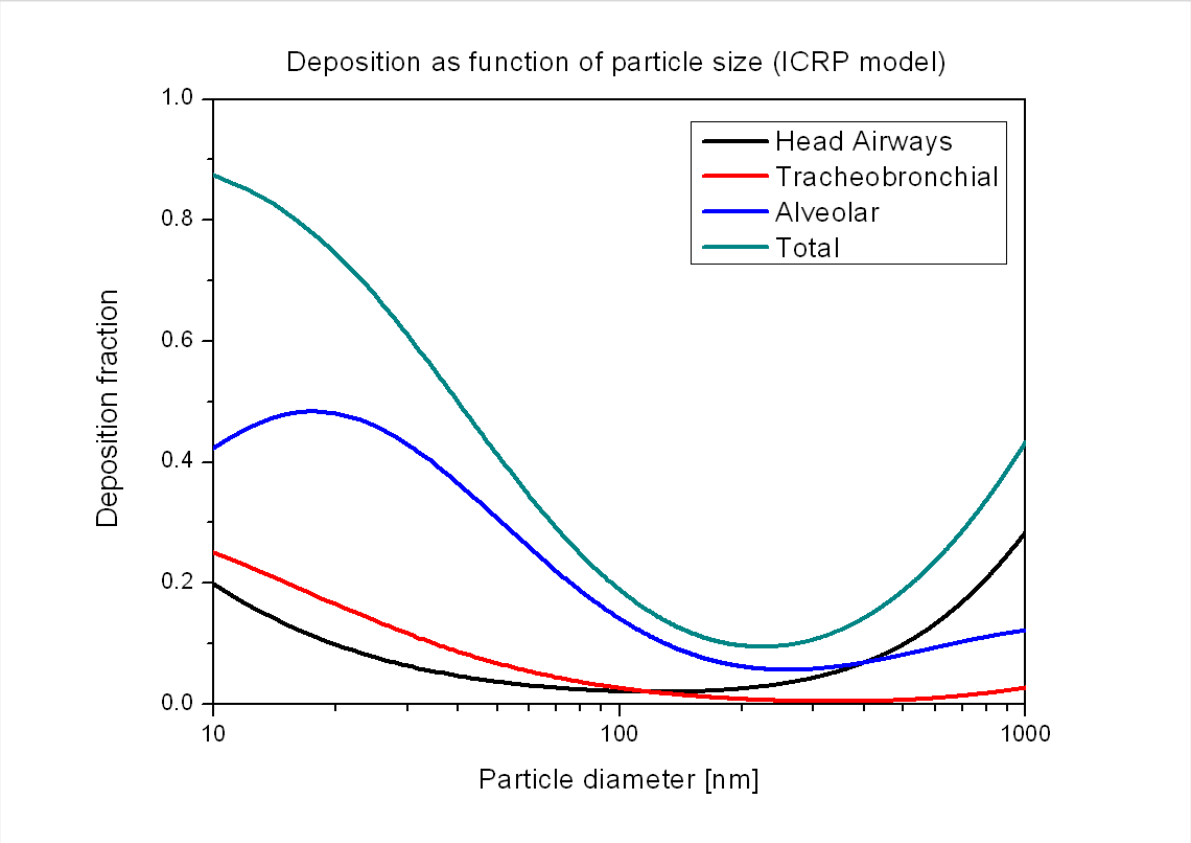
Lung-deposited surface area

How do we quantify particulate matter?

Particulate matter can be measured in many different ways. Traditionally, the particle mass per unit air volume is measured, usually with an upper size limit of x microns (PMx - with PM10, PM2.5, PM1 most common). Alternative metrics are e.g. particle number, or particle surface area per volume. These are purely physical metrics. Chemistry can also be taken into account, e.g. by measuring the amount of black carbon, or polyaromatic hydrocarbons (PAH). There is no such thing as the "best" metric to use - it always depends on the application or the question you want to answer. However, some things are much easier to measure than others. The lung-deposited surface area can be measured approximately very easily.

Lung deposition of particles

A general observation when discussing health effects caused by particles is that the traditional reporting of a quantity per unit volume of air is not very meaningful. Only those particles that end up in the human body can cause health effects, so that is what should be measured. The deposition fraction as function of particle size for three different areas of our airways is shown in the figure below.



The total deposition has a clear minimum at about 200-300nm, where only about 10% of the particles present in the air are deposited in our body, while at 40nm diameter, about half the particles end up in our body. On a mass basis, a single 200nm particle (unit density, spherical) is 125 times heavier than a 40nm particle, and contributes 125 times more to the measured PMx, although it contributes "only" 20 times more to the mass ending up in the human body, because its deposition is much less probable. We can thus conclude that - at least concerning health effects - we should look at deposited particles only.

Particle surface area is relevant

Several laboratory studies have demonstrated that on a mass-basis, smaller particles appear to be more toxic than larger particles. This is explained by the larger surface area of the smaller particles: the particle surface is the place where our body interacts with the particles. Particles can transport adsorbed toxins on their surface, or their surface can act as catalyst inside a cell, creating reactive oxygen species (ROS). It has been shown that toxic effects scale well with particle surface area in both *in-vitro* and *in-vivo* experiments (see appendix for details). Of course, these statements only apply to biopersistent particles, and not to soluble particles. The general feeling in the medical community appears to be that soluble particles are mostly harmless compared to biopersistent particles.

In our opinion, there is clear evidence that the surface area is a more important metric than particle mass or particle number for biopersistent particles. We should therefore measure the **lung-deposited surface area (LDSA),** as it appears to be the most relevant physical metric for quantifying exposure to particles.

Measuring lung-deposited surface area

Measuring the LDSA in principle requires a measurement of the entire particle size distribution, followed by a summation of particle surface in each size bin weighted by its lung-deposition probability, i.e. this would require a complex measurement and some calculation. However, by a lucky coincidence, LDSA can be measured directly by diffusion charging. Diffusion chargers impart a size-dependent charge q on particles passing through them, which can be well described by

$$q \cong const \cdot d^{1.1}$$

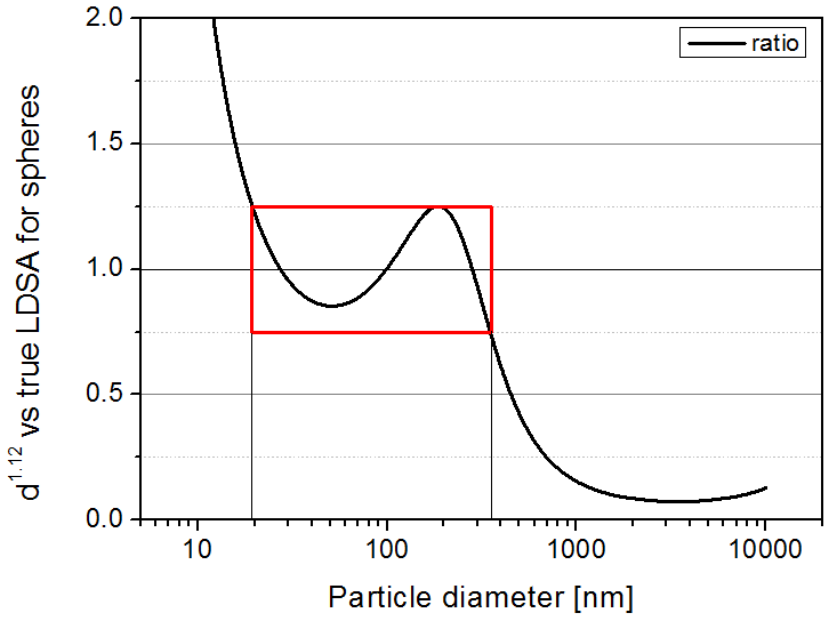
where d is the particle diameter. In the lung deposition curve, one can see that the particle deposition in the lower airways is approximately inversely proportional to particle diameter in the diameter range of 20-300nm. LDSA is thus approximately proportional to the diffusion charger signal:

$$LDSA = surface\ area \cdot deposition\ probability \cong d^2 \cdot d^{-1} = d^1 \approx q$$

This calculation is only approximately true and only true for spherical particles – however, it is clear that no physical metric can really measure particle toxicity and that if we have to choose among physical metrics, the LDSA is the best option (see appendix for further information).

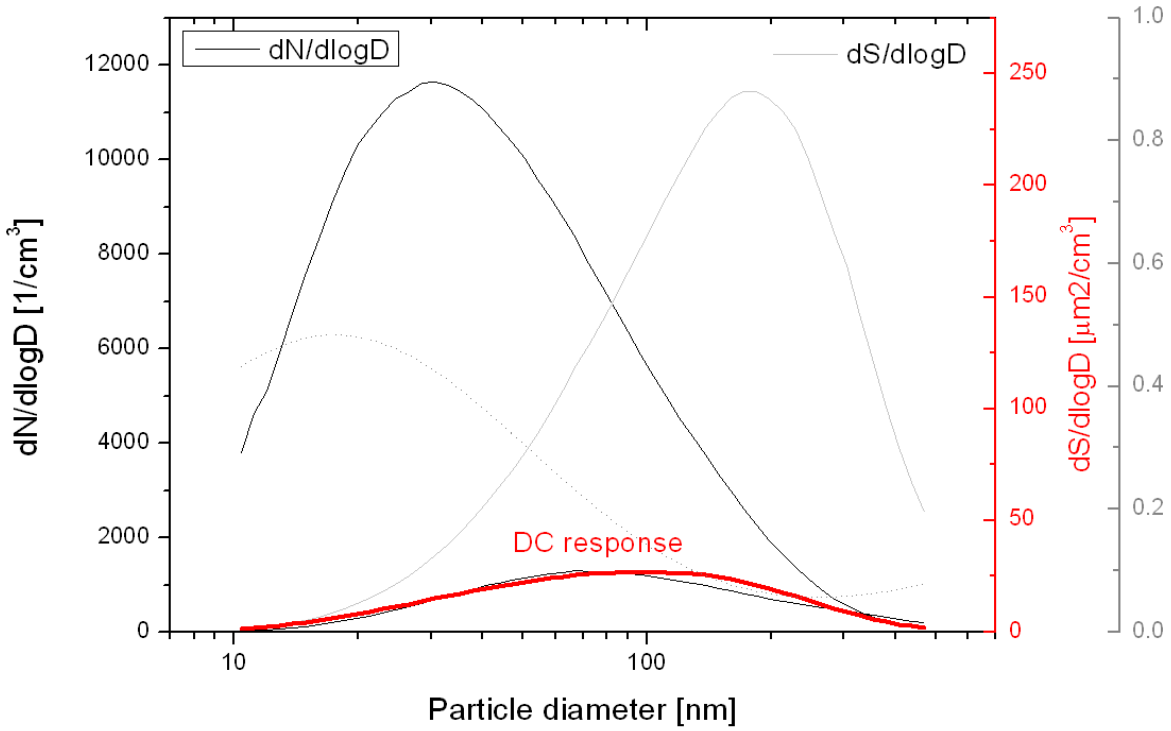
How accurate is this LDSA measurement

When discussing the accuracy of the LDSA measurement via unipolar charging (as in the partector), two things are important: (1) how accurate is the indicated LDSA as function of particle size and (2) what particles are being measured – are they in the size range where the instrument is accurate, or not? The figure below gives the answer to the first question: In it, the ratio of the signal produced by unipolar charging is compared to the calculated LDSA for spherical-uniform-density spheres according to the ICRP model.



At first glance, this graph is rather disappointing, but please note the logarithmic x-axis, whereas the y-axis is not logarithmic. Between 20 and 350nm, the LDSA indicated by the partector is accurate to within 25%. For smaller particles, it is overestimated, for larger particles it is underestimated. If the majority of the lung-deposited surface area comes from particles between 20 and 350nm, the partector will be accurate; if it is measuring much smaller or much larger particles it will be significantly off the true LDSA value (it still measures particle charging accurately, but the interpretation as LDSA is incorrect).

The next graph shows the particle size distribution measured by an SMPS in Zürich, Switzerland, averaged over an entire year, along with the calculated particle surface area, the lung-deposited particle surface area and a typical unipolar charger response:



The particle number size distribution has a maximum at about 30nm; the calculated particle surface area size distribution has its maximum around 200nm. The lung-deposited surface area distribution (which is the interesting part) and the charging response have their maximum around 100nm. Particles below 20nm and above 350nm hardly contribute to the total signal (area under the curve), and thus in this case, the LDSA measurement is accurate. We expect this to be the case in most urban environments in general.

Further reading

For a further discussion on particle surface area and health effects, as well as LDSA measurement, we recommend the following papers:

W. Wilson et al: *Use of the Electrical Aerosol Detector as an Indicator of the Surface Area of Fine Particles Deposited in the Lung*. J. Air & Waste Manage. Assoc. **57**:211-220 (2007)
M.Auffan et al: *Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nature Nanotechnology*, **4**:634-640 (2009).
C. Asbach et al: *Conceptual limitations and extensions of lung-deposited Nanoparticle Surface Area Monitor (NSAM)*. Journal of Nanoparticle Research, **11**:101-109 (2009).

FAQ on LDSA

Q1: Does the LDSA measurement work for all people?
A1: No. The breathing patterns of individuals are not identical, and therefore, the lung deposition function is not exactly the same for everyone, however, it is similar for most people.

Q2: Is the LDSA measurement correct for all types of particles?
A2: No. There are different deposition mechanisms for particles in the lung. For the smallest particles, deposition by diffusion is dominant, which is simply a function of particle size. For larger particles, impaction is another important deposition mechanism. Impaction depends both on particle size and particle density. In our instrument's unipolar charger, the particles acquire a charge that depends only on particle size, not on particle density. Therefore, the instrument response cannot distinguish between particles of different density.

Q3: Is LDSA at least correct for compact particles of unit density for a healthy individual at rest?
A3: No. The signal measured in our instrument is a good approximation of LDSA under the above conditions, but this is really just a lucky coincidence. For particle diameters larger than about 400nm, this nearly 1-to-1 correspondence breaks down, and our instrument underestimates LDSA of larger particles. In typical urban environments, most of the LDSA is however in the size range where the approximation holds true, so this is not a serious limitation.

Q4: What about chemical composition of the nanoparticles – isn't that important too?
A4: Of course. Salt particles found near the seaside are quite harmless, while some nanoparticles are particularly dangerous (e.g. metals, particles with lots of PAH on the surface). Therefore, any physical measurement (particle mass, number, surface area, even particle size distribution) is insufficient for an assessment of nanoparticle toxicity.

Q5: So the LDSA reported by your instrument is not accurate due to breathing patterns, particle composition, particle morphology and larger particles. Besides, it doesn't differentiate between harmless and dangerous particles. Aren't you just wasting my time??
A5: Not at all. Whereas all those limitations are true, you should not forget that similar limitations also apply to all other nanoparticle measurements you can make! Imagine for instance that you have a perfect particle number counter – an ideal CPC. It will tell you exactly how many particles there are in per cm^3 of the air. However, due to differences in breathing patterns between individuals, once again the actual dose of each individual is slightly different. And the CPC is also unable to detect differences in particle chemistry, density or morphology. The most popular measure of particles, PM10, also suffers from the fact that it cannot distinguish different types of particles. Nevertheless, PM10 has been found to be associated with all kinds of detrimental health effects, and thus can serve as a surrogate measurement for particle toxicity. In our opinion, it is likely that LDSA will turn out to be similar – a good surrogate for nanoparticle toxicity, where PM10 underestimates the toxicity due to the very low mass of nanoparticles.

Appendix: Figures& Quotes from selected publications

On the following pages, I have assembled a couple of figures and quotes from 8 newish scientific publications on the relevance of surface area as dose metric for nanoparticles. Please note that this is not an unbiased literature review, but rather a selection of publications that support my own point of view, and the use of our instrument. There are also dissenting voices in the scientific community (e.g. Wittmaack, K., 2007. In search of most relevant parameters for quantifying lung inflammatory response to nanoparticle exposure: particle number, surface area or what? Environ. Health Perspect. 115, 187–194., and Warheit, D.B.,et al., 2006. Pulmonary instillation studies with nanoscale TiO_2 rods and dots in rats: toxicity is not dependent upon particle size and surface area. Toxicol. Sci. 91, 227–236.). For the quotes, I have sometimes added a short explanation on the context of the quote in italics. I have made direct references to the role of surface area bold.

When looking through studies on the effects of nanoparticles, it is worth remembering that there are very many different so called "biological endpoints" that can be measured (e.g. inflammation markers, cell death etc), studies can be done *in vitro* on different types of cells, or *in vivo* in different animals, with all kinds of different particles, possibly with unrealistically high concentrations or ill-defined doses. One must therefore be very careful in comparing different studies.

Macrophage Responses to Silica Nanoparticles are Highly Conserved Across Particle Sizes
Katrina M. Waters et al.
TOXICOLOGICAL SCIENCES 107(2), 553–569 (2009)

*From the conclusions: "*Our collective results are in agreement with Oberdorster et al. (2007), in that **for AS, particle surface area is the most informative dose metric for comparing results across different particle sizes**. Dose-response relationships for cytotoxicity and protein secretion responses based on particle number or mass varied by more than an order of magnitude across different particles sizes. In contrast, these relationships expressed on a surface area basis showed an excellent fit (coefficient of determination = 21% for macrophage cytotoxicity) across a wide range of AS sizes and different particle manufacturers. Similarly, among more than 750 early gene expression changes identified, the majority (~76%) displayed a tighter correlation with surface area dose than with mass dose. These results suggest the inherent particle surface chemistry properties of AS that are responsible for these biological effects do not significantly change as particle size decreases to the nanoscale."

To the right: biological response as function of particle number, mass and surface area for different sizes of amorphous silica nanoparticles. The figure shows that the response is well explained by surface area.

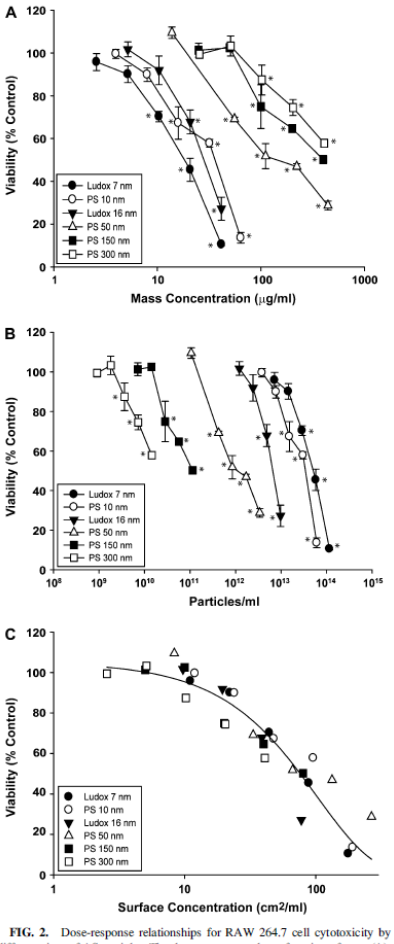


FIG. 2. Dose-response relationships for RAW 264.7 cell cytotoxicity by different sizes of AS particles. The data are presented as a function of mass (A), particle number (B), or total administered particle surface area (C) as alternative dose metrics. Values represent the mean (± SE) of at least three replicates normalized to untreated controls and were determined after 24-h treatment. *Differs from untreated controls ($p < 0.05$).

Nanotoxicology: An Emerging Discipline Evolving from Studies of Ultrafine Particles
Günter Oberdörster et al.
Environ Health Perspect 113:823–839 (2005).

Explaining Figure 4, to the right: "However, when the instilled dose was expressed as particle surface area, it became obvious that the neutrophil response in the lung for both ultrafine and fine TiO2 fitted the same dose– response curve (Figure 4B,D), **suggesting that particle surface area for particles of different sizes but of the same chemistry, such as TiO2, is a better dosemetric than is particle mass or particle number** (Oberdörster G 2000)."

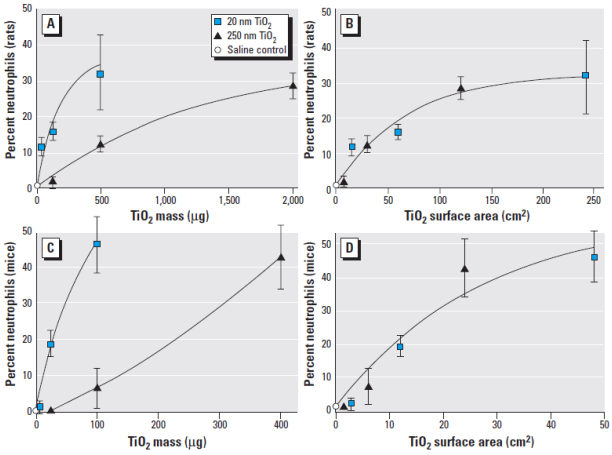


Figure 4. Percentage of neutrophils in lung lavage of rats (*A,B*) and mice (*C,D*) as indicators of inflammation 24 hr after intratracheal instillation of different mass doses of 20-nm and 250-nm TiO_2 particles in rats and mice. (*A,C*) The steeper dose response of nanosized TiO_2 is obvious when the dose is expressed as mass. (*B,D*) The same dose response relationship as in (*A,C*) but with dose expressed as particle surface area; this indicates that particle surface area seems to be a more appropriate dosemetric for comparing effects of different-sized particles, provided they are of the same chemical structure (anatase TiO_2 in this case). Data show mean ± SD.

VOLUME 113 | NUMBER 7 | July 2005 • Environmental Health Perspectives

From the conclusions: "Results of older biokinetic studies and some new toxicology studies with NSPs (mostly ambient UFPs) can be viewed as the basis for the expanding field of nanotoxicology. These studies showed that the greater surface area per mass renders NSPs more active biologically than larger-sized particles of the same chemistry, and that **particle surface area and number appear to be better predictors for NSPs-induced inflammatory and oxidative stress responses**."

R. Aitken et al: Manufacture and use of nanomaterials: current status in the UK and global trends. Occupational Medicine 2006, 56, 300-306.

From a section titled "exposure to nanoparticles": "Typically, airborne exposures in the workplace are assessed in terms of mass concentration. **Current evidence suggests that the most appropriate metric for exposure by inhalation for NPs is surface area**. This appears to fit best with current toxicological evidence relating to mechanisms of harm. It would also address directly the issue of agglomeration. Ideally a personal sampler should be available which could assess this metric. However, none currently exists. For those NPs that could be considered as fibres, such as CNTs, particle number may be a more appropriate metric than surface area."

D.M.Brown et al: Size-Dependent Proinflammatory Effects of Ultrafine Polystyrene Particles: A Role for Surface Area and Oxidative Stress in the Enhanced Activity of Ultrafines.
Toxicology and Applied Pharmacology 175, 191–199 (2001)

From the abstract:
"The aim of the present study was to investigate proinflammatory responses to various sizes of polystyrene particles as a simple model of particles of varying size including ultrafine. In the animal model, we demonstrated that there was a significantly greater neutrophil influx into the rat lung after instillation of 64-nm polystyrene particles compared with 202- and 535-nm particles and this was mirrored in other parameters of lung inflammation, such as increased protein and lactate

dehydrogenase in bronchoalveolar lavage. When surface area instilled was plotted against inflammation, these two variables were directly proportional and the line passed through zero. **This suggests that surface area drives inflammation in the short term and that ultrafine particles cause a greater inflammatory response because of the greater surface area they possess** …. These findings suggest that ultrafine particles composed of low-toxicity material such as polystyrene have proinflammatory activity as a consequence of their large surface area. This supports a role for such particles in the adverse health effects of PM10."

Ann. occup. Hyg., Vol. 47, No. 2, pp. 123–144, 2003
Estimating Aerosol Surface Area from Number and Mass Concentration Measurements
Andrew D. Maynard

"As a result, there has been considerable interest in examining whether characterizing occupational ultrafine aerosol exposure against some metric other than mass is more appropriate. Although research has indicated that particle number concentration may be important, in most cases it appears that both particle number concentration and size play a role in determining response following inhalation. However interpretation of ultrafine particle data in terms of particle surface area leads to a dose response that is independent of particle diameter in many cases (Oberdörster, 2000; Brown et al., 2001). A similar trend has been observed at larger particle diameters (Lison et al., 1997; Driscoll, 1999; Tran et al., 2000), indicating **that for low-solubility particles characterizing exposure in terms of surface area will lead to more appropriate exposure limits and evaluation methods**."

Toxic Potential of Materials at the Nanolevel
Andre Nel, et al.
Science 311, 622 (2006);

"There is a direct relationship between the surface area, ROS-generating capability, and proinflammatory effects of nanoparticles in the lung (4–8). From a mechanistic perspective, ROS generation and oxidative stress is the bestdeveloped paradigm to explain the toxic effects of inhaled nanoparticles (3–10). "

Surface area of particle administered versus mass in determining the pulmonary toxicity of ultrafine and fine carbon black: comparison to ultrafine titanium dioxide
Tina M Sager and Vincent Castranova
Particle and Fibre Toxicology 2009, 6:15

Ultrafine and fine carbon black particles were instilled in vivo in rats.

From the Abstract, results section:
"Ultrafine carbon black particles caused a dose dependent but transient inflammatory and cytotoxic response. On a mass basis, these responses were significantly (65 fold) greater than those for fine sized carbon black. However, **when doses were equalized based on surface area of particles given, the ultrafine carbon black particles were only slightly (non-significantly) more inflammogenic and cytotoxic compared to the fine sized carbon black.** At one day post-exposure, inflammatory potencies of the ultrafine carbon black and

ultrafine titanium dioxide particles were similar. However, while the pulmonary reaction to ultrafine carbon black resolved with time, the inflammatory effects of ultrafine titanium dioxide were more persistent over a 42 day postexposure period. "

From the Abstract, conclusions section:
"These results indicate that for low toxicity low solubility materials, **surface area of particles administered rather than mass burden of particles may be a more appropriate dose metric for pulmonary toxicity studies**. In addition, ultrafine titanium dioxide appears to be more bioactive than ultrafine carbon black on an equivalent surface area of particles delivered basis."

From the introduction
"In general, for a fixed mass of particles, surface area increases as particle size becomes smaller. Thus, a dose dependence on particle surface area may explain the greater toxicity of nanoparticles compared with an equal mass of fine particles of the same material [10,11]. **The finding that particle surface area rather than mass appears to be a more appropriate metric of dose for predicting pulmonary inflammation may imply a need to reconsider exposure assessment practices for workplaces producing or using nanoparticles**. Currently, occupational exposure limits for airborne dusts are defined in terms of mass per m3 of air [11]"

Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: Role of particle surface area and internalized amount
Salik Hussain et al.
Toxicology 260 (2009) 142–149

From the discussion section on "Role of surface area and oxidative stress in pro-inflammatory effects of NPs":
"There have been discussions in the recent past about the most relevant parametric for the inflammatory effects induced by NP.In many in vivo and in vitro studies surface area of NPs has been described as the most relevant parametric for studying NP induced pro-inflammatory responses (Monteiller et al., 2007; Singh et al., 2007; Stoeger et al., 2006; Oberdörster et al., 2005; Duffin et al., 2002) although some other studies have not confirmed this statement (Wittmaack, 2007; Warheit et al., 2006). **Our study clearly indicates the surface area dependent nature of a NP induced proinflammatory response.We observed a strong correlation between the BET surface area and in vitro pro-inflammatory effects (GM-CSF mRNA expression) of the NPs.** Furthermore, the dose-dependent pro-inflammatory response was correlated with the endocytosis (cellular granularity) for CB 13 nm and TiO2 15nm NPs showing the importance of the internalized amount in this cellular response."