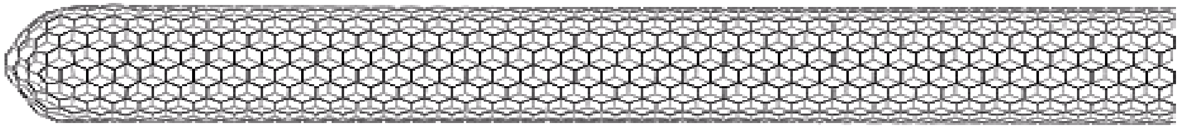

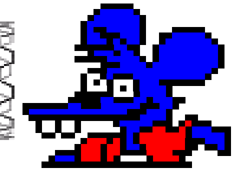

PULMONARY TOXICITY OF SINGLE-WALLED NANOTUBES IN MICE

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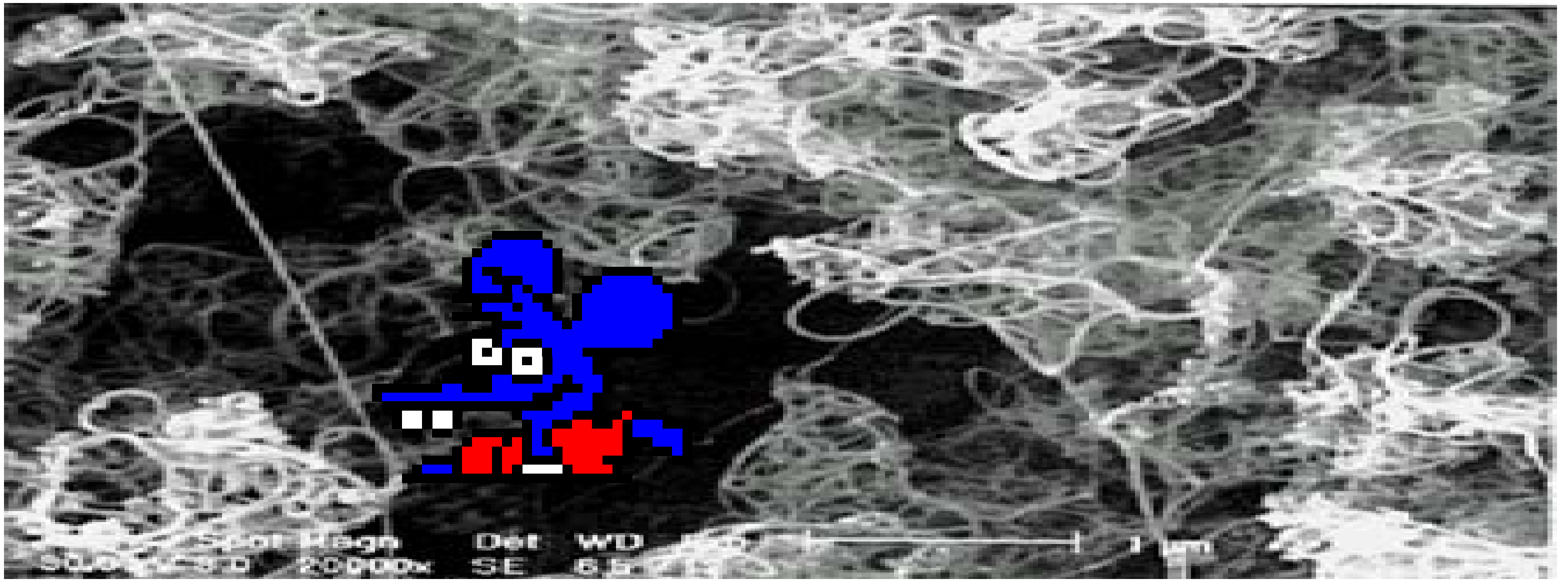
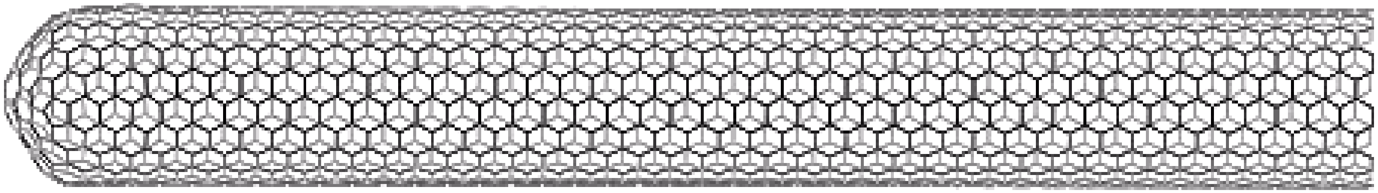
JSC Toxicology Group, and Wyle Laboratories
NASA Johnson Space Center, Houston, TX

John T. James, Ph.D. /NASA
Richard McCluskey, M.D., Ph.D/NASA
Robert L. Hunter, M.D.,Ph.D./UT Med. Ctr.



PROPERTIES AND USES



- SWNTs are structurally rolled-up graphite sheets
- ~ 1.5 nm diameter and several microns long
- Generally pack tightly and parallel to form ropes or rods
- Have highly desirable electrical, mechanical, and thermal properties
- Potentially wide applications in electronic, computer, and aerospace industries

SYNTHESIS OF SWNT

SWNTs are Commonly Synthesized by:

- **Carbon arc discharge**

  (Carbon source: graphite)

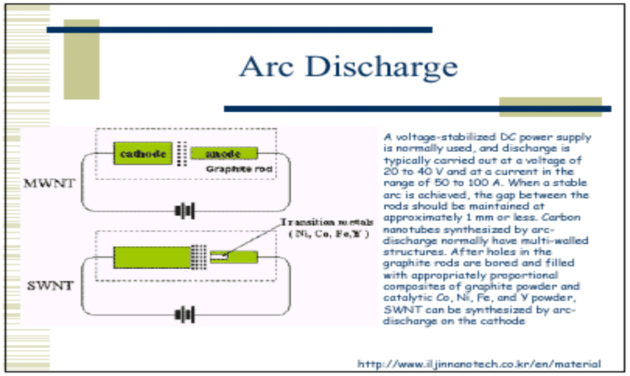
- **Laser evaporation**

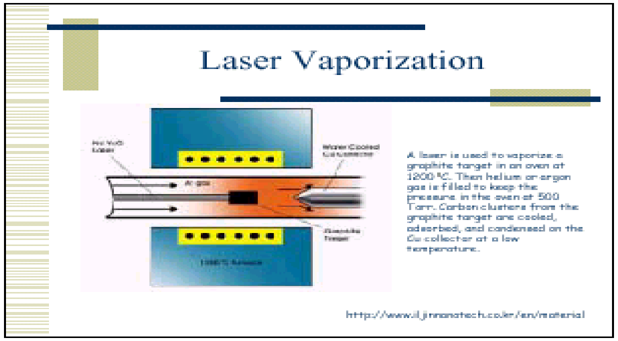
  (Carbon source: graphite)

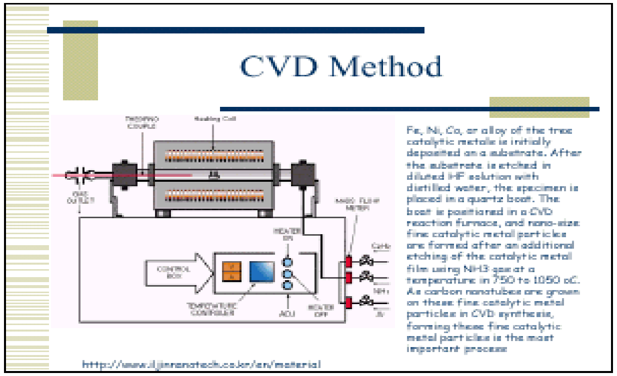
- **Chemical vapor deposition**

  (Carbon source: CO as in HiPco Process)

(All these process require catalytic metals, such as Fe, Ni, and Co; **products contain residual metals**)







Why Do We Need Toxicity Information?

Pouring HiPco-NT between Containers at Rice Facility
(Courtesy of Drs. Maynard & Baron, NIOSH)

Richard Smalley of Rice Univ. has predicted that hundreds or thousands of tons of NTs could be produced in 5 to 10 years and "in time, millions of tonnes of nanotubes will be produced worldwide every year " (Ball 2001; ISI 2002).

➔Human exposures will increase

JSC has a Nanolab that makes carbon nanotubes and workers could potentially be exposed to NT.

Toxicity of such an important material is unknown

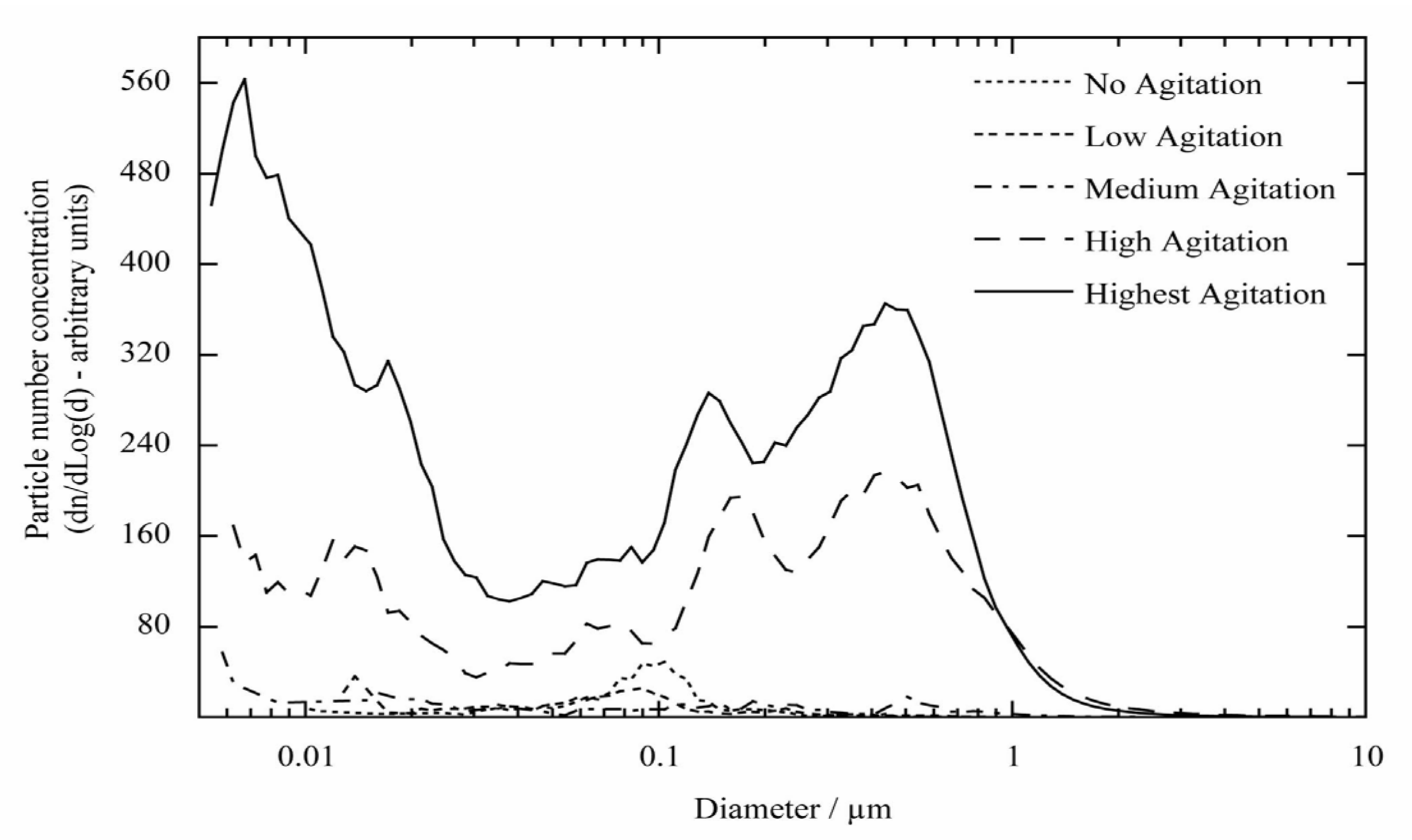
Results of Air sampling of nanotube particles at Rice, CNI and NASA facilities

(Courtesy of Dr. Maynard and Dr. Baron, NIOSH, 2002)

Table 2. **Analysis results of personal air samples from each of the four field sites**.
Nanotube concentrations have been estimated assuming 30% Fe by mass.
Fe: Limit of detection: 0.0643 µg. Limit of quantification: 0.212 µg.
Ni: Limit of detection: 0.0182 µg. Limit of quantification: 0.0601 µg.

Sampling site	Mass Fe (µg)	Mass Ni (µg)	Estimated nanotube concentration from Fe mass (µg/m^3)
Field Blank B1	2.06	[0.022]	
Field Blank B2	1.72	[0.038]	
NASA JSC (Laser)	**1.30**	**[0.059]**	**31.1**
Rice (HiPCO)	**2.75**	**[0.020]**	**116.8**
CNI (Laser)	**1.55**	**0.314**	**53.6**
CNI (HiPCO)	**3.80**	**0.208**	**96.0**

Particle size distribution of a HiPCo sample under agitation courtesy of Baron et al. of NIOSH (2003)



Fine NT particles can potentially reach the lungs. How can we assess NT toxicity in the lungs ?

- Inhalation Exposures

  Need to isolate dust of respirable size

  Need a large amount of material

  Experimentally difficult (aerosol generation and monitoring)

  NTP study: comprehensive [2 yrs, 2 doses, Rats and Mice (M&F), all organs]

  >$10, 000, 000

- Intratracheal Instillation

  Need little material

  Relatively easy

  Less expensive (we received a total of only $70,000 CDDF for the project)

  Allow for comparing several dusts in the same experiment

  Disadvantages – General single treatment - not way humans are exposed

  -- Uneven distribution of dust in lungs

SWNT PRODUCTS WE STUDIED

Raw HiPco NT

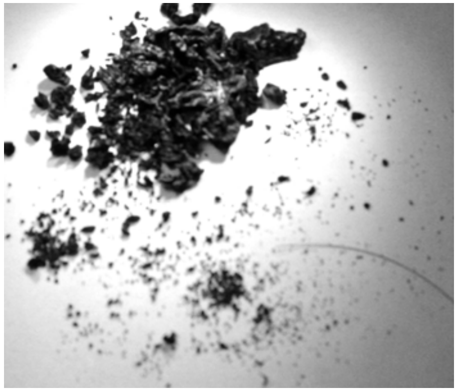
27.0% Fe, 0.8% Ni

Purified HiPco NT
2.1% Fe

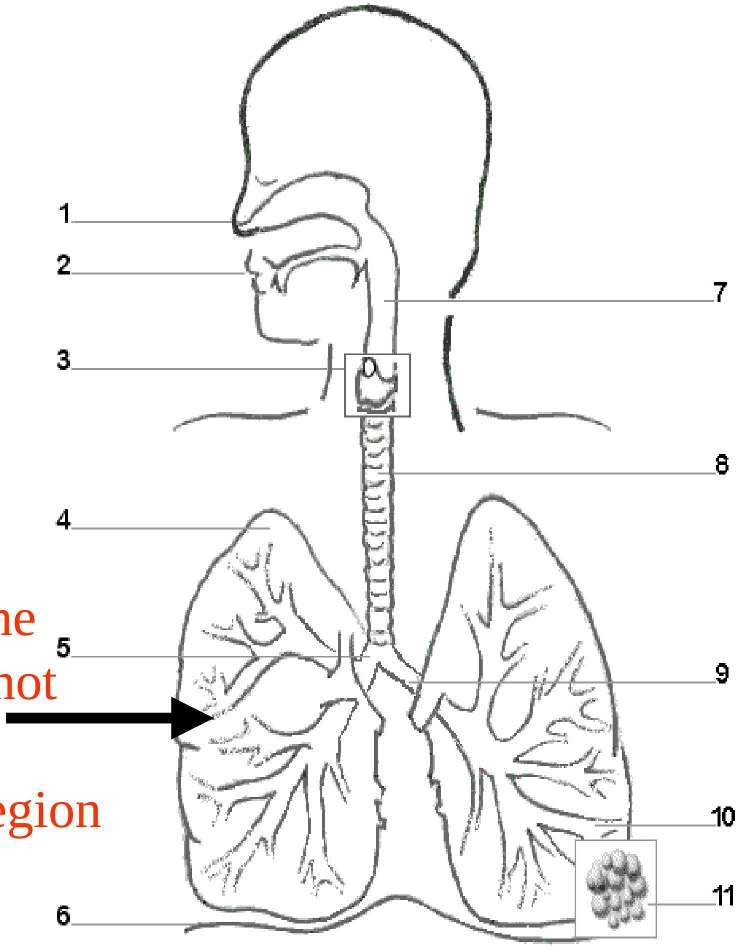
Carbolex Electric-arc NT

26% Ni, 5%Y, 0.5% Fe

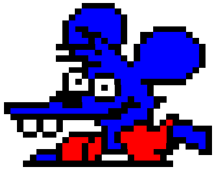
Need Fine (Respirable) Particle for studying effects in the lungs



Large airborne particles cannot reach the pulmonary region



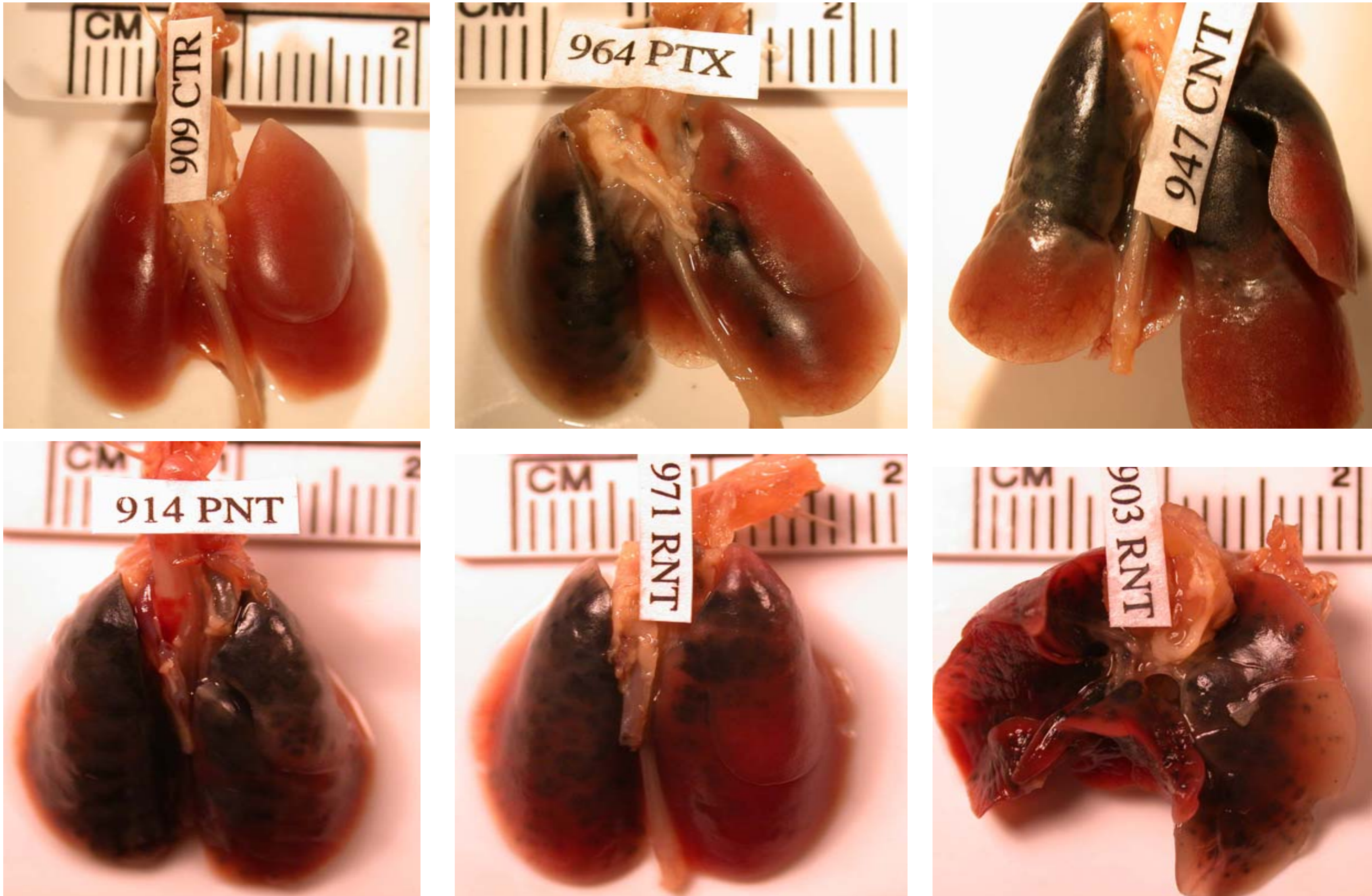
LUNG HISTOPATHOLOGY STUDIES OF MICE INTRATRACHEALY INSTILLED WITH NTs



MATERIALS	Metal	Dose	Duration	
	%	mg	7-d	90-D
Raw HiPco NT	Fe 27%, Ni 0.8%	0.5	4	5
Raw HiPco NT	Fe 27%, Ni 0.8%	0.1	4	5
Purified HiPco NT	Fe 2%	0.5	4	5
Purified HiPco NT	Fe 2%	0.1	4	5
Carbolex Arc NT	Ni 26% ,Y 5%	0.5	4	5
Carbolex Arc NT	Ni 26% ,Y 5%	0.1	4	5
Carbon Black	Not detected	0.5	4	5
Carbon Black	Not detected	0.1	4	5
Quartz	Not determ'd	0.5	4	5
Quartz	Not determ'd	0.1	4	5
Serum Control	--	--	4	5
Serum Control	--	--	4	5
Total			48	60

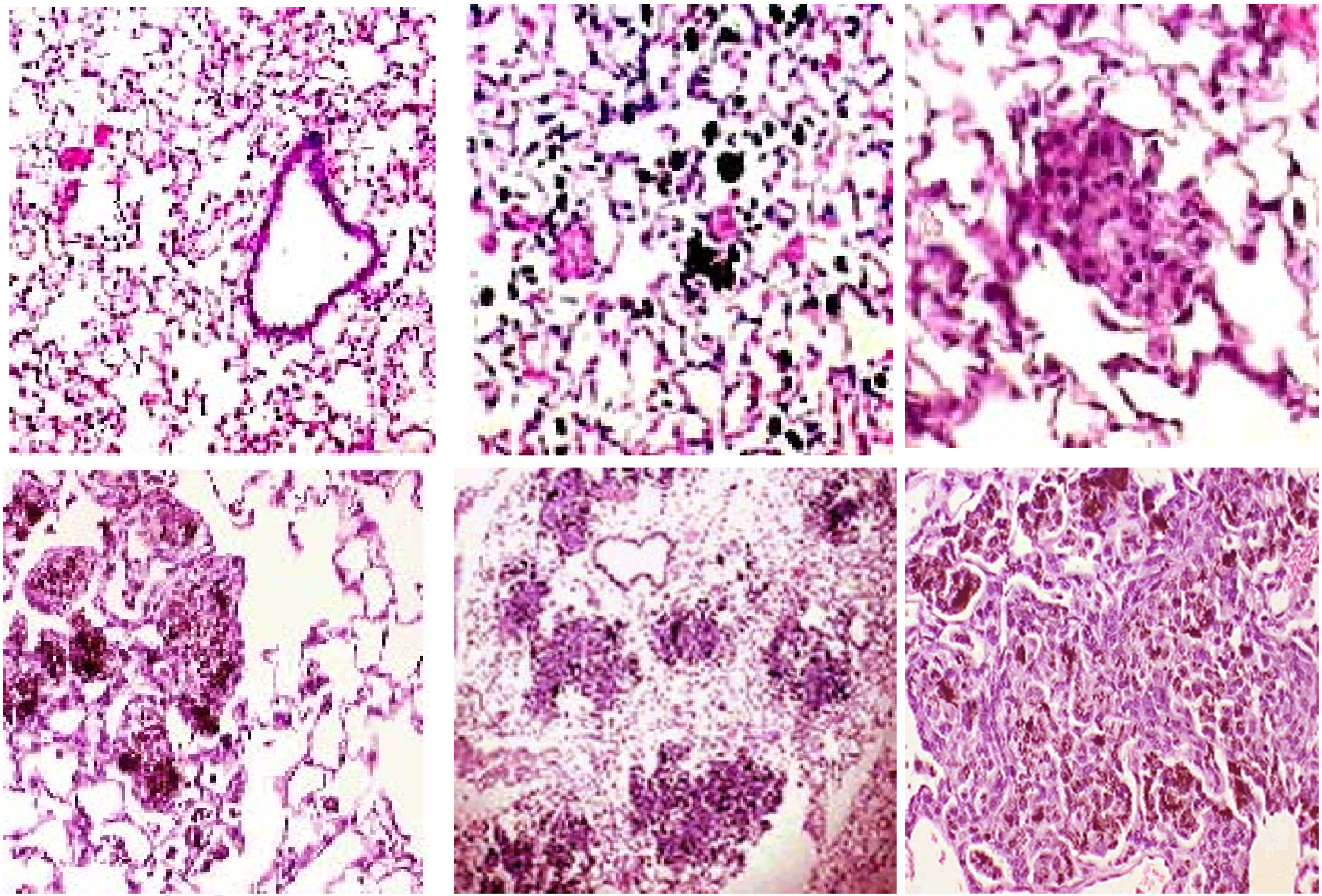
A major manufacturer regards NTs as graphite and recommends to use OSHA exposure limit on graphite (5 mg/m^3) for NT. If a 30-g mouse breathe 30 ml/min at 5 mg/m^3 of respirable-size NT particles and assuming 40% of the dust deposits in the lung, it will take 17 days to reach a lung burden of 0.5 mg

Lungs from Mice of HD-90d Groups



Histopathologic Micrographs of Lungs from Mice of the HD-7d Groups

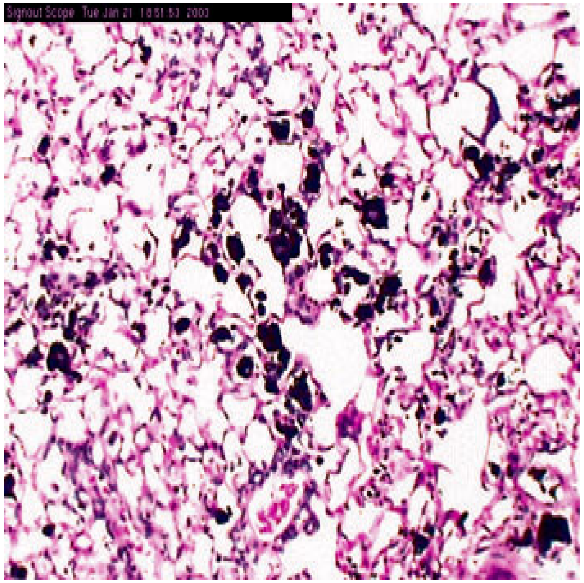
Serum | Carbon Black | Quartz



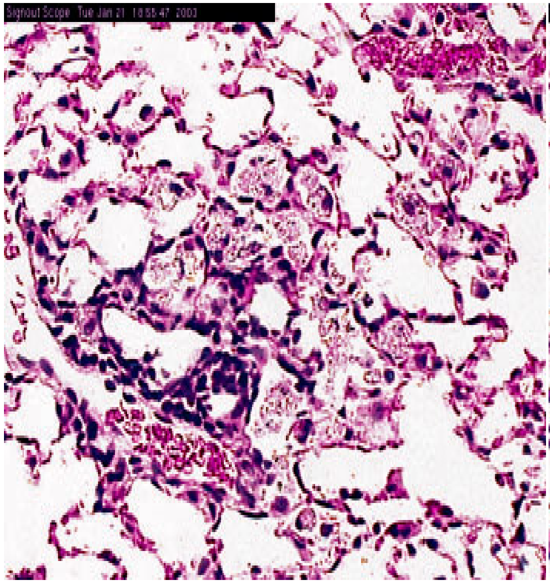
Carbolex NT | Raw NT | Purified NT

Histopathologic Micrographs of Lungs from Mice of the HD-90d Groups

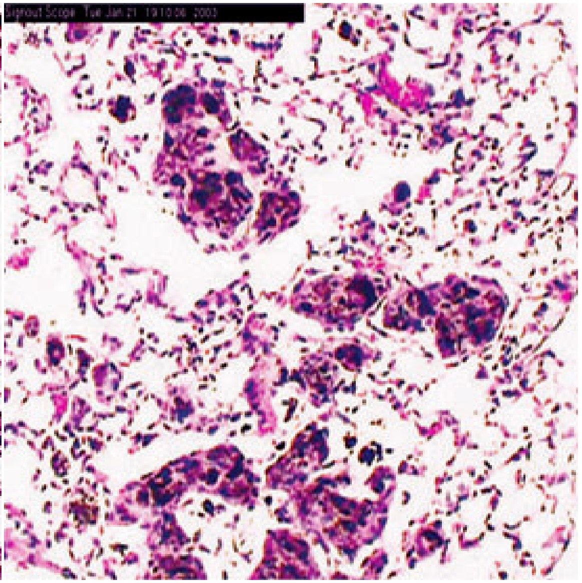
Carbon Black

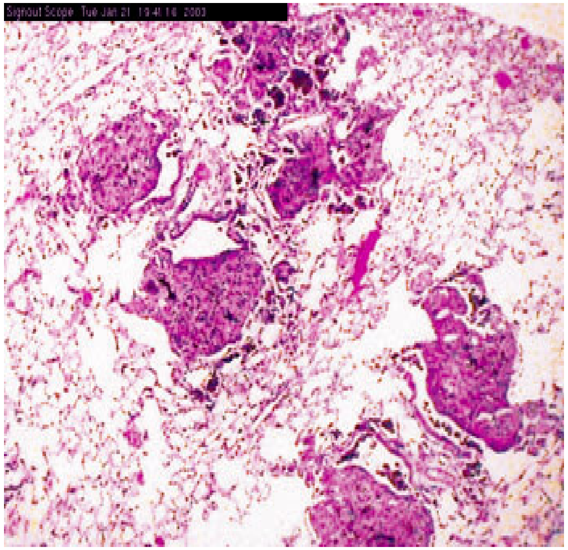


Quartz

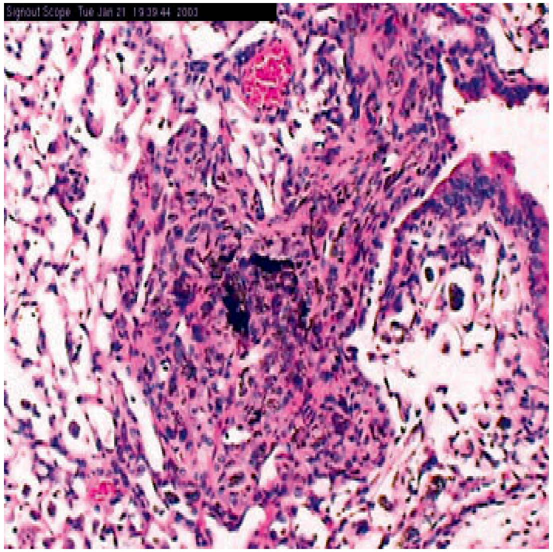


Carbolex NT

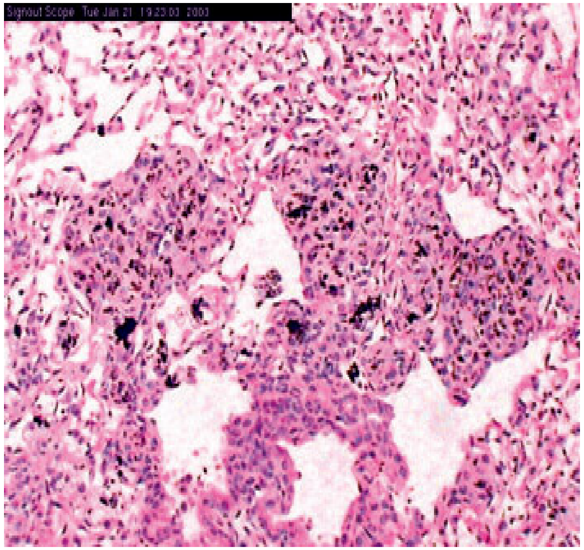




Raw NT (low m.)

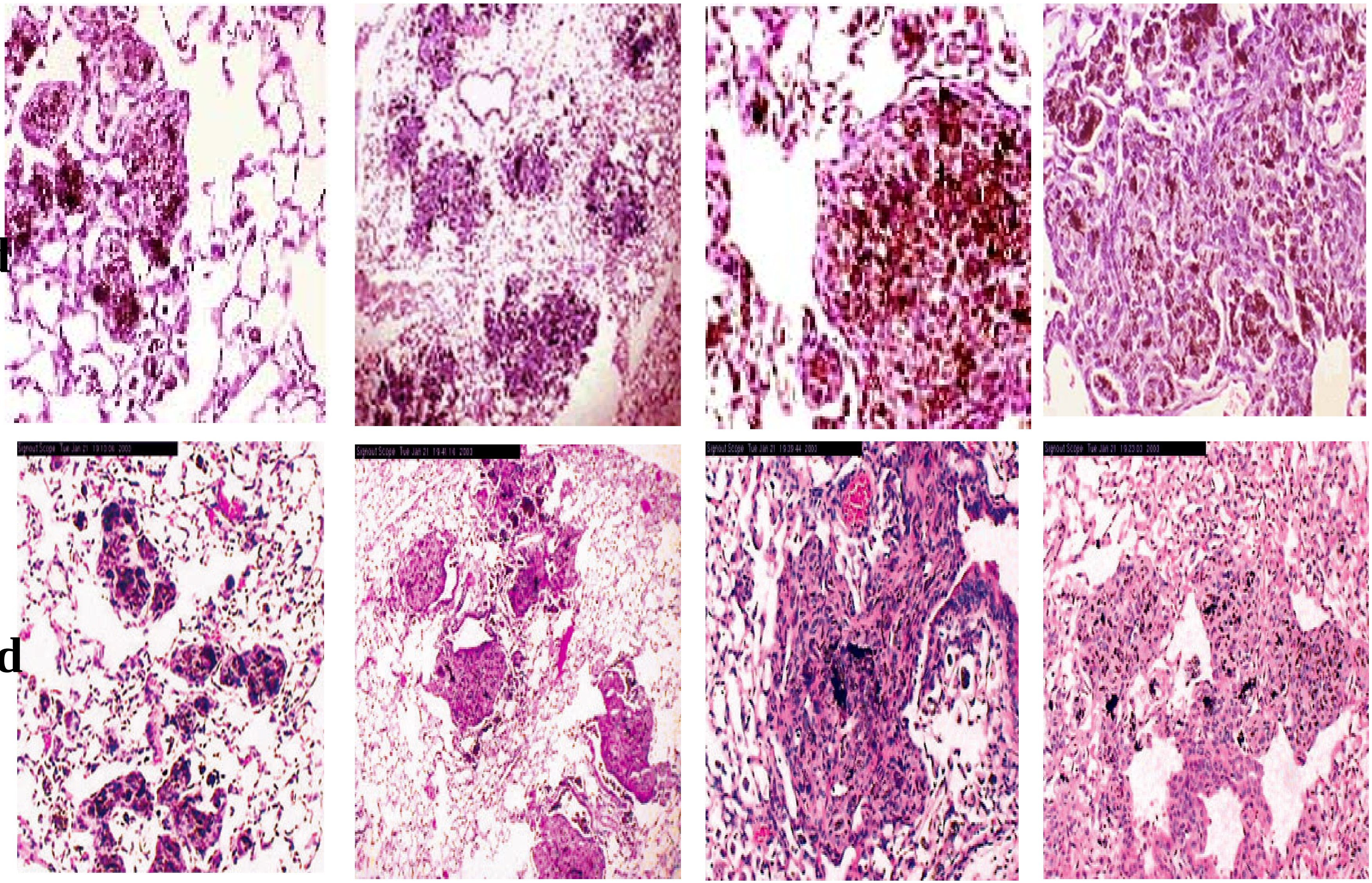


Raw NT (high m.)



Purified NT

Lung Micrographs from Mice of the HD-7d and HD-90d Groups



Science

11 April 2003 issue, p. 243

AMERICAN CHEMICAL SOCIETY MEETING

Nanomaterials Show Signs of Toxicity

Robert F. Service

"… the nanotubes--with or without metal particles--triggered the formation of **granulomas**, a combination of dead and live tissue surrounding the material that's a significant sign of toxicity, **Lam** says. **Warheit (Du Pont)** reported seeing **granuloma** formation in a similar study but noted that the inflammation seemed to tail off after 3 months…"

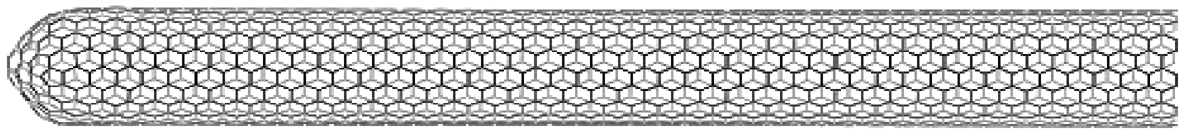
DuPont - Warheit study used a laser-evaporated NTs in rats

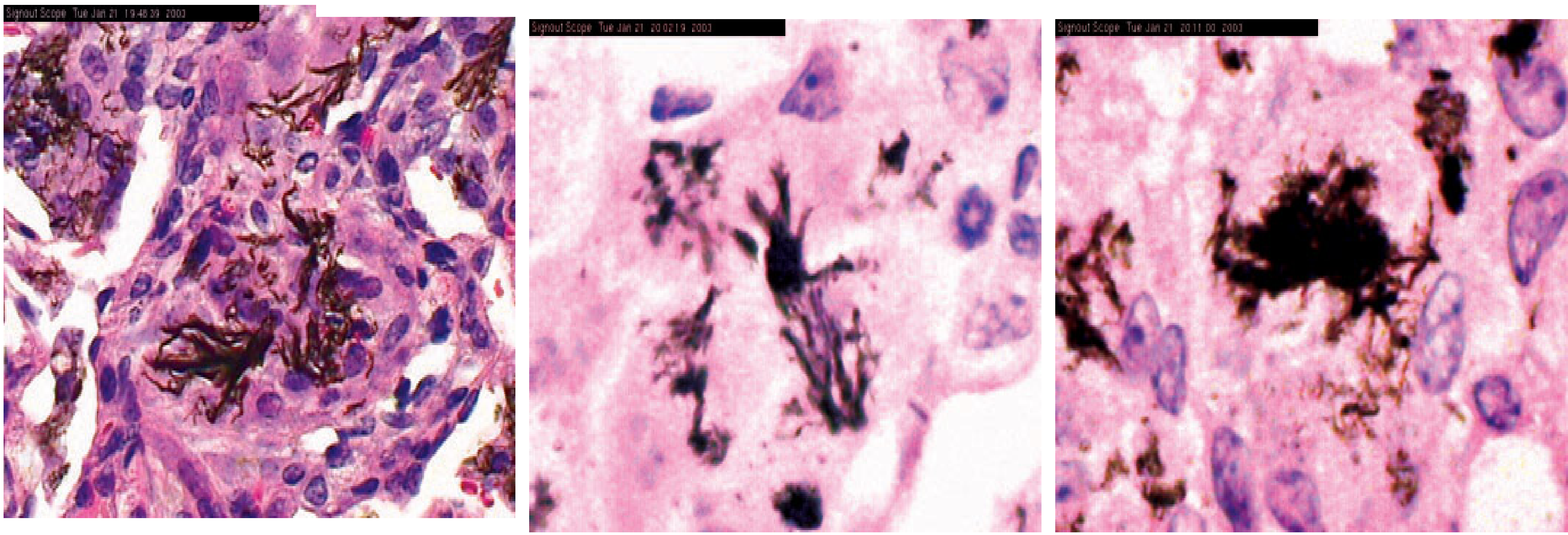
NASA Study used HiPco and Carbon arc NTs in mice

SWNT and Carbon Black are carbon, but

Why did SWNT, but not carbon black, produce granulomas ?

SWNTs vs. Carbon Black





Individual tube: ~ 1.5 nm diameter and several microns long

Bundles: NTs pack tightly and parallel to form ropes or rods

Structurally: Individual tubes or bundles are fibers, CB is amorphous

Surface chemistry of SWNTs and CB are also different

Why Do We Concern About Granulomas and Fibers?

Granulomas (miscropic nodules), consisting particles, live and dead cells, and debris and could impair cellular and physiological (gas exchange) lung functions and give rise to fibrosis, more defined nodules, and other lesions.

Fibers are generally of more health hazard than other forms of particulates. It is well established that the pathogenicity of a fiber in the lungs directly correlates with its biopersistency (Oberdorster 2000). **NTs are totally insoluble and probably one of the most biologically nondegradable man-made materials.** Determining how the NT-induced granulomas progress would require a longer-duration study with this biopersistent material.

CONCLUSIONS

- Granulomas were observed in lungs 7 d or 90 d after an instillation of 0.5 mg NT per mouse (also in some with 0.1 mg);
- NT, regardless synthetic methods, types and amounts of residual catalytic metals, produced granulomas;
- Lung lesions in the 90-d NT groups, in most cases, more pronounced than those in the 7-d groups.

CONCLUSIONS

- Our study shows that, on an equal-weight basis, if carbon nanotubes reach the lungs, they are much more toxic than carbon black and can be more toxic than quartz, which is considered a serious occupational health hazard in chronic inhalation exposures.

- If fine NT dusts are present in a work environment, exposure protection strategies should be implemented to minimize human exposures.

ACKNOWLEDGEMENTS

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PROJECT INITIATION:	Ms. A. Lee, Dr. D. Colbert, Dr. R. Hauge
STUDY CONTRIBUTION:	Dr. M. Kuo, Dr.T. Blasdel, S. Bassett, M. Nelman, J. Nelle, B. Whitaker, and N. Hudson.
CONTRUCTIVE COMMENTS:	Staff at Rice CNST
ACADEMIC SUPPORTS:	Dr. V. Colvin and her colleagues

STUDY PRIMARILY OF RESPIRABLE-SIZE PARTICLES

PROBABLE OF LITTLE NANOPARTICLES

•Rice “aggressive sonication” prepared fine-particle suspensions (10 mg/L) containing mostly individual fibers and a few small bundles.

•Our brief sonicated suspension were 200 to 1000x more concentrated and contained particle aggregates.

The high NT concentration and brief sonication minimized deaggregation of NT samples to individual fibers.

Dr. Robert Hauge of Rice U advised us that brief sonication would not shorten or change the fundamental nature of SWNTs.

Environmental Fate of Nanomaterials ???

- 1) Nanomaterials in Soil
- Biopersistency
- Disolution
- Biodegradation
- Aggregation (adsorption to environmental matrix)
- 2) Nanomaterials in aquatic environment
- Dissolution and suspension in aqueous media
- Sedimentation
- 3) Bioaccumulation

Earth worms and Aquatic animals