Health & Safety Executive NanoAlert Service

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Bulletin Contents:

1. MEASUREMENT, EXPOSURE AND CONTROL

In this bulletin, the search included a comprehensive search of the literature as described in Issue 1 and an additional search from specific relevant journals. Those articles considering engineered nanoparticles were assigned a higher priority than those related to ambient ultrafine particles. A breakdown of the number of papers per topic is shown in Figure 1.

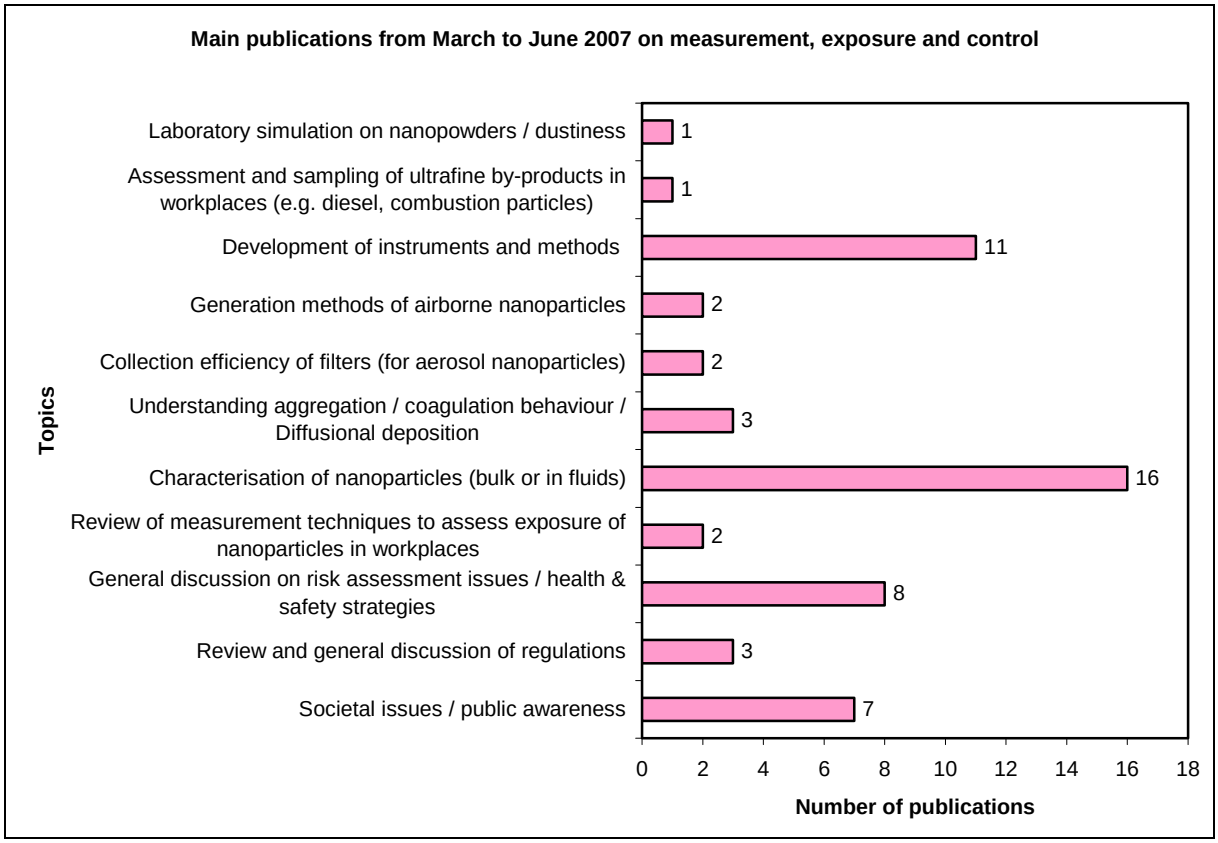

Figure 1: Breakdown of the number of papers per topic (measurement, exposure and control) retrieved in the four months from March to June 2007.

The search did not identify any studies reporting exposure measurement of nanoparticles in workplaces. A review on the assessment and sampling of ultrafine by-products in workplaces and a paper reporting on a test method to study the behaviour of bulk nanopowder were retrieved by the search.

Eleven papers on evaluation, development of instruments and methods to measure exposure to nanoparticles / ultrafines as well as two review papers on measurement techniques were identified.

This current search did not identify any studies on the effectiveness of collective or personal control devices to reduce exposure from engineered nanoparticles. However two articles investigating nanoparticle penetration through filters have been retrieved.

The search found 16 papers of relevance to characterisation of nanoparticles.

A number of reviews or general articles (18) on regulations (3), risk assessment (8) and societal issues / public awareness (7) were also identified.

1.1 Exposure data

Workplace exposure

This current search did not identify any studies on workplace exposure or dispersion of nanoparticles.

Agglomeration / nanopowder behaviour

The dustiness behaviour of nanoparticles is an important property. In 2006, the European Committee for Standardization (CEN/TC137/WG3) produced a document providing standardisation in measurement of dustiness of bulk powders (EN15051) [1]. This standard establishes two reference test methods (single drop or rotating drum method) that classify dustiness in terms of health-related fractions of bulk solid materials.

Gas fluidisation technique has also been applied as a means of studying the behaviour of a powder.

As mentioned in the previous bulletin, very few studies have explored the behaviour of nanopowders. For materials where nanoparticles do not become readily airborne under normal handling procedures, the associated risk from inhalation will be considerably reduced. A paper on nanoparticle fluidisation in model 2D and 3D beds using high speed X-ray imaging and microtomography has been published [2]. In this article, the authors reported on the design of a high spatial (down to 400 nm) and temporal resolution (down to 1 ms) X-ray imaging apparatus to determine dynamically the nanoparticle agglomerates volume, density and porosity in fluidised beds under different gas flow velocities.

1.2 Measuring and monitoring of airborne nanoparticles

Until it has been agreed which are the most appropriate metrics (such as mass, number, surface area) for assessing exposure to nanoparticles in relation to potential adverse effects, a range of instruments may be required to fully characterise and monitor release of nanoparticles in the workplace. In addition to concentration levels, the physical and chemical characteristics of the engineered nanoparticles are important parameters for discrimination against natural ambient ultrafine particles or those produced from combustion.

The International Organisation for Standardisation has published the ISO standard TR 27628:2007 (Workplace atmospheres – Ultrafine, nanoparticle and nano-structured aerosols – Exposure characterization and assessment) [3]. This report provides terms and definition as well as guidelines on characterising and monitoring, against a range of metrics, nanoaerosol exposure in real-time or using off-line analysis. With regards to definition and terms, an approved ASTM E 2456-06 (Standard terminology for nanotechnology) is also available [4].

Assessing exposure to airborne nanomaterials: Current abilities and future requirements. Maynard and Aitken (2007) [5]

From a possible classification scheme for nanostructured particles and a list of attributes thought to be relevant for determining potential health effects following inhalation, the authors have considered the relevance of different physical metrics for exposure measurements. They also have reviewed techniques available to measure airborne nanoparticle concentrations in term of number, surface area and mass as well as discussed the features of a possible universal aerosol monitor.

Evaluation of instruments or methodologies

It is important that the performance and detection limit of instruments used in workplaces for assessing exposure to airborne engineered nanoparticles are investigated. Recent toxicological studies have shown the importance of measuring particle surface area for assessing exposure to nanoparticles in relation to potential health effects. Few instruments are available to measure directly particle surface area (eg. Diffusion chargers: LQ1-DC Matter Engineering and TSI 3070a Electrical Aerosol Detector (EAD)). Recently TSI have developed a Nanoparticle Surface Area Monitor (NSAM) Model 3550, which measures total surface area deposited in tracheobronchial and alveolar regions of human lung.

Application of a diffusion charger for the measurement of particle surface concentration in different environments. Ntziachristos et al (2007) [6]

Ntziachristos et al have investigated the performance of the NSAM for the measurement of nanoparticles' surface area concentrations in different urban environments. They concluded that:

- The particle surface area concentrations of the NSAM correlated well with the surface area concentrations derived from the particle number size distribution measured with a scanning mobility particle sizer (SMPS).
- The calculated mean surface area diameter (derived from the particle surface area concentrations measured with the NSAM and from the total particle number concentration measured by the condensation particle counter) was in reasonable agreement with the arithmetic mean SMPS diameter.

HSL has found similar relationships in a recent research study.

Standards and generation of airborne nanoparticles

It is important that the performance and detection limit of instruments used in workplaces for assessing exposure to airborne engineered nanoparticles are investigated. This is a need to generate stable and reproducible nanoparticle aerosols in the laboratory environment for the calibration and testing of instruments measuring airborne nanoparticles. A paper on the use of glowing wire for the production of charged, uniformly sized nanoparticles (from a few nanometers to some tens of nanometers in diameter) has been published [7].

Development of instruments

In recent years, a number of improvements have been carried out to currently available instruments and to techniques for monitoring nanoaerosol exposure (mass, number, surface area concentrations). It is important that the performance of new or improved instruments is systematically investigated against test aerosols and current instruments. A number of papers on the development or improvement of static instruments and their evaluation has been published.

Development of fast response instruments to size particles by their electrical mobility equivalent diameters.

The most common instruments used for sizing nanoparticles are scanning mobility particle sizers (SPMS). They size particles by their electrical mobility equivalent diameters. In conventional SMPS, the scan time ranges from 3 to 5 minutes and recently a number of fast response instruments have been developed. These fast response scanning mobility spectrometers could be very valuable when measuring exposure to engineered nanoparticles in workplaces from processes likely to generate random and short time scale high

concentrations. A number of papers have been published on the development and performance of fast response scanning mobility instruments.

Development and performance test of a unipolar diffusion charger for real-time measurements of submicron aerosol particles having a log-normal size distribution. Park et al (2007) [8]

The authors have published an article on a unipolar diffusion charger for SMPS, operating at high flow rate, to reduce residence time of particles in the instrument and measurement time. The performance of the charger was assessed at different applied voltages, using NaCl (< 100 nm) and dioctyl sebacate (DOS) (100 – 700 nm) and was evaluated in terms of particle loss and charging characteristics. The authors found a total particle loss below 15%. The number of charges was almost linear with respect to particle diameter. By using the diffusion charger with a condensation particle counter (CPC) and an aerosol electrometer, the authors calculated the geometric mean diameter and size distribution, and compared these results with the size distribution measured with an SMPS. The estimated geometric mean diameters of 80.5 nm for NaCl and 427 nm for DOS particles were 33% larger than those measured by SMPS. The authors concluded that: " *However, for less than 5 s we were able to obtain the particle size distribution by using the estimation of geometric mean*."

Development of a multi-stage differential mobility analyser (MDMA). Da-Ren Chen et al (2007) [9]

The authors reported on the development and assessment of a multi-stage differential mobility analyser (MDMA). In a SMPS, DMA separates particles based on their electrical mobility. This new MDMA was designed to enable the fast measurement of a wide particle size range with a good size resolution. It has a three modular sampling stage, each having a DMA classification function, which can simultaneously classify monodisperse particle at three different sizes. The MDMA can operate at high sheath flows, enabling high-resolution particle size measurement and/or reduction of the lower sizing limit. The performance of this new instrument was assessed with a tandem DMA at different ratios of aerosol and sheath flow-rates. The transmission efficiency was reduced for particles of smaller sizes especially at high sheath to aerosol ratio leading to particle loss. Further improvement on the MDMA is needed.

Performance Comparison of Scanning Electrical Mobility Spectrometers. Rodrigue et al (2007) [10]

The authors report on the performance of two fast response scanning mobility spectrometers (SEMS) (TSI SMPS 3936 and MSP WPS XP1000) for particles in the range 20 to 300 nm. The particle sizing characteristics of the two instruments were in good agreement with each other over a range of particle size studied. The number concentrations were generally consistent with a reference instrument counter only for particles greater than 90nm. The authors suggested that a net particle detection efficiency (determined from experiments with monodisperse aerosol) can be used to reconcile measurements made with different SEMS operating at different flow-rates or scan times.

Improvement of charging performance for instruments measuring aerosol particles

Instruments, such as the diffusion charger (DC), Scanning Mobility Particle Sizer (SMPS) or Electrical Low Pressure Impactor (ELPI) used for measuring aerosols, modify the electrical charge on particles before detection. Particle charging performance depends greatly on

particle diameter and decreases rapidly as particle diameter decreases especially for nanoparticles.

A paper on the development and assessment of a uniform charging method for submicron and nanoparticles using condensation has been published [11]. In this method, the particles are grown to micrometer sized droplets through condensation, which are charged and dried. The authors claim that this charging method performs much better than conventional corona.

Development of instrument for physical and chemical characterisation

In addition to concentration levels of airborne nanoparticles, the physical and chemical characteristics of engineered nanoparticles are important parameters for discrimination against natural ultrafine particles or those produced from combustion. Few papers reported development or improvement of instruments to measure and characterise nanoparticles in workplaces.

Off-line electron microscopy (EM) analysis is a technique of choice to investigate nanoparticles' size, shape and chemical composition as well as agglomerates' structure. This analytical technique can be complementary to real-time techniques measuring mass, number, and surface area concentrations. For analysis by TEM, thermal precipitation and electrostatic precipitation provide one of the most suitable techniques for collecting airborne nanoparticles with a thin carbon film. Common problems with electrostatic precipitator are that sampling efficiency depends on the particle size and deposition on substrate may be spatially non-uniform. Two papers have been published on the development or improvement of precipitators (electrostatic and thermal precipitators) for the collection and chemical analysis of nanoparticles.

Thermophoretic sampler and its application in ultrafine particle collection. Wen and Wexler (2007) [12]

The authors reported on the development and validation of a modified thermal precipitator designed by Maynard (1995) for the collection of particles below 10 nm on TEM grids. The collection efficiency was improved by adding:

- a bypass channel before particles entering the temperature gradient region in to the inlet high flow rate and minimise the diffusional loss of nanoparticles
- a thermal electric cooler on the surface supporting the TEM grid to increase the temperature gradient and to maximise thermophoresis deposition without increasing particle volatisation.

The authors theoretically calculated that a maximum collection efficiency of 41% could be achieved for particles of 1 nm for an inlet flow rate of 1.5l/min, thermophoretic flow rate of 0.01 l/min and thermal gradient of 5.10^5 K/m. The sampler collection efficiency was assessed with laboratory generated silver nanoparticles (median size of 6 nm). The authors measured a collection efficiency of 2% for a total < 5nm particle concentration of 10^5 cm^3 at a thermophoretic flow rate of 0.015 l/min and collection time of 90 minutes. The particles were not uniformly deposited on the grid. The authors claim that it is *"a substantial achievement of collecting particles less than 10 nm in diameter with a short sampling time"*.

Theoretical and experimental evaluation of a portable electrostatic TEM sampler. Fierz et al (2007) [13]

The authors reported on the development calibration and performance evaluation of a portable electrostatic precipitator for the collection of nanoparticles on TEM grids. The battery-powered sampler measures 35 x 25 x 15 cm and weighs 7.5 kg. The design of this sampler is similar to the design by Dixkens and Fissan (1999). The sampler consists of an

aerosol charger, a deposition zone and an aerosol electrometer. The grid is placed on a conductive rod and held in place by a cap, which is electrically conductive to prevent the build-up of charge. The electrometer can be used to estimate the sampling times for an appropriate coverage of the grid (about 1%). The authors calibrated this sampler with six monodisperse particle sizes in the range from 20 to 320 nm and found that the efficiency was equivalent to 1700 x (diameter)$^{-1.78}$; with diameter in nm. The numerical model agreed qualitatively with the experimental data but showed a 30% systematic deviation. With polydisperse particles the shape and size distribution from TEM analysis were very similar to the SMPS measurements, but showed deviations of up to 40% in number concentration. The numerical model has shown a collection efficiency of 100% for charged particles below 10 nm but these particles mainly deposited in a circular region with a diameter of 1.2 mm (these numerical results have not been confirmed experimentally).

Several papers have been identified suggesting and exploring other approaches, which maybe relevant for the measurement and characterisation of airborne nanoparticles.

The condensation particle counter battery (CPCB): A new tool to investigate the activation properties of nanoparticles. Kulmala et al (2007) [14]

The authors present a condensation particle counter battery (CPCB). The CPCB consists of two water based and two butanol based CPCs. The objective was to design an instrument able to distinguish ultrafine atmospheric aerosol particles of different chemical composition through their activation properties in different vapours. The CPCs' detection efficiency and cut-off sizes were characterised under laboratory conditions using $(NH_4)_2SO_4$, NaCl nanoparticles and for different temperature differences between the condensator and the saturator. The authors showed that water soluble and water insoluble as well as butanol soluble and butanol insoluble nanoparticles may be discriminated in the CPCB through different activation diameters.

This paper may be relevant for obtaining a crude discrimination between engineered nanoparticles and ultrafine ambient particles.

On the effect of particle alignment in the DMA. Zelenyuk and Imre (2007) [15]

The differential mobility analyser (DMA) measures particle mobility diameter. For spherical particles, the mobility diameter is equal to the particle volume equivalent diameter. Aspherical particles can become aligned to the electric field of the DMA and their mobility diameter is then a function of their orientation and shape. The authors report on a study investigating the relationship between particle shape, alignment in the DMA and mobility diameter in order to obtain real-time information on particle shape. A tandem DMA (TDAM) system was used to measure the changes of mobility diameter as a function of the DMA electric field/sheath flow rate. The first DMA selected particles with narrow distribution of mobility diameter and the second DMA assessed the effect of the electric field/sheath flow rate on the particle alignment and on the mobility diameter. Measurements were carried out with polystyrene latex (PLS) agglomerates (primary particle diameter 199 nm) and a number of polydisperse aerosols with particles of non-uniform shapes and unknown volume equivalent diameters (primary particle diameter particles $\geqslant$ 200nm).

The authors concluded that:

- measurement of changes in particle mobility diameters as a function of the electric field/sheath flow rate provides information on the particle shape and symmetry;
- measurement of aerodynamic diameters in addition to the measurement of particle mobility diameters enables calculation of an effective density and an approximate dynamic shape factor.

Two other papers have been identified:

- Deriving the mean primary particle diameter and related quantities from the size distribution and the gravimetric mass of spark-generated nanoparticles [16].
- On the effective density of non-spherical particles as derived from combined measurements of aerodynamic and mobility equivalent size [17]. The quantity linking mass and mobility or dynamic diameter of non-spherical particles is the effective density of the particle, which take into account the particle density, particle shape, and porosity. The authors have investigated the effective density concept as a tool for characterisation of non-spherical particles.

1.4 Control

This current search did not identify studies on the effectiveness of collective or personal control devices to reduce exposure from engineered nanoparticles. However two articles investigating nanoparticle penetration through filters have been identified and reported in the above section on filtration.

Filtration is used in diverse control methods such as air cleaning or personal respiratory protection. It is important that filter penetration efficiency is tested for nanoparticle aerosols. This current search identified two articles investigating nanoparticle penetration through filters:

- A simulation of unsteady-state filtration via nanofibre media at reduced operating pressures [18]. The filter collection efficiency of electrospun fibre-web was simulated for nanoparticle aerosols from 50 to 500 nm at reduced operating pressures. The authors' mathematical simulation model suggested that:
    - by decreasing the fibre diameter of the web, the collection efficiency (of the filtration media having identical pressure drops) will increase and the diameter associated with the most penetrating particles (minimum efficiencies) will decrease;
    - by increasing the flow temperature, the collection efficiency will increase.

  The authors also simulated the caking process (one of the major problems of electrospun filters).
- Physical collection efficiency of commercial available filters (polytetrafluoroethylene (PTFE), polycarbonate (PC) and gelatine) for collecting airborne bacteria, viruses and other particles in the 10 - 900 nm size range including polystyrene latex (350 nm) and polydisperse NaCl aerosol in the 10 – 600 nm range [19]. The authors concluded that the PTFE and gelatine filters had excellent collection efficiency (>93%) for all of test particles. They also suggested that PC filters had lower collection efficiency especially for small particles (<100 nm).

1.5 Characterisation

Characterisation of bulk nanomaterials

It is recognised that complete and accurate particle characterisation is essential for understanding the potential toxicological properties of nanoparticles. Furthermore, characterisation of nanomaterials is fundamental to ensure consistency and reproducibility of any tests.

Several papers have been published on the characterization of nanoparticles in their bulk form, in fluids (biological or water / solvent) or for toxicological evaluation.

Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. Borm et al (2007) [20]

The European Centre for Ecotoxicology and Toxicity of Chemicals (ECETOC) organised a workshop in November 2006 to develop testing strategies for nanoparticles in terms of heath and safety issues (Warheit et al). One workshop focussed on the need for enhanced efforts in nanomaterial characterisation. The experts suggested that the most important physical chemical parameters of nanomaterials for any target organ toxicity assessment were: nanoparticle composition, dissolution, surface area and surface characteristics, size, size distribution and shape. They also proposed additional parameters depending on the type of target organ toxicological studies (e.g. for brain studies, hydrophobicity and surface charge are other parameters to be characterised).

Powers et al discussed possible solutions to some of the issues relating to measurement of nanoparticles size, shape and dispersion for toxicological studies [21].

Rawle reports and discusses the characterisation of a ceramic powder using Brunauer Emmett and Teller (BET) gas adsorption measurement technique and laser diffraction technique. BET measures the specific surface area and laser diffraction measures particle size distribution [22]. The authors suggested that the ratio between BET and laser diffraction results enables a measure of the agglomeration of nanopowders to be determined.

Ortner has reviewed the possibilities of space-resolved solid-state speciation. Information such as elemental composition and distribution as well as crystallographic structure and oxidation states can be obtained by solid state speciation techniques [23]. In this paper, the author discusses the photon probe techniques (laser induced techniques, X-ray absorption techniques, UV and γ-ray techniques) and the electron probe techniques in terms of lateral resolution, depth resolution and information obtained by the techniques. Ion probe techniques and stylus techniques, which do not generally give information on speciation, are also included in this paper.

The search also identified several papers reporting on techniques to characterise and visualize nanoparticle agglomeration and uptake in cells including:

- Conventional transmission electron microscopy (TEM) provides very little contrast between unstained cells and carbon-based nanoparticles. Porter et al report on the use of energy filtered transmission electron microscopy (EFTEM) to image the distribution of carbon-based nanoparticle aggregates (C_{60} Buckminster fullerene) [24]. They showed that images recorded using low loss EFTEM provided a significant improvement in obtaining contrast between the organelles of the cells and the carbon-based C_{60} nanoparticles in disorded and crystalline form. They also demonstrated that the low loss spectra enabled the visualisation of C_{60} within the cytoplasm of the cell. Primary C_{60} particle diameters ranged between 60 and 270 nm and clusters diameters between 420 and 1300 nm.
- Skebo et al report on the use of an ultra high-resolution imaging system (URI) to visualise nanoparticle agglomerates (as small as 90 - 100 nm in size) inside cells or on the surface of cells [25]. The URI system (CytoViva150 URI system) uses an advanced illumination system attached to an inverted transmission optical microscope.
- Suzuki et al report on a method to evaluate uptake of potential nanoparticles in cultured cells using flow cytometric light scatter analysis [26]. They claim that the intensity of the side-scattered light revealed uptake of TiO_2 nanoparticles into cells and that the uptake of other nanoparticles such as silver and iron oxide could also be detected.

1.5 Bibliography of key papers

Measuring and monitoring of airborne nanoparticles

1. European Committee for Standardization. EN 15051. Workplace atmospheres - Measurement of the dustiness of bulk materials - Requirements and reference test methods, 2006.
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3. International Organization for Standardization. ISO/TR 27628:2007. Workplace atmospheres - Ultrafine, nanoparticle and nano-structured aerosols - Inhalation exposure characterization and assessment, 34 pages, 2007.
4. ASTM International. E 2456-06. Terminology for Nanotechnology, 2006.
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7. Peineke C, Attoui MB and Schmidt Ott A. Using a glowing wire generator for production of charged, uniformly sized nanoparticles at high concentrations. Journal of Aerosol Science, 37, 1651-1661, 2007.
8. Park D, An M, and Hwang J. Development and performance test of a unipolar diffusion charger for real-time measurements of submicron aerosol particles having a log-normal size distribution. Journal of Aerosol Science, 38, 420-430, 2007.
9. Chen DR, Li W and Cheng MD. Development of a multiple-stage differential mobility analyzer (MDMA). Aerosol Science and Technology, 41, 217-230, 2007.
10. Rodrigue J, Ranjan M, Hopke PK and Dhaniyala S. Performance comparison of scanning electrical mobility spectrometers. Aerosol Science and Technology, 41, 360-368, 2007.
11. Choi Youngjoo and Kim Sangsoo. An improved method for charging submicron and nano particles with uniform charging performance. Aerosol Science and Technology, 41, 259-265, 2007.
12. Wen J and Wexler AS. Thermophoretic sampler and its application in ultrafine particle collection. Aerosol Science and Technology, 41, 624 - 629, 2007.
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14. Kulmala M, Mordas G, Petäjä T, Grönholm T, Aalto PP, Vehkamäki H, Hienola AI, Herrmann E, Sipilä M, Riipinen I et al. The condensation particle counter battery (CPCB): A new tool to investigate the activation properties of nanoparticles, Journal of Aerosol Science, 38 , 257-376, 2007.
15. Zelenyuk A and Imre D. On the Effect of Particle Alignment in the DMA. Aerosol Science and Technology, 41, 112 - 124, 2007.

16. Wittmaack K. Deriving the mean primary-particle diameter and related quantities from the size distribution and the gravimetric mass of spark generated nanoparticles. Journal of Nanoparticle Research, 9, 191-200, 2007.

17. Schmid O, Karg E, Hagen DE, Whitefield PD, and Ferron GA. On the effective density of non-spherical particles as derived from combined measurements of aerodynamic and mobility equivalent size. Journal of aerosol science, 38, 431-443, 2007.

Control

18. Maze B, Vahedi Tafreshi H, Wang Q and Pourdeyhimi B. A simulation of unsteady-state filtration via nanofiber media at reduced operating pressures. Journal of Aerosol Science, 38, 550-571, 2007.

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Characterisation

20. Borm PJ, Robbins D, Haubold S, Kuhlbusch T, Fissan H, Donaldson K, Schins R, Stone V, Kreyling W, Lademann J, Krutmann J, Warheit D and Oberdorster E. The potential risks of nanomaterials: a review carried out for ECETOC. Part Fibre Toxicol, 3, 11, 2006.

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2. HEALTH EFFECTS

A new source of information was searched for the preparation of this bulletin: the Nano EHS virtual journal at http://icon.rice.edu/virtualJournal.cfm, as an addition to the strategy described in the Appendix to Issue 1 of the NanoAlert bulletin. This source of information will also be included in subsequent searches.

The majority of the publications (46%) retrieved by the health effects searches in the four months from March to June 2007 described effects of engineered nanoparticles in *in vitro* systems, continuing a trend observed in the previous bulletin (Issue 2)(Figure 2). Of these publications, almost equal numbers of papers described effects of nanoparticles on human cells (24%) and animal cells (22%) grown *in vitro*. The proportion of publications (16%) describing the effects of engineered nanoparticles in animals was very similar to the previous bulletin. However, a larger number of reviews (38%) were published in March to June 2007 compared to the previous four months.

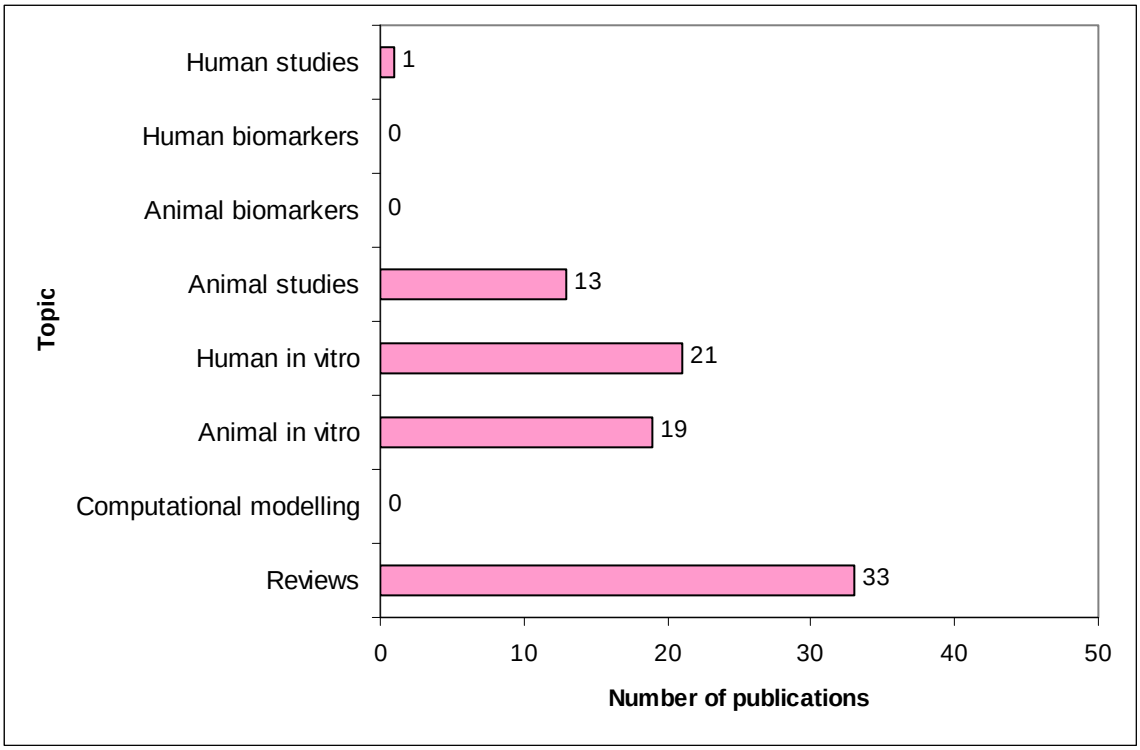

Figure 2: Breakdown per topic of the numbers of publications retrieved in the four months from March to June 2007 on the human health effects of *engineered* nanoparticles.

2.1 Human studies and epidemiology

One study retrieved in the searches examined the effects of ambient ultrafine particles on levels of a marker for platelet activation in humans [1]. This study is described here as the only recent study in humans, albeit one on ambient not engineered nanoparticles:

Ultrafine particles and platelet activation in patients with coronary heart disease - Results from a prospective panel study. Rueckerl et al (2007) [1]

In a prospective panel study in a susceptible population, conducted in Erfurt, Germany, blood cell counts and plasma levels of soluble CD40 ligand (sCD40L), a marker of platelet activation, coagulation and inflammation, were analysed in 57 male patients with coronary

heart disease during the winter of 2000-2001. Hourly data were recorded on mass and number concentration of ultrafine particles, elemental and organic carbon, gaseous pollutants and meteorological conditions. Increases in sCD40L and decreases in platelet counts were observed in association with ultrafine particles, supporting previous studies that have shown adverse cardiovascular effects of ambient pollution. **The authors conclude that the increased sCD40L levels support the hypothesis that elevated ambient air pollution can enhance inflammatory conditions, potentially explaining the observed association between air pollution and cardiovascular morbidity and mortality in susceptible individuals.**

2.2 Animal *in vivo* studies

One study retrieved in the searches considered the potential effects of nanoparticles on embryonic development, albeit indirectly since the goal of the work appeared to be development of nanoparticle tags for embryos *in vitro* [2]. Mouse one-cell embryos were either injected or co-incubated with polystyrene or polyacrylonitrile nanoparticles (neither size nor dose stated in abstract). Externally applied polyacrylonitrile but not polystyrene nanoparticles altered embryo development after two days, but not after six days. Injection of nanoparticles inhibited development, such that fewer embryos reached the blastocyst stage on day six.

Of the other twelve animal studies of the potential health effects of nanoparticles, three considered the outcomes of engineered nanoparticle inhalation and three examined the effects of ambient nanoparticle inhalation. Since inhalation is likely to be the major route of entry for nanoparticles in many exposure scenarios, all three papers on engineered nanoparticles are considered in detail.

Inhalation exposure study of titanium dioxide nanoparticles with a primary particle size of 2 to 5 nm. Grassian et al (2007) [3]

Mice were exposed by inhalation to an aerosol of TiO_2 nanoparticles, with a primary size of 2-5 nm, in a whole-body exposure chamber, either acutely for 4 hr or sub-acutely for 4 hr per day for 10 days. Following acute exposure to 0.77 or 7.22 mg/m^3 TiO_2 nanoparticles, there were minimal toxic effects. Sub-acute exposure to 8.88 mg/m^3 TiO_2 nanoparticles increased the numbers of cells in the bronchoalveolar lavage fluid (BAL) immediately and after 1 or 2 weeks, but the effect had resolved by 3 weeks. **The authors conclude that acute or sub-acute exposure of mice by inhalation to 2-5 nm nanoparticles of TiO_2 induces moderate but transient pulmonary inflammation.**

This study differs from one discussed in the previous bulletin (Issue 2) in which larger TiO_2 nanoparticles (19-21 nm) given in a single intratracheal dose induced emphysema-like lung injury [4].

Building on earlier studies, which concluded that inhalation of carbon nanotubes (CNTs) can induce pulmonary inflammation, there has been one recent report of the potential for CNTs to induce cardiovascular effects in wild-type and two genetically modified strains of mice: heme oxygenase (HO-1) reporter and ApoE knockout mice.

Cardiovascular effects of pulmonary exposure to single-wall carbon nanotubes. Li et al (2007) [5]

A single intrapharyngeal instillation of single-walled carbon nanotubes (SWCNTs) induced (i) activation of HO-1, an oxidative stress marker, in lung, aorta and heart of HO-1 mice, and (ii) aortic mitochondrial DNA damage after 7, 28, and 60 days in wild-type mice exposed to 10 / 40 μg SWCNTs. The mitochondrial DNA damage was accompanied by changes in aortic mitochondrial glutathione and protein carbonyl levels, both changes that are linked to cardiovascular diseases. Repeated exposure to 20 μg SWCNT per mouse once every 2

weeks for 8 weeks accelerated plaque formation in the ApoE knockout mice fed an atherogenic diet, and also increased mitochondrial DNA damage, but not inflammation. **The authors conclude that respiratory exposure to CNTs may contribute to adverse cardiovascular effects.**

Lung retention and clearance of nanoparticles has been studied in rats:

Efficient elimination of inhaled nanoparticles from the alveolar region: evidence for interstitial uptake and subsequent re-entrainment onto airways epithelium. Semmler-Behnke et al (2007) [6]

Rats were given a single intratracheal dose of iridium-radiolabelled nanoparticles (size and dose not provided in the abstract), and the distribution of the particles analysed over the following 6 months. Free nanoparticles were initially found in BAL fluid, and subsequently within alveolar macrophages. However, in contrast to micron-sized particles, which remain at 0.8, nanoparticle levels decreased to 0.06 of the total lung burden after 3 weeks; at this time point, the nanoparticles had mostly (80%) translocated into the epithelium and lung interstitium. **The authors conclude that nanoparticles can be removed from the lung surface into the interstitium, and whilst macrophages phagocytose nanoparticles less than larger particles, they can nevertheless clear them from these interstitial sites to the larynx.**

Three other publications examined the pulmonary effects of ultrafine carbon or ambient rather than engineered nanoparticle inhalation or injection, and are therefore only summarised here, being of lower priority for this bulletin. The effects of intratracheal administration of urban air particulates from six European cities were examined in mice [7]. The ultrafine ($PM_{0.2}$) samples induced negligible lung inflammation, in terms of BAL markers. Differential time-dependent effects were seen with larger particles, and generally, the authors concluded that the $PM_{10\text{-}2.5}$ samples were more inflammatory than the $PM_{2.5\text{-}0.2}$. In the second publication, the role of the vagus nerve in inflammatory and cardiorespiratory effects of diesel particles was examined in the rat [8]. Either mid-cervical vagotomy or administration of atropine reduced the particle-induced neutrophilia in the lung, implicating muscarinic receptors in the inflammatory response, and suggesting that receptor antagonists might be clinically useful for reducing particle-induced inflammation in susceptible individuals. One further report on ambient nanoparticles examined the effects of diesel exhaust particles (DEP), administered intraperitoneally (not via inhalation), on endothelial function in wild-type and ApoE knockout mice, and found that DEP only affected vascular function in the ApoE mice with slight atherosclerosis [9].

A key issue for researchers and regulators alike is the most effective approach for studying the potential toxicity of nanoparticles. Much emphasis has been placed on developing, optimising and validating *in vitro* methods; however few papers have directly compared results from *in vitro* studies with *in vivo*. One paper published recently has assessed the ability of *in vitro* tests to predict *in vivo* lung toxicity of several particles [10]:

Assessing toxicity of fine and nanoparticles: comparing *in vitro* measurements to *in vivo* pulmonary toxicity profiles. Sayes et al (2007) [10]

Two series of experiments were carried out: the pulmonary toxicity in rats of 1 or 5 mg/kg of five different particles was compared with the *in vitro* responses of rat lung epithelial cells, rat alveolar macrophages or a co-culture of the two. The particles tested were: carbonyl iron (CI, 358 nm in dry state), crystalline silica (CS, 452 nm), amorphous silica (AS, 354 nm), fine ZnO (111 nm) and nano-ZnO (90 nm); all the particles showed increased mean particle size when suspended in PBS or cell culture medium, due to aggregation.

Rats were intratracheally instilled with 1 or 5 mg/kg of the particles in PBS, and after 24h, 1 week, 1 or 3 months, the concentrations of LDH and cells in BAL fluid were analysed. All the

particles induced transient increases in LDH and pulmonary inflammation, only CS inducing sustained responses.

The *in vitro* responses to the particles were cell, time and dose-dependent, complicating their interpretation and comparison with the *in vivo* data. The lung epithelial cells were the most sensitive cells in terms of LDH responses. All the particles induced some LDH release at the highest dose (after 1, 4, and 24h); whilst the nano- and fine ZnO were the most potent in terms of inducing significant toxicity at the lowest doses, CS and AS induced the greatest LDH releases overall. The results of the MTT assays differed from the LDH results (either *in vivo* or *in vitro*). Three cytokines were studied as *in vitro* indicators of inflammation. MIP-2 was only produced by macrophages in response to AS and CS. Significant TNF-α was only produced in the co-cultures in response to AS, and IL-6 was released from macrophages and the co-cultures in response to CS, AS and nano-ZnO.

The authors conclude that there was little correlation between the *in vivo* and *in vitro* measurements, and that the *in vitro* systems require further development, standardization and validation.

A second paper from Warheit's group then proposes a minimum base set of toxicity studies for nano-products, which they have used to evaluate the toxicity of titanium dioxide:

Development of a base set of toxicity tests using ultrafine TiO_2 particles as a component of nanoparticle risk management. Warheit et al (2007) [11]

A base set of ten toxicity tests were evaluated with different forms of TiO_2 (two rutile forms of 136 and 149 nm in water; 79:21 anatase: rutile TiO_2 of 140 nm; and 80:20 anatase: rutile TiO_2 of 380 nm) and crystalline silica (480 nm) as a control. The tests comprised five mammalian hazard tests: a pulmonary toxicity test in rats using intratracheal instillation, with assessment of inflammation and histopathology after 24h, 1 week, 1 or 3 months; acute dermal irritation study in rabbits with assessment after 1, 24, 48 and 72h; dermal sensitisation / local lymph node assay in mice; acute oral toxicity in rats (observations for up to 14 days) and acute eye irritation study in rabbits, with assessment after 1, 24, 48 and 72h. Two genotoxicity tests were done: the bacterial reverse mutation test and the chromosomal aberration test in CHO cells. Three aquatic toxicity studies were done with the rainbow trout, *Daphnia magna* and green algae, *Pseudokirchneriella subcapitata*. The results suggest that the different forms of TiO_2 have low pulmonary and acute oral toxicity; they are neither skin irritants nor sensitisers, and they only induce minor ocular redness. Both genotoxicity tests were negative. The aquatic toxicity tests showed that TiO_2 is of low (trout and *D. magna*) or medium (algae) concern. **The authors conclude that the forms of TiO_2 tested have low hazard potential in mammals and aquatic species following acute exposures.**

One paper has considered the effects of injection of nanoparticles in animals:

Migration of intradermally injected quantum dots to sentinel organs in mice. Gopee et al (2007) [12]

To evaluate the distribution of nanoparticles following intradermal infiltration (e.g. as would occur after topical exposure), quantum dots (QD, cadmium-selenium core, 37 nm) were used as surrogates for other similarly sized nanoparticles and were injected intradermally into mice. Their distribution was analysed after 0, 4, 8, 12 or 24h using fluorescence microscopy and inductively coupled plasma mass spectrometry (ICP-MS). There was a time-dependent loss of cadmium from the injection site, although residual fluorescence remained, and 7.5% accumulated in liver, lymph nodes, and kidneys in the first 24h. **The authors conclude that regional lymph nodes and liver can be used as sentinel organs for analysis of dermal penetration of nanoparticles, such as QD.**

Two papers consider the influence of nanoscale on bioavailability of elements for gut absorption, and are viewed as lower priority for this bulletin since they do not directly

consider potential health effects. Reducing the particle size of poorly soluble iron compounds to nanoscale (10 and 30 nm) appears to improve their bioavailability, with no indications of toxicity [13]. Similarly, nano-selenium acts as an antioxidant with equal efficacy compared to bulk selenomethionine (the usual supplemental form), but has much lower toxicity (in terms of median lethal dose, acute hepatic injury and short-term toxicity) [14]. The final paper identified in the health effects searches described the long-term efficacy of a small ceramic heater for generating silver nanoparticles (13-14 nm) for inhalation studies [15].

2.3 *In vitro* studies

Of the 40 publications identified in this area by the human health effects searches, 21 analysed the effects of engineered nanoparticles in human cells *in vitro*, and will be considered first, since they are considered to be of higher priority than studies in animal cells *in vitro*.

In the previous bulletin (Issue 2), we noted one paper that reported the significant cyto- and genotoxicity of nanoparticles of TiO_2 in human lymphoblastoid cells [16]. These authors have now considered the potential genotoxicity of ultrafine silica in the same cells:

Cytotoxicity and genotoxicity of ultrafine crystalline SiO_2 particulate in cultured human lymphoblastoid cells. Wang et al (2007) [17]

The cytotoxicity and genotoxicity of nano-sized (<100 nm) SiO_2 were analysed in WIL2-NS cells (0-120 μg/ml for 6, 24 or 48h). There was a dose-dependent increase in cytotoxicity by MTT assay. In the genotoxicity assays, a dose-dependent decrease in the proliferation index was seen in the cytokinesis block micronucleus assay, with 120 μg/ml SiO_2 inducing a four-fold increase in micronucleated binucleated cells after 24h. The lowest dose that had a significant effect in this assay was 30 μg/ml after 24h. 120 μg/ml SiO_2 caused a significant increase in induced mutant frequency in the hypoxanthine guanine phosphoribosyltransferase mutation assay, but there was no difference in DNA strand breakage in the Comet assay. **The authors conclude that like ultrafine TiO_2, nanosized SiO_2 can also cause significant cyto- and genotoxicity in human cells.**

A second paper reported dose-dependent changes in viability of normal fibroblasts and tumour cells in response to nanoparticles of silica and chitosan, the fibroblasts with a longer doubling time being more susceptible to injury than the tumour cells [18]. Synthesising silica with chitosan significantly reduced its toxicity.

Three reports of the effects of carbon nanotubes (CNTs) on human cells were identified in the human health effects searches [19-21]. Exposure of human lung fibroblasts to CNTs, sorted on the basis of length, revealed that the cells do not take up CNTs that are longer than about 200 nm [19]. Below this threshold, the CNTs are taken up and induce cytotoxicity. The effects of the aggregation state of CNTs on toxicity have been studied in the human mesothelioma cell line MSTO-211H; CNTs dispersed using polyoxyethylene sorbitan monooleate were less toxic than asbestos, but rope-like agglomerated CNTs were more cytotoxic than asbestos or dispersed CNTs at equal concentrations [20]. The third study examined the interactions between human skin keratinocytes and SWCNTs that had been derivatized with 6-aminohexanoic acid [21]. The SWCNTs were taken up into intracytoplasmic vacuoles, and dose-dependently induced loss of viability by MTT assay; they also induced production of IL-6 and IL-8, but not IL-1β, IL-10 nor TNFα. Dispersion of the CNTs (with Pluronic F127) again reduced the toxicity.

Two papers report the effects of nanoparticles on human lung cells *in vitro*. One focussed on the effects of chemical composition and catalytic activity on oxidative stress generation in human lung epithelial cells, using iron, cobalt, silica and manganese nanoparticles and their

oxides of the same size, shape, morphology and agglomeration state, to remove these as variables (although these characteristics were not given in the abstract) [22]. The nanoparticles entered cells by a Trojan-horse mechanism, Co and Mn inducing up to eight times more oxidative stress than these metals in solution, and catalytic activity significantly influenced their effects. The second paper compared the cytotoxicity of Ag, TiO_2, Fe_2O_3, Al_2O_3, ZrO_2, Si_3N_4 nanoparticles with chrysotile asbestos, CNTs and carbon black (ranging in size from 2 nm to 20 μm) in mouse alveolar macrophage, human macrophage and human lung epithelial cell lines [23]. The results were similar for the macrophage cell lines, and the authors suggest that simple macrophage cell line assays represent a useful approach for gaining preliminary information on the potential human respiratory health risks of nanoparticles. A critique was also identified of one paper [24] that was considered in the second bulletin (Issue 2) [25].

Several publications mentioned in the first bulletin (Issue 1) discussed results that contradict the conventional opinion that nanoparticles cannot penetrate intact human skin (e.g. [26]). A further study identified in the latest searches reveals that metal nanoparticles (less than 10 nm) could penetrate hair follicles and stratum corneum in excised human skin samples, occasionally reaching the viable epidermis, but they did not permeate the skin [27]. In contrast, less than 0.03% of applied ZnO nanoparticles in a sunscreen formulation were reported to penetrate beyond the upper layers of stratum corneum of human skin [28]. The third publication of lower priority considered the cytotoxicity of different emulsions and lipid nanoparticles in human HaCaT skin cells, mouse macrophages and fibroblasts, reporting that stearylamine and stearic acid significantly affected viability [29].

Two publications have analysed the inflammatory effects of nanoparticles *in vitro*, building on the large numbers of papers reporting inflammatory effects *in vivo* noted in previous bulletins. In human aortic endothelial cells, Fe_2O_3 nanoparticles (size not given in abstract) did not alter production of messenger RNA or protein of ICAM-1, IL-8 or MCP-1, whilst Y_2O_3 or ZnO nanoparticles induced an inflammatory response at concentrations above 10 μg/ml; at 50 μg/ml, ZnO nanoparticles were cytotoxic [30]. The second paper is of lower priority for the bulletin as it considers the toxicity and proinflammatory effects of superparamagnetic iron oxide nanoparticles (to be used for imaging), noting in human monocyte-macrophages that the nanoparticles were not toxic *in vitro*, nor did they induce release of proinflammatory cytokines or superoxide anions, nor interfere with phagocytosis [31].

Examining uptake and cellular distribution of carbon-based nanoparticles is problematic, but one report was identified in the searches in which C60 nanoparticles were differentiated from cellular components in human monocyte-macrophages using energy filtered transmission electron microscopy [32]. Two other publications have examined uptake or penetration of nanoparticles into human cells *in vitro*, for the purposes of transport into cells, or labelling cells, rather than examination of toxicity, and are therefore of low priority for this bulletin [33; 34].

There have been several reports of techniques for examining nanoparticle health effects *in vitro* (or *ex vivo*). A new method has been described for estimating the concentration of nanoparticles in whole blood, based on dynamic light scattering; the authors state that this approach could be used to estimate the actual dose of injected nanoparticles delivered *in vivo*, and their rate of clearance [35]. The other methodological reports all used human or human with animal cell line systems. An improved model for studying nanoparticle transport by M cells in gut epithelium has been developed, in which human Caco-2 cultures were inverted after seeding, increasing conversion into M cells; this approach has been used to examine transport of 200 nm polystyrene nanoparticles [36], and a nanoparticle-encapsulated peptide [37]. A further paper describes a molecular approach for analysing nanoparticle toxicity, in which differential display was used to analyse changes in messenger RNA expression following exposure of mouse fibroblasts to micro- or nanoparticles, or ions [38]. A proinflammatory cytokine cell sensor has also been developed, based on the mouse

macrophage cell line, RAW 264.7, and HeLa cells, engineered to respond to cytokines produced by the macrophages with nuclear transport of the transcription factor, NF-KB, and ensuing reconstitution of luciferase from split fragments [39]. Incubation of the sensor with nanoparticles therefore leads to cytokine release and light emission in proportion to the concentration of particles, with a detection limit of approximately 1 μg/ml for 40 nm TiO_2 nanoparticles.

Studies in animal *in vitro* systems are considered to be of low priority for this bulletin. Of the 19 papers in this category, one paper however considered the effects of flexing on penetration of fullerene nanoparticles through intact pig skin. This work builds on previous studies of particle penetration into skin and since pig skin closely resembles human skin, it is considered in detail.

Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. Rouse et al (2007) [40]

Dermatomed pig skin (400 μm thick) was treated with a fullerene substituted derivative of a nuclear localisation peptide sequence for 8 or 24h. The skin was also flexed (45°, 20 flexes per minute) for 60 or 90 minutes. Absorption was assessed in a flow-through diffusion cell. After 8h, the particles (approximately 3.5 nm) had penetrated only into the epidermal layers of non-flexed skin, but had penetrated into the epidermis and dermis of flexed skin. Penetration was not due to skin damage, since the stratum corneum appeared intact by confocal scanning microscopy. After 24h, penetration was increased in all experimental groups. Transmission electron microscopy confirmed that the particles had penetrated intact skin and were found between keratinocytes.

The remaining papers on animal *in vitro* systems fall into two major categories: there were ten reports of the potential effects of nanoparticles in animal cells *in vitro*, whilst the other eight papers considered interactions between nanoparticles and proteins or lipid bilayers. Both of these categories of publication are considered to be of low priority for this bulletin and will be only briefly summarised. One publication examined the effects of fluorescent nanoparticles (of mixed sizes) on the viability and morphology of cryopreserved mouse embryonic stem cells [41]. After 6 days, the nanoparticles entered the cytoplasm and perinuclear region of the cells, and reduced cell size and viability; they also induced dose-dependent changes in cellular differentiation. Two reports noted cytotoxicity and decreased phagocytic ability of macrophages in response to C60 fullerenes, aluminium nanoparticles (50, 80, 120 nm, with a 2-3 nm oxide coating) and to a lesser extent Al_2O_3 (30/40 nm) nanoparticles, suggestive of effects on the cytoskeleton [42; 43]. C60 also facilitated ADP-induced platelet aggregation [42]. In brain phagocytic cells, namely microglia (BV2 and GL261 glioma cell lines), multi-walled CNTs were not cytotoxic, nor did they induce proliferative or cytokine changes, and the authors conclude that they therefore potentially represent biodegradable nano-vehicles for brain tumour therapeutics [44]. However, SWCNTs in culture medium (either filtered to remove SWCNT aggregates or unfiltered) inhibited growth of rat aortic smooth muscle cells at doses above 0.1 mg/ml, whilst at lower doses, removal of the aggregates reduced the inhibitory effects [45]. Another paper described a dose-dependent decrease in viability and ability to extend neurites in rat neuronal cells (PC12 cells) in response to Fe_2O_3 nanoparticles [46]. Uptake of magnetic nanoparticles has been observed in mouse red blood cells [47], as noted for human red blood cells in the previous bulletin (Issue 2), and uptake of individual and agglomerated metal nanoparticles (Ag: 25, 80, 130 nm; Al: 80 nm; Mn: 40 nm) into rat liver cells (BRL 3A), rat alveolar macrophages (MACs) or rat neuroendocrine cells (PC12) has been studied using an ultra-high resolution system [48]. Another study has used confocal laser scanning microscopy to demonstrate dose, time and size-dependent uptake of TiO_2 particles (5, 23, 5000 nm) into the cytoplasm of Chinese hamster ovary (CHO) cells [49]. One paper

identified in the searches has examined adhesion of cells to nanomaterials, showing that surface changes can induce large changes in cell signalling and inflammation [50].

In biological fluids, nanoparticles and proteins interact, leading to generation of what has been termed a "protein corona" around the particle that may significantly influence the physiological responses to particles *in vivo* [51]. Several publications report approaches for studying these nanoparticle – protein interactions: matrix-assisted laser desorption / ionisation (MALDI) mass spectrometry [52], reflectometry [53], isothermal titration calorimetry, surface plasmon resonance and size exclusion chromatography [54]. These and other techniques have been used to study: adsorption of human serum albumin to copolymer nanoparticles [55]; adsorption – desorption of bovine serum albumin on CNTs [53]; and the kinetics of association and dissociation of albumin and fibrinogen with copolymer nanoparticles [54]. One key paper in this area has reported that nanoparticles can increase the probability of nucleation of protein fibrils, by locally increasing protein concentrations, and decreasing the lag time for nucleation [56]. These effects result from both the enhanced surface charge and surface area of nanoparticles, and have implications for the potential effects of human exposure to nanoparticles, since protein fibrillation is implicated in many human diseases [57]. One further paper has examined the formation of lipid bilayers on SWCNT transistors, and protein binding to the supported bilayer [58].

2.4 Reviews

A large number of reviews were identified in the human health searches (33), a significant increase on the six identified in the last bulletin (Issue 2). Of these, 39% (13) considered the risks that nanoparticles may potentially present to human health and regulatory approaches for managing these risks [59-71]. Four reviews covered specialist health issues, namely applications of nanoparticles in paediatric respiratory disease [72], the impact of nanotechnology on neurology [73], as well as how human exposure might be assessed [74] and a new approach for testing nanoparticles *in vitro*, in which human cells are directly exposed to airborne agents, giving results that so far correspond well with animal data [75]. There were two reviews of current knowledge of the mechanisms of action of nanoparticles in cells and tissues [76; 77], and one considering how CNTs might be used to probe cellular activities [78]. The Royal Society of Chemistry has published a second book in its Nanoscience and Nanotechnology Series on fullerenes (the first being on Nanotubes and Nanowires [79]), summarising their reactivity, chemistry and applications [80]. Five reviews discussed the societal and ethical implications of nanotechnology [81-85], one focussing on the public perceptions of nanotechnology and food [86]. The remaining articles consisted of announcements of new nanotechnology centres [87], national plans [88], reports of progress with initiatives, specifically, the UK's nanotechnology voluntary reporting scheme [89] or general reviews / interviews on nanotechnology [90-92].

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