Invited presentation at the Workshop *"Heath Risk of Carbon Nanotubes, Can We Learn from Mineral Fibers or Ultra-fine Particulates ?"* during the annual meeting of the Society of Toxicology

March 26, 2007

TOXICITY OF CARBON NANOTUBES AND ITS IMPLICATIONS FOR OCCUPATIONAL AND ENVIRONMENTAL HEALTH

Chiu-wing Lam, Ph.D.,[1,2] and John T. James, Ph.D.[1]

[1]NASA Johnson Space Center Toxicology Group and [2]Wyle Laboratories
Houston, TX

GENERAL CLASSES OF MATERIALS OF NANO OR ULTRAFINE SIZES

1 **Natural Nano Particles**

those that are generated by naturally occurring events such as **volcanoes** (Halloysites) and **forest fires**.

2. **Incidental/Anthropogenic Ultrafine Particulates**:

those generated as an unintentional by-product of human industrial activities and include things like **diesel engine exhaust, Automotive exhaust**, **grinding particulates**, and **welding fumes**

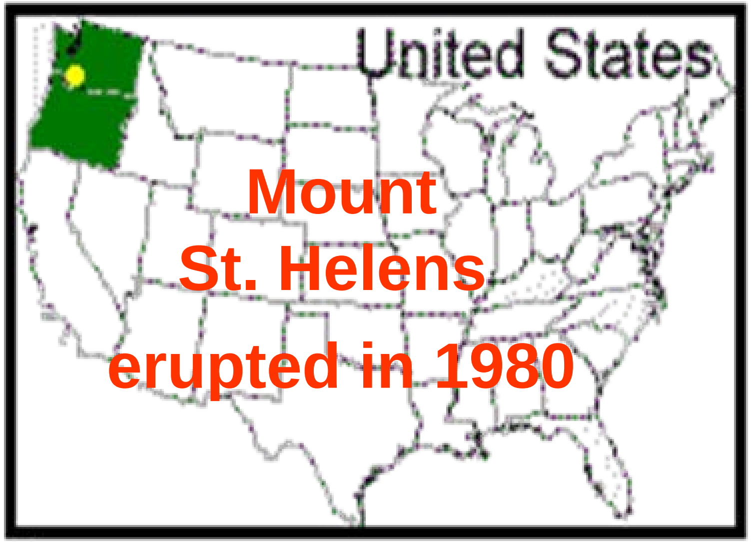
3. **Industrial Nanosize powders**

Mined or synthesized: carbon black, alumina, titanium dioxide, silica, etc.

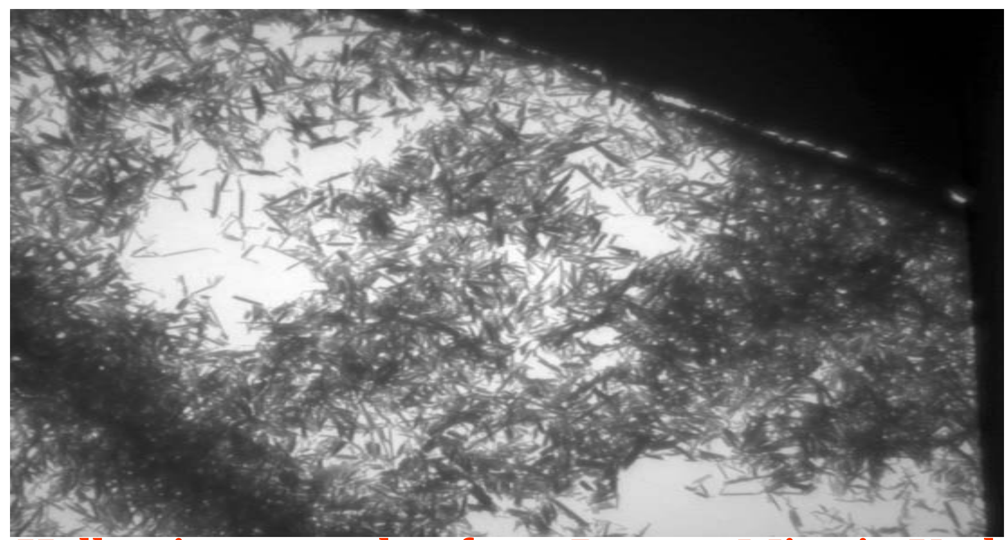
4. **Engineered or Manufactured nanomaterials**

synthesized recently associated with the nanotechnology developments: **fullerenes, carbon nanotubes, quantum dots**, etc.

MOTHER NATURE MAKES NANOMATERIALS



Halloysite clay (Nanoclay, or China clay) deposit at Matauri Bay in New Zealand (particle sizes ~10 nm)



Halloysite nanotubes from Dragon Mine in Utah (tube size ~30nm dia x 0.5 to 10 micron long)

COAL-FIRED POWER PLANTS PRODUCE LARGE AMOUNTS OF ATMOSPHERIC NANO/ULTRAFINE PARTICULATE MATTER

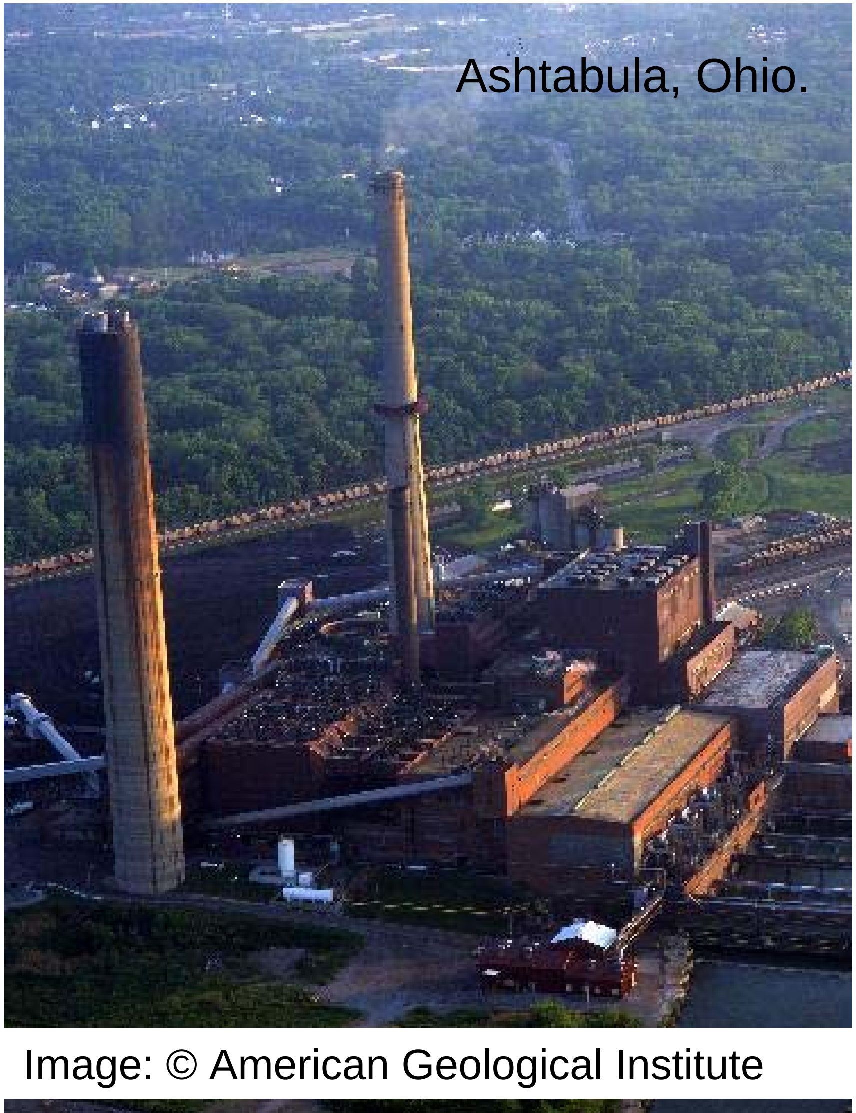


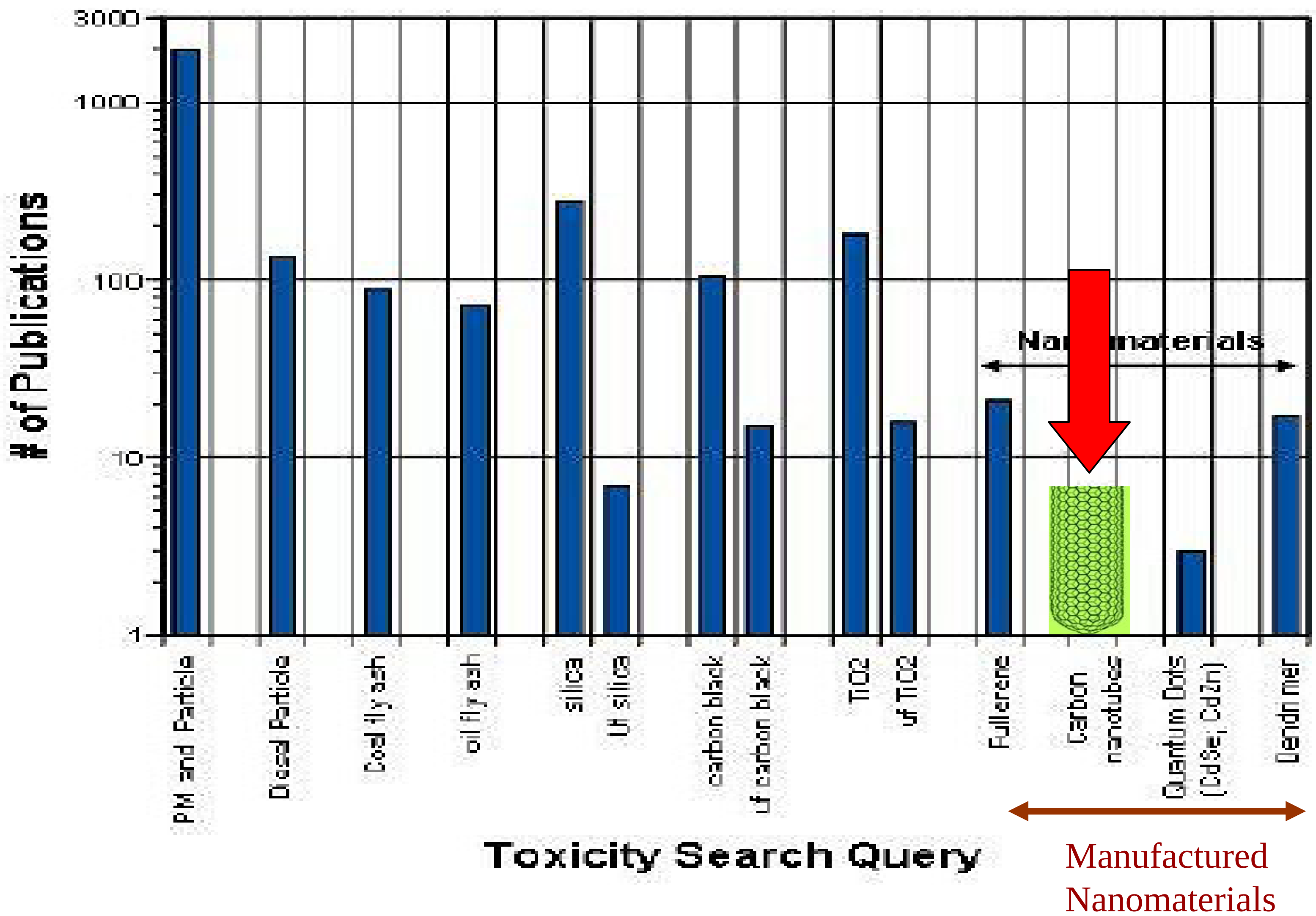
Image: © American Geological Institute

Image: © CORBIS

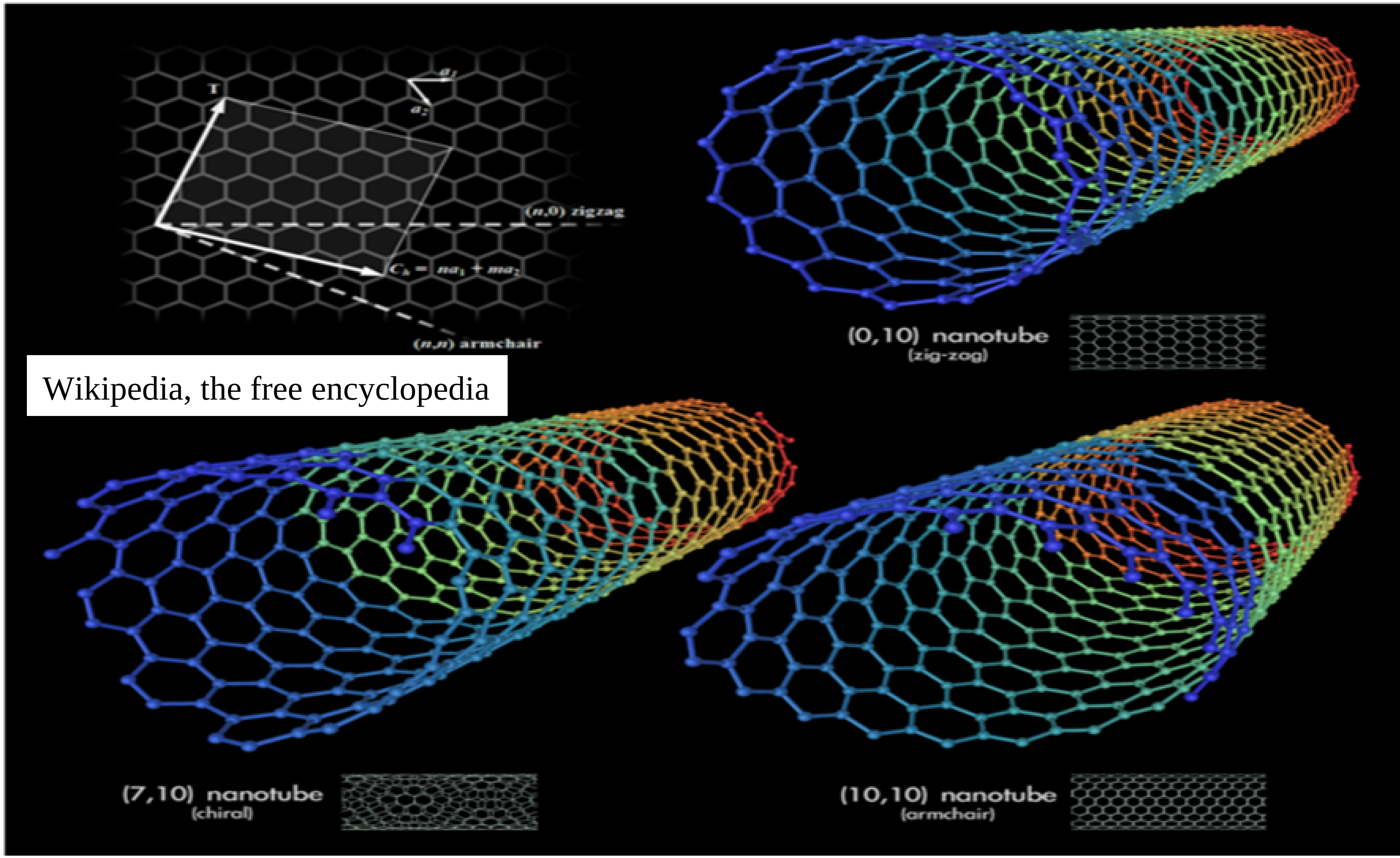
INDUSTRIAL PROCESSES ALSO PRODUCE A LARGE AMOUNT OF ATMOSPHERIC NANO/ULTRAFINE PARTICULATE MATTER

YOU AND I ALSO CONTRIBUTE NANOPARTICLES TO THE ENVIRONMENT

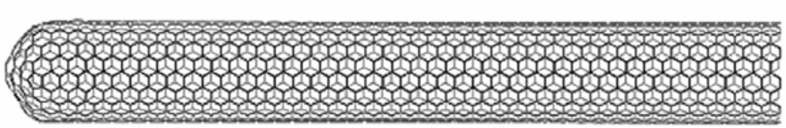
TOXICOLOGICAL STUDIES OF PARTICLES

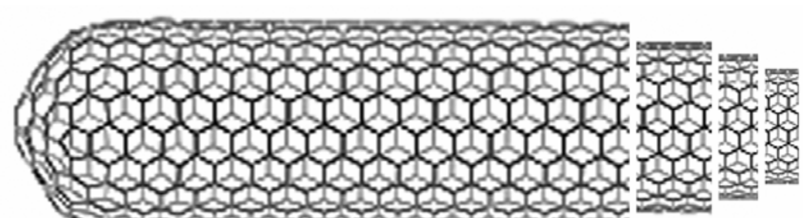


PubMed Search up to 2005: Info from EPA 2005 (modified by C. Lam)

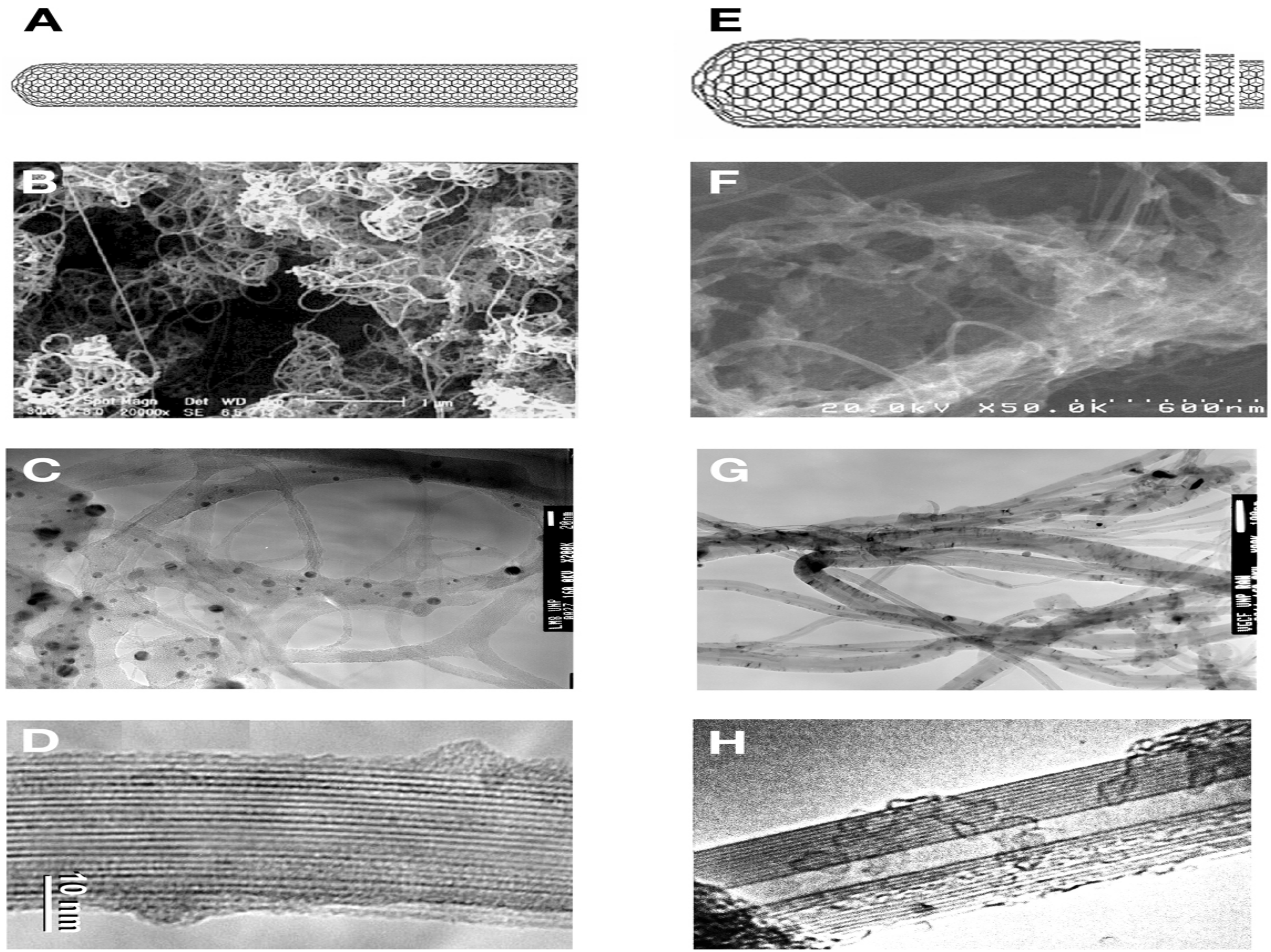


Wikipedia, the free encyclopedia



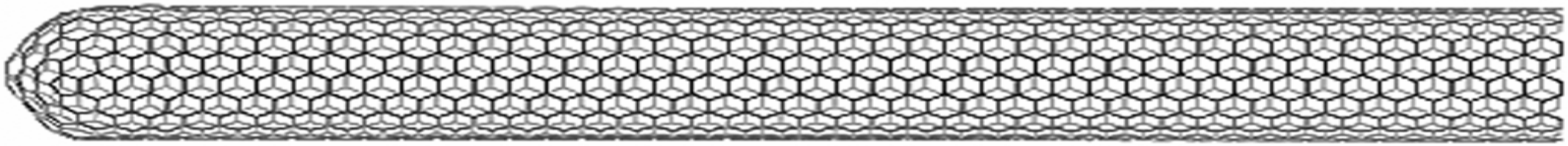


STRUCTURES OF CARBON NANOTUBES

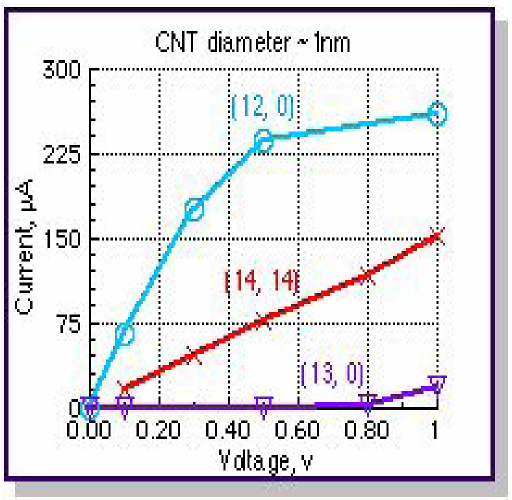


Lam et al. Crit. Rev. Tox. 36:189-217, 2006

ELECTRICAL CONDUCTIVITY OF CARBON NANOTUBES



Armchair CNTs conduct electricity >**1000x** better than **copper**



Zigzag CNTs do not conduct electricity

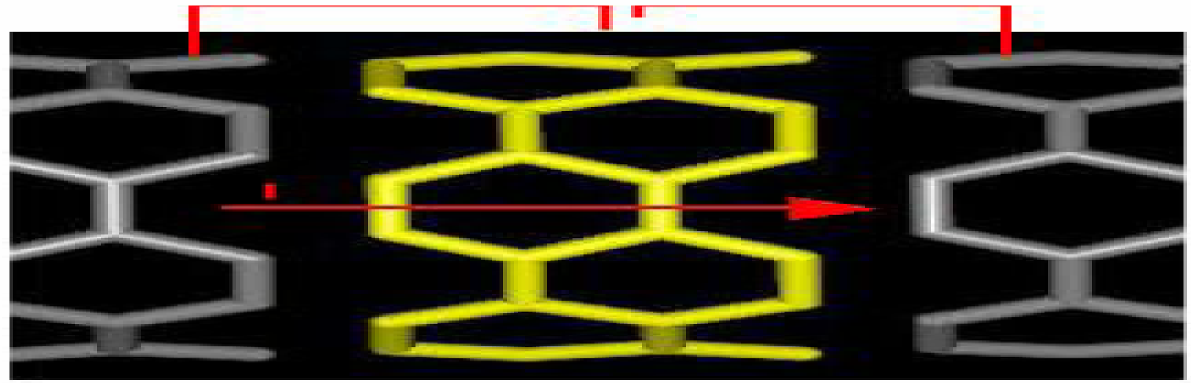


Fig. 2. Simulation model showing current (I) through the central region in response to voltage (V) applied as shown.

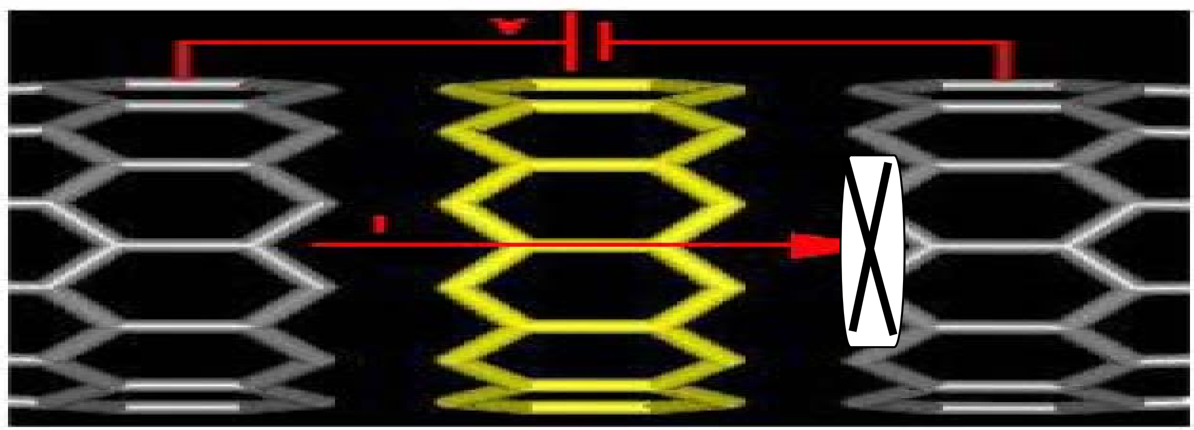


Fig. 4. Simulation model of the zigzag nanotube.

Simulated model and data by Gokturk H.S. (Matsushita Electric) 2005

CNTS (ARMCHAIR) ARE EXCELLENT ELECTRICAL CONDUCTOR

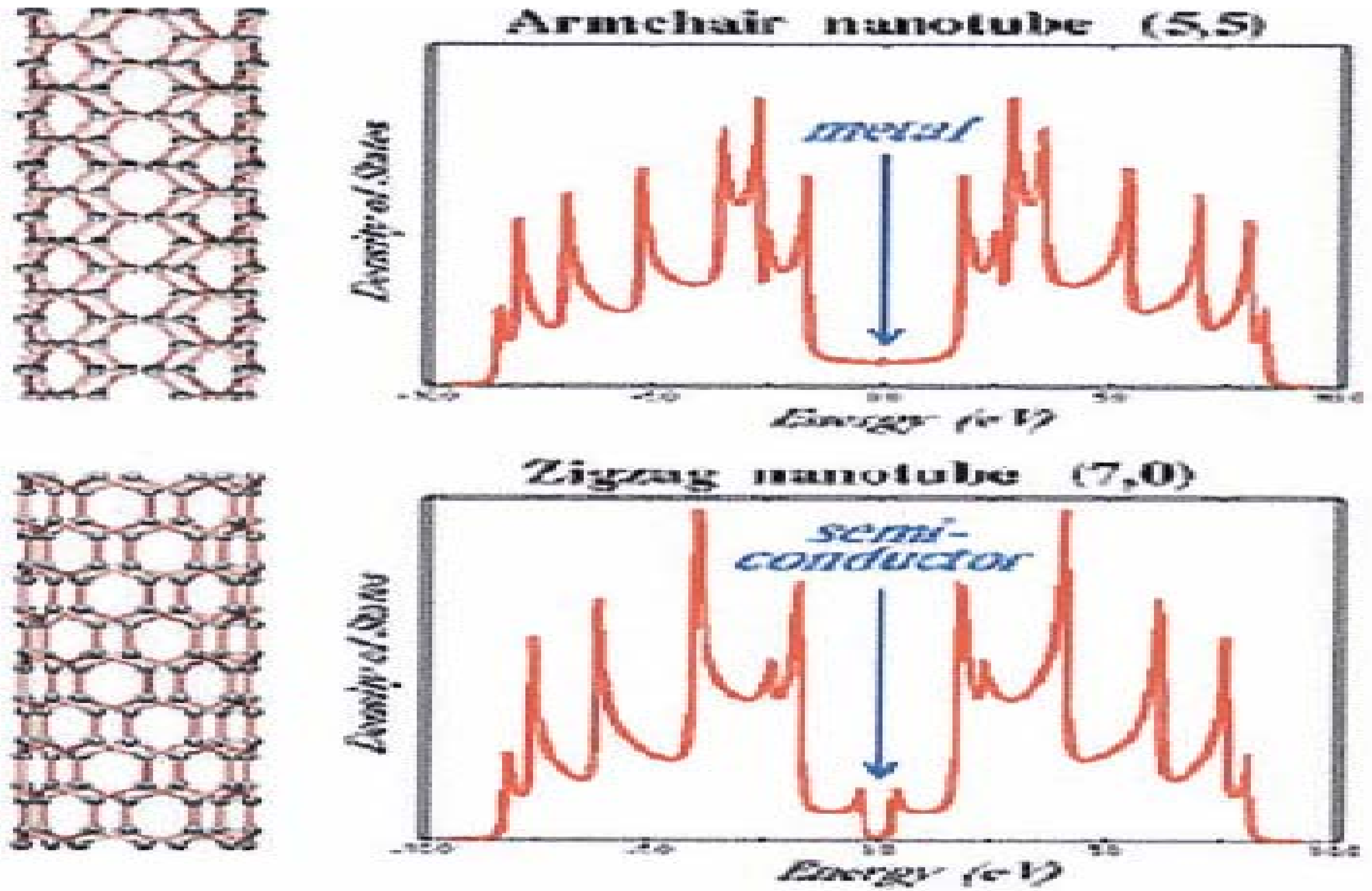
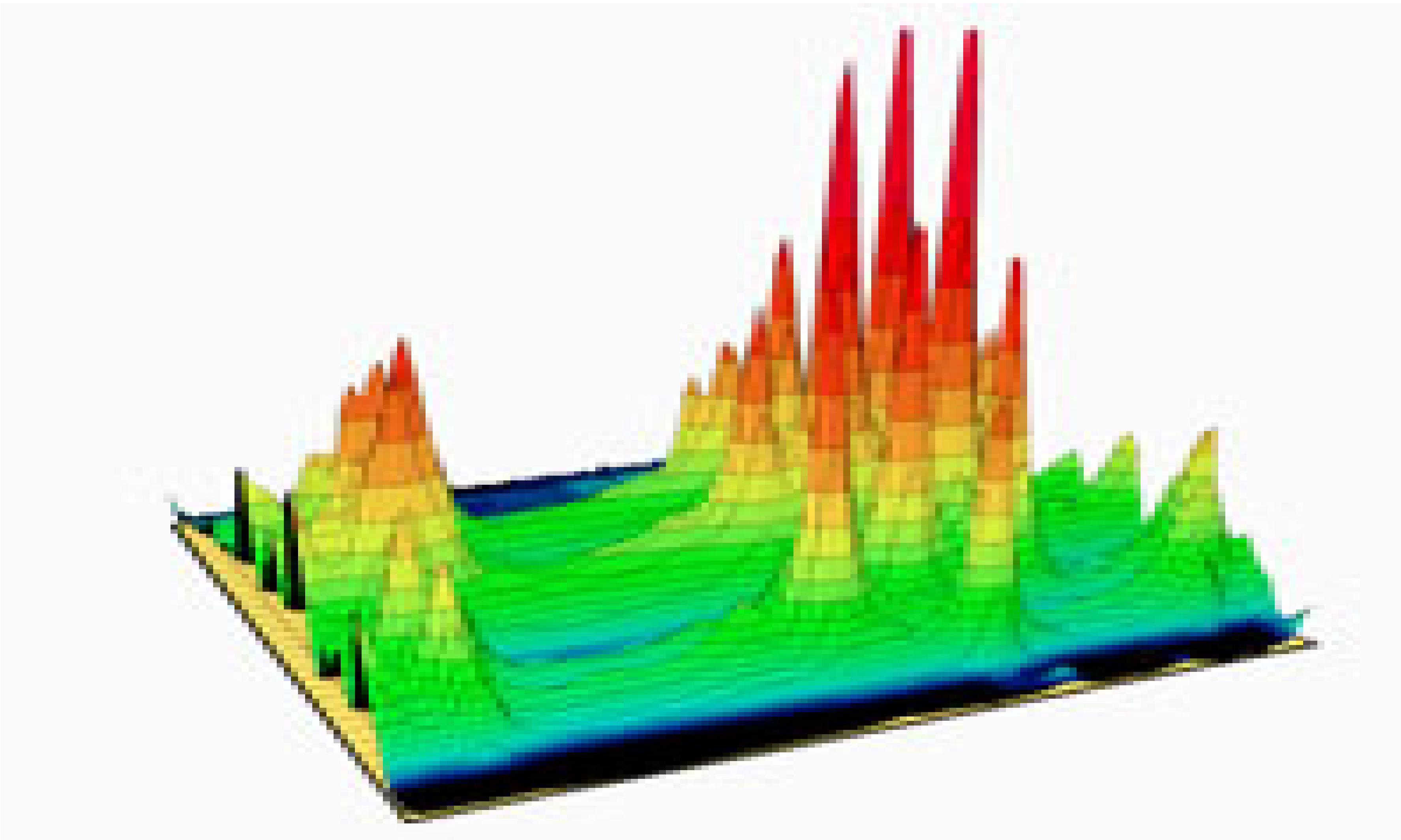


Fig. 2. Electronic properties of two different carbon nanotubes. The Armchair (5,5) nanotube (*Upper*) exhibits a metallic behavior (finite value of charge carriers in the DOS at the Fermi energy). The Zigzag (7,0) nanotube (*Lower*) is a small gap semiconductor (no charge carriers in the DOS at the Fermi energy). Sharp spikes in the DOS are Van Hove singularities.

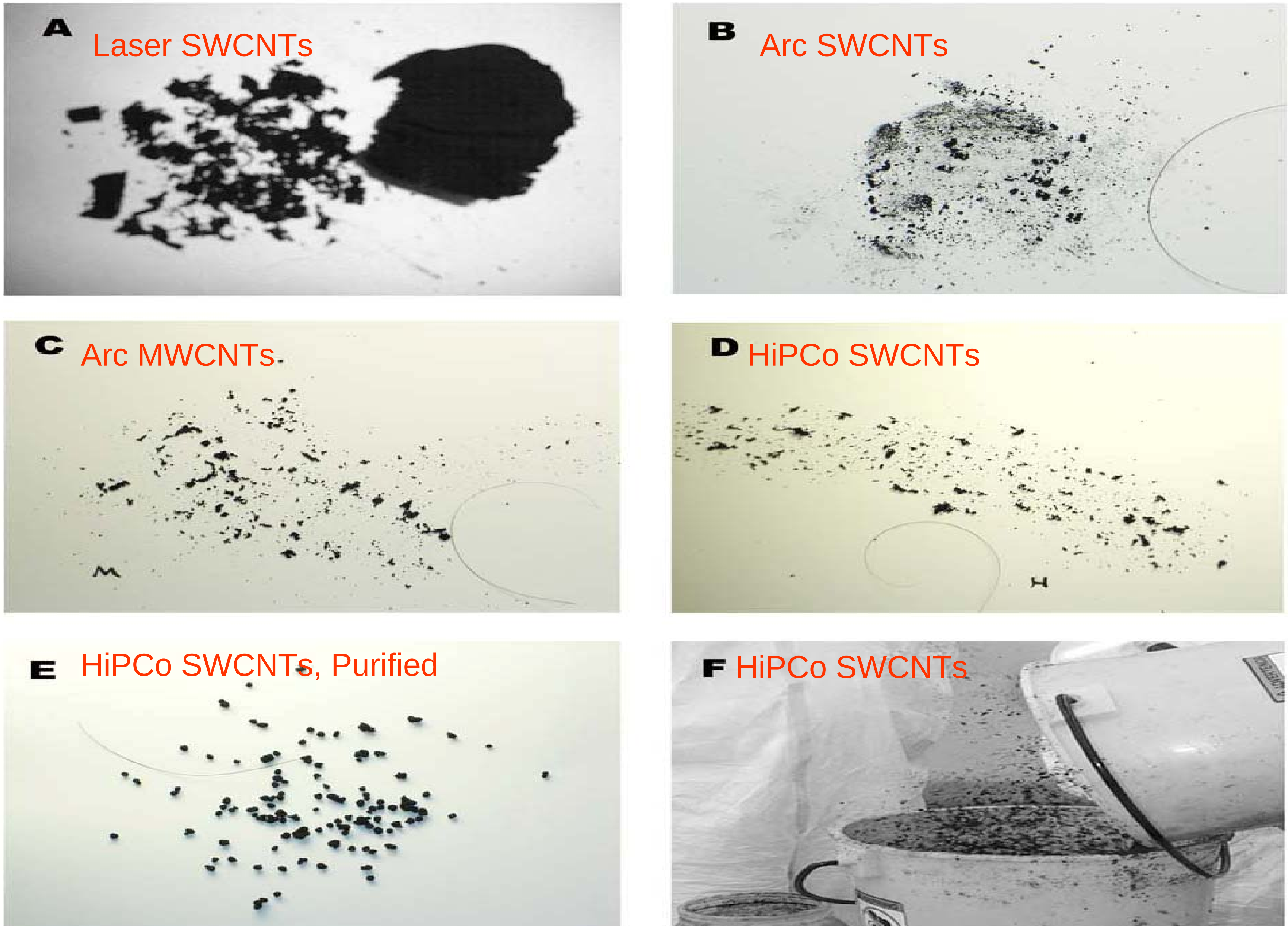
Ajayan et al (1999), *PNAS* 96:14199-14200

>30 "SPECIES" OF SWCNTs DUE TO SLIGHT VARIATIONS IN NANOTUBE STRUCTURE AND DIAMETER WERE ETECTED



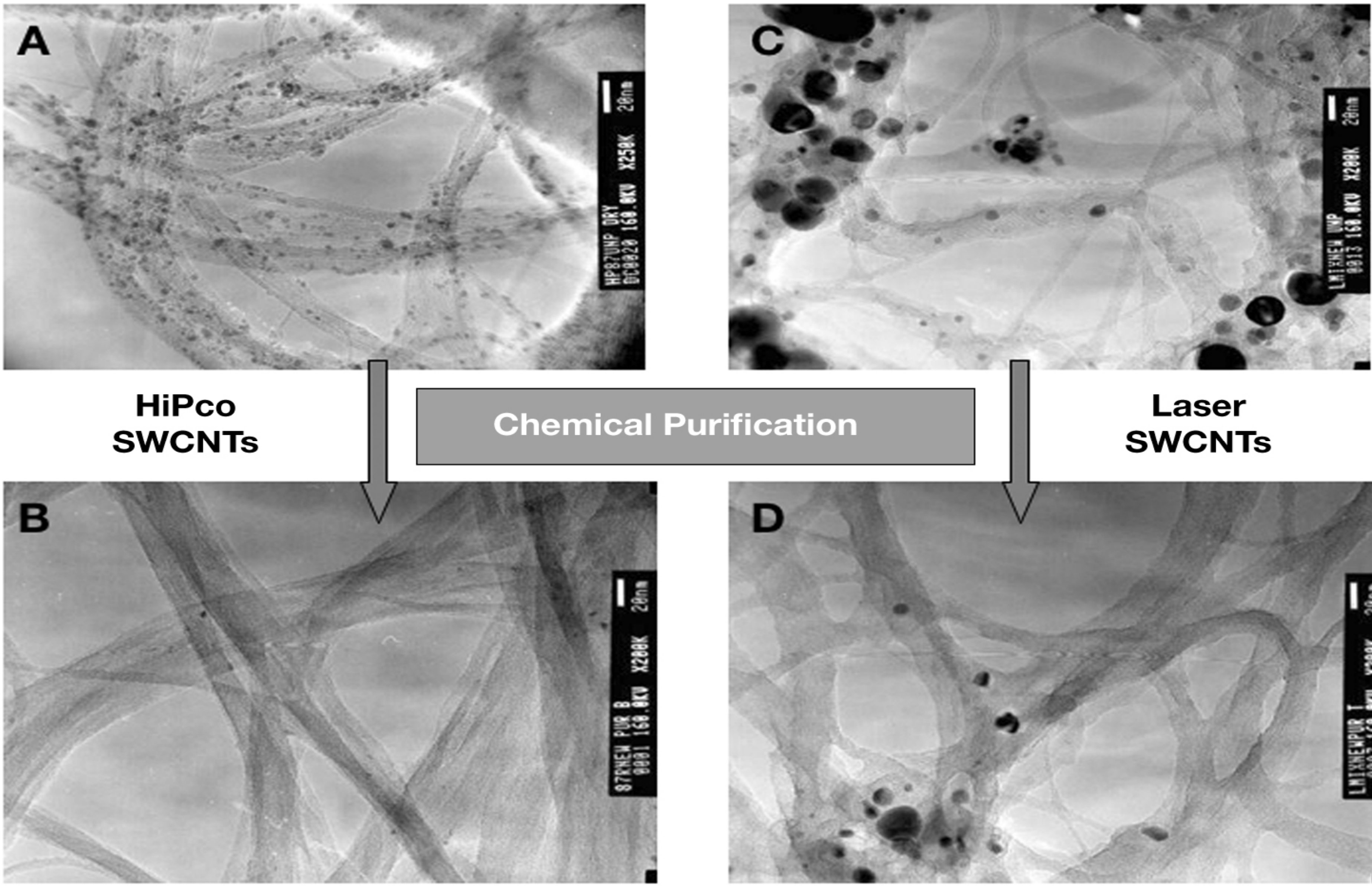
Bachilo et al. Science 298: 2361-2366, 2002

PHYSICAL CHARACTERISTICS OF BULK CNT MATERIALS



Lam et al. Crit. Rev. Tox. 36:189-217, 2006

PURIFICATION OF CNTS TO REMOVE CATALYST METAL RESIDUE



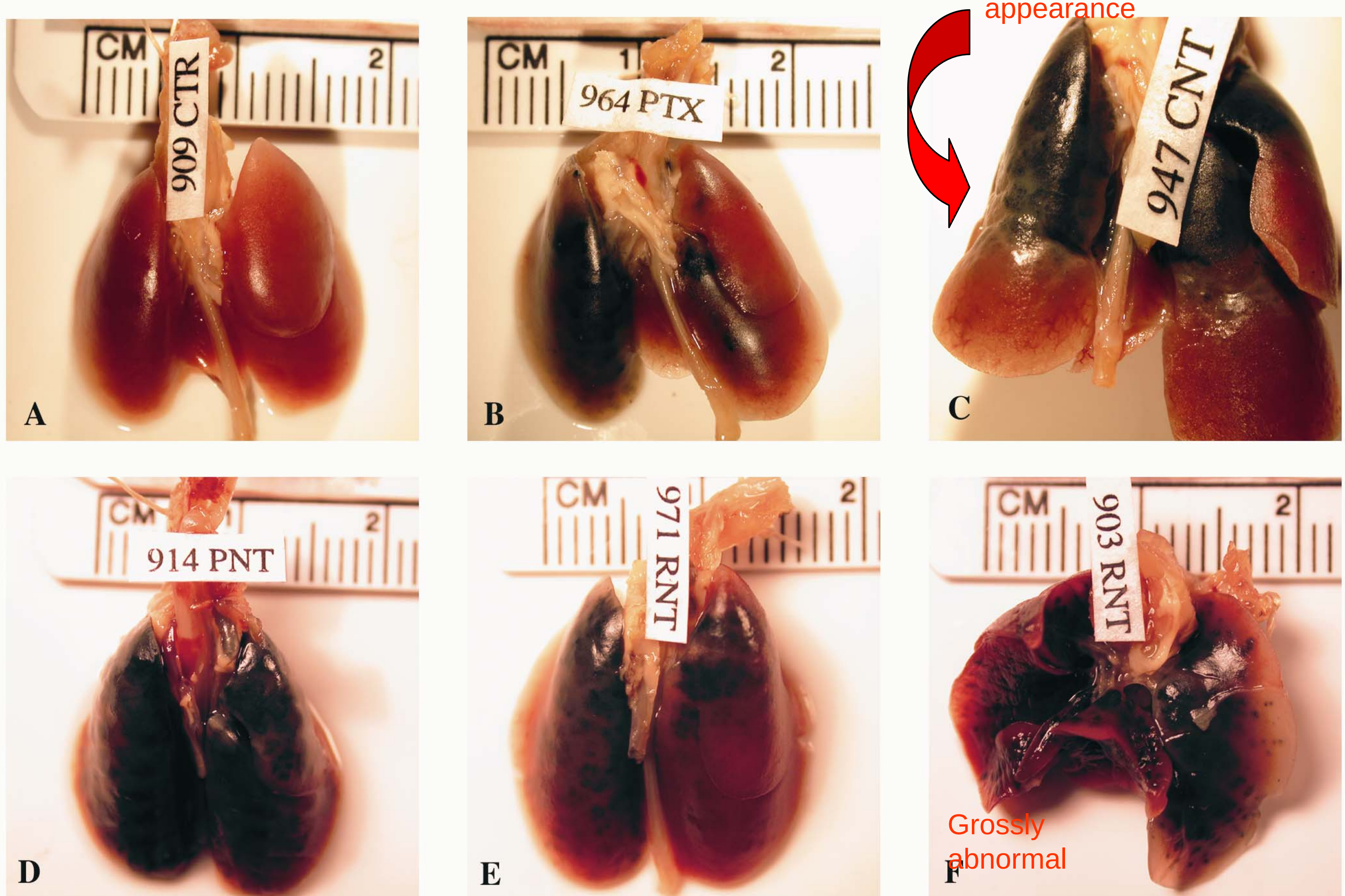
Lam et al. Crit. Rev. Tox. 36:189-217, 2006

POTENTIAL EXPOSURE HAZARD OF MANUFACTURED CARBON NANOTUBES

(Courtesy of Drs. Maynard & Baron, NIOSH)

LUNGS OF MICE INSTILLED WITH CARBON BLACK & CNTS

(0.5 mg/LUNG IN MICE SACRIFICED 90 DAYS LATER)



Lam et al Tox. Sci. 77:126-134, 2004

PULMONARY LESIONS IN MICE PRODUCED BY CNTS MADE BY DIFFERENT PROCESSES AND CONTAINED DIFFERENT METALS AND AMOUNTS

Carbon Black, 90d
(Nanosize)

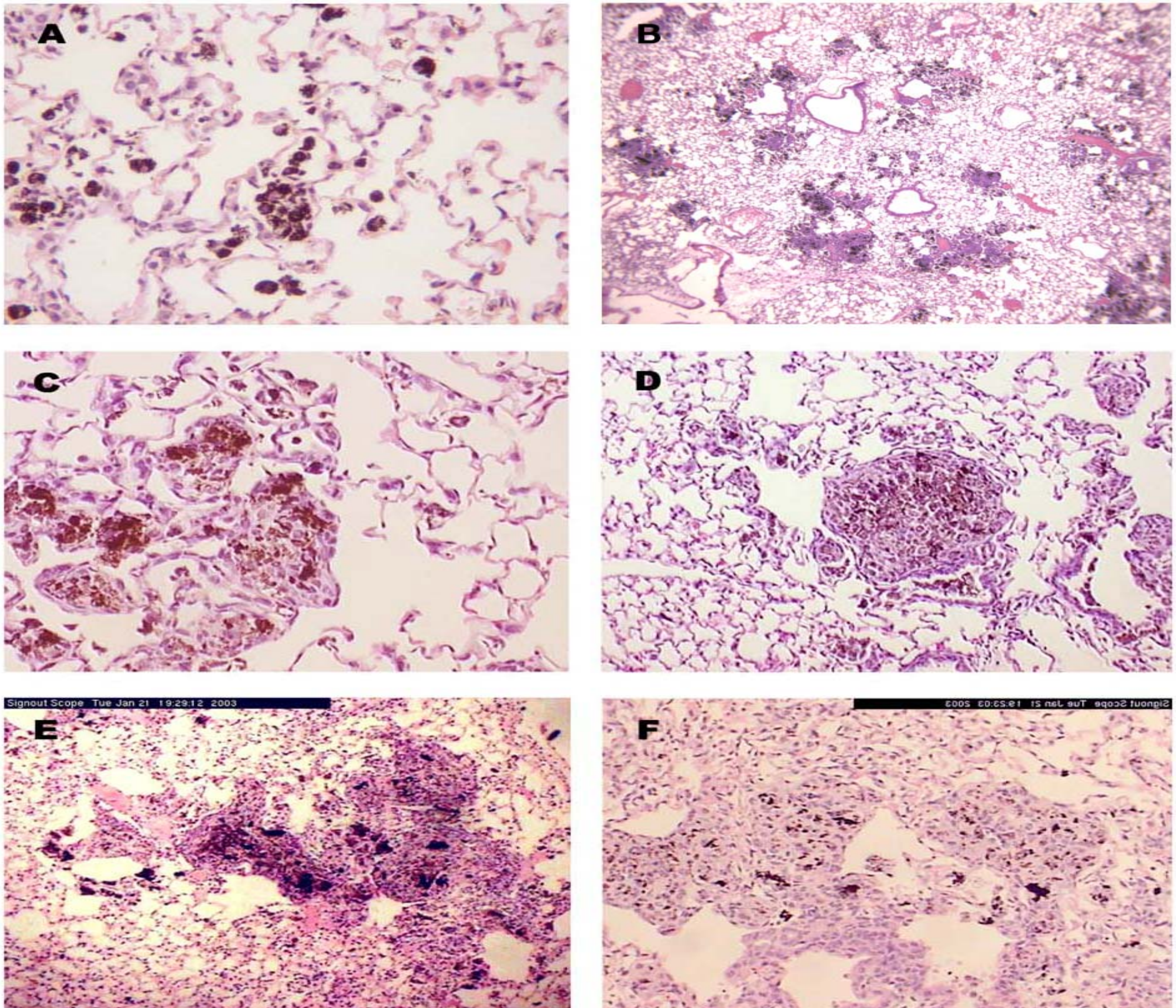
CNTs, 7d
Laser
Ni

CNTs, 7d
Electric Arc
Ni, Y

CNTs 7d
HiPco,
Fe

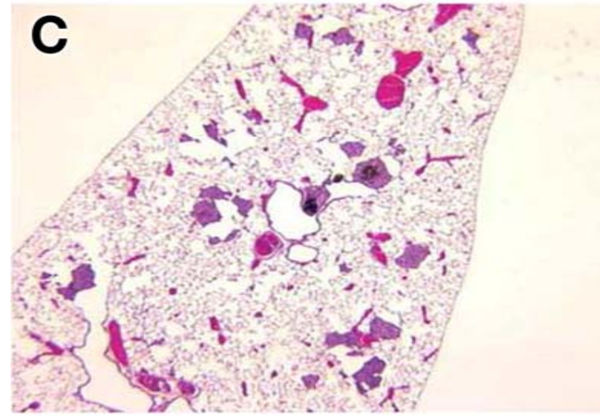
CNTs 90d
HiPco
Fe

Purified CNTs 90d
HiPco
Fe (trace)



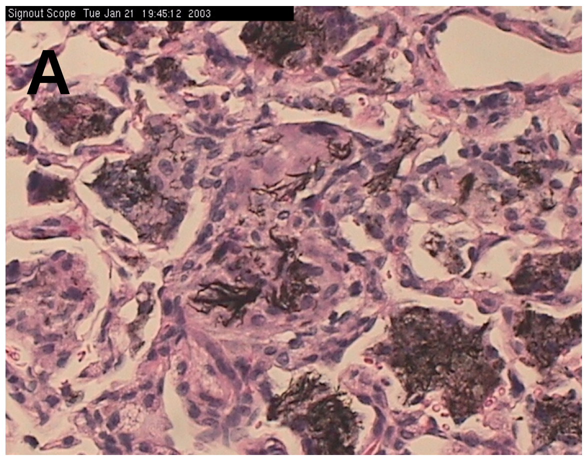
(Lam et al Tox. Sci 77:126-134, 2004; Lam et al Crit. Rev. Tox 36:189-217, 2006)

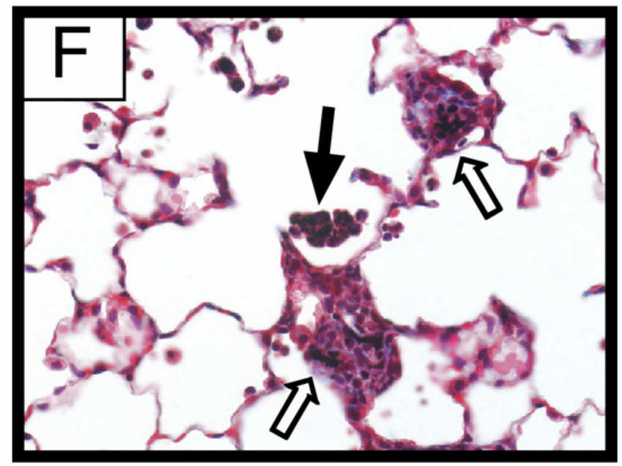
SWCNTS AND MWCNTS PRODUCED GRANULOMAS AND OTHER LUNG LESIONS IN RODENTS



Warheit et al. 04
(Du Pont)
SWCNTs
Rats

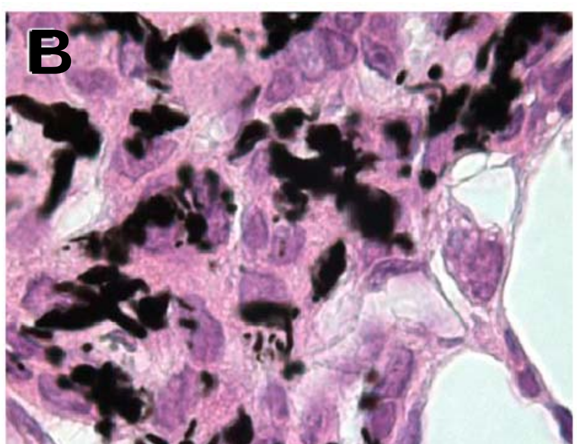
Lam et al. 04
(NASA)
SWCNTs
Mice

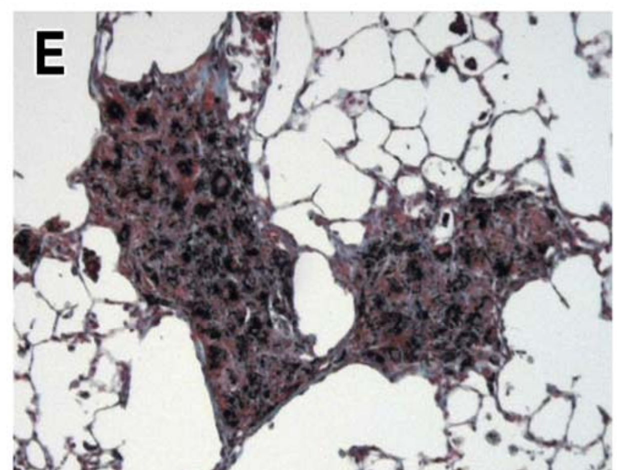




Mangum et al. 06
(CIIT)
SWCNTs
Rats

Shvedova et al. 05
(NIOSH)
SWCNTs
Mice

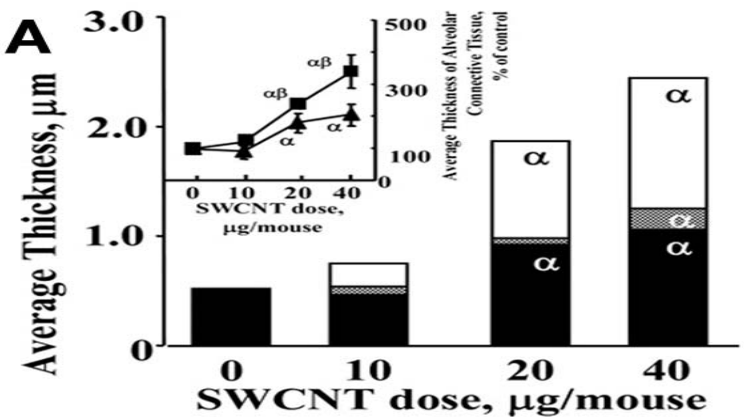


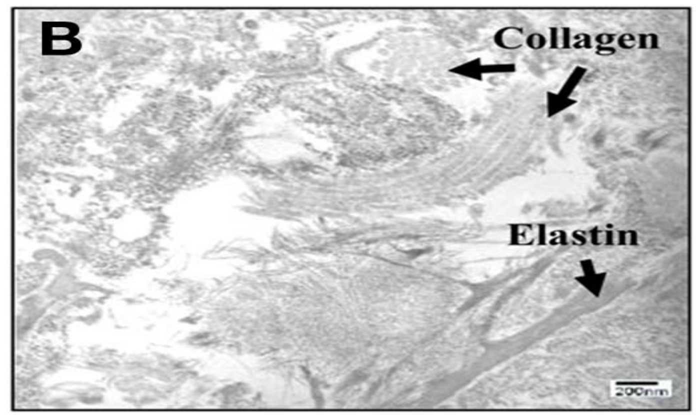


Muller et al. 05
(Belgium)
MWCNTs
Rats

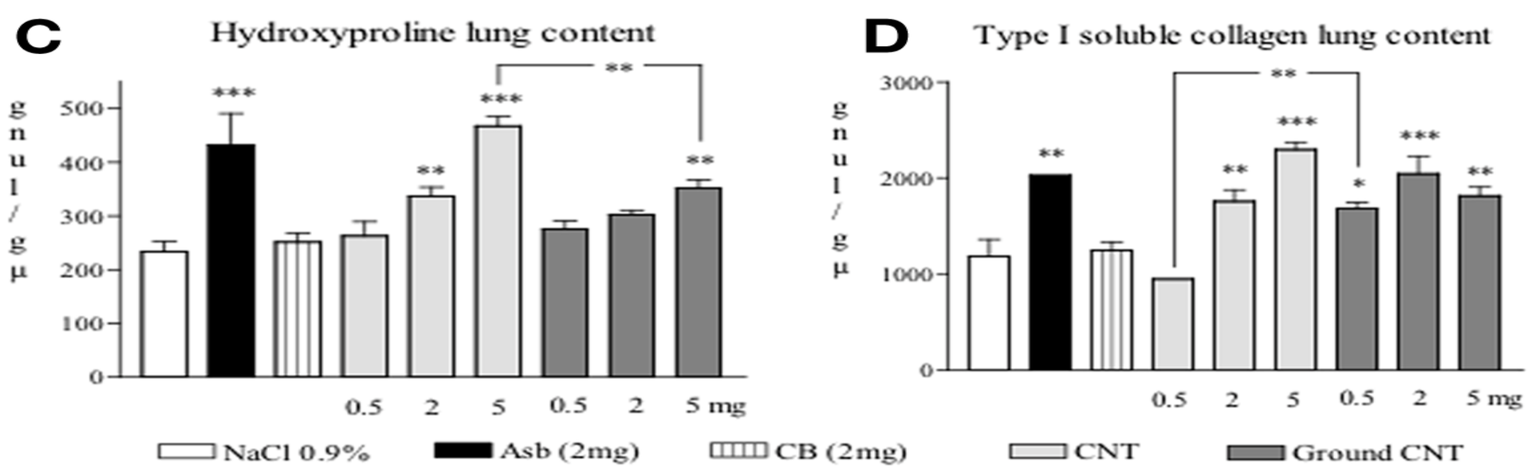
FIBROGENIC ACTIVITY OF CNTS WAS DOSE DEPENDENT

Shvedova: "SWCNTs induced "**an unusually robust pulmonary inflammatory responses with a very early onset of fibrosis**, which is accompanied by a significant oxidative stress and antioxidant depletion"



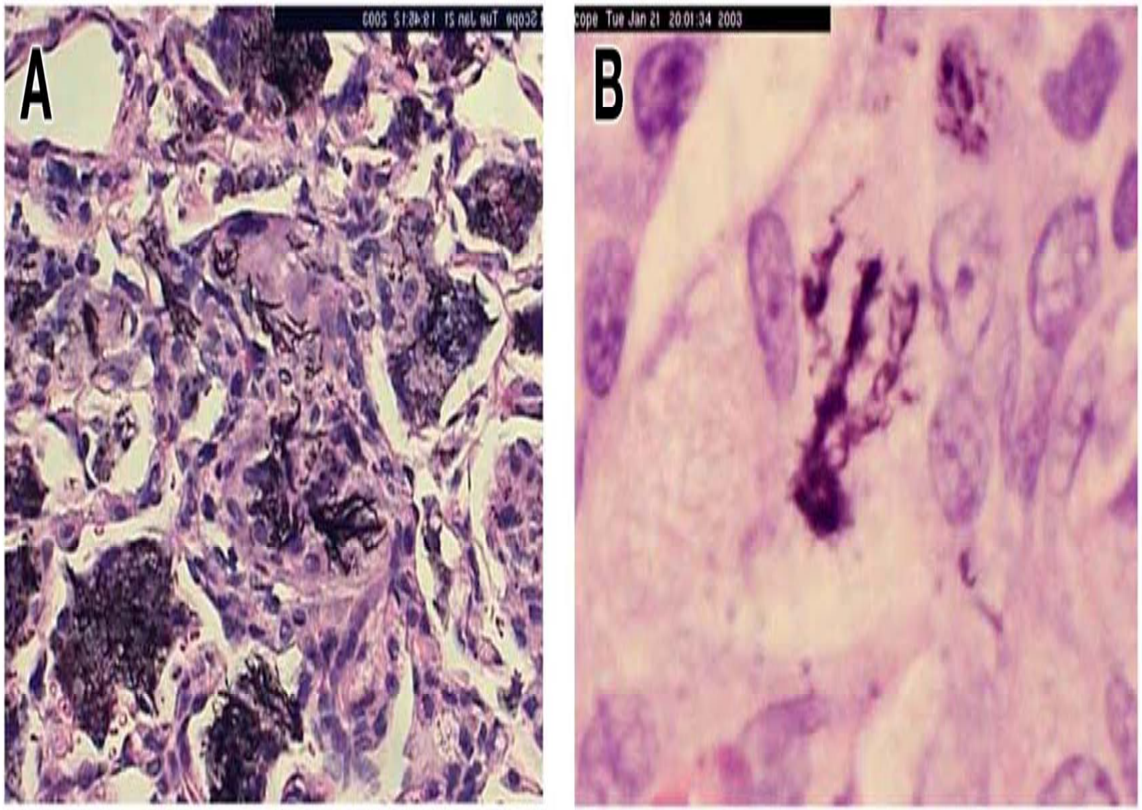


SWCNTs
(Shvedova et al, 2005)

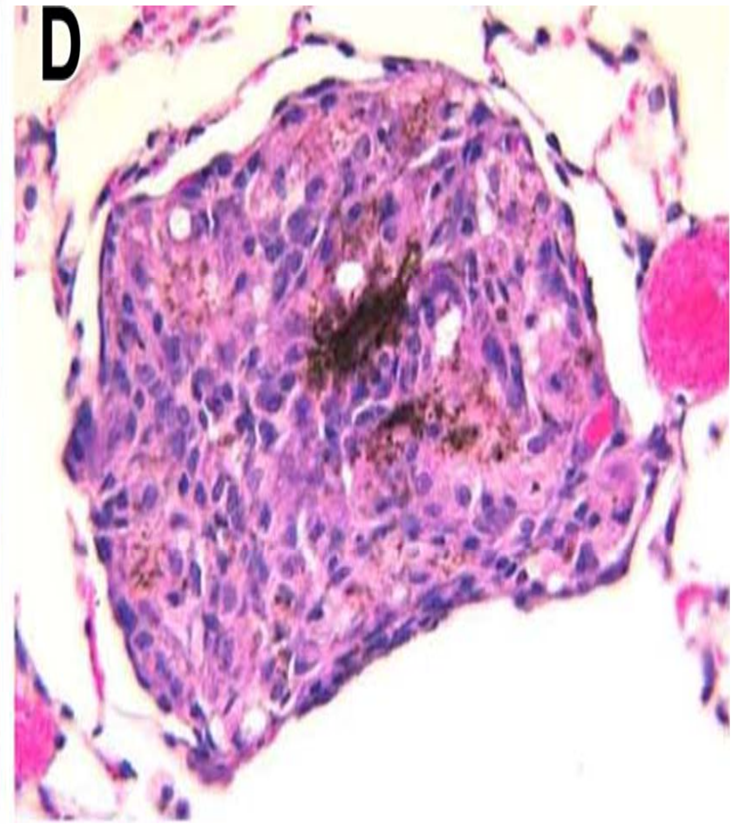


MWCNTs
(Muller et al, 2005)

GRANULOMAS IN THE LUNGS OF CNT-TREATED RODENTS CONTAIN FIBROUS CNTS

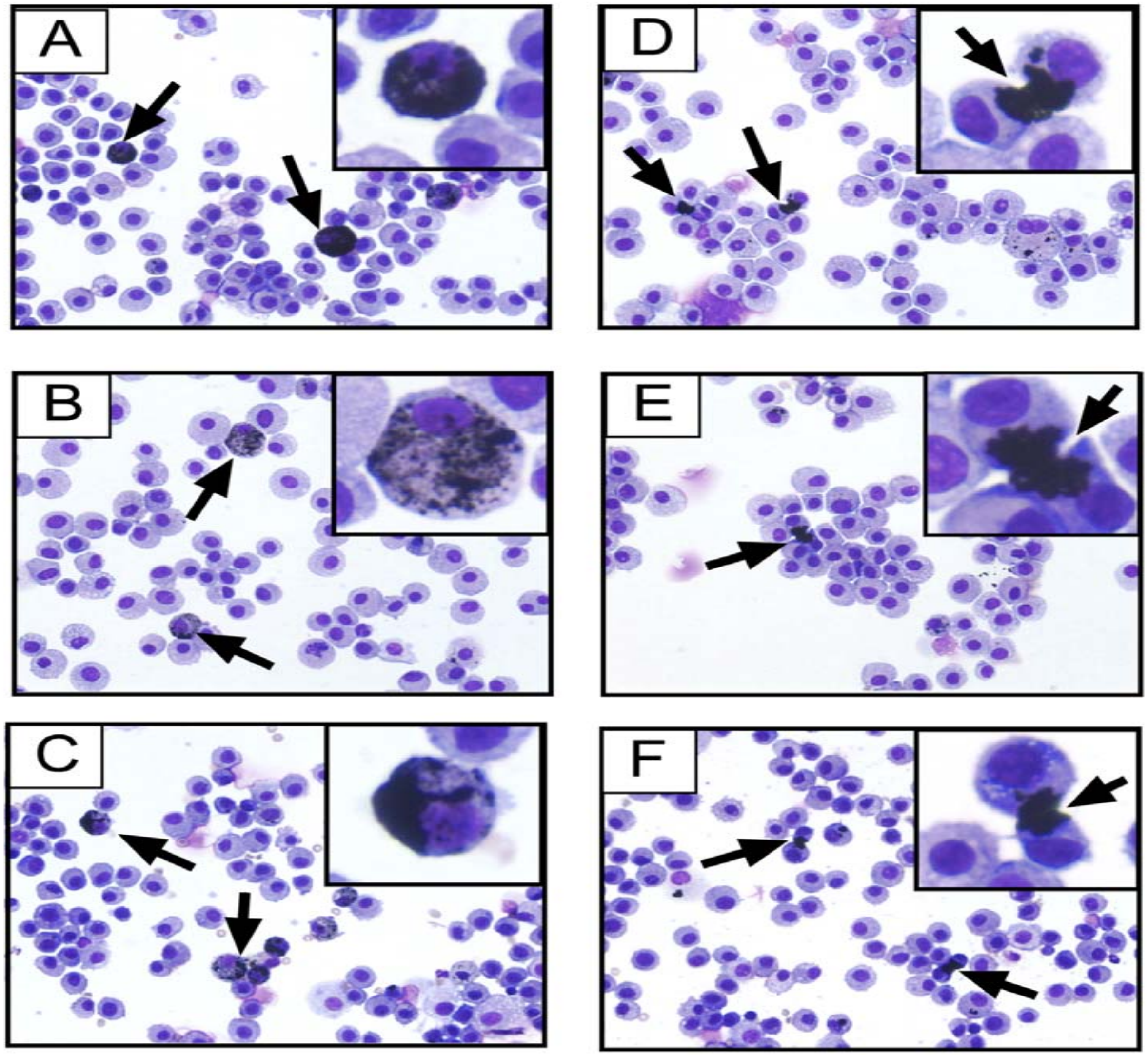


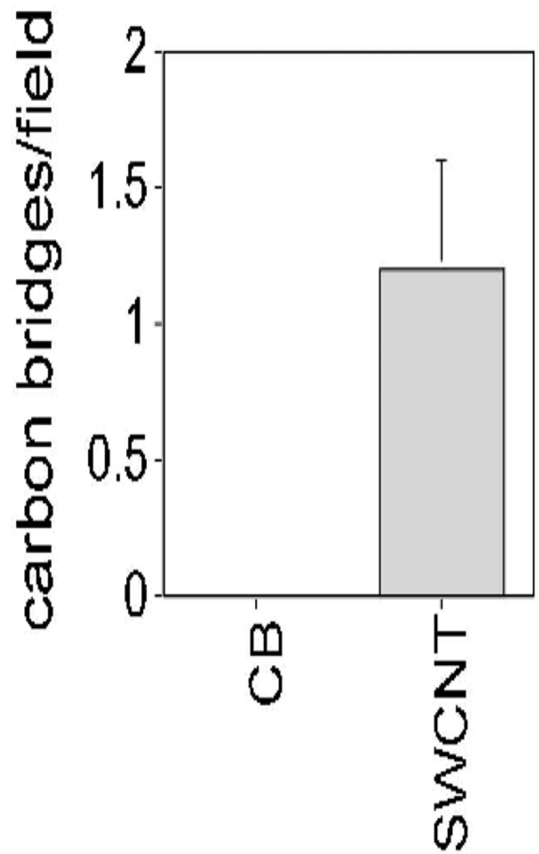
(Lam et al., Tox. Sci. 77, 2004)



(Warheit et al., Tox. Sci 77, 2004)

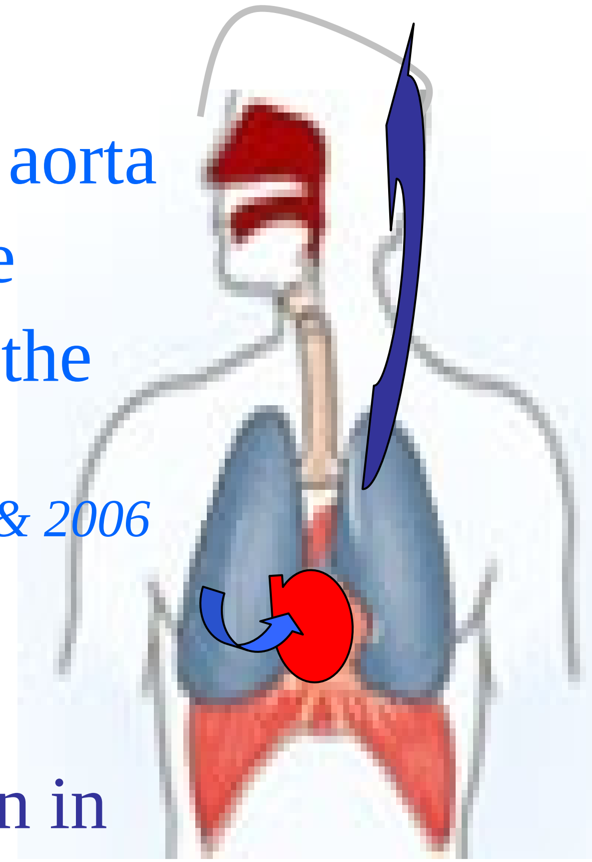
AGGREGATES OF CNTS TAKEN UP BY MULTIPLE CELLS





(Mangum et al. Particle and Fiber Tox. 2006)

EXTRAPULMONARY TOXICITY OF CNTS GIVEN TO THE MOUSE LUNG



SWCNTs toxicity in the Heart

- Damage in the mitochondrial DNA in aorta
- increase in the percent of aortic plaque
- induction of atherosclerotic lesions in the brachiocephalic artery of the heart

NIOSH studies, 2005 & 2006

MWCNTs in the Brain

- Elicited neuroinflammation
- alter inflammatory cytokine expression in brain areas

NIOSH Study (Sriram et al.) 2007

SUMMARY OF CNT TOXICITY IN RODENTS

All conducted by non-inhalation exposures and examined ≤90d after instillation

Lung

- Inflammation, fibrosis, epithelioid granulomas
- Biochemical and toxicological changes in BALF
- Respiratory function impairments
- retarded bacterial clearance after bacterial inoculation

Heart

- Damage in the mitochondrial DNA in aorta,
- increase in the percent of aortic plaque, and induction of atherosclerotic lesions in the brachiocephalic artery of the heart

Brain

- Inflammation and increase inflammatory cytokines in the brain.

Relative Toxicity

- Toxicity: CNTs > quartz > nanosize carbon black

U.S. Department of Health and Human Services
NIH News
National Institutes of Health
National Institute of Environmental Health Sciences (NIEHS)

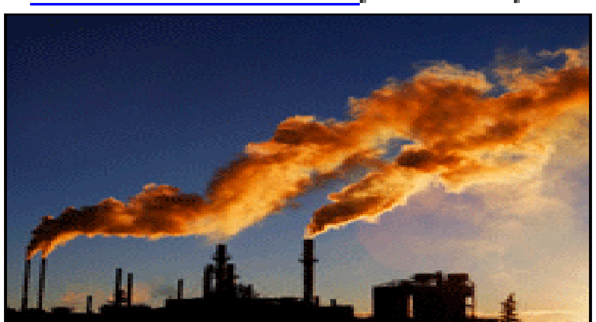
March 5, 2002

BYU Contact: Michael Smart
(801) 378-7320

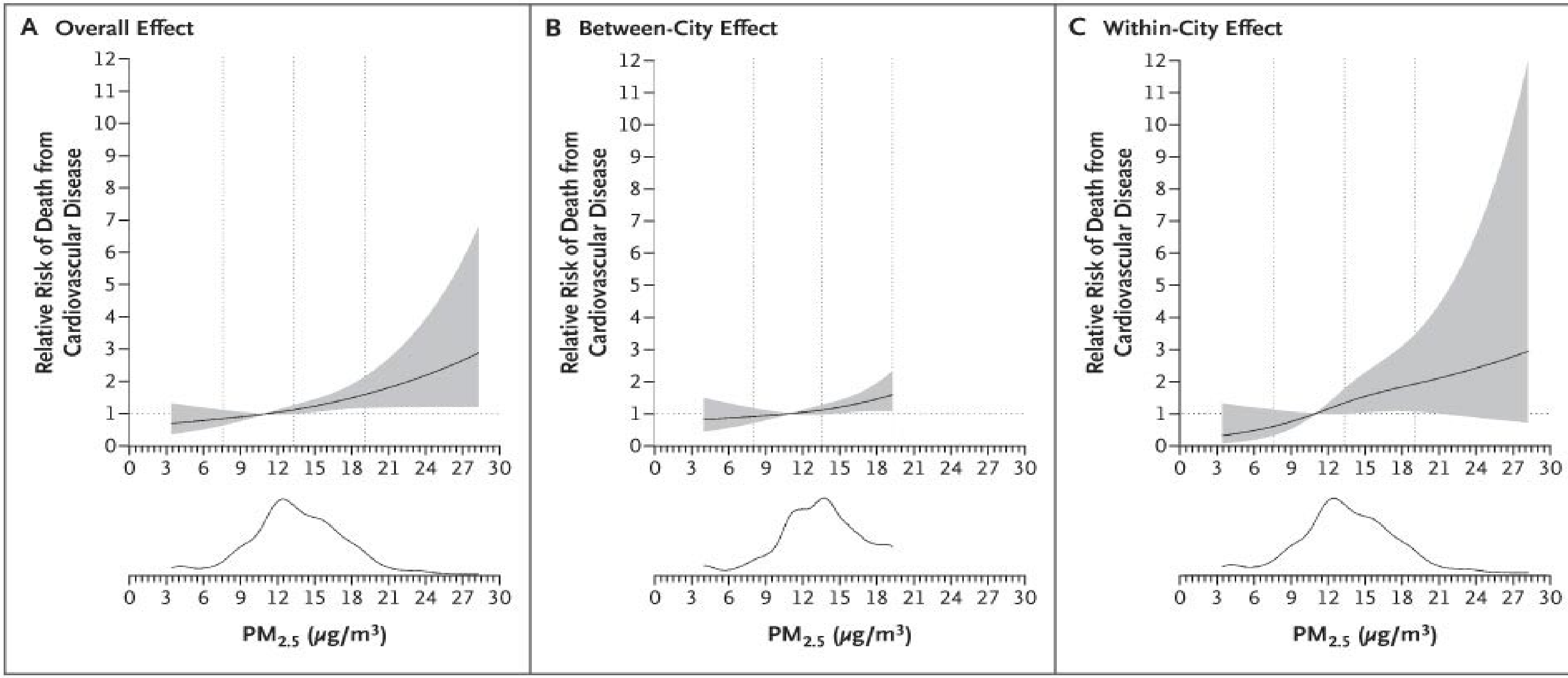
NIEHS Contact: Bill Grigg
grigg@niehs.nih.gov
(301) 402-3378

Link Strengthened Between Lung Cancer, Heart Deaths and Tiny Particles of Soot, Dust

Years of exposure to the high concentrations of tiny particles of soot and dust from cars, power plants and factories in some metropolitan areas of the United States significantly increase residents' risk of dying from lung cancer and heart disease, according to a study financed largely by the National Institute of Environmental Health Sciences and conducted by scientists at Brigham Young University, Provo, Utah; the University of Ottawa, Ontario, the American Cancer Society and New York University School of Medicine, Tuxedo, N.Y.



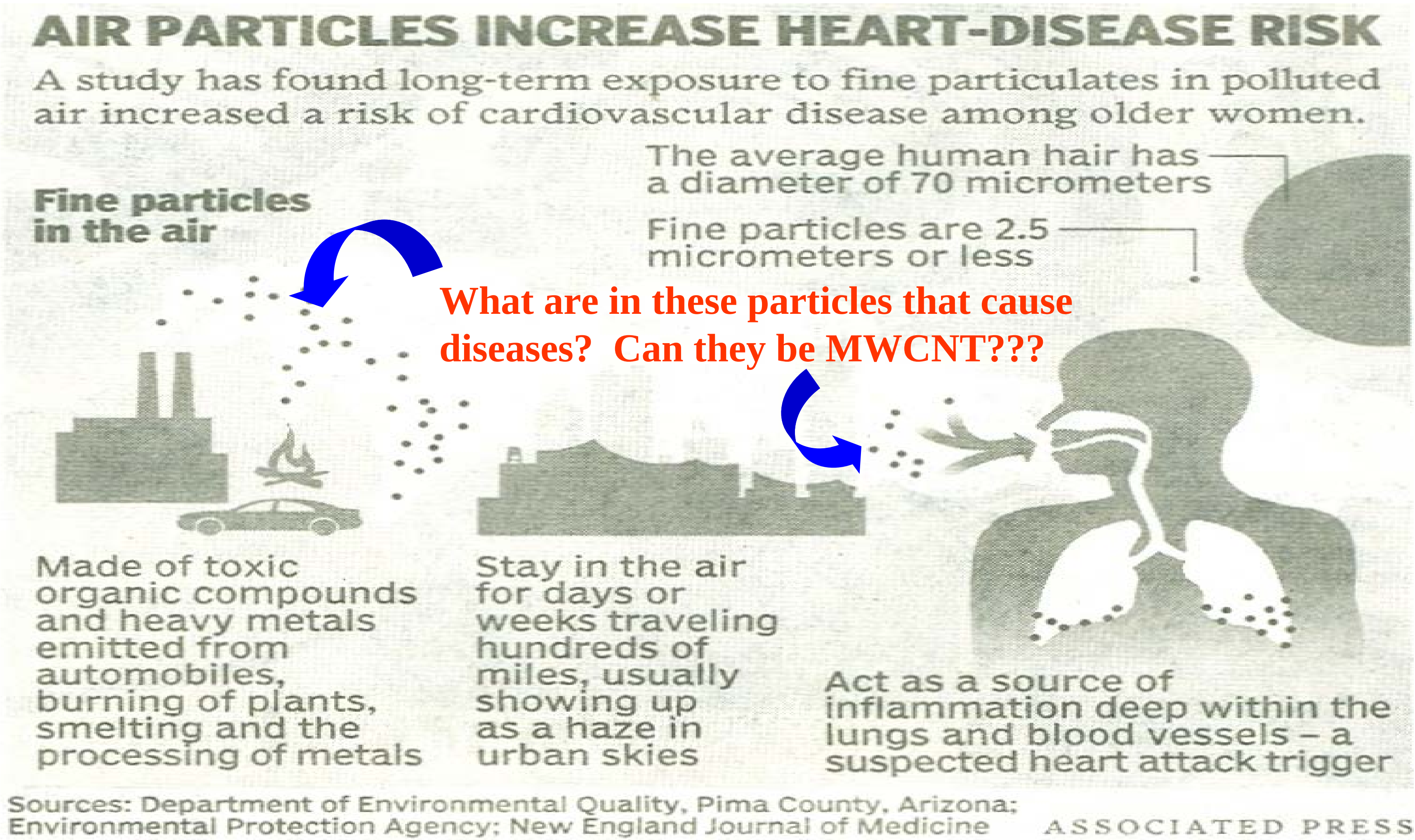
LONG-TERM EXPOSURES TO AIRBORNE PARTICULATE MATTER INCREASED INCIDENCE OF CARDIOVASCULAR EVENTS IN WOMEN



Each increase of 10 µg/m³ was associated with a 24% increase in the risk of a cardiovascular (CV) event (hazard ratio, 1.24 [95% CI:, 1.09 to 1.41]) **and a 76% increase in the risk of death from CV disease** (hazard ratio, 1.76; 95% CI: 1.25 to 2.47).

(*Miller et al. NEJM 356:447-458,2007*)

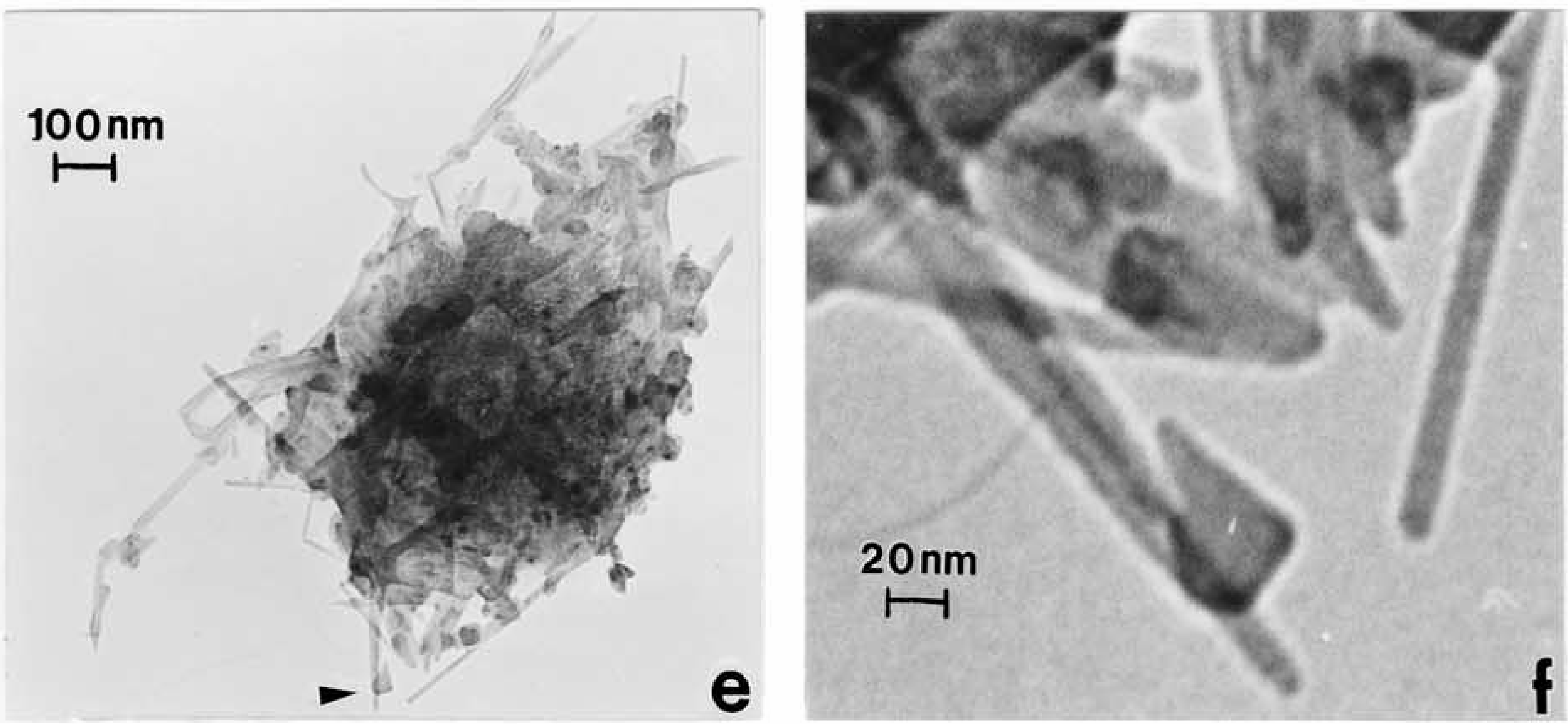
CARBON NANOTUBES IN THE ENVIRONMENT: IMPLICATIONS TO POLLUTION-INDUCED CARDIOPULMONARY DISEASES??



MWCNTS

IN THE ENVIRONMENT ???

MWCNTS FOUND IN PREHISTORICAL ICE SAMPLES FROM GREENLAND



Figures (e) and (f) show 10000 year-old particulate trapped in a Greenland ice core. (f) shows a magnified image at arrow in (e)

Murr et al., J. Mater. Sci. 39:2199

Carbon nanotubes in wood soot

Atmos. Sci. Let. **7**: 93–95 (2006)

L. E. Murr* and P. A. Guerrero
Department of Metallurgical and Materials Engineering, The University of Texas at El Paso, El Paso, TX

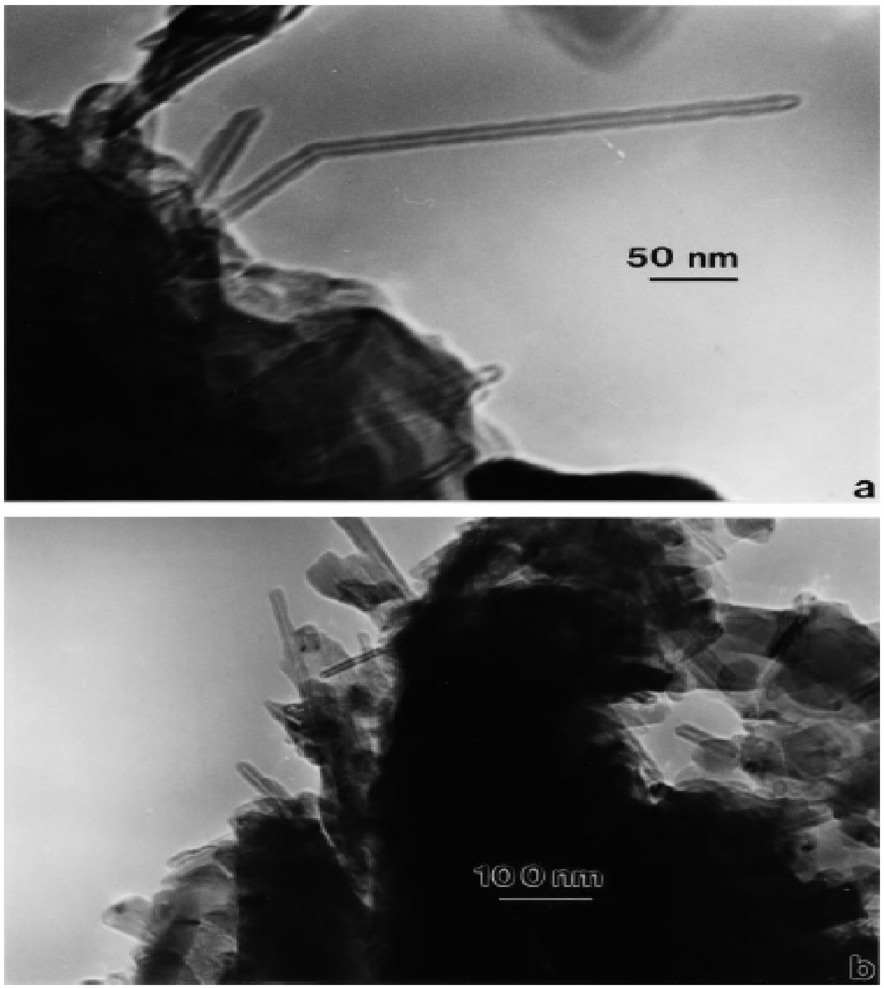


Figure 3. (a) and (b) show TEM examples of multiwall carbon nanotubes aggregated with graphite nanofragments and fullerene-like nanoforms

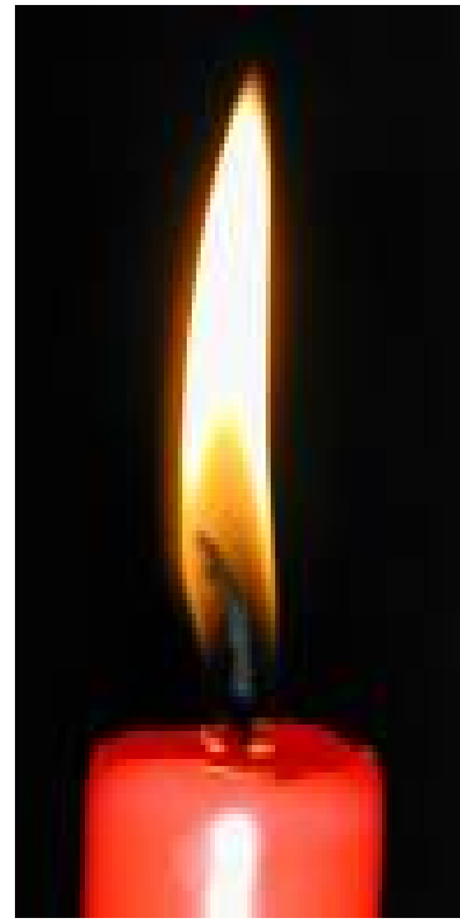
www.for.gov.bc.ca

MWCNTs PRODUCED BY CANDLE FLAMES

"Carbon nanotubes can be found naturally in places like candle flames, but these nanotubes have many defects and have more than one wall (imagine taking a stack of several papers, rolling them all up together, and adding some tears and bends)."

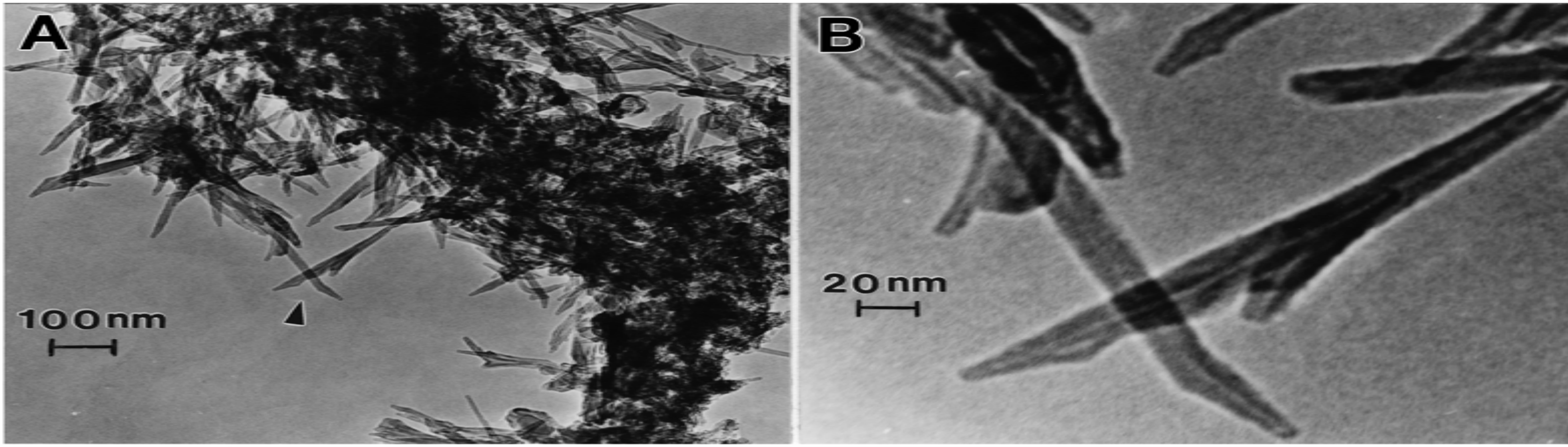
Cornell Center for Materials Research

http://www.ccmr.cornell.edu/education/ask/?quid=801

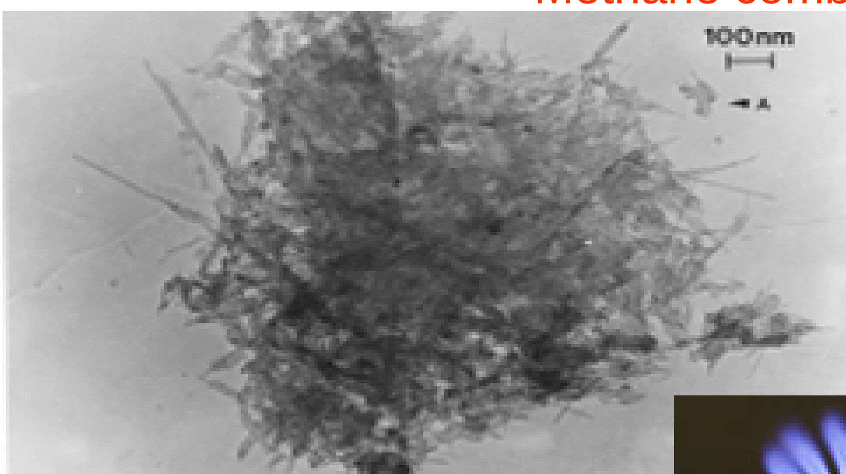


www.interactives.co.uk

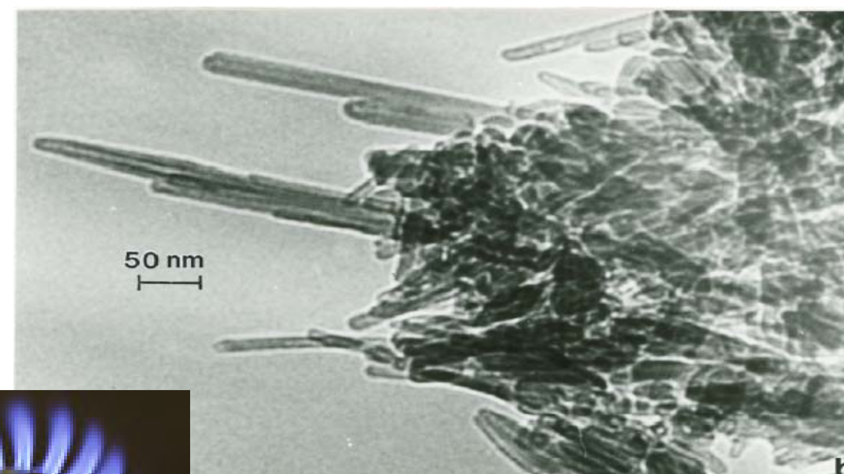
MWCNTs GENERATED FROM FUEL COMBUSTION



Methane combustion Exhaust



Propane combustion Exhaust



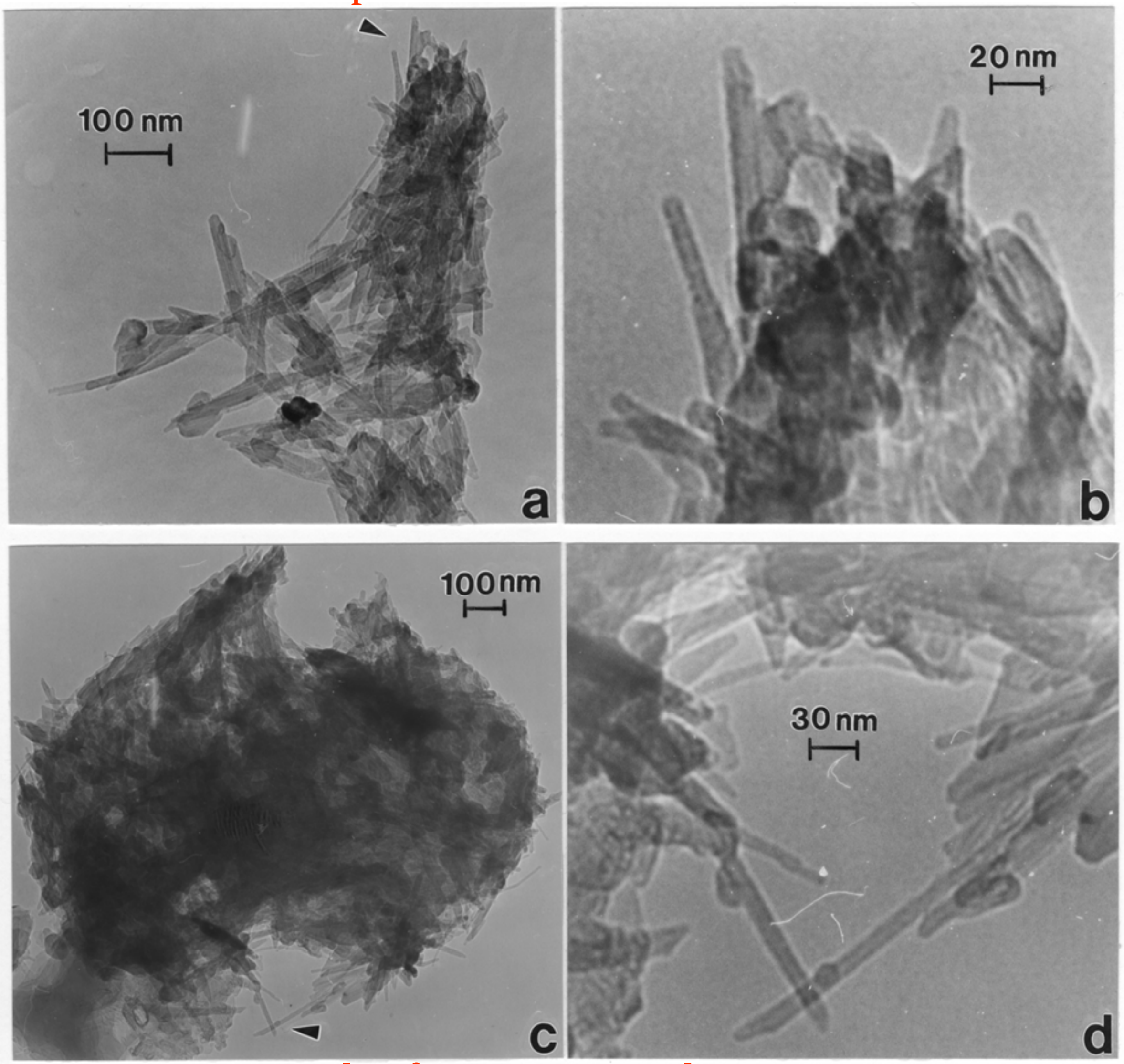
Natural gas combustion Exhaust

Source: PhotoSearch

Courtesy of L. Murr and A. Holian

MWCNTs FOUND IN FINE PARTICULATE MATTER IN URBAN AIR

PM samples from El Paso downtown

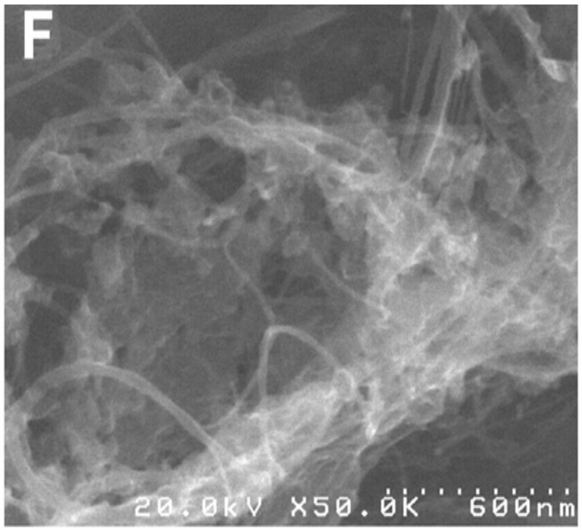


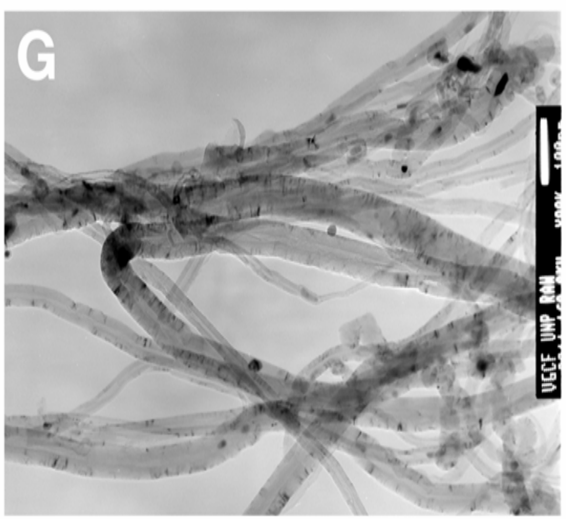
PM samples from Houston downtown

Courtesy of L. Murr and A. Holian

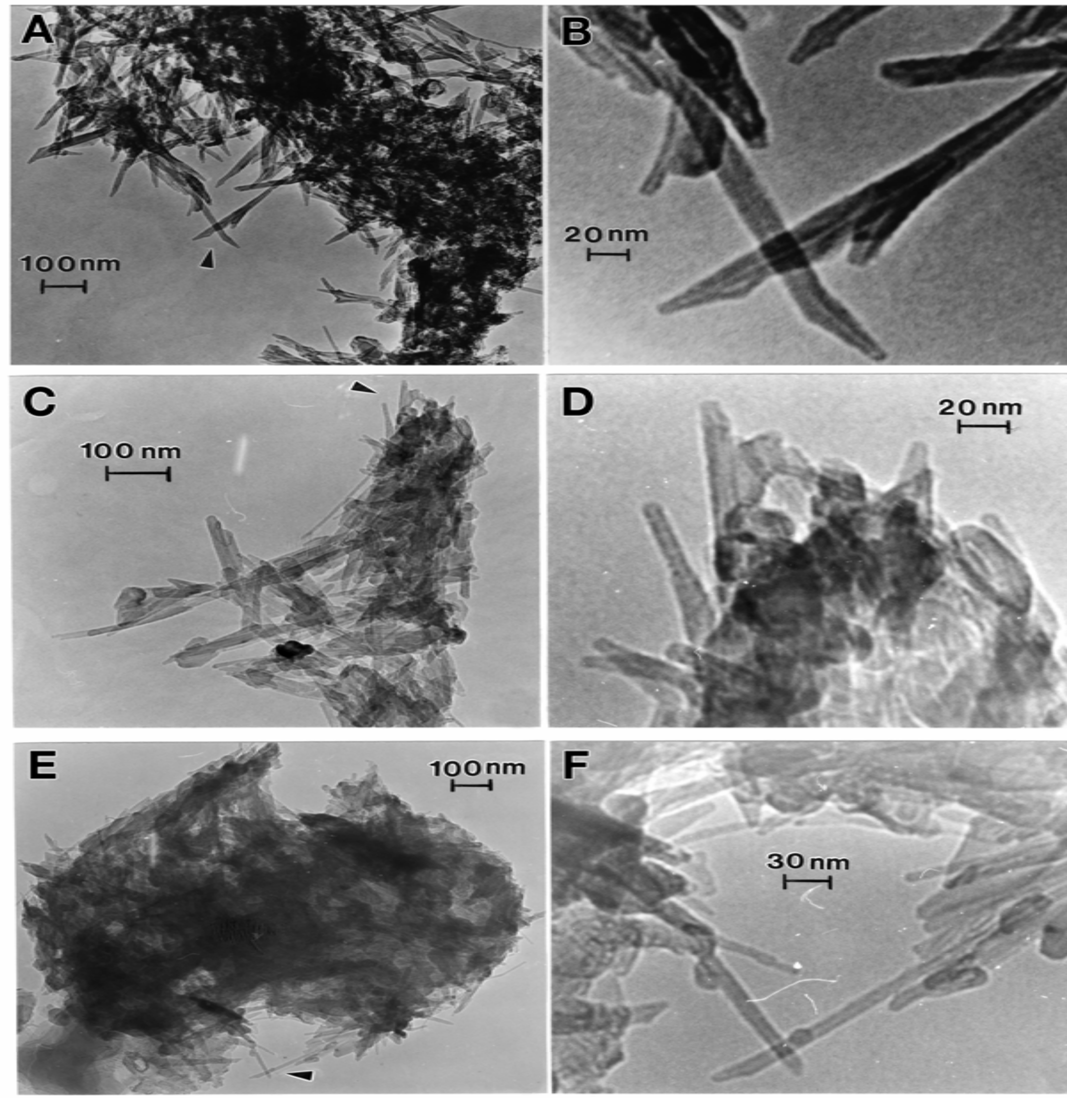
MANUFACTURED vs COMBUSTION-GENERATED MWCNTS

Manufactured MWCNTS





Fuel Combustion-generated MWCNTs

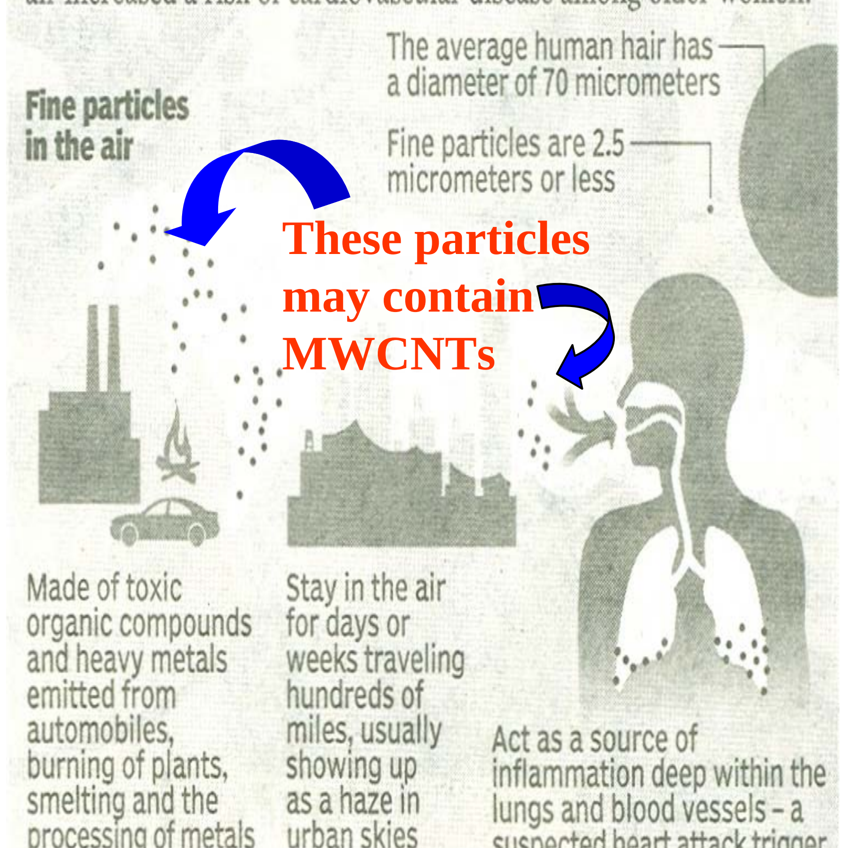


Lam et al. Crit. Rev. Tox. 36:189-217, 2006

COMPARISON OF MANUFACTURED MWCNTS AND FUEL COMBUSTION-GENERATED MWCNTS

	Manufactured	**Fuel Combustion-Generated**
Synthesis Conditions		
Fuel/Materials	Purified feed stock	Heterogeneous
Temperature	Controlled, optimized	Vary
Environment	Confined	Not confined
Metal Catalysts	Generally: yes, but not needed	Generally: no
Aggregation	More orderly	Random
Products	**Longer, and more uniform in size**	**Very short, highly irregular in size, contain many defects on walls**

FUEL-COMBUSTION GENERATED MWCNTS IN THE ENVIRONMENT

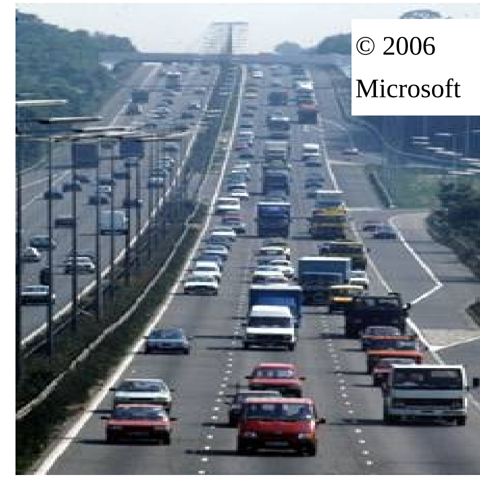


AIR PARTICLES INCREASE HEART-DISEASE RISK

A study has found long-term exposure to fine particulates in polluted air increased a risk of cardiovascular disease among older women.

Sources: Department of Environmental Quality, Pima County, Arizona; Environmental Protection Agency; New England Journal of Medicine

ASSOCIATED PRESS



CONCLUSIONS

ANIMAL STUDIES (≤90 DAYS) SHOWED

- Inflammation, fibrosis, epithelioid granulomas
- Biochemical and toxicological changes in BALF
- Respiratory function impairments, retarded bacterial clearance
- Damaged mitochondrial DNA in aorta, increased % of aortic plaque, and induce atherosclerotic lesions in the heart
- Toxicity: CNTs > quartz > nanosize carbon black

ENVIRONMENTAL STUDIES SHOWED

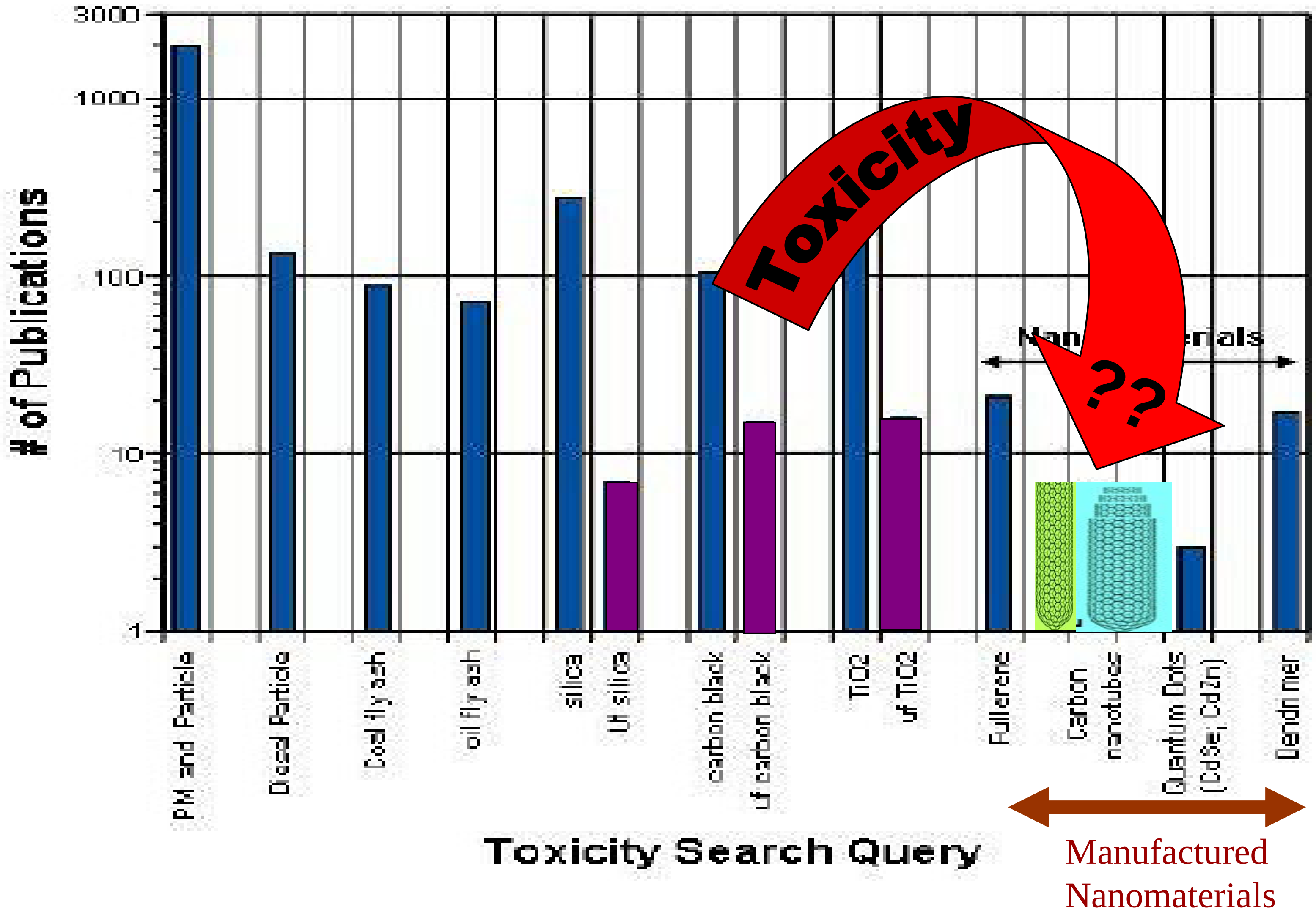
- Atmospheric fine PM aggregates shown to contain MWCNT nanoaggregates
- Association between exposures to PM2.5 & cardiopulmonary diseases
- Highest correlation between impairment of lung functions in children and elemental carbon in environmental samples (Gauderman et al., 2004).

PROPOSED ROLE OF MWCNTs IN PATHOGENESIS OF CARDIOPULMONARY DISEASES

- **We postulate that MWCNTs may play a significant role of pollution- induced cardiopulmonary diseases**

(Lam et al., *Crit. Rev. Tox.* 36: 189-217, 2006)

TOXICITY OF CNTS: WHAT CAN WE LEARN FROM TOXICITIES OF ULTRAFINE PARTICLES

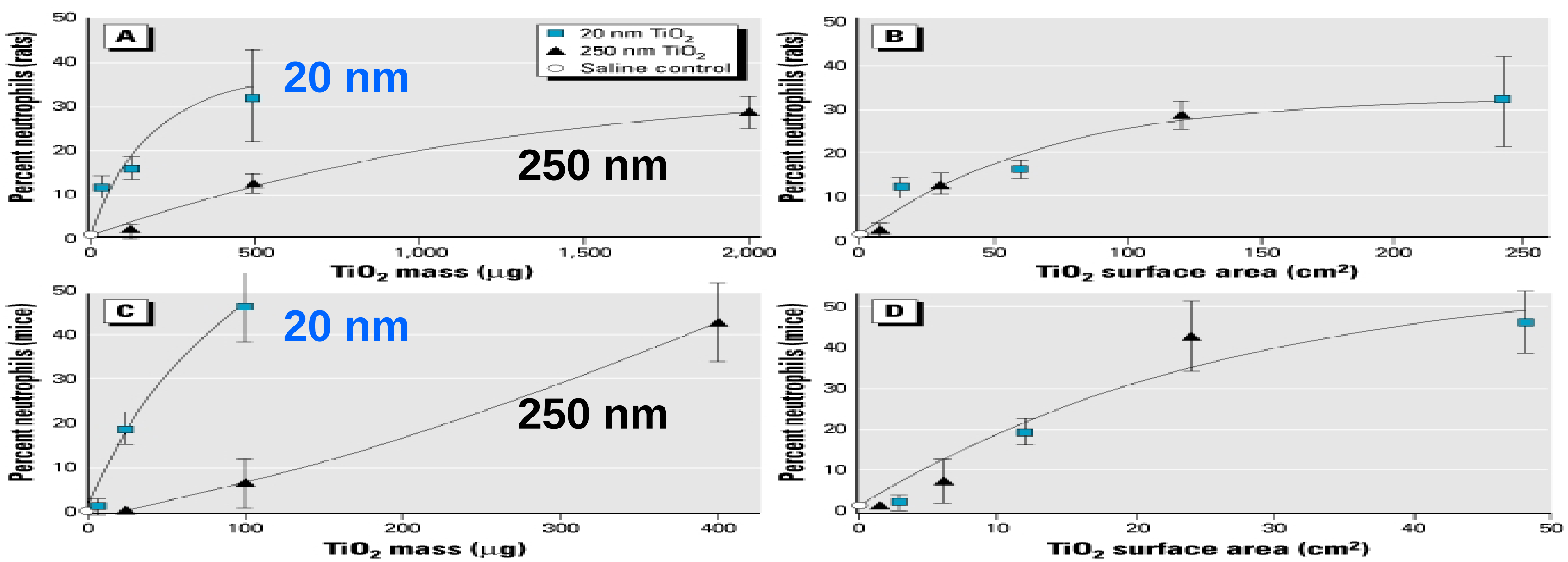


PubMed Search up to 2005: Info from EPA 2005 (modified by C. Lam)

G. Oberdorster

(Environ. Hlth Perspec., 2005)

Toxicity **increased** **With ⇓ Size or ⇑ Surface Area**



D. Warheit

(Tox. Sci. 95(1):270-280, 2007)

Toxicity **did not increase with ⇓ size or ⇑ surface area**

"*Pulmonary bioassay studies with nanoscale and fine-quartz particles in rats: toxicity* ***is not dependent upon particle size but on surface characteristics***"

TOXICITY OF CNTS: WHAT CAN WE LEARN FROM ULTRAFINE PARTICLE TOXICITY??

- Toxicity of carbon nanotube >> nanosize carbon black (or graphite)
- CNTs are electrically and thermally conductive, fibrous, biopersistent and very complicated in structures

Factors affecting toxicity of CNTs are more than size and surface area !

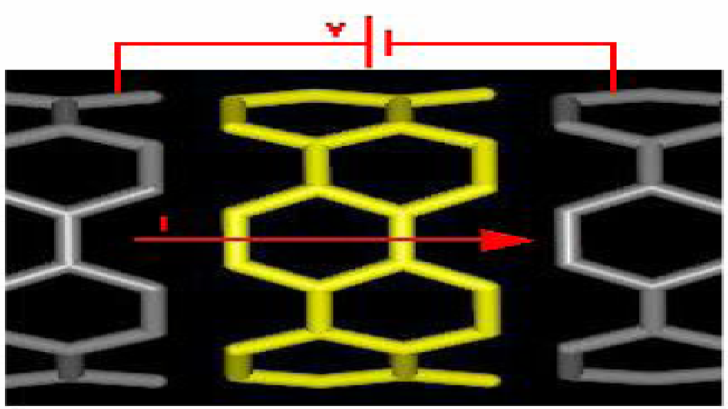
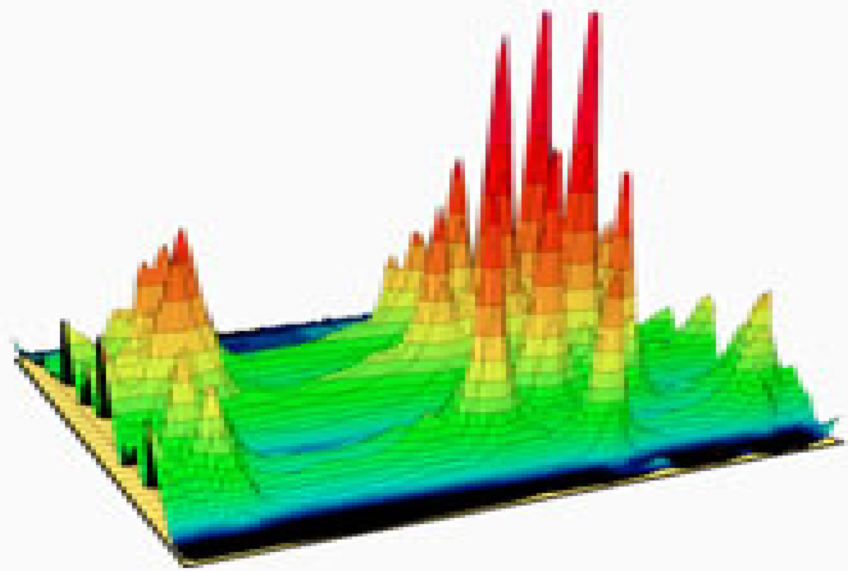
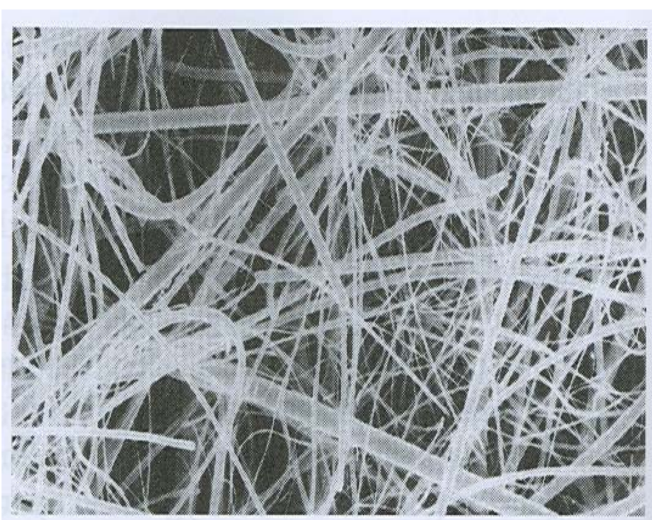


Fig. 2. Simulation model showing current (I) through the central region in response to voltage (V) applied as shown.



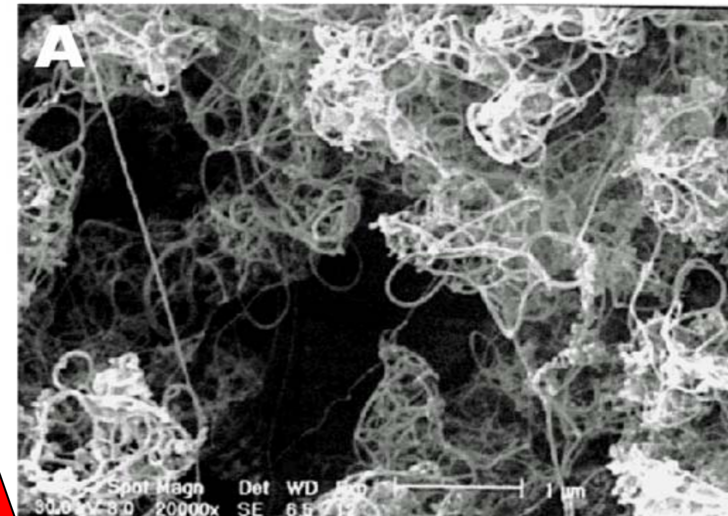
TOXICITY OF CNTS: WHAT CAN WE LEARN FROM FIBER TOXICITY ??

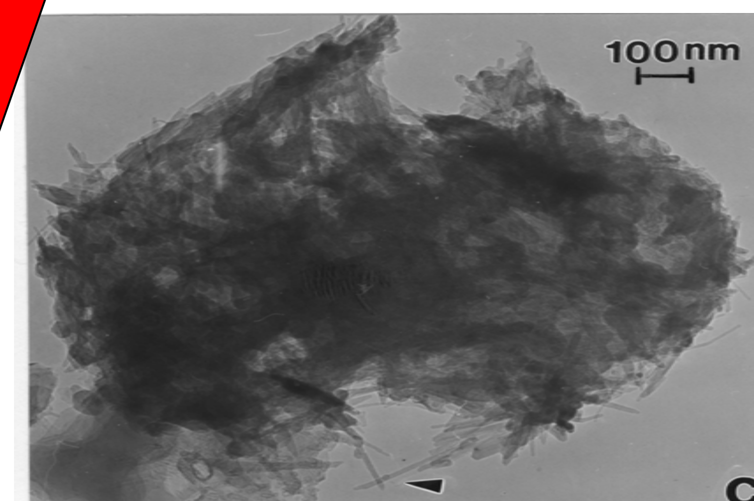
Asbestos (Chrysotile)



Glass fibers

Manufactured SWNTs





MWCNTs in Houston Air

?? TOXICITY

Girard, Prin. Environ. Chem p526, 2005

TABLE 2-1 Typical Chemical Composition of Some Commercial MVF[a]

Composition %

Name[b]	11	A	C	21	F	G	22	RCF-1	X-607	Insofrax
Class	Glass	Glass	Glass	Rock	Rock	Rock	Slag	RCF	RCFsub.	RCF sub.[c]
SiO_2	63.40	65.00	61.70	46.30	56.30	60.10	38.40	[illegible]	58.30	76.20
Fe_2O_3	0.30	0.10	0.10	13.20	0.30	6.10	0.00	[illegible]	0.10	0.30
TiO_2	0.06	0.02	0.02	2.60	0.10	0.05	[illegible]	[illegible]	[illegible]	0.08
Al_2O_3	3.90	1.90	1.00	13.50	3.20	0.40	[illegible]	[illegible]	[illegible]	1.40
CaO	7.40	7.40	7.20	10.00	26.10	[illegible]	[illegible]	[illegible]	[illegible]	0.20
MgO	2.80	2.60	2.90	9.10	[illegible]	[illegible]	[illegible]	[illegible]	0.40	21.50
Na_2O	15.40	16.10	16.10	3.10	[illegible]	[illegible]	[illegible]	[illegible]	0.30	0.07
K_2O	1.30	0.70	0.60	[illegible]	[illegible]	[illegible]	[illegible]	0.20	0.10	0.10
B_2O_2	4.50	4.70	[illegible]	[illegible]	[illegible]	[illegible]	0.00	0.01	0.00	0.00
P_2O_5	0.00	1.10	[illegible]	[illegible]	[illegible]	[illegible]	0.00	0.10	0.40	0.03
SO_3	0.30	[illegible]	[illegible]	[illegible]	[illegible]	0.05	1.80	0.00	0.00	0.00
Cr_2O_3	0.00	0.00	[illegible]	[illegible]	[illegible]	0.00	0.00	0.03	0.00	0.00
MnO	0.01	0.00	[illegible]	[illegible]	0.00	0.00	0.70	0.00	0.00	0.01
ZrO_2	0.00	0.00	[illegible]	0.00	0.00	0.00	0.00	0.10	0.00	0.00
Total	99.40	99.60	[illegible]	99.80	99.10	99.50	100.80	99.40	99.30	99.90

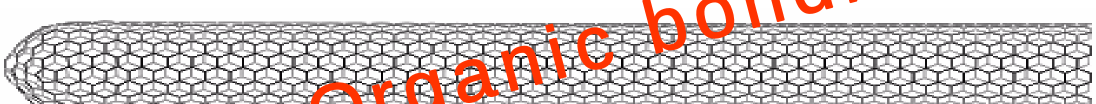
[a]Data derived from Bernstein et al. (1996), Maxim et al. (1999a), and McConnell et al. (1994, 1995, and 1999); Material Safety Data sheets for Isofrax fibers from the Unifrax Corporation, Niagara Falls, NY.

[b]Name: 11, Certain Teed B glass wool fiber; A, new glass wool; C, new glass wool; 21, rock wool; F, rock wool; G, rock wool; 22, slag wool; RCF-1 - kaolin-based refractory ceramic fiber; X-607, rock wool produced by Unifrax; Isofrax, refractory ceramic fiber.

cSubstituted RCF.

Source: *NRC 2000*

Bonds in mineral fibers are inorganic



CNTs: Pure carbon

TABLE 2-1 Typical Chemical Composition of Some Commercial MVF[a]

Composition %

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Class	Glass	Glass	Glass	Rock	Rock	Rock	Slag	RCF	RCFsub.	RCF sub.[c]
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Fe_2O_3	0.30	0.10	0.10	13.20	0.30	6.10	0.00	1.00	0.10	
TiO_2	0.06	0.02	0.02	2.60	0.10	0.05	0.50	2.10	[illegible]	[illegible]
Al_2O_3	3.90	1.90	1.00	13.50	3.20	0.40	[illegible]	[illegible]	[illegible]	1.40
CaO	7.40	7.40	7.20	10.00	26.10	[illegible]	[illegible]	[illegible]	38.70	0.20
MgO	2.80	2.60	2.90	9.10	[illegible]	[illegible]	[illegible]	0.08	0.40	21.50
Na_2O	15.40	16.10	16.10	[illegible]	[illegible]	[illegible]	0.40	0.00	0.30	0.07
K_2O	1.30	0.70	[illegible]	[illegible]	0.70	0.20	0.50	0.20	0.10	0.10
B_2O_2	[illegible]	[illegible]	[illegible]	0.00	0.00	0.00	0.00	0.01	0.00	0.00
P_2O_5	[illegible]	[illegible]	1.10	0.40	2.90	0.08	0.00	0.10	0.40	0.03
SO_3	[illegible]	0.03	0.20	0.00	0.00	0.05	1.80	0.00	0.00	0.00
Cr_2O_3	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.03	0.00	0.00
MnO	0.01	0.00	0.01	0.20	0.00	0.00	0.70	0.00	0.00	0.01
ZrO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
Total	99.40	99.60	100.00	99.80	99.10	99.50	100.80	99.40	99.30	99.90

Mineral fibers are nonconductive & used as insulators

CNTs have excellent electrical and unusual high thermal conductivity

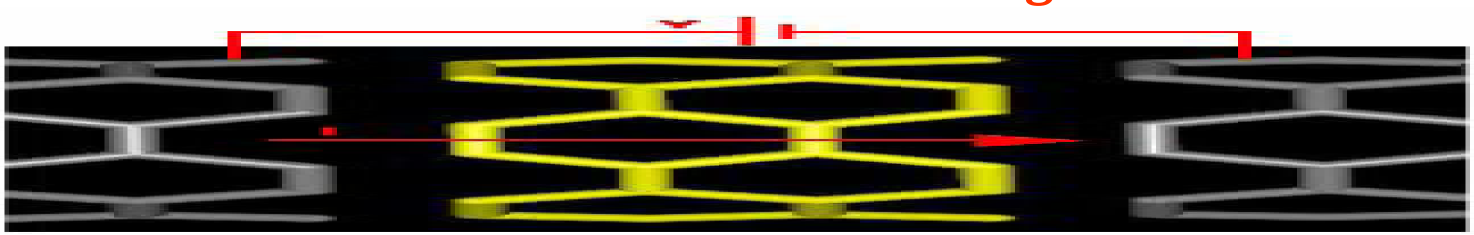


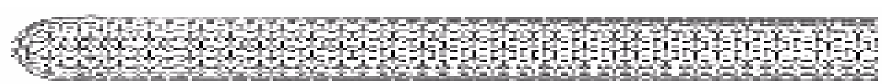
Fig. 2. Simulation model showing current (I) through the central region in response to voltage (V) applied as shown.

In Vitro Solubility and In Vivo Biopersistence of Asbestos and Various Types of MVF in Lung

Fiber class	Fiber type and use[a]	K_{dis}[b] at pH 7.4	Time to dissolve[c], days	WT $½$,[d] days	Ref.
Asbestos	Amosite	<1	>5,000	~400	1
Asbestos	Crocidolite	<1	>5,000	~800	1
RCF	High temperature applications	3	~1,700	~50	1
Other-RCF substitute	Magnesium silicate	>150	~35	~6	2
Glass	MMVF 32, specialty applications	9	~550	~80	1
Glass	MMVF 33, filtration	12	~400	~50	1
Glass	MMVF 11, building insulation	100	~50	~10	1
Glass	A, building insulation	250	~20	~4	1
Glass	MMVF 10, building insulation	300	~20	~35	1
Glass	P, building insulation	>500	<10	~5	1
Glass	C, building insulation	>500	<10	~4	1
Glass	B, building insulation	>500	<10	~2	1
Rock	MMVF 21, building insulation	20	~250	~60	1
Rock	MMVF 34[a], building insulation	60	~80	~5	1

Ref: NRC 2000

Carbon Nanotubes



Time to dissolve → ∞
Half Life in Lung? Would be long!

Lam (2004): CNTs lung lesions got worse with time.

Mercer (2007): Alveolar macrophage clearance is not a significant component of the lung response.

MVF biopersistence → pathogenicity & carcinogenicity

CNTs would be very biopersistent → pathogenicity ?? → carcinogenicity

TOXICOLOGICAL RISK OF CNT EXPOSURES

CNT TOXICITY

- Severe lung lesions, CNTs are rather toxic
- Lung lesions got worse with time (NASA and NIOSH Studies)
- Produced Pulmonary function decrement & Cardiovascular tox.
- Carcinogenicity: don't know???

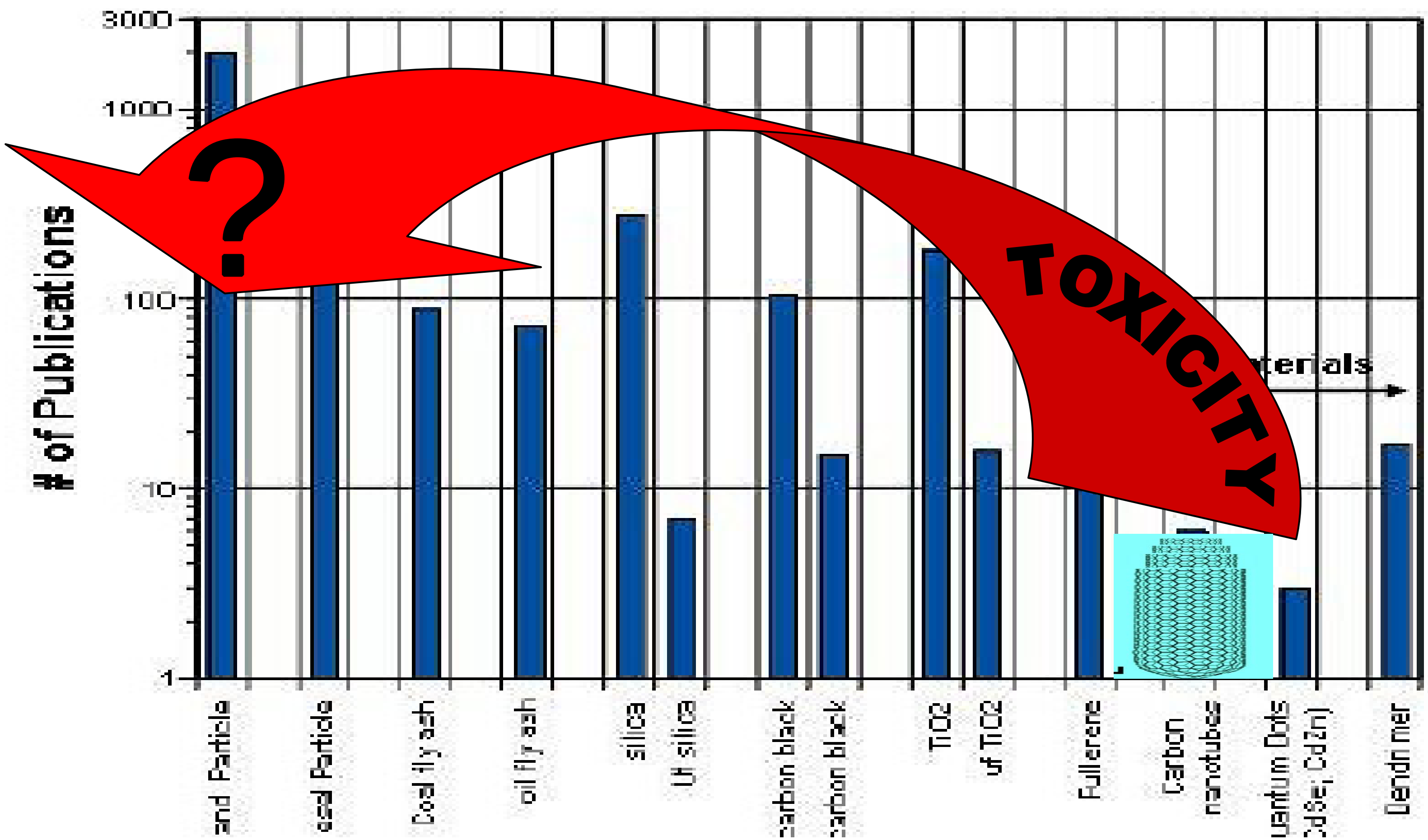
OCCUPATIONAL RISK AND RECOMMENDATIONS

- Recommend setting industrial CNT exposure limits:
  CNT << carbon black or graphite
- Implement industrial hygiene to minimize exposures

ENVIRONMENTAL HEALTH

- MWCNTs are present in environment
- We postulate that exposure to combustion-generated MWCNTs in fine PM may play a significant role in air pollution-related cardiopulmonary diseases

A BIG QUESTION FOR TOXICOLOGICAL RESEARCH



PM2.5 related cardiopulmonary diseases: can we learn from CNT toxicity?

U.S. Department of Health and Human Services

NIH News

National Institutes of Health

National Institute of Environmental Health Sciences (NIEHS)

Do MWCNTs play an important role ??

March 5, 2002

BYU Contact: Michael Smart
(801) 378-7320

NIEHS Contact: Bill Grigg
grigg@niehs.nih.gov
(301) 402-3378

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Diseases

Link Strengthened Between Lung Cancer, Heart Deaths and Tiny Particles of Soot, Dust

Years of exposure to the high concentrations of tiny particles of soot and dust from cars, power plants and factories in some metropolitan areas of the United States significantly increase residents' risk of dying from lung cancer and heart disease, according to a study financed largely by the National Institute of Environmental Health Sciences and conducted by scientists at Brigham Young University, Provo, Utah; the University of Ottawa, Ontario, the American Cancer Society and New York University School of Medicine, Tuxedo, N.Y.