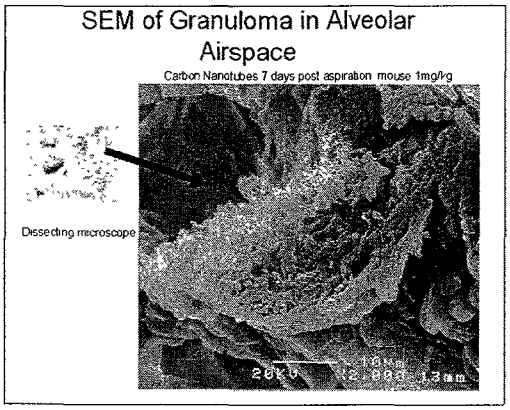

IL-1β, pg/ml
Post exposure, days

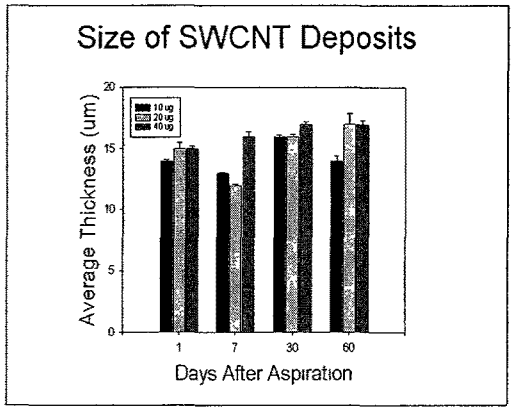

TGF-β1
fold increase vs control
Post exposure, days

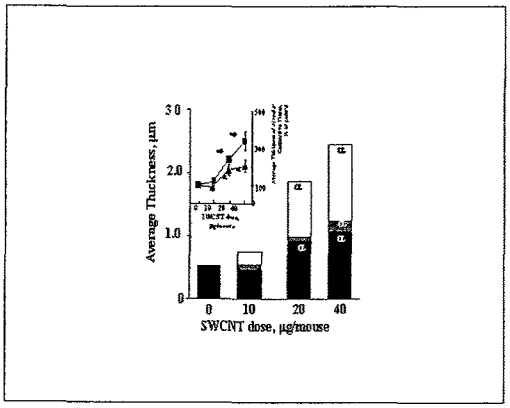

SWCNT Inflammatory Response 1Hour
1hr PF
PMN
PMN
SWCNT

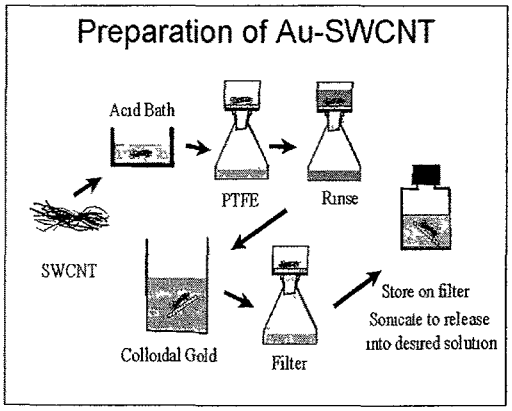

H&E SWCNT 30 days
20μm


SWCNT Response 7 Days
Collagen
20 μm
SWCNT
Pharyngeal aspiration of 40ug SWCNT in C57BL/6 mice.

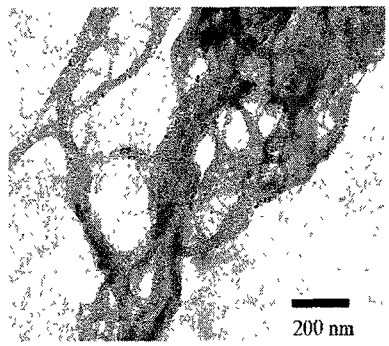

SEM of Granuloma in Alveolar Airspace
Carbon Nanotubes 7 days post aspiration mouse 1mg/kg
Dissecting microscope


Size of SWCNT Deposits
Average Thickness (um)
10 ug
20 ug
40 ug
0
5
10
15
20
1
7
30
60
Days After Aspiration

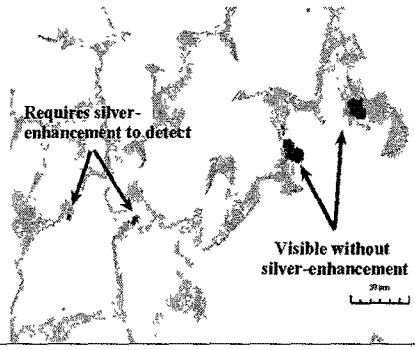

Average Thickness, μm
0
1.0
2.0
3.0
0
10
20
40
SWCNT dose, μg/mouse


Preparation of Au-SWCNT
Acid Bath
PTFE
Rinse
SWCNT
Colloidal Gold
Filter
Store on filter
Sonicate to release
into desired solution

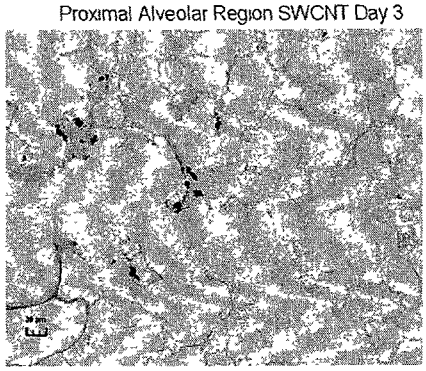
10nm Colloidal Gold-Labeled SWCNT



Detection of Sub-Micron SWCNT by Silver Enhancement

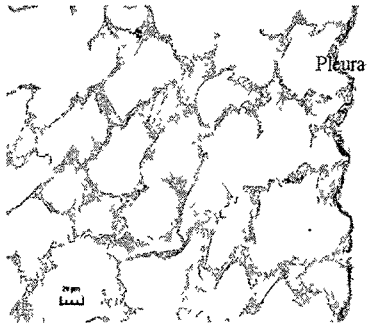


Proximal Alveolar Region SWCNT Day 3



Silver-enhanced gold-labeled SWCNT, 40 ug aspiraton, perfusion fixed

Pleural AU-SWCNT 1 day



Summary

A. Nebulized SWCNT dispersed as aggregates and nanotubes

B. Aspiration causes transient oxidant stress, damage and inflammation, peaking by 7 days post-exposure

C. Histology visualizes aggregates in the terminal bronchials and proximal alveoli with no visible material in distal alveoli

D. Size of aggregates doesn't change with time

E. Rapid fibrosis – begins in 7 days and progresses through 60 day post-exposure
   1) Fibrosis in granulomatous lesions containing aggregates
   2) Diffuse interstitial fibrosis in distal alveolar walls with no visible SWCNT

F. Used silver enhancement of gold-labeled SWCNT
   1) See aggregates in proximal alveoli and terminal bronchials
   2) See nanoropes in walls of distal alveoli

Conclusions

A. See granulomatous lesions at deposition sites of aggregates

B. See interstitial fibrosis in sites of deposition of nanoropes