Safety evaluation of engineered nanoparticles: Relevance for ambient ultrafine particles?

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Results of numerous studies, *in vitro* and *in vivo*, have revealed that engineered nanomaterials (ENM) can induce significant toxicity. However, because most of these studies were designed using very high doses/concentrations, their usefulness for risk assessment purposes can be questioned. The sole reliance on dose–response data falls short of enabling a comprehensive safety assessment of ENM. With respect to inhalation as the route of exposure, the availability of exposure–dose–response data based on a subchronic or chronic rodent inhalation study would be an appropriate basis for quantitative risk assessment. A case study in rats of 3 month inhalation exposures to multiwalled carbon nanotubes (MWCNT) is briefly discussed to illustrate how both hazard and risk characterization can be derived from such subchronic study. Ideally, a minimum of three exposure concentrations plus sham-exposed controls should be used, with detailed characterization of the aerosol and including measurement of biokinetic data. Essential for a comparative approach would be the availability of results from subchronic inhalation of positive and negative control materials against which the materials to be tested can be ranked. Also, expressing results by different dosemetrics such as particle mass, surface area, volume, or number, can provide important information about potential underlying mechanisms. Analyzing dose-response relationships with respect to hazard ranking could be done by identifying the steepest slope of the dose–response relationship (Rushton *et al*., 2010). For risk characterization, the exposure–dose–response relationship can be analyzed by using a benchmark dose approach (BMD, Davis *et al*., 2011) in order to derive an associated benchmark concentration (BMC) as a safe exposure level. Results derived from the rodent study will be the basis for an extrapolation of risk to human exposure scenarios, provided species differences in respiratory tract dosimetry are considered. This concept will be illustrated using two subchronic inhalation studies in rats with MWCNT (Ma-Hock *et al*., 2009; Pauluhn, 2010). Results of previously published subchronic inhalation studies with negative reference particles (carbon black, nano-TiO_2 and micro-TiO_2) and positive reference particles (crystalline silica, Ni_3S_2, both known human carcinogens inducing significant acute and chronic adverse effects) were selected for comparison. Using different dosemetrics for hazard characterization showed that the retained particle surface area as well as the retained particle volume at the end of the 3-month rat inhalation studies appeared to be the best metrics to rank the MWCNT against the reference particles. Hazard groupings of low, medium and high could be established, with MWCNT ranking in the medium group. With respect to deriving a BMC for subchronic exposures of rats, it turned out that carbon black, as the more benign particle type, required a much higher exposure concentration than the tested MWCNT to reach the benchmark response (BMR), whereas Ni_3S_2 needed only to be inhaled at a very low concentration to reach its BMR at the 3-month timepoint. In order to extrapolate the rat to a human equivalent concentration (HEC), dosimetric extrapolation with rat-specific and human-specific particle deposition models should finally be carried out.

Can a similar approach be used to derive a “safe” exposure concentration for ambient ultrafine particles (UFP)? Although engineered nanomaterials and ambient ultrafine particles are of the same size

category, there are still significant differences in terms of chemistry and surface properties between ENM (purposefully designed by well-controlled processes) and ambient UFP (generated by numerous anthropogenic combustion sources which generate also gaseous compounds; and generated by non-anthropogenic gas-to-particle conversions in the ambient atmosphere). Even "clean" natural gas fueled power plants emit ultrafine particles at high concentrations, similar to oil and coal-fired power plants (Chang *et al*., 2004). A most often discussed source of ambient UFP is exhaust from traditional diesel engines because of its toxic nature; with the introduction of very efficient filtration devices to retain UFP, the new technology diesel engines are extremely clean with respect to particle emissions (Mayer *et al*., 2008; Hesterberg *et al*., 2012). However, despite the efficient removal of exhaust UFP and of chemical constituents, and despite the absence of epidemiological studies or long-term animal inhalation studies for an in depth risk assessment of new technology diesel exhaust, the International Agency for Research on Cancer (IARC, 2012) in its recent carcinogen evaluation meeting determined that there is sufficient evidence in humans for carcinogenicity of diesel engine exhaust as a cause for lung cancer – including the new technology diesel. IARC classifications are solely based on a hazard potential rather than on a risk analysis which most likely would not be significant due to the very low particle emissions of new technology diesel exhaust. On the issue of establishing an ambient ultrafine particle standard, EPA in its most recent proposed rule regarding a national ambient air quality standard for particulate matter gave no indication that a standard for UFP is being considered (EPA, 2012). EPA proposed, though, to lower the annual health standard for $PM_{2.5}$ and set a separate standard to improve visibility for a 24-hr. standard, but otherwise retained the 24-hr. standard for coarse particles and existing secondary standards for $PM_{2.5}$ and PM_{10}.

When considering an ultrafine particle standard, the question how to derive a standard needs to be discussed. Studies at Rochester (unpublished) have shown that ambient UFP-bound reactive oxygen species (ROS) vary widely from day-to-day and within a given day. Of interest, there was no consistent correlation between number of ambient UFP and the ROS activity associated with these particles. It appears that episodic nucleation processes, including gas-to-particle conversions which are not necessarily seasonally restricted, may play a role. In separate studies using engineered nanoparticles it was found that the ROS-inducing potential of these nanoparticles correlated well with acute *in vivo* responses (Rushton *et al*., 2010); if such correlation can also be established for ambient UFP, measurement of UFP-bound ROS could be a simple means to predict the *in vivo* reactivity of UFP in exposed humans and compare it to other well characterized materials. For example, Zhao and Hopke (2012) compared the ROS inducing capacity of the particulate phase and the gaseous phase of cigarettes with those of ROS bound to particles in urban settings. They suggested to express urban particulate exposure in terms of ROS equivalency of cigarette smoke and presented examples of wide variations of cigarette equivalencies of particulate air pollution among different cities world-wide.

With respect to establishing a general ambient ultrafine particle standard, one could consider to base this on a specific dosemetric for expressing the intrinsic UFP bound ROS activity. However, a general UFP standard (targeting all UFP) based on any dosemetric does not make sense because of the enormous differences in UFP chemistry from different specific sources (controllable anthropogenic, uncontrollable natural) which cause significant differences in toxicity. Therefore, a UFP standard should best be source-specific and should be based on the number concentration of emitted UFP, including also the smaller UFP down to <10 nm. A suggested strategy is to identify those sources which emit the most reactive UFP, which could be based on the measurement of particle-bound ROS as an initial screening tool. It would then be justifiable to regulate these most reactive sources rather than ambient UFP

immissions by introducing an emission number standard. Such standard could be derived according to a risk assessment concept as discussed at the beginning of this presentation. Obviously, co-pollutants (particulate, gaseous) have to be considered as well. For toxicology, this will involve source-specific hazard identification and ranking which could be based on validated *in vitro* approaches, complemented by a source-specific risk characterization based on *in vivo* rodent inhalation assays. Obviously, availability of results of epidemiological studies from exposures to specific UFP sources would be most suitable for a human risk assessment.

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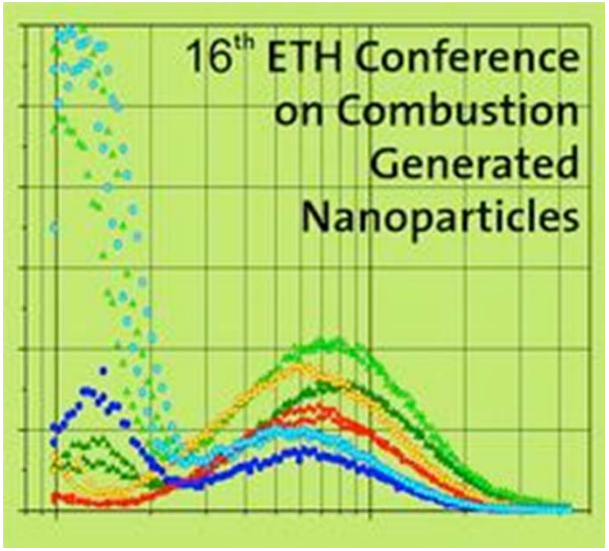

ETH ZÜRICH LABORATORIUM FÜR AEROTHERMOCHEMIE UND VERBRENNUNGSSYSTEME

16th ETH-Conference on Combustion Generated Nanoparticles

24 – 27 June 2012

ETH Zentrum, Zürich,

Session 5b: Health Effects

Safety Evaluation of Engineered Nanoparticles: Relevant for Ambient ultrafine Particles?

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University of Rochester

27 June 2012

UNIVERSITY of ROCHESTER MEDICAL CENTER

A Case Study:

Risk Assessment Based on Subchronic (3 months) Rodent Inhalation Study

- subchronic multi-concentration inhalation studies with MWCNT in rats
  - *important: aerosol characteristics; biokinetics (lung burden);post exposure period*
- use results of "positive" and "negative" reference materials
- select sensitive endpoints of response *(quantitative best)*
- establish Exposure – Dose - Response correlations
- express by different dosemetrics *(particle-mass, -surface area, -volume, -number)*
- evaluate results to establish:
  - ***hazard ranking*** *against pos. and neg. control, by different dosemetrics*
  - *subchronic no effect level for rat:* ***NOAEL; BMD/BMR/BMC***
- estimate chronic no effect level *(based on accumulated lung burden)*
- use dosimetric extrapolation to estimate HEC *(Human Equivalent Concentration)*

Two Subchronic MWCNT Inhalation Sudies in Rats

Inhalation Toxicity of Multiwall Carbon Nanotubes in Rats Exposed for 3 Months

Lan Ma-Hock,* Silke Treumann,* Volker Strauss,* Sandra Brill,* Frederic Luizi,† Michael Mertler,‡ Karin Wiench,* Armin O. Gamer,* Bennard van Ravenzwaay,*,[1] and Robert Landsiedel*

**Product Safety, BASF SE, 67056 Ludwigshafen, Germany; †Nanocyl S. A., 5060 Sambreville, Belgium; and ‡Process Engineering, BASF SE, 67056 Ludwigshafen, Germany*

TOXICOLOGICAL SCIENCES **112(2)**, 468–481 (2009)

Subchronic 13-Week Inhalation Exposure of Rats to Multiwalled Carbon Nanotubes: Toxic Effects Are Determined by Density of Agglomerate Structures, Not Fibrillar Structures

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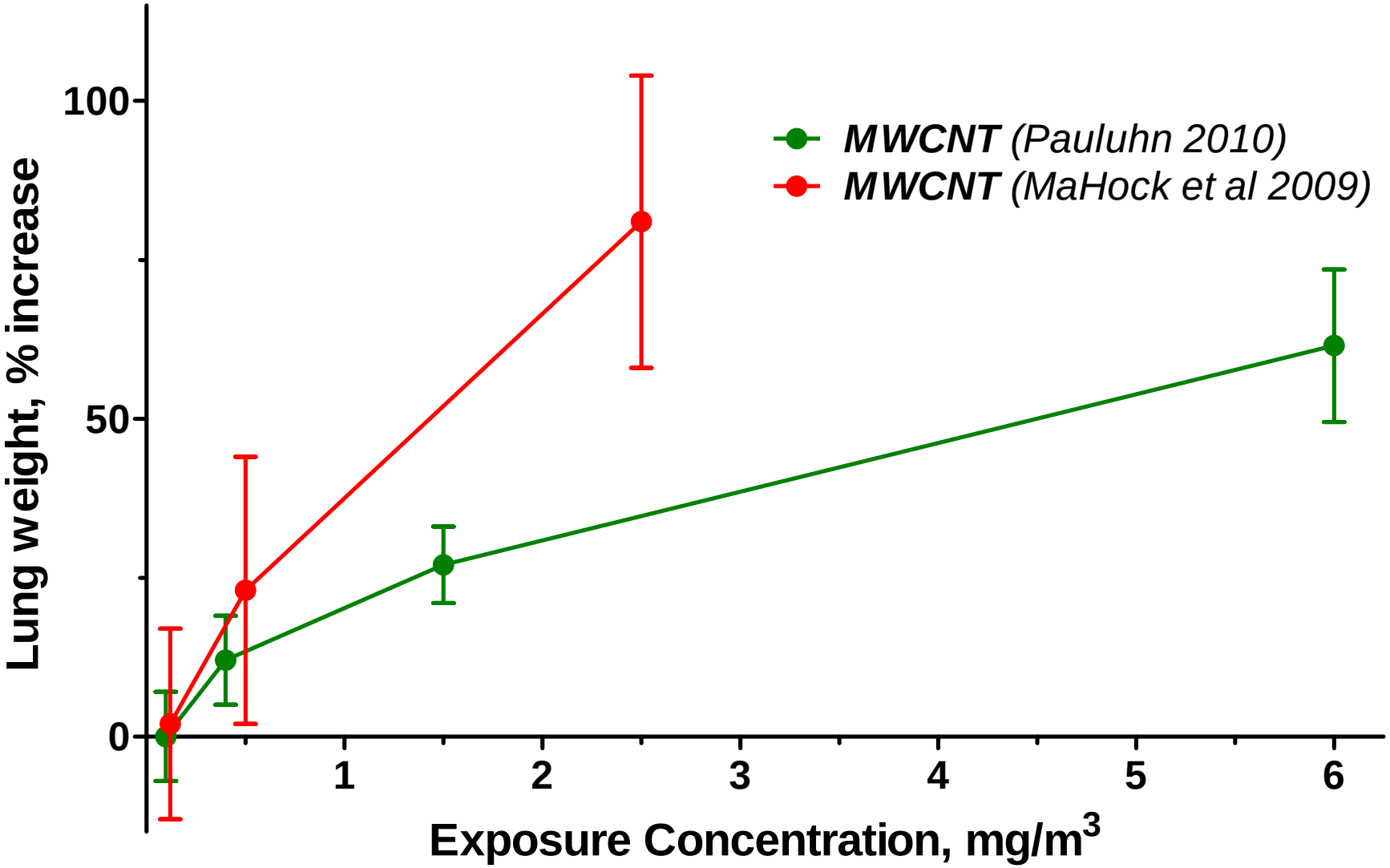
TOXICOLOGICAL SCIENCES **113(1)**, 226–242 (2010)

90 - Day Inhalation, Male Rats: MWCNT

Percent Increase of Lung Weight Above Controls

As Function of **Exposure Concentration**



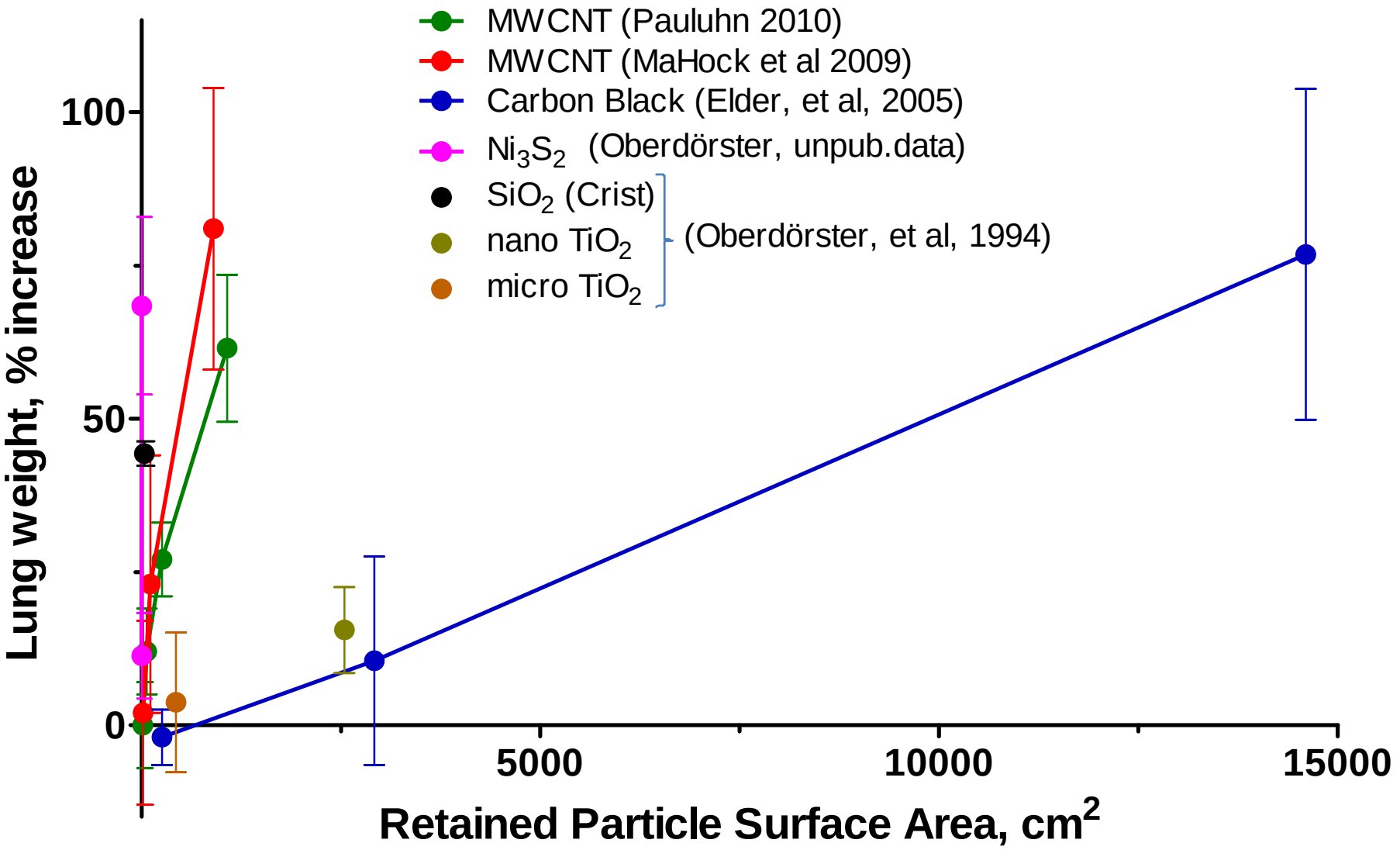
Comparing MWCNT results with 5 other subchronic rat inhalation studies:

ultrafine carbon black	
nano TiO_2	*negative*
micro TiO_2	***Reference materials***
cristalline silica	*positive*
nickel subsulfide	

90 - Day Inhalation, Male Rats: MWCNT, SiO_2, Ni_3S_2, TiO_2

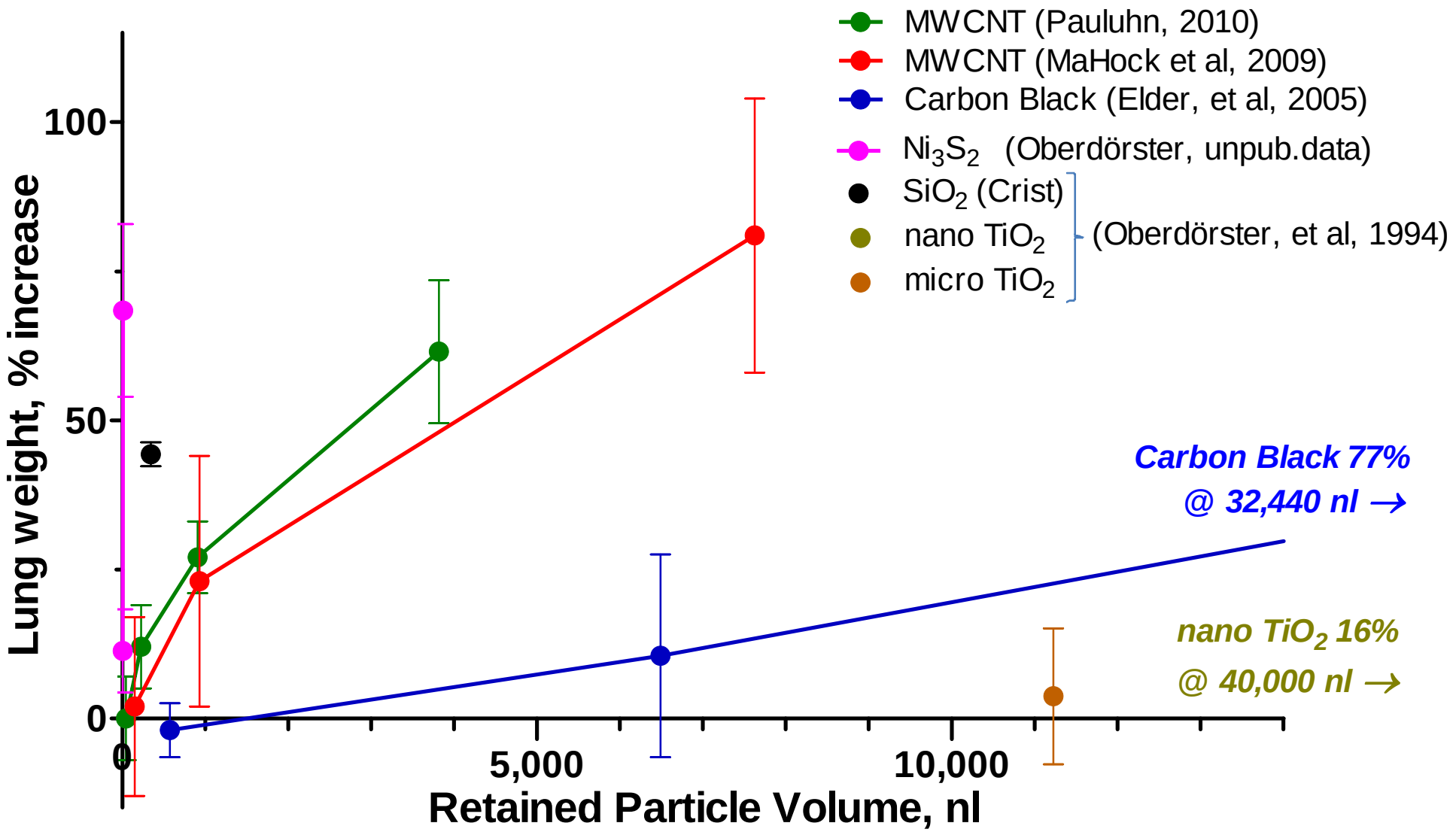
Percent Increase of Lung Weight Above Controls

As Function of Retained Particle **Surface Area**



90 - Day Inhalation, Male Rats: MWCNT, SiO_2, Ni_3S_2, TiO_2

Percent Increase of Lung Weight Above Controls
As Function of Retained Lung Burden (**Volume**)
*(based on **bulk** density)*



Hazard Ranking of Different (Nano)-Materials Based on Different Metrics and Steepest Slope of Exposure-Dose-Response Relationships from Subchronic Rat Inhalation Studies (endpoint: lungweight increase)

Metric	Ranking
Exposure Conc. **:**	micro**TiO_2** < nano**TiO_2** < **CB** < **MWCNT**-P < **MWCNT**-MH = **SiO_2** < **Ni_3S_2**
Retained Lung Burden:	
Mass:	micro**TiO_2** < nano**TiO_2** < **CB** < **SiO_2** = **MWCNT**-P = **MWCNT**-MH < **Ni_3S_2**
Surface area:	**CB** < nano**TiO_2** = micro**TiO_2** < **MWCNT**-P = **MWCNT**-MH < **SiO_2** < **Ni_3S_2**
Volume (bulk dens)**:**	micro**TiO_2** = nano**TiO_2** < **CB** < **MWCNT**-MH = **MWCNT**-P < **SiO_2** < **Ni_3S_2**
Volume (mat. dens)**:**	micro**TiO_2** < nano**TiO_2** < **CB** < **SiO_2** = **MWCNT**-P = **MWCNT**-MH < **Ni_3S_2**

Hazard Ranking of Different (Nano)-Materials Based on Different Metrics and Steepest Slope of Exposure-Dose-Response Relationships from Subchronic Rat Inhalation Studies (endpoint: lungweight increase)

Three Hazard Groupings (based on BET surface area):

Low: ***CB; TiO_2 ⟶ < 0.3 % lungwt. incr./cm^2***

Medium: ***MWCNT ⟶ 0.3 – 1 % lungwt. incr./cm^2***

High: ***SiO_2; Ni_3S_2 ⟶ >1 % lungwt. incr./cm^2***

Rat subchronic exposure concentration to reach BMD-L based on increase in lungweight:

Carbon black: 3700 – 5700 µg/m^3

MWCNT: 140 – 250 µg/m^3

Ni_3S_2: 30 – 35 µg/m^3

NEXT STEPS

Specific:

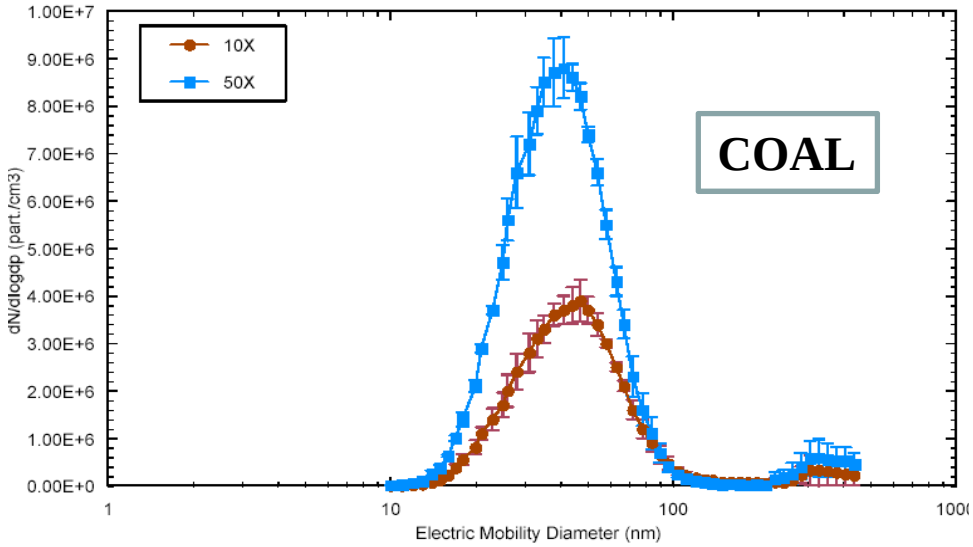
Dosimetric extrapolation of rat to human BMC-L to obtain human equivalent concentration (HEC)

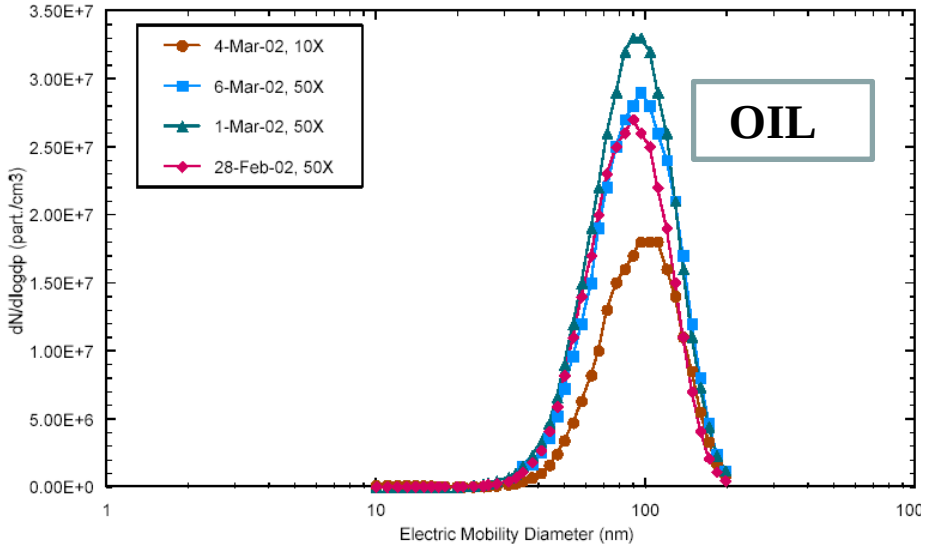
Dosimetric extrapolation of subchronic BMC-L to chronic BMC-L

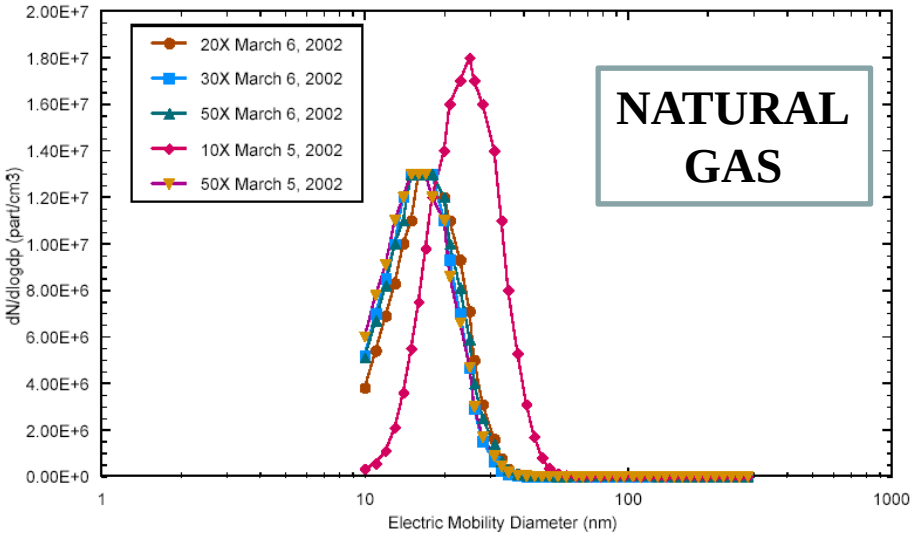
Powerplants: Ultrafine Particle Size Distribution at 10, 20, 30 and 50 X Dilution Air Ratios

(Exhaust temp. 450°K; residence time 80 sec)

(Chang et al., 2003)







International Agency for Research on Cancer

World Health Organization

June 12, 2012

Evaluation:

Sufficient evidence in humans for carcinogenicity: Group 1

- **as cause for lung cancer: sufficient evidence**
- **positive association** (limited evidence) **for increased risk of bladder cancer**

GASOLINE ENGINE EXHAUST**: Possibly carcinogenic to humans: Group 2B**

New Technology Diesel Exhaust: While the amount of particles and chemicals are reduced with these changes, it is not yet clear how the quantitative and qualitative changes may translate into altered health effects; research into this question is needed.

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Hazard vs Risk

Evaluation:

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New Proposed Rule, June 14, 2012

ENVIRONMENTAL PROTECTION AGENCY

National Ambient Air Quality Standards for Particulate Matter

SUMMARY:

- **Strengthen annual health standard for fine particles, $PM_{2.5}$: within range 12-13 µg/m^3** (current: 15 µg/m^3)

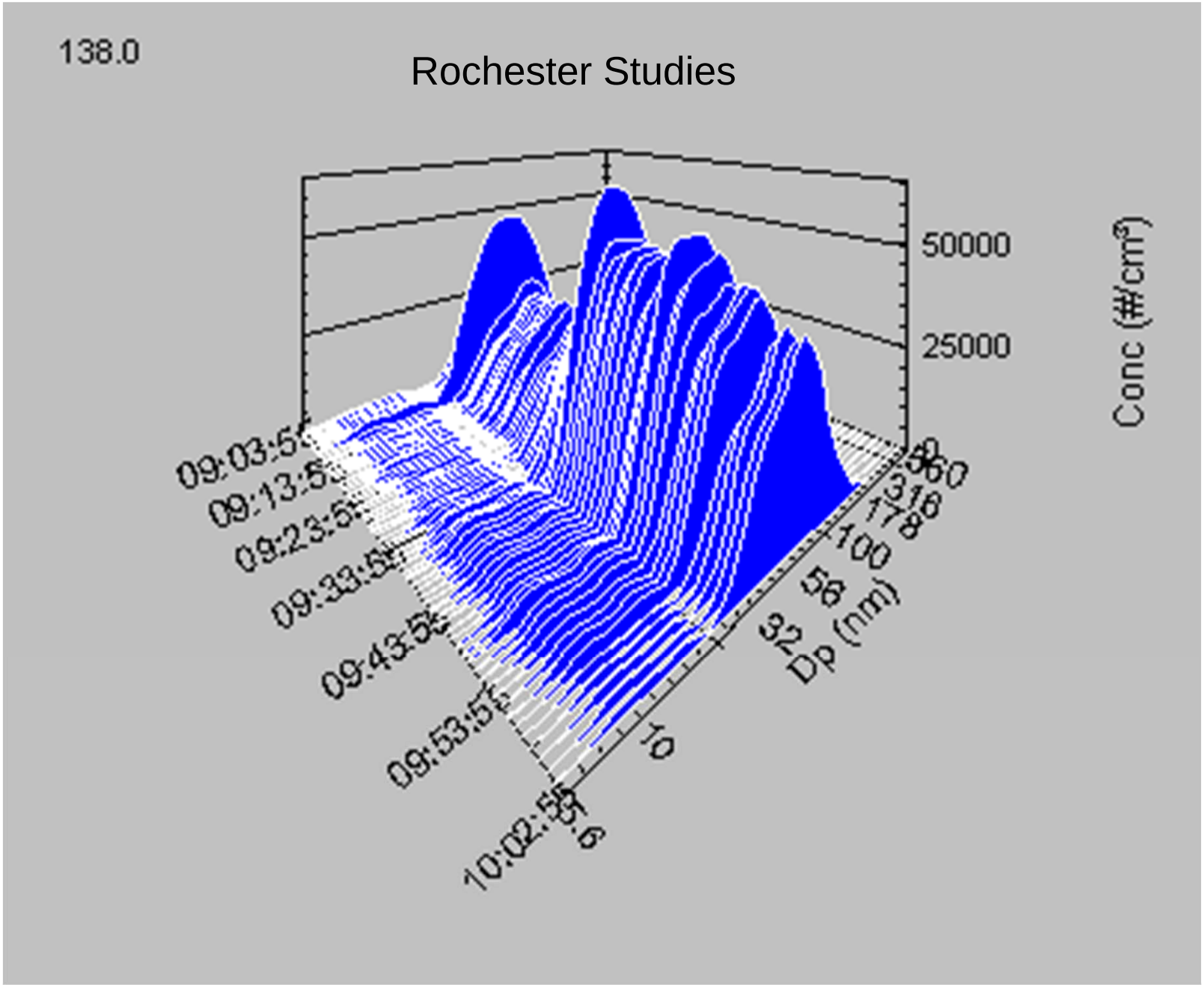
- **Retain existing 24-hr. fine standard at 35 µg/m^3**

- **Set separate standard to improve visibility for 24 hr. standard** (30 deciviews or 28 deciviews)

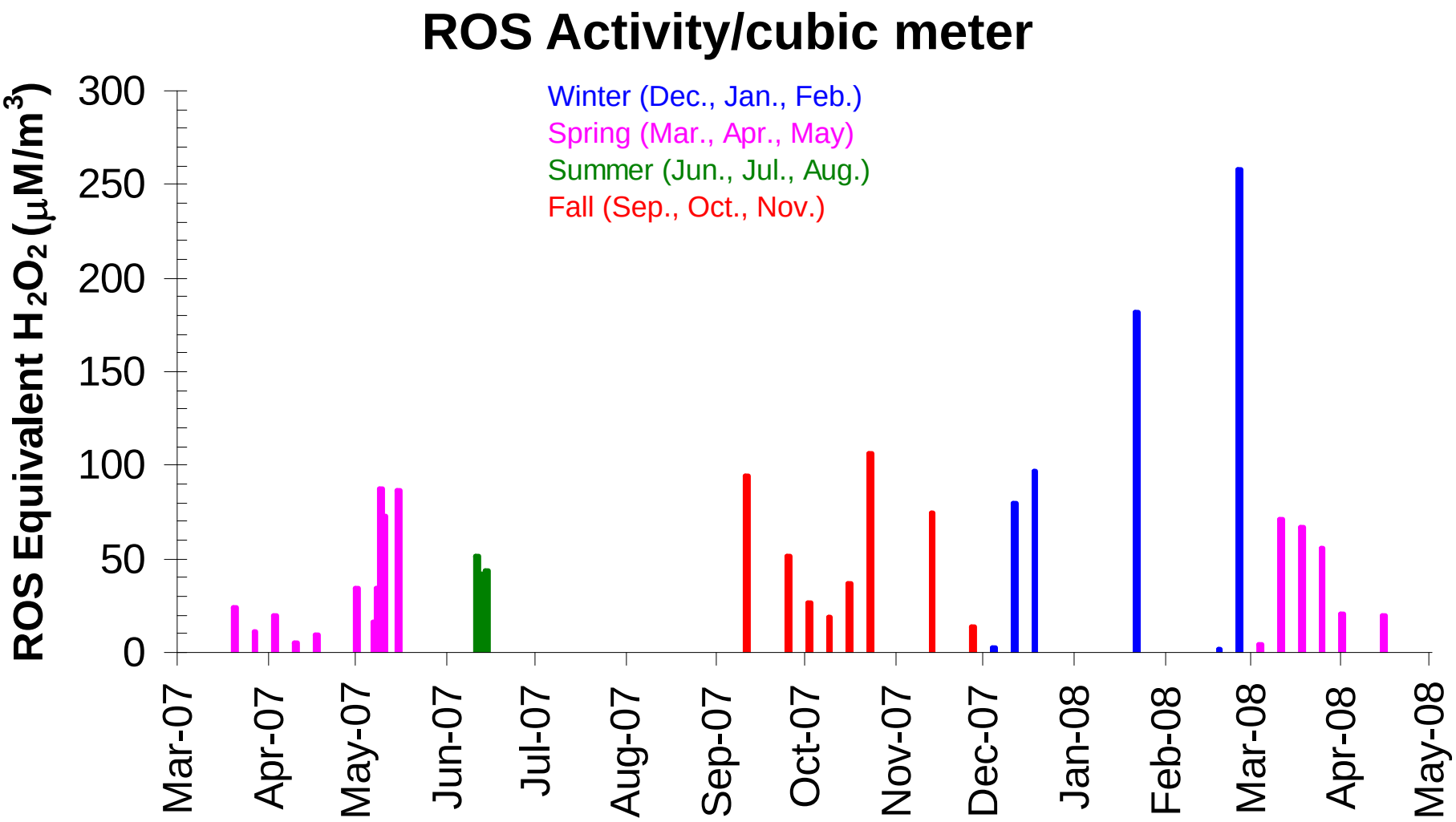
- **Retain existing secondary standards for $PM_{2.5}$ and PM_{10} identical to primary standards** (protecting against ecological effects, effects on materials and climate impacts)

- **Retain existing 24 hr. standard for coarse particles** (150 µg/m^3)

Ultrafine Particle Concentrator output, FMPS Data (June 3, 2008)

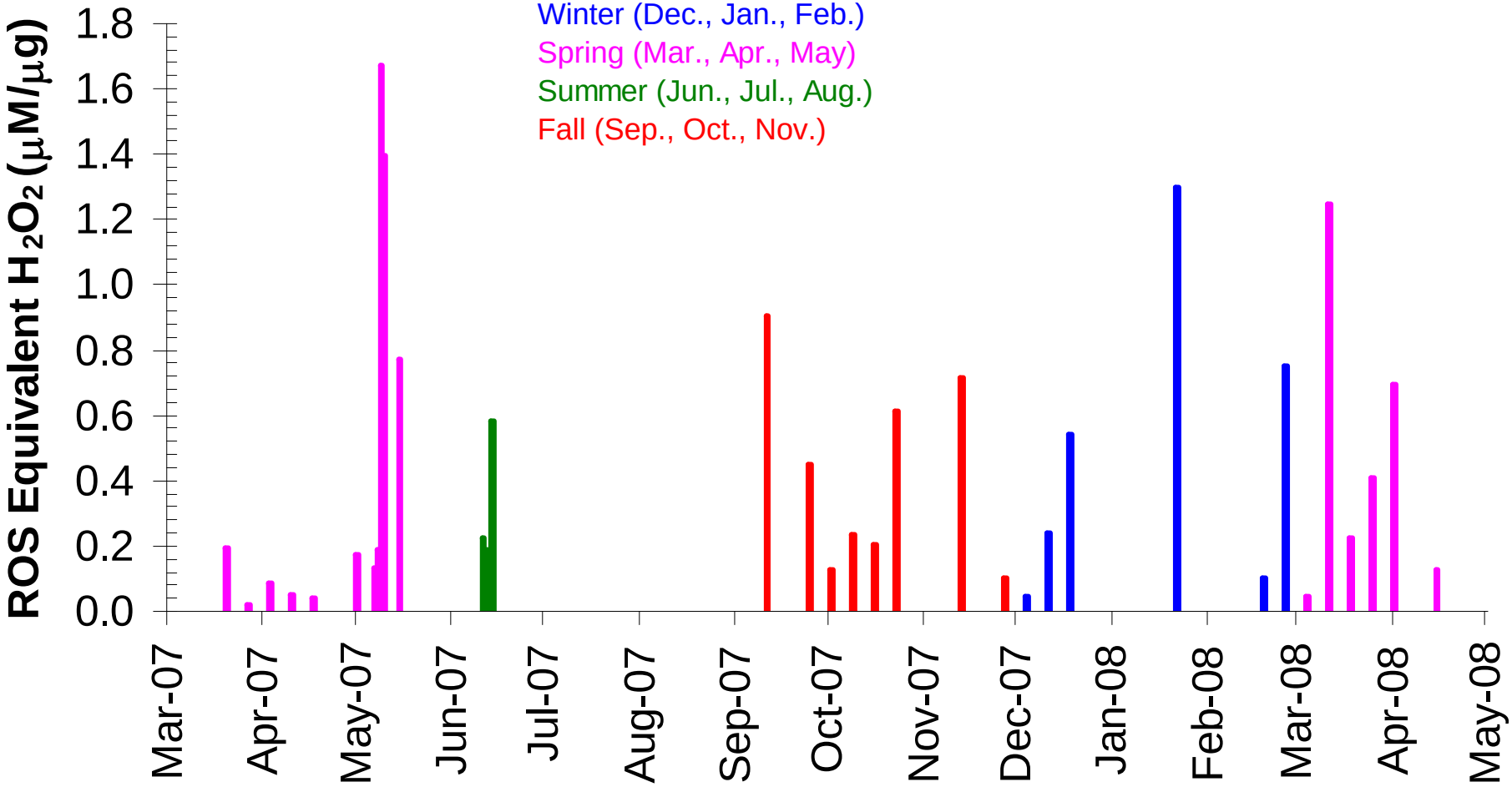


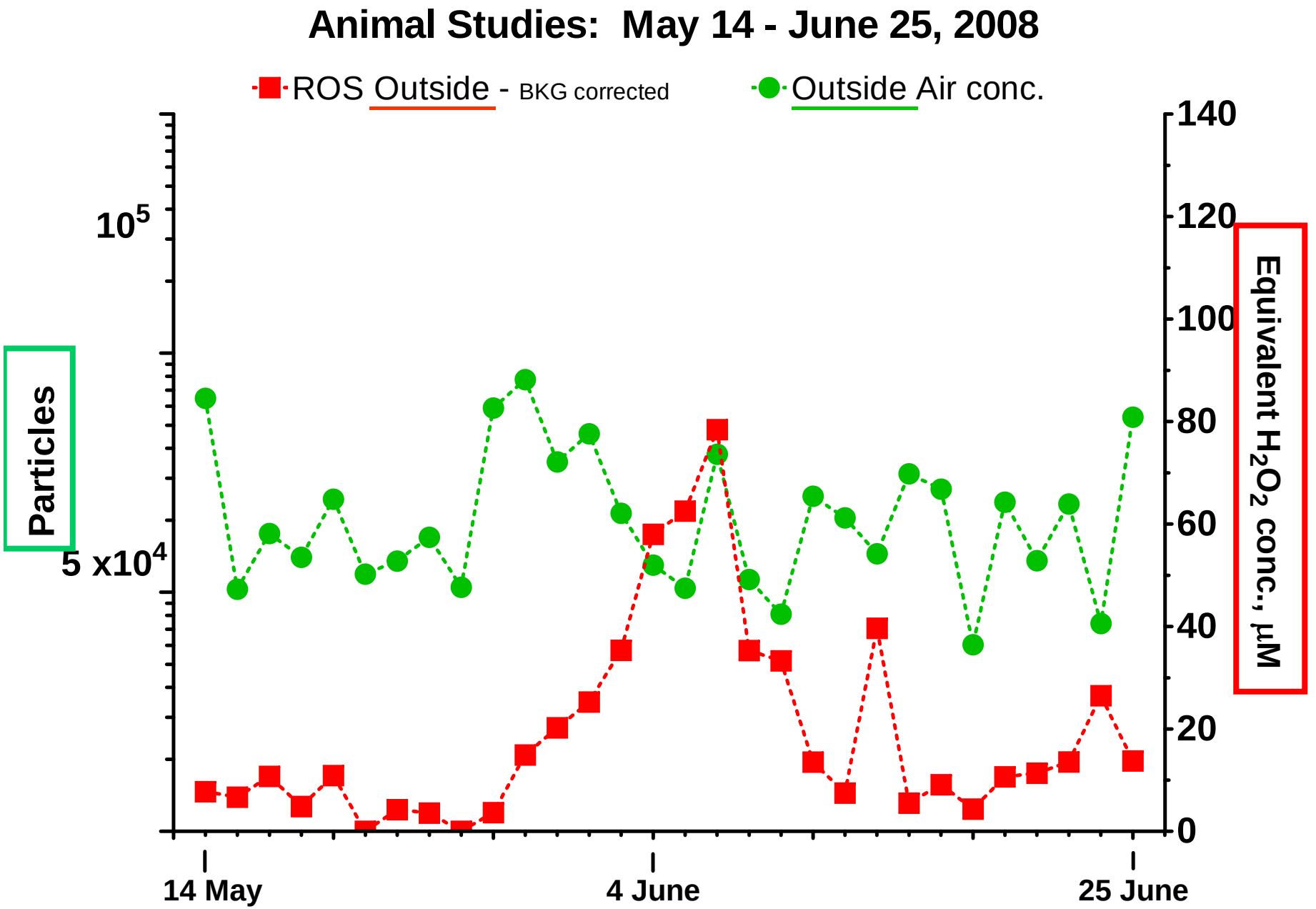
Particle bound ROS:



Particle bound ROS:

ROS Activity/microgram




Animal Studies: May 14 - June 25, 2008
ROS Outside - BKG corrected
Outside Air conc.
Particles
10^5
5 x10^4
140
120
100
80
60
40
20
0
Equivalent H2O2 conc., µM
14 May
4 June
25 June

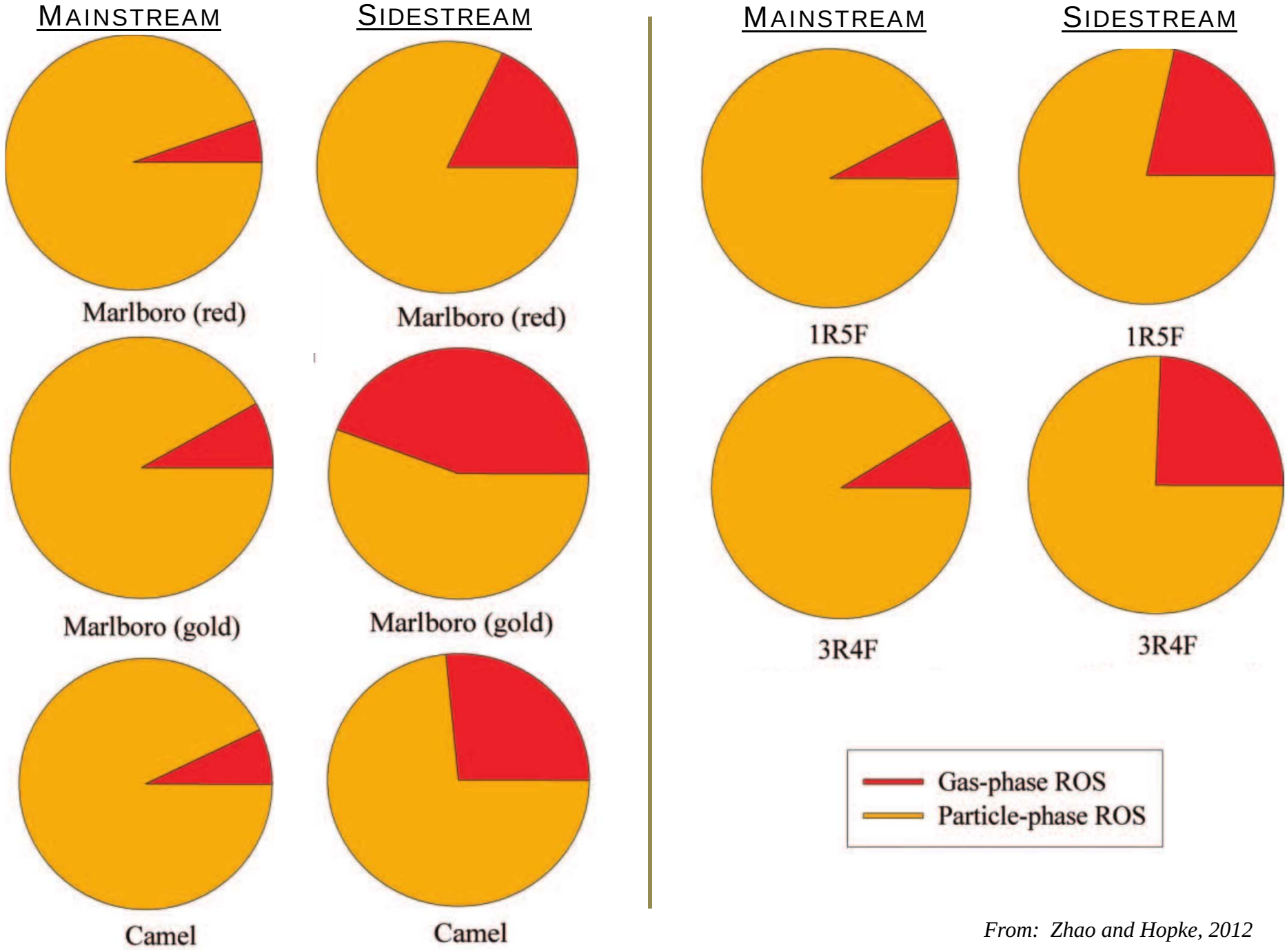
CONCLUSIONS

- **ROS activity/m^3 of ambient UFP can vary widely**
  (sources; seasonal; Episodes of natural UFP [gas to particle conversions]?)

- **ROS activity of ambient UFP does not always parallel their**
  number or mass concentration *(chem. composition?)*

- **ROS activity of lab-generated UFP** (nanoparticles) **seems to**
  reasonably well predict acute in vivo responses *(chronic exposure?)***;**
  for ambient UFP: correlation still to be established.

Cigarette gas-phase ROS and particle-phase ROS partitioning ratio



From: Zhao and Hopke, 2012

Particle-bound ROS concentrations measured in previous studies

Source location and type	ROS concentration (nmol H_2O_2/m^3-air)	Inhaled ROS (nmol/h)	Exposed length equals to one Marlboro (red)	
			Mainstream smoke (h)	Sidestream smoke (h)
Taipei (Taiwan) sidewalk	0.54	0.19	720	355
Singapore (Singapore) ambient	5.71	2.06	68	34
Singapore (Singapore) traffic	15.10	5.44	26	13
Rubidoux, CA (USA) ambient	5.89	2.12	66	33
Flushing, NY (USA) ambient	0.87	0.31	447	220
Rochester, NY (USA) ambient	8.30	2.99	47	23

Continuous exposure for 2-3 days to urban air is equivalent to smoking one Marlboro (red).

Under heavy traffic conditions (Singapore traffic) it is only a one day exposure.

From: Zhao and Hopke, 2012

Ambient UFP Standard:

Physico-chemical properties of UFP are different from different sources:

Elemental carbon
Organic carbon compounds
Inorganics (metals)
Coagulation
Surface properties
Solubility
Volatility

} **influence Toxicity**

UFP source A ≠ UFP source B ⟹ different biol./toxicol. effects

Discussion points:

Given the day-to-day variation in ultrafine particle levels and exogenous ROS activity:

- ***Does it make sense to consider an ambient UFP standard based on daily particle mass, or number concentration, or particle ROS activity?***

UFP Standard

Which Dosemetric?

- **particle mass:** ***too low, probably not meaningful***
- **particle surface area:** ***more difficult to measure***
- **particle number:** ***relatively easy to measure***
- **other:** ***ROS inducing potential? Need to define methods***

A general UFP standard (targeting all UFP) **based on any dosemetric would not make sense because of origin specific chemistry differences** *(source specific UFP: anthropogenic, natural)* **which cause significant differences in toxicity**

Proposed Concept for UFP Standard

- **UFP standard should best be source-specific**
- **Based on number concentration of emitted UFP**
  consider UFP down to < 10nm
- **Need to identify sources that emit most reactive UFP**
- **Regulate these sources *(rather than all UFP)* by introducing a number emission standard, based on UFP risk assessment**
- **Consider co-pollutants** *(particulate, gaseous)*

Establishing an UFP Number Standard?

- Zero emissions for all sources *(or as low as technically feasible)*

- Epidemiology *(how source specific?)*

- Toxicology
  - in vitro: hazard identification and ranking, source specific
  - in vivo: risk characterization *(subchronic rodent inhalation*)
    many challenges: endpoints, extrapolation of NOAEL…

- Other?

Establishing an UFP Number Standard?

- Zero emissions for all sources *(or as low as technically feasible)*

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    many challenges: endpoints, extrapolation of NOAEL…

- Other?

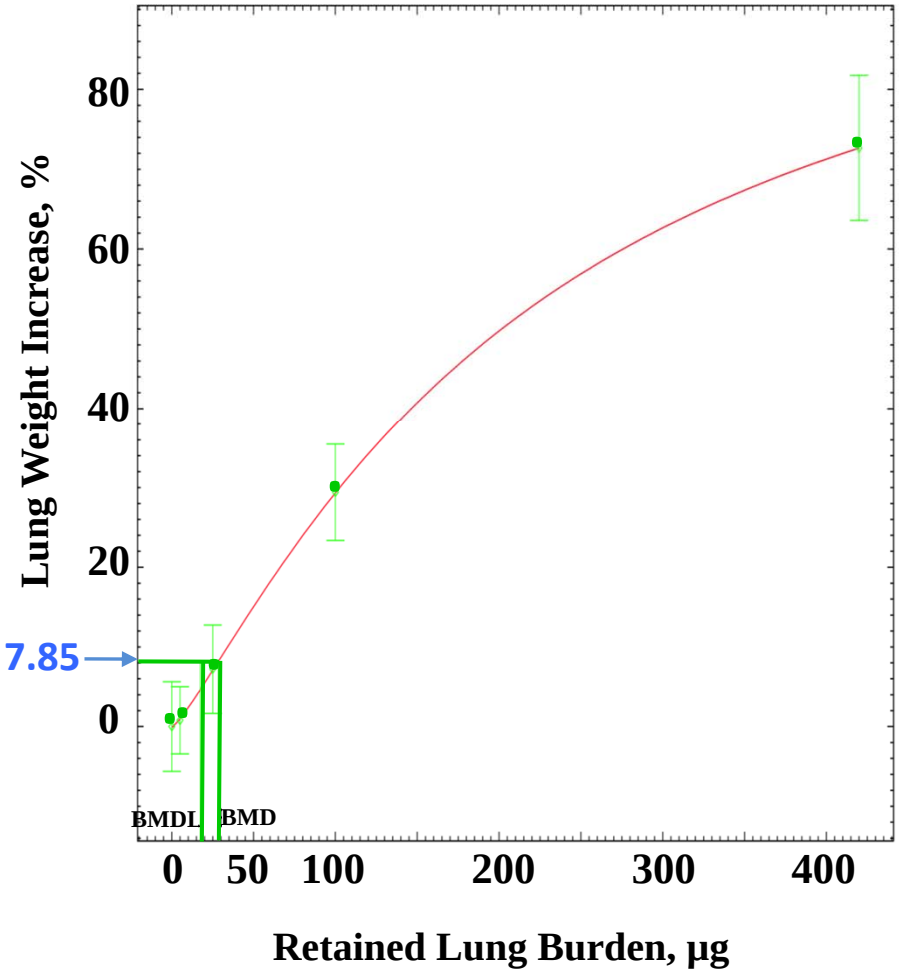
Future Goal:

develop and validate in vitro or in silico methods
that allow to predict and characterize human risk

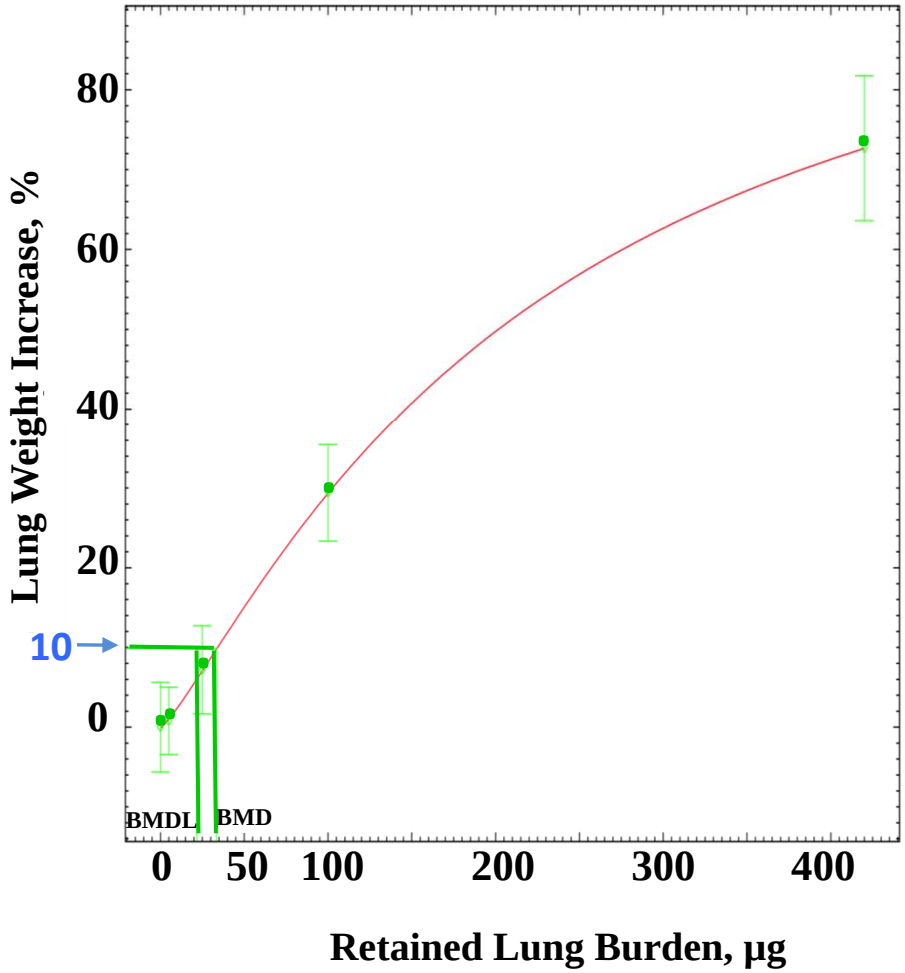
Benchmark Response (BMR) With Two Different Criteria for Response:

Hill Model for Pauluhn (2010) data based on lung burden (µg)

1 SD above control response



10% increase above control response



Note*: *After normalization, data are % increase relative to control with mean = 0.