Health & Safety Executive NanoAlert Service

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Bulletin Contents:

1. MEASUREMENT, EXPOSURE AND CONTROL

In this bulletin, the strategy included a comprehensive search of the literature as described in Issue 1 and an additional search from relevant journals. Those articles considering engineered nanoparticles were assigned a higher priority than those related to ambient ultrafine particles. A breakdown per topic of the number of papers published over the four months from November 2007 to February 2008 within the scope of measurement, exposure and control of nanoparticles is shown in Figure 1. As observed in previous bulletins, a significant number of papers have been published on the development, improvement and assessment of instruments for the measurement of airborne nanoparticles, as well as on the characterisation of nanoparticles in their bulk form, in fluids or in biological tissues. A few papers were identified on the generation of standards nanoparticles for toxicity and instruments testing, an important topic in the field of health and safety. This issue did not retrieve any papers on the design and development of compact monitors, which are much sought after by occupational hygienists. As in previous bulletins, very few studies on the assessment of exposure to engineered nanoparticles in the workplace have been published. A paper from the USA has been identified as well as two surveys using questionnaires to German and Swiss companies gathering information on the manufacture and handling of nanomaterials in workplaces. The same observation can be drawn on engineering control measures. However, a few papers have been published reporting on the efficiency of filters but there were no studies on face-fit testing of respirators.

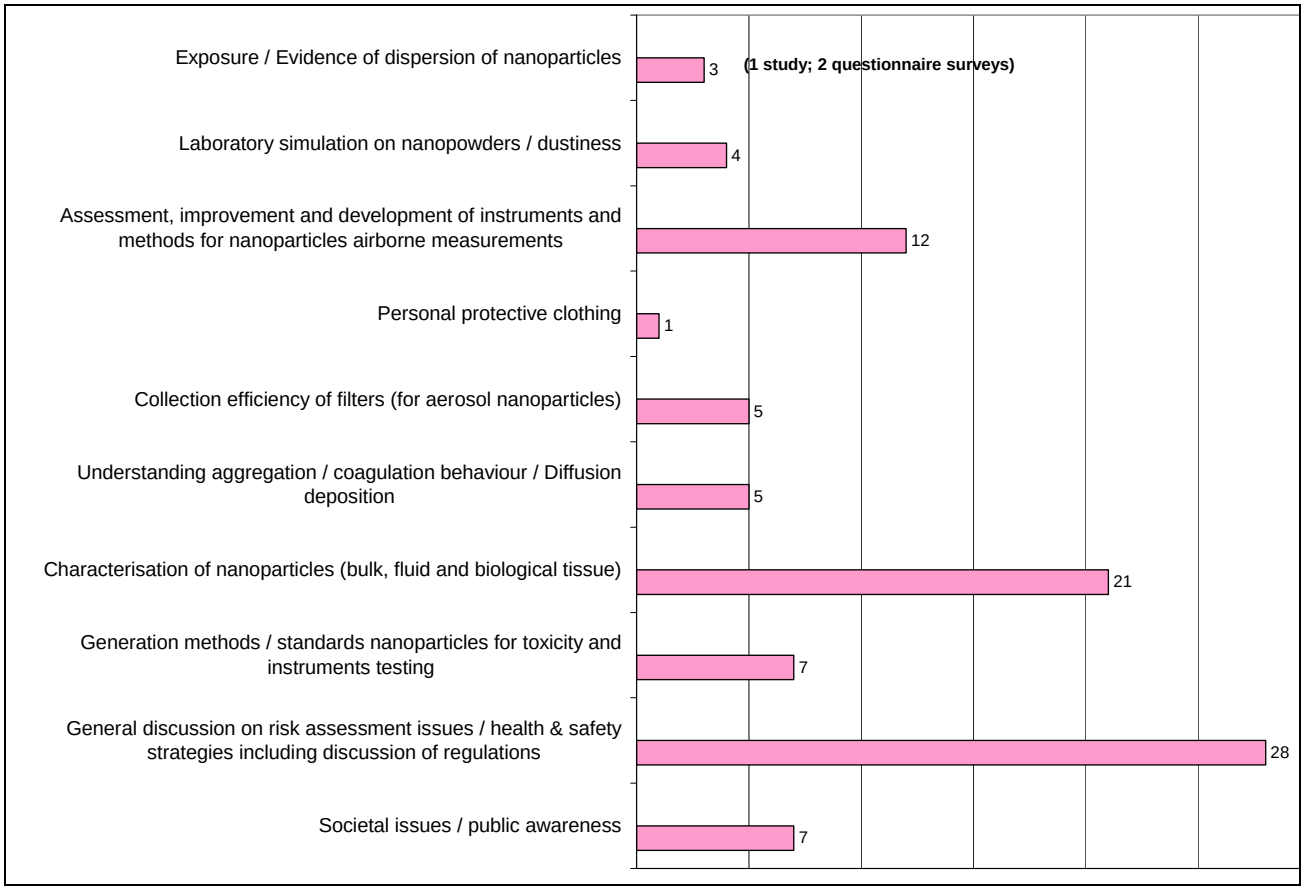

Figure 1: Breakdown of the number of papers per topic (measurement, exposure and control) retrieved in the four months from November 2007 to February 2008.

1.1 Exposure data

Workplace exposure

Toxicology studies have suggested that the monitoring of nanoparticle exposure in terms of mass concentration alone is not sufficient and that it is also necessary to measure particle surface area and number concentration. Recent studies have usually included measurement of all three metrics.

As observed in the previous bulletins, very few studies on the assessment of exposure level to engineered nanoparticles in the workplace have been published in peer-reviewed journals. A paper published by the National Institute for Occupational Safety and Health (NIOSH) in the USA has been identified:

Case Study. Identification and characterization of potential sources of worker exposure to carbon nanofibers during polymer composite laboratory operations. Methner et al (2007) [1]

NIOSH has carried out measurements at a university-based research laboratory using carbon nanofibres (CNFs) to produce high-performance polymer composite materials. Various operations were monitored, including:

- "Chopping of extruded composite material containing CNFs"
- "Transferring CNFs from a plastic receptacle outside a laboratory hood to a small beaker for weighing inside the hood"
- "Transferring and mechanically mixing CNFs with acetone inside a 5 gallon mixing vessel positioned on the floor outside the hood without local exhaust ventilation (LEV)"
- "Cutting composite material using a wet saw"
- "Manually sifting oven-dried, epoxy-coated CNFs on an open bench-top to remove large clumps"

PM10 mass, particle number, active surface area concentrations and size distributions were monitored using real-time instruments. Samples were collected on quartz filters for carbon analysis using NMAM method 5040 and on carbon grids using a point-to-plane electrostatic precipitator (ESP) for transmission electron microscopy analysis. Inhalable dust and surface samples were also collected. A ventilation assessment was evaluated using smoke tubes and by visual inspection of LEV controllers and rooftop air handler.

The authors found:

- The processes described above did not produce substantial sources of CNF emissions in the laboratory.
- Two processes (weighing / mixing CNFs in an unventilated area within a cage and wet-saw cutting of a composite material) showed slight increases in airborne particle concentrations for mass and number compared to background concentrations.
- Few fibre bundles were observed on the ESP samples. From air and surface area analysis using total carbon, CNFs are being released and seemed to migrate from the laboratory to a nearby office (probably by foot-ware).

In addition, two papers reported surveys using questionnaires to German and Swiss companies gathering information on the manufacture and handling of nanomaterials in workplaces.

Exposure to nanomaterials in Germany - Corporate survey of the Federal Institute for Occupational Health and Safety (BAuA) and the Association of the Chemical Industry (VCI) using questionnaires. Plitzko and Gierke (2007) [2]

This paper reported on a survey using questionnaires to German companies in order to gather information on the manufacture and handling of nanomaterials in workplaces. The questionnaire consisted of "a general part with general cross-product questions" and "a specific part with product-specific questions on individual nanomaterials" with a focus on potential exposure by inhalation. Twenty one percent (n=45) of the 217 participating companies performed activities (production, use or processing) involving nanomaterials. For this questionnaire, the definition of nanomaterials was "particles manufactured as powders which have, in at least two dimensions, an extension of under 0.1 µm, as well as their aggregates and agglomerates". The definition of activities was "activities involving nanomaterials of 10 kg/year". The questionnaire revealed that:

- 40% of the companies (n=18) carried out activities in volumes of 10 to 100 kg/years.
- 11% of the companies (n=5) produced nanomaterials in volumes of over 100 tons/year.
- 71% (n=32) of the companies had 1 to 9 workers performing activities involving nanomaterials; 16% (n=7) had 10 to 49 workers; 2% (n=1) had 50 to 249 workers; 9% (n=4) had > 250 workers.
- 31% (n=14) of the companies carried out regular or exploratory measurements (gravimetric analysis of inhalable and respirable fraction as well as particle number concentration).
- 58% (n=26) of the companies declared having information on potential health effects. Companies without information (40%; n=18) were mainly companies performing activities in volumes of <100 kg/year.
- Few measurements were reported. The inhalation dust concentration reported was between 0.2 and 10 mg/m^3; the respirable dust concentration between 0.1 and 3 mg/m^3 and the particle number concentration between 0.1x10^5 to 6.0x10^5 particles/cm^3.
- Protection measures included process and ventilation technical measures as well as personal protective measures (respiratory protection).

Helland et al (2008) reported on a written survey of 40 companies involved in production and application of nanomaterials in Germany and Switzerland between December 2005 and February 2006 [3]. The questionnaire consisted of three sections: 1) material properties, 2) exposure and hazard assessment, and 3) risk assessment. Nanomaterials were defined as engineered materials having one or more dimensions below 100 nm. The authors found that:

- Four companies (10%) reported investigating the potential uptake of nanomaterials by organisms during the life cycle (including human exposure).
- One third of companies reporting carrying out risk assessments.
- 62.5% of the nanomaterials were reported as spherical, 20% as sheet like structures, 5% as fibres, 7.5% as other.

Agglomeration / nanopowder behaviour

The dustiness behaviour of nanoparticles is an important property. When nanoparticles do not become readily airborne under normal handling procedures, the associated risk from inhalation will be considerably reduced. Dustiness testing enables the investigation and quantification of the propensity of a powder to become airborne when handled. In 2006, the European Committee for Standardization (CEN/TC137/WG3) produced a document

providing standardisation in the measurement of dustiness of bulk powders (EN15051). 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.

In this issue, a paper on the dustiness behaviour of nanoparticles has been identified: Schneider and Jensen (2008) have developed a dustiness test in which both single drop and rotating drum tests can be performed on small amounts of samples [4].

Combined single-drop and rotating drum dustiness test of fine to nanosize powders using a small drum. Schneider and Jensen (2008) [4]

The authors have designed a dustiness test in which both the single drop and rotating drum tests can be performed using small amounts of samples (6 g of material). The test begins with a single drop test challenge followed by a rotation test using a smaller version of the EN 15051 rotating drum (cylinder length: 23 cm; internal diameter: 16.3 cm; volume flow rate of 11 L/min). The rotation tests were carried out at a rotation speed of 11 rpm. The size distributions and number concentrations were measured in the drum using a fast mobility particle sizer and an aerodynamic particle sizer. The dustiness indexes in mg / kg of test material were calculated from the mass of dust collected on a filter.

The authors tested: pigment-grade and ultrafine TiO_2, two grades of corundum (aloxite), yttrium-stabilised zirconia (Y-zirconia) granules, fumed silica, goethite, talc and bentonite. They found that:

- Reproducible results were obtained.
- Most of the samples produced two more or less separate size modes >0.9 μm and, except for TiO_2 pigment grade and aloxite, also a size mode at 100 to 200 nm.
- The total number of generated particles tended to decrease with increased crystallite size during the rotating drum test.
- Pigment grade TiO_2 had the lowest dustiness (31 ± 21) and ultrafine TiO_2 the highest (8338 ± 233) (a factor of 300 times). This dustiness index is between the thoracic and inhalable dustiness as defined in EN15051.

Other methods have been applied as a means of studying the behaviour of nanopowders:

- Debrincat et al (2008) [5] have determined the strength of inter-particle forces within agglomerates of nickel flash furnace concentrate and dust from experimental observations combined with mathematical equations from the literature. They found that the agglomerates had a tensile strength ranging from 0.01 Pa to 38.7 Pa and inter-particle forces ranged from 2.2×10^{-12} N to 1.5×10^{-10} N.
- Teleki et al (2008) [6] investigated the potential of high pressure dispersion and dynamic light scattering (DLS) for rapid and quantitative estimation of particle aggregation and agglomeration. An aggregate is made of particles bounded by strong chemical forces and an agglomerate is made of particles bounded by weak physical forces. By dispersing titanium dioxide powder in electrostatically stabilised aqueous suspensions through a nozzle at high pressure, the authors showed a reduction of the size of agglomerates and obtained a bimodal size distribution composed of primary particles and aggregates of particles.

A paper describing a new method to study the de-agglomeration of powders has been identified. Kurkela et al (2008) reported on a new method to study the de-agglomeration of powders with minimal particle deposition in the system [7]. Powders were fed at a low continuous rate into an apparatus where they met an adjustable dispersion main gas flow. The particle size distribution of the agglomerates was measured downstream. This method has been tested with micron-size silica and glass spheres, and might be useful for the testing nanopowders.

1.2 Measuring and monitoring of airborne nanoparticles

Moss (2008) recently reported that nanoparticle number does matter when estimating risk and that both nanoparticle number and surface area are relevant [8]. 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. A number of papers have been published on the evaluation and development of such instruments.

Evaluation of instruments or methodologies

It is important that the performance and detection limits of instruments used in workplaces for assessing exposure to airborne engineered nanoparticles are investigated.

Condensation Particle Counters (CPCs) are used to measure number concentrations of aerosol particles and can detect submicron particles. A paper on the performance of the ultra-fine water based CPC has been published:

On operation of the ultra-fine water-based CPC TSI 3786 and comparison with other TSI models (TSI 3776, TSI 3772, TSI 3025, TSI 3010, TSI 3007). Mordas, Manninen, Petaja, Aalto, Hameri, Kulmala (2008) [9]

The authors assessed the performance of the ultra-fine water based condensation particle counter (UWCPC TSI3786). The influence of temperature differences between the saturator and the tube growth and of particle chemical composition on the detection efficiency were investigated. The authors showed that:

- For silver particles, the cut size D50 varied from 2.9 to 4.5 nm during scanning of the temperature difference from 53 to 70^{0}C. The authors concluded that "UWCPC can be applied for sub 3 nm studies, even for non-hygroscopic particles".
- Using a temperature difference between the saturator and the growth tube of 70 ^{0}C, the D50 cut-sizes for silver, ammonium sulfate and sodium chloride were 2.9, 2.3 and 1.8 nm respectively.
- Using 15 and 30 nm silver particles, the counting efficiency was very good when concentrations were in the range 3,000 – 50,000 cm^{-3}. The maximum observable number concentration (within 10% accuracy) was 100,000 cm^{-3}.
- Against other TSI models (TSI 3785, TSI 3776, TSI 3772, TSI 3025, TSI 3010, TSI 3007) and butanol based CPCs (TSI 3776, TSI 3025), UWCP has a larger cut size for silver particles than butanol-based CPCs but smaller cut size than other TSI CPCs.
- In default operation regime using silver particles, the TSI 3776 had the lowest detection limit (D50 of 3.2 nm) (e.g. compared to 3.6 nm for TSI 3025).

Determination of mean particle size using the electrical aerosol detector and the condensation particle counter: Comparison with the scanning mobility particle sizer. Frank, Saltiel, Hogrefe, Grygas, Garland (2008) [10]

The authors compared two methods for the measurement of airborne nanoparticle mean size, using a combustion aerosol source of particle sizes from 13 to 183 nm:

- The scanning mobility particle sizer (SMPS) sizes particles by their electrical mobility equivalent diameters but requires 3 to 5 minutes to perform a full scan.

- The electrical aerosol detector (EAD) combined with the condensation particle counter (CPC) provides rapid measurements. The EAD measures the aerosol diameter concentration or total length in mm/cm^3. The mean particle size (nm) is then equal to: 10^6 x EAD total aerosol length (mm/cm^3) / CPC concentration (cm^3). The EAD and CPC have low sample flow rates (2.5 and 1.5 L/min respectively).

The authors found that:

- There was poor agreement between EAD/CPC and SMPS mean particle size data.
- There was good agreement between EAD/CPC and SMPS total aerosol length data.
- Differences in measurements may be due to differences in the concentrations measured by the CPC and SMPS.

The difference in particle mean size might be due to diffusion losses and nanoparticle aggregate mobility as well as the morphology, charging and composition of the particles. The authors concluded that it is unlikely that the EAD combined with the CPC could be used as an independent method for measuring the mean particle sizes for sources of rapidly changing particle behaviour.

A low pressure impactor can sample and size classify airborne particles. Wang et al (2007) compared the coal ash particle mass size distributions determined by the Dekati Low Pressure Impactor (DLPI) with those from the Berner Low Pressure Impactor (BLPI) [11]. BLPI and DLPI are low-pressure impactors which consist of 11 and 13 collection stages and a particle size range of 30 nm – 16 µm and 30 nm – 10 µm respectively. The authors found that:

- The DLPI collected larger mass fraction of sub micron particles than the BLPI.
- The DLPI (which has two additional collection stages) may give a better resolution of sub micron particles than BLPI.

Development of instruments and methodologies

A number of papers have been published on the development or improvement of instruments or methodology (better resolution, faster response, improved charging performance) for measuring exposure to nanoparticles.

Development of instruments with improved resolution and faster response

Fast response instruments could be very valuable when measuring exposure to engineered nanoparticles in workplaces from processes likely to generate high concentrations over a random and short time scale. The most common instruments used for sizing nanoparticles are SMPS, which size particles by their electrical mobility equivalent diameters. In conventional SMPS, the scan time ranges from 3 to 5 minutes and in the last few years a number of fast response instruments have been developed. A paper on improvement and performance of a fast response instrument for sizing airborne nanoparticles has been identified:

Real-time measurement of submicron aerosol particles having a log-normal size distribution by simultaneously using unipolar diffusion charger and unipolar field charger. Park, Kim, An and Hwang (2007) [12]

Park et al (2007) have published an article on the development and performance of a unipolar diffusion charger for fast real-time measurements of submicron aerosol particles having a log-normal size distribution. By using the unipolar diffusion charger with an electrometer and a condensation particle counter (CPC), the authors found that the

estimated geometric mean diameters of 80.5 nm for NaCl and 427 nm for dioctyl sebacate (DOS) particles were 33% larger than those measured by SMPS. In this article they have improved this method by using a unipolar field charger and another electrometer instead of a CPC and found that:

- The estimated results for NaCl (<100 nm) and DOS (100-700 nm) particles were within 10% of the data measured with a SMPS.
- The detection time with improved set-up (a unipolar field charger and two electrometers) was faster (<3s) than the 5s obtained with the previous set-up (a unipolar field charger, an electrometer and a CPC).

The mass concentration of airborne nanoparticles can be difficult to measure especially when low levels are present in the workplace. Dohn et al (2007) have derived an analytical expression relating mass and position of a particle attached on a cantilever to the resulting change in cantilever resonant frequency [13]. The authors claimed that this method could be applied for very accurate particle mass measurement.

Development of multifunctional instruments

As shown by Wake (2006), the relationships between the mass, number and active surface area concentrations of particles of different morphology may not be simple [14]. Therefore, a range of instruments has to be deployed in workplaces to assess exposure levels, based on all three metrics, and ideally, a single instrument measuring all three metrics is required. The current searches did not retrieve any papers on the development of such multifunctional instruments. At the present time, there is no such instrument, but scientists have tried to develop empirical relationships between surface area and mobility diameter in order to derive surface area information from size measurements.

Cho et al (2007) have established experimentally a relationship between the mobility diameter of agglomerated nanoparticles and the number and size of primary particles [15]. They used these results to determine the agglomerate surface area as a function of the measured mobility diameter, and developed an empirical relationship between surface area and mobility diameter. This is only valid for agglomerates with a limited number of primary particles and in the transition regime (at standard conditions).

Improvement of charging performance for instruments measuring aerosol particles

Instruments, such as the diffusion charger (DC), SMPS or Electrical Low Pressure Impactor (ELPI), used for sizing and measuring aerosols, modify the electrical charge on particles before detection. Particle charging performance depends greatly on particle diameter. Two papers on the development or improvement of aerosol chargers for sizing instruments have been identified:

- Kleefsman et al (2008) derived a numerical model to compare different aerosol chargers based on the total charge that an aerosol particle acquires [16].
- Vivas et al (2008) have redesigned an existing corona unipolar charger (Büscher et al 1994) in order to reduce multiple charging of sub micron particles (particles of > 20 nm can acquire more than one charge) [17]. The authors claim that this corona charger is suitable for size selection of sub micron particles by differential mobility analysis (DMA). The advantage over the bipolar diffusion charging is the absence of a radioactive source, commonly used in connection with a SMPS.

Evaluation of instrument for physical and chemical characterisation

In addition to the concentration levels of airborne nanoparticles, physical and chemical characteristics are important for differentiating between engineered nanoparticles and ambient ultrafine particles. Real-time instruments measuring mass, number, surface area concentrations do not provide chemical or morphological information, and it is recognised that in workplaces discrimination between engineered nanoparticles and background sources of ultrafines is difficult. One approach is to collect particles for off-line physical and chemical characterisation using electron microscopy.

Semi-quantitative characterisation of ambient ultrafine aerosols resulting from emissions of coal fired power stations. Hinkley, Bridgman, Buhre, Gupta, Nelson, Wall (2007) [18]

Hinkley et al reported on methods and results of the sampling and chemical analysis of ultrafine aerosols from emissions of coal fired power stations. Particles of > 1 μm were collected with a Burkard spore sampler for analysis by Scanning Electron Microscopy (SEM). The 0.3 – 1.0 μm particles were collected in size fractions using cascade impactors for Ion Beam Analysis and principal component analysis. The <300 nm particles were collected using the TSI Nanometer Aerosol Sampler (NAS) for Transmission Electron Microscopy (TEM) with Energy Dispersive X-Ray (EDX) analysis.

The NAS allows charged particles to be sampled onto substrates (e.g. TEM grids) for further analysis. It consists of a sampling chamber with an electrode at the bottom and a pump that draws airborne particles into the chamber. An electric field (of up to 10,000 V) between the chamber and the electrode focuses the charged particles onto the substrate. In this study, the authors placed a cascade impactor (to remove larger particles) and a charge neutraliser before the NAS. Particles were collected onto TEM grids consisting of a thin film of formvar. The TEM images provided information on types (shape, size) of particles and agglomerates, while EDX gave qualitative chemical analysis.

Tantra et al (2008) have reviewed the analytical techniques available and potentially suitable for the detection of CNTs and discussed some of the challenges in the measurement of CNTs in the workplace [19]. These techniques are microscopic techniques (electron microscopy, scanning probe microscopy) and spectroscopic techniques (Raman spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, fluorescence spectroscopy and Terahertz spectroscopy).

A few papers reported development of non-conventional instruments. Bogan et al (2008) reported on a technique for introducing single particles in free flight into a pulsed X-ray beam for substrate free imaging [20]. They demonstrated an imaging resolution of better than 40 nm by diffractive imaging (or lensless imaging) with single X-ray pulse. The intercepted particles can further be characterised for chemical composition using a time-of-flight mass spectrometer (TOFMS).

Standards and generation of airborne nanoparticles

It is important that the performance and detection limits of instruments used in workplaces for assessing exposure to airborne engineered nanoparticles are investigated. There is a need to generate stable and reproducible, well-characterised nanoparticle aerosols in the laboratory environment for the calibration and testing of instruments measuring airborne nanoparticles. An interesting paper highlighted below has been published:

A reference number concentration generator for ultrafine aerosols based on Brownian coagulation. Koch, Pohlmann and Schwarz (2008) [21]

The authors have developed a system delivering a reference number concentration (calibration source) to calibrate instruments and set-ups for the measurement of number

concentrations of ultrafine or nanoparticles. This system is based on aerosol formation by homogenous nucleation of an organic vapour and particle growth by Brownian coagulation. The authors designed a system able to generate predictable reference number concentrations (from 10^6 to 10^7 particles/cm^3) of liquid nanoparticles with a high degree of reproducibility. The system can be used for calibration during a limited period of time (100s to 1000s).

1.3 Control

Control plays a crucial part in the protection of workers' health. Legislation requires the hazards and risks to be controlled. If it is not practicable to eliminate the risks, then the risks need to be reduced through substitution or engineering controls, the last level of control being the provision of personal protective equipment (PPE). As observed in the previous bulletins, very few articles on the performance of engineering control for nanoparticles have been published. The current search did not identify any papers on engineering control, but a few studies on performance efficiency of filters and protective clothing have been published.

Engineering control

This search did not retrieve any paper on engineering control of nanoparticles.

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. A few papers have been identified. However no studies on 'face-fit testing' of respirators have been retrieved from this search.

Nanoparticle penetration through NIOSH approved N95 filtering facepiece respirators. Rengasamy, Verbofsky, King, Shaffer (2007) [22]

The authors carried out penetration measurements on five commercially available, NIOSH approved N95 filtering-facepiece respirator models (half-masks) using two test methods:

- polydisperse aerosol test method (using TSI 8130 automated filter test, which is similar to the NIOSH respirator certification test)
- monodisperse NaCl aerosol test method (using TSI 3160 fractional efficiency tester).

For the monodisperse aerosol test method, data were collected using NaCl particles in the size range of 20-400 nm and a flow rate of 85 L/min.

The authors found that:

- The average initial particle penetration levels measured for the five respirators, using the polydisperse aerosol test method, ranged from 0.61% to 1.24%.
- The most penetrating particle size was around 40 nm.
- The mean particle penetration levels measured for the 40 nm monodisperse aerosol ranged from 1.4 to 5.2%. The maximum penetration reported in papers occurs in the range 100-400 nm for most fibrous filters, but the authors noted that it can vary depending on filter types and characteristics as well as experimental conditions (e.g. flow rates, particle charges).
- The rank of ordering of the filtration performances of the five respirators was consistent between the two test methods.

Nanosafe2 (EU funded project) has published a dissemination report on **the efficiency of fibrous filters and personal protective equipments against aerosols [23]**. The key messages are:

- ***"Fibrous filters are even more efficient for nanoparticles"***. The maximum penetrating particle size is around 150 – 300 nm. Wang and Kasper had predicted a limitation in the efficiency of filtration of fibrous filters for nanoparticles by thermal rebound effect but it has not yet been verified for particle diameters above 2 nm.
- ***"HEPA filters, respirator cartridges and fibrous filters for masks are more efficient for nanoparticles".*** The Nanosafe2 consortium performed tests on HEPA filters at a face velocity of 9.6 cm/s using graphite nanoparticles. They found that the nanoparticle aerosol penetration rates decreased considerably with particle size.

Boskovic et al (2007) carried out a further study to understand why the filter efficiency may be different for the removal of nanoparticles of different shape but of the same electrical mobility (e.g. higher filtration efficiency for spherical polystyrene latex (PSL) compared to cubic magnesium oxide (MgO)) [24]. They investigated the filtration of spherical PSL and cubic MgO in the size range of 50 – 300 nm for filtration efficiency of 10 – 20 cm/s on a polypropylene filter coated with mineral oil. They found similar filtration efficiencies for the particles of different shapes, concluding that the oil coating applied to the filter minimises the motion of particles along fibres of the filter after initial collision.

Nanofibrous filtering media: Filtration problems and solutions from tiny materials. Barhate and Ramakrishna (2008) [25]

The authors have reviewed nanofibrous filtering media. These filtering media, made of fibres of diameter ranging from 100 to 1000 nm have a low weight, high permeability and small pore size making them attractive for filtration applications and the collection of the most penetrating aerosol particles (reported in the literature to be between 100 and 500 nm for conventional filters). The recommended High Efficiency Particulate Air filters or H type filters for airborne particulate matters have a minimal removal efficiency of 99.97% of particles greater than or equal to 300 nm in diameter.

Personal protective clothing and gloves

Personal protective clothing and gloves are used to protect workers from skin contact to chemical substances or dust. It is important that the penetration of clothing materials and gloves is tested for nanoparticle aerosols.

The key messages of the Nanosafe2 (EU funded project) dissemination report on **the efficiency of fibrous filters and personal protective equipments against aerosols [23]** are:

- ***"Non-woven fabrics seem much more efficient (airtight materials) against nanoparticle penetration. Avoid the use of protective clothing made of cotton fabrics"***. The Nanosafe2 consortium carried out protective clothing performance tests using methods with airflow and without airflow. Using the through diffusion cell and graphite nanoparticles (30 and 80 nm), the diffusion coefficient was smaller for non-woven Tychem and non-woven Tyvek (<1-2 10^{-7} m^2/s) compared to cotton (>2.5 10^{-6} m^2/s for 80 nm nanoparticles) or paper (~1.8 10^{-6} m^2/s for 30 nm nanoparticles).
- ***"Warning: Nanoparticles may penetrate through commercially available gloves! Use at least two gloves."*** Some nitrile, latex, neoprene and vinyl gloves were tested. Using the through diffusion method and graphite nanoparticles, the consortium found that:

- The 80 and 30 nm particles diffused through the gloves.
- The 80 nm particles diffused more readily through gloves than the 30 nm particles. For 80 nm nanoparticles, the diffusion coefficient ranged from <1 10^{-11} to ~ 4 10^{-11} m^2/s.

1.4 Characterisation

Characterisation of bulk nanomaterials

Generation of nanoparticles

For inhalation toxicology studies, it is important that reproducible and stable aerosols of defined particle size distribution and concentration are generated for the duration of exposure. This can be very challenging. A few papers addressing this issue have been published:

Synthesis of nanoparticles in a flame aerosol reactor with independent and strict control of their size, crystal phase and morphology. Jiang, Chen, Biswas (2007) [26]

The authors have developed a flame aerosol reactor (FLAR) system to produce nanoparticles with controlled physicochemical characteristics including size, crystal, structure, morphology and degree of agglomeration in quantities of about 10 mg/hour for biological effect studies. The methodology was demonstrated for TiO_2.

Guo et al (2007) synthesized iron oxide nanoparticles for health effects studies in a H_2/air diffusion flame [27]. The authors measured two size modes in the particles. The large size mode contained crystalline, non-agglomerated particles with a median diameter of ~ 45 nm; the small size mode contained mostly amorphous particles with a size range of 3 to 8 nm. The nanoparticles were characterised by TEM, X-ray diffraction, surface area measurement, inductively coupled plasma mass spectroscopy and a spectrophotometric speciation method.

Smart et al (2007) investigated the effectiveness of shortening double-walled carbon nanotubes (CNT) by high-energy ball milling for polymer nanocomposite and toxicology studies [28]. Ball milling increased amorphous carbon content and severely damaged the CNTs when the process time was greater than 4 minutes. Their characterisation included TEM, high resolution TEM (to observe the structure, morphology, side wall damage and to estimate diameters), SEM (to characterise the morphology), Raman (to investigate the diameter and types of nanotube), thermogravic analysis (TGA) (to quantitatively determine residual metal catalysts levels) and X-Ray photoelectron spectroscopy (to determine the oxidation of the DWCNT side walls and measure quantitatively the amount of different carbonaceous species).

Characterisation of nanoparticles in their bulk form, in fluids or biological tissues

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 characterisation of nanoparticles in their bulk form, in fluids (biological or water / solvent) or for toxicological evaluation.

Does nanoparticle activity depend upon size and crystal phase? Jiang, Oberdorster, Elder, Gelein, Mercer and Biswas (2008) [29]

The authors reported on a method to investigate the dependence of the physicochemical properties of TiO_2 nanoparticles on their reactive oxidant species (ROS) generating

capabilities. The TiO_2 nanoparticles were produced by well controlled aerosol routes using several gas phase synthesis methods. The physicochemical characteristics analysed were:

- Particle mobility size distributions measured during the synthesis process using a SMPS;
- Morphology and primary size distribution measured by TEM and SEM after collection on a filter;
- Crystallite size, crystal structure and weight fraction of the different crystal phases measured by X-Ray diffraction;
- Specific surface area and equivalent particle diameter measured by BET.

Ziegler et al (2007) reported a method for the length measurement of single-walled carbon nanotubes (SWCNTs) [30]. The functionalisation of SWCNTs with dodecyl chains via a Birch reaction provided a high dispersion of the nanotubes in chloroform. The suspension was mildly sonicated and spin-coated onto freshly cleaved mica substrates. The nanotubes were visualised by atomic force microscopy (AFM) and the length measured using software, capable of differentiating between individual nanotubes, nanotube ropes and particles, with minimum user intervention.

Microscopy is an important tool for characterisation. It can be used to localise nanoparticles in tissues and cells, and to investigate how nanoparticles enter cells and their fate after uptake. A number of papers in this area have been selected for this bulletin:

Visualization and quantitative analysis of nanoparticles in the respiratory tract by transmission electron microscopy. Muhlfeld, Rothen-Rutishauser, Vanhecke, Blank, Gehr, Ochs (2007) [31]

The authors reported on advanced TEM for the visualisation and quantitative analysis of nanoparticles in tissues and cells. They reviewed the applicability, advantages and disadvantages of:

- Preparation methods:

  Chemical fixation

  Physical fixation

- TEM techniques:

Conventional TEM. With very few exceptions, conventional TEM should be used with other methods to identify nanoparticles and to prevent technical bias (cellular structures being mistaken for nanoparticles and vice versa).

Immuno TEM offers visualisation, co-localisation and quantification of nanoparticles and antigens.

Energy filtered TEM combines high resolution TEM with analytical electron energy loss spectroscopy (EELS) and imaging. It offers elemental mapping and the possibility to distinguish unambiguously between cellular structures and nanoparticles.

Electron tomography is an emerging technique that allows the analysis of nanoparticle shape, volume and surface in 3D. It has been recently applied to study the interactions of nanoparticles with cellular structures. It is anticipated that this technique will be useful to study contact sites between nanoparticles and macromolecules.

The authors also discussed:

- The development of approaches to correlate light and electron microscopy (e.g. combining live cell microscopy with HRTEM).

- Stereological methods for the quantitative analysis of the distribution of nanoparticles in tissues and cells.

The search identified a few other papers reporting on techniques to characterise and visualise nanoparticle uptake into cells:

- Porter et al (2007) reported on the visualisation of individual SWCNTs in the cell using energy-filtered transmission electron microscopy (EFTEM) in combination with electron energy loss (EEL) spectrum imaging [32]. The authors found that this technique gave good image contrast without staining, which cannot be achieved using conventional imaging techniques. The authors also present a method for imaging intracellular SWCNTs by encapsulating silver iodide inside SWCNTs without changing their surface chemistry and visualising them using confocal microscopy.
- Rothen-Rutishauser et al (2007) [33] used microscopy techniques for the intracellular localisation of nanoparticles:
  - Energy filtering TEM (EFTEM) combined with electron energy loss spectroscopy (EELS) for the presence and intracellular localisation of gold particles coated with silver (25 nm) and TiO_2 (20 and 30 nm) nanoparticles.
  - Laser scanning microscopy microscopy combined with image restoration for the intracellular localisation of fluorescently labelled polystyrene particles (1 μm and 78 nm). The particles were counted using Diacount software and the quantitative distribution of the different particles among the different cells labelled with specific cell surface markers was compared using a contingency table analysis.
- Chiellini et al (2007) used confocal laser scanning microscopy to study the intracellular fate of bio-eliminable polymeric nanoparticles co-precipitated with fluorescein labelled human serum albumin [34].
- Kwon et al (2008) used Magnetic Resonance Imaging (MRI) and Confocal Laser Scanning Microscopy (CLSM) techniques to study the distribution of 50 nm inhaled fluorescent magnetic nanoparticles in mice [35]. For fluorescent image analysis, the nanoparticles were visualised in slides of fixed tissues from organs. MRI images were obtained from the brains of the mice.
- Choi et al (2007) reported on the synthesis of perylene diimide dye for use as a fingerprint detection powder and absorbed onto TiO_2 nanoparticles [36]. This dye could be used for live cell imaging.

An interesting paper describing a quantitative microscopic method for the evaluation of particle distributions in tissues and cells has been retrieved from the searches:

A novel quantitative method for analyzing the distributions of nanoparticles between different tissue and intracellular compartments. Muhlfeld, Mayhew, Gehr, Rothen Rutishauser (2007) [37]

The authors described a quantitative microscopic method for the evaluation of particle distributions within sectional images of tissues and cells. The method is based on counting the number of particles associated with a tissue or intracellular compartment and a size estimation of each of these compartments. A relative deposition index is then derived which indicates whether a compartment contains more (RDI>1), the same (RDI=1) or less particles (RDI<1) than would be expected from its compartment size. The total chi-squared value can then indicate whether the observed distribution is random or not, and the partial chi-squared values can help to identify the compartments that are preferential targets of particles.

A number of papers on other techniques have been reported including Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for the quantitative detection of metal quantum dots in tissues and organs of animals after exposure to nanoparticles [38] [39] [40].

Isaacson et al (2007) have coupled quantitative liquid-liquid extraction with liquid chromatography electrospray ionization mass spectrometry for the quantitative determination of fullerenes (C60 to C98) in water and uptake of C60 by embryonic zebrafish [41]. The authors claimed that the quantification limit of this method was 0.4 µg/l and the detection limit was 0.02 µg/l.

Nanoparticles tend to agglomerate and clump in solutions. Inadequate dispersion and unsatisfactory characterisation of nanoparticles in liquid for *in vivo* and *in vitro* experiments may lead to inaccurate toxicity assessments. The searches identified several papers reporting on dispersion media and techniques to characterise nanoparticle agglomeration in solution, including:

- Deguchi et al (2007) have studied the dispersion stability of C60 nanoparticles in a simulated physiological environment using dynamic light scattering (DLS) [42]. It was noticed that DLS has power limitations to resolve multi-modal particle size distributions. Couteau and Roebben have found that DLS is not able to resolve modes with a diameter ratio lower than 4 [43]. DLS was also used to observe the adsorption of proteins molecules at the surface of C60 nanoparticles.
- Liu et al (2007) reported a rapid and high efficiency capillary electrophoresis based method for determining the size of gold nanoparticles (between 5 and 60 nm) in solution [44].
- Elgrabli et al (2007) proposed a method to disperse carbon nanotubes (CNTs) for toxicological studies using a saline solution containing albumin [45].

Protein adsorption on nanoparticles will influence their uptake by cells. A few papers investigating protein adsorption on the surface of nanoparticles using techniques such as DLS or UV-visible spectroscopy have been published [46] [42].

1.5 Bibliography of key papers

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2. HEALTH EFFECTS

The publications retrieved by the health effects searches in the four months from November 2007 to February 2008 showed a broadly similar pattern of distribution amongst the different topics to previous bulletins. Many of the primary publications described effects of engineered nanoparticles in *in vitro* systems (28% of the total) (Figure 2), almost equal numbers of papers describing the effects of nanoparticles on human cells (13%) and animal cells (14%) grown *in vitro*. The proportion of publications (9%) describing the effects of engineered nanoparticles in animals was smaller than in previous bulletins. A large number of reviews (57%) was retrieved by the health effects searches for this period.

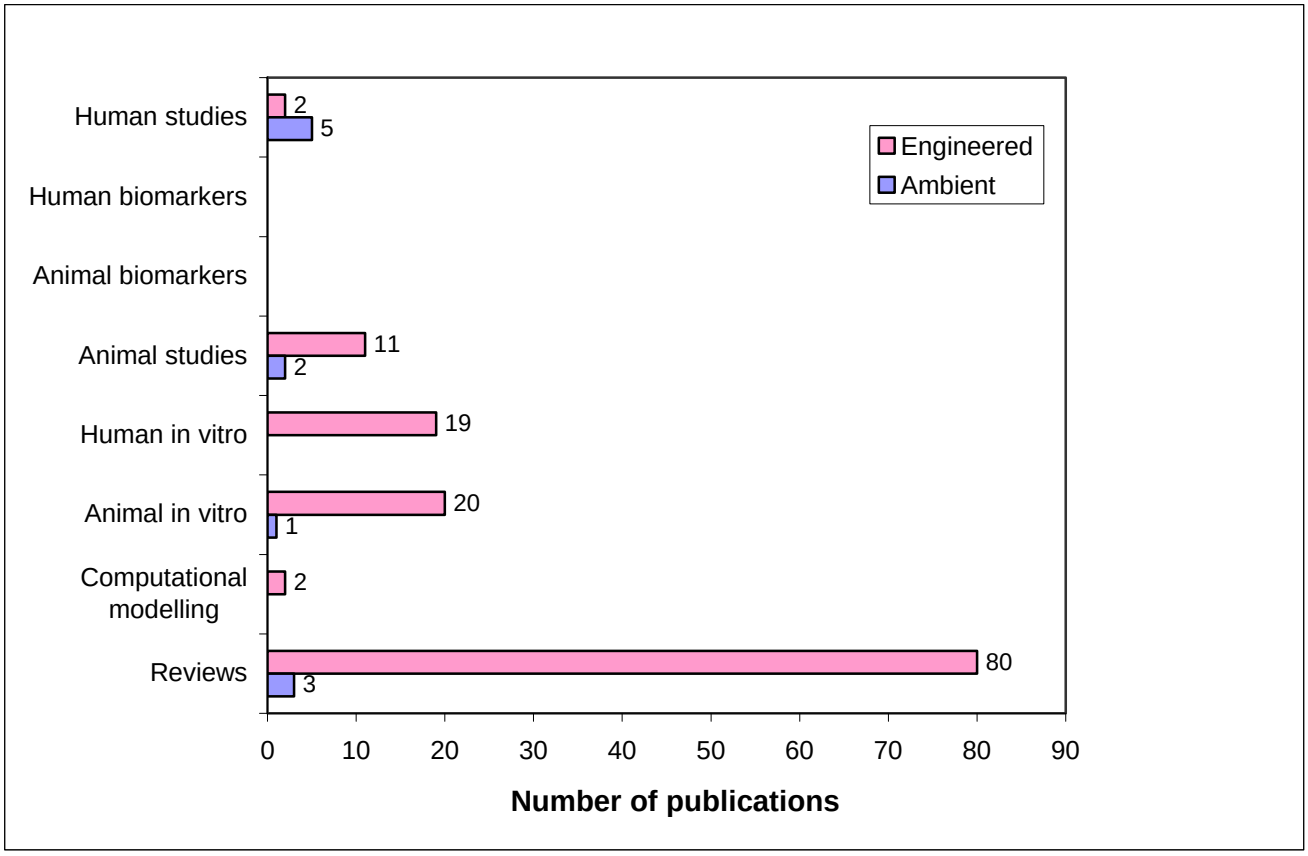

Figure 2: Breakdown per topic of the numbers of publications retrieved in the four months from November 2007 to February 2008 on the human health effects of *ambient* and *engineered* nanoparticles.

2.1 Human studies and epidemiology

The searches retrieved two and five studies of the potential human health effects of engineered and ambient nanoparticles respectively. The first of the two studies which analysed the effects of engineered nanoparticles summarises the conclusions from IARC on the carcinogenic hazards of inhaled particles of carbon black, titanium dioxide and talc, which is of less relevance to this bulletin. Although the particle size of these chemicals is not restricted to nanoscale, IARC's considerations are nevertheless relevant to this bulletin, since in previous NanoAlert issues the potential carcinogenicity of carbon black and titanium dioxide has been discussed, including both epidemiological and animal studies.

Carcinogenic hazards from inhaled carbon black, titanium dioxide, and talc not containing asbestos or asbestiform fibers: recent evaluations by an IARC Monographs Working Group. Baan (2007) [1]

IARC has reviewed the data on the carcinogenic hazards presented to humans by exposure to titanium dioxide and carbon black (and talc, which will not be considered here), and their conclusions will be published in volume 93 of the IARC Monographs Series. Although epidemiological studies of both chemicals provide inadequate evidence of carcinogenicity, there is sufficient evidence in rodent experiments. Therefore the **IARC Working Group concluded that carbon black and titanium dioxide are possibly carcinogenic to humans, Group 2B**. Interestingly, the Working Group noted that impairment of lung clearance of inhaled particles, leading to their accumulation, with ensuing inflammation, cell injury, fibrosis and potentially cancer, may occur in both laboratory animals and in humans in dusty workplaces, so that the data from the former are relevant to humans.

The second study of relevance to the potential human health effects of engineered nanoparticles considered possible sources of worker exposure to carbon nanofibres, and has been considered in the Exposure section of this issue of NanoAlert [2].

The five human studies on ambient particles are less relevant for this bulletin and are therefore only summarised briefly. Two studies have examined the effects of exposure of volunteers to ambient nanoparticles, and are both consistent with the proposal that inhalation of ambient ultrafine particles is associated with adverse health effects. Frampton (2007) demonstrated that ultrafine particles (<50 nm diameter) deposit efficiently in the respiratory tract, and deposition is enhanced by exercise or asthma; the changes observed in blood leukocytes and pulmonary gas diffusion are best explained by effects on pulmonary vascular function [3]. Rundell and co-workers have shown that inhalation of high levels of ultrafine particles during exercise in non-asthmatic males leads to decreased lung function and changes in exhaled breath condensate (specifically total nitrate was decreased by 43%) [4]. Furthermore, Samet et al (2007) analysed cardiovascular endpoints and multiple markers in bronchoalveolar lavage fluid and blood from healthy volunteers who were exposed to concentrated ambient particulate matter. They reported modest size-dependent effects on cardiovascular, pulmonary and haematological parameters [5]. Traffic-related air pollution (not specifically particulate matter) has been independently associated with mortality, although the relative risks for cardiovascular, respiratory or lung cancer mortality were generally small [6]. Similarly, Schwartz and co-workers conclude from their analysis of the dose-response relationship between urban airborne particles and survival that a reduction in particle concentrations below current US Environmental Protection Agency (EPA) standards would increase life expectancy [7].

2.2 Animal *in vivo* studies

Thirteen studies of the effects of nanoparticles in experimental animals were identified in the searches. Of these, six papers considered the potential effects of carbon nanotubes (CNTs). These papers build on the growing literature on the potential health effects of CNTs, which in summary to date, suggest that administration of these novel structures to animals by inhalation-type routes of delivery lead to pulmonary inflammation, fibrosis and the formation of granulomas.

There has been some debate in the field over the interpretation and relevance of experimental data generated by instillation of nanoparticles into animals versus inhalation, and the first paper highlighted here reports a preliminary comparison of the two routes of administration:

Comparative study of pathological lesions induced by multiwalled carbon nanotubes in lungs of mice by intratracheal instillation and inhalation. Li et al (2007) [8]

When 0.05 mg multi-walled CNTs (MWCNTs) were instilled into mice, accumulation of clumps of the CNTs in the bronchi and alveoli was accompanied by inflammation and damage to the alveoli. In contrast, when mice inhaled an aerosol of MWCNTs (32.6 mg/m^3, deposition dose 0.07, 0.14 or 0.21 mg over 8, 16 or 24 days), more aggregates of CNTs were observed in the alveoli than in the bronchi, and although there was proliferation and thickening of the alveolar walls, there was less damage than observed following instillation. **The authors suggest that the differences in lung lesions may arise due to differences in the size of the aggregates of CNTs and how they deposit in the respiratory tract in response to instillation or inhalation.**

Methods for dispersion of nanoparticles for both animal and *in vitro* experiments vary, a variety of vehicles currently being used: cell culture medium or phosphate buffered saline (PBS), with detergents (e.g. Tween), solvents (e.g. tetrahydrofuran) or proteins (e.g. serum) added to promote dispersion. However, although the issue of protein adsorption of nanoparticles is being investigated and relevant publications have been summarized in previous issues of NanoAlert, there have been few systematic evaluations of the effects of different media on the outcome of the experiments. Two reports have now addressed this important issue, which may significantly affect experimental results, for carbon nanoparticles:

A comparison of dispersing media for various engineered carbon nanoparticles. Buford et al (2007) [9]

A variety of different carbon nanoparticles (fullerenes, single- (SW) and multi-walled (MW) CNTs) from different commercial sources were dispersed (at 5 mg/ml) in seven vehicles:

- cell culture medium + 10% foetal calf serum (FCS)
- 100% FCS (± delipidation)
- phosphate buffered saline (PBS)
- PBS + 7.5% bovine serum albumin (BSA)
- PBS + 1% Tween 80
- 100% DMSO

The extent of dispersion was assessed by light microscopy (400x). Although the median size area of the particles in the different vehicles was quite consistent, the area of the largest agglomerates varied widely, according to type and source of the particle and the vehicle. The 'worst' vehicles for dispersion of all the particles were PBS alone, PBS + 1% Tween 80, and 100% DMSO. The effects of two dispersants (PBS versus 100% FCS) were compared following instillation into mice, by histological comparison of the lungs after 24h and 7 days. The SWCNTs were better dispersed in the lungs using FCS, consistent with the observation that this vehicle was one of the most effective *in vitro*; the particles dispersed in FCS also persisted in the lung tissue for 7 days, at which time, there were signs of sustained inflammation.

The authors conclude that complete dispersion is not practical. Although SWCNTs and MWCNTs are more prone to agglomeration than fullerenes, the most effective vehicles for dispersion contain protein and/or lipids.

Effect of BSA on carbon nanotube dispersion for *in vivo* and *in vitro* studies. Elgrabli et al (2007) [10]

The second paper compared dispersion of SWCNTs and MWCNTs from different commercial sources by sonication in saline ± BSA, FCS or Tween 80. When assessed by optical microscopy, dispersion was greatest in medium containing BSA, in which suspensions were more homogeneous with smaller agglomerates.

When the cytotoxicity of the CNTs suspended in saline ± BSA was analysed *in vitro* (using respiratory epithelial cells, A549, or myeloid cells, U937), the results were more reproducible (smaller standard deviations) in the presence of the BSA. The authors suggest this is due to the greater homogeneity of the suspension; the presence of the BSA did not affect the overall cytotoxicity.

Rats were intratracheally instilled with MWCNTs suspended in saline ± BSA; after 24h, the type of cells and amount of protein in the bronchoalveolar lavage (BAL) fluid were not different between the two vehicles, but inclusion of BSA increased both the number of cells in BAL fluid induced by the CNTs, and the percentage of CNT-containing cells, suggesting that better dispersion of the CNTs promotes phagocytosis. There were no significant alterations in lung structure between the different preparations of CNTs.

The authors of both studies conclude that the type of vehicle can significantly influence experimental outcomes. Acid functionalization (which derivatizes the carbon backbone with carboxylic acid and sulfonate groups) can also increase dispersion of SWCNTs, and Saxena et al (2007) have compared the biological effects of normal and acid functionalized SWCNTs, *in vivo* and *vitro*:

Enhanced *in vitro* and *in vivo* toxicity of poly-dispersed acid-functionalized single-wall carbon nanotubes. Saxena et al (2007) [11]

In LA4 lung epithelial cells, acid-functionalised SWCNTs (AF-SWCNTs; 0-50 μg/ml) caused significantly greater cytotoxicity than SWCNTs (or controls), the numbers of viable cells remaining after 4 days being reduced by 85%, compared to 35% for SWCNTs. This could be explained by reduced cell proliferation or increased cell death. Cell cycle analysis showed that the AF-SWCNTs inhibited proliferation more than SWCNTs, whilst both types of CNTs induced approximately equal amounts of apoptosis. If the AF-SWCNTs were pre-treated with poly-L-lysine to neutralize the negative charge (and promote re-aggregation), the differences between the two CNTs were reduced.

When administered to mice via pharyngeal aspiration, the AF-SWCNTs induced a stronger inflammatory response, in terms of BAL cell count, protein concentration and cytokine levels after 18h, although the differences between the two CNTs were reduced after 72h.

Three further publications have considered the effects of different vehicles on *in vitro* experimental results. Deguchi et al (2007) have examined stabilization of C60 fullerenes using human serum albumin (HSA), and found that in PBS, fullerenes coagulated and precipitated rapidly, but >1 mg/ml HSA prevented this [12]. Dynamic light scattering showed that the HSA forms a protective layer on the nanoparticles, preventing the salt-induced coagulation. Generation of reactive oxygen species (ROS), depolarization of mitochondria and necrosis of cells *in vitro* in response to C60 fullerenes can be ranked according to the suspension medium: tetrahydrofuran > ethanol > water [13]. Similarly, in a cell-free system, formation of reactive species was greater when 14 nm carbon black particles were suspended in 0.025% dipalmitoyl phosphatidyl choline (DPPC) than saline or 1% BSA, whilst in cells, ROS production was higher when nanoparticles were suspended in medium containing BSA and/or DPPC [14].

Two reports retrieved in the latest searches have investigated the distribution of CNTs in mice following injection: Wang et al (2008) injected ^{131}iodine-labelled hydroxylated SWCNTs

into mice and analysed their distribution over the course of the following hour [15]. Within 2 min, radioactivity was detected throughout the body (except for the brain), with the greatest levels observed in liver, kidneys, stomach and lungs. In the second paper, radiolabelled MWCNTs, modified with glucosamine, were injected into mice, and the resultant distribution also suggested that the CNTs moved readily throughout the body [16].

A further five papers have examined the effects of engineered nanoparticles other than CNTs in experimental animals, with two examining the effects of ambient ultrafine particles *in vivo*. The first paper has examined the effects *in vivo* of synthetic ultrafine amorphous silica:

Inflammatory mediators induced by intratracheal instillation of ultrafine amorphous silica particles. Cho et al (2007) [17]

The pulmonary toxicity of crystalline silica has been studied, but there is less information on the toxicity of amorphous silica, especially ultrafine amorphous silica (UFAS). In this study, 0, 2, 10 and 50 mg/kg of UFAS, with a primary particle size of 14 nm and resuspended in PBS, were intratracheally instilled into mice. After 24h and 1 week, there was a significant increase in absolute and relative lung weights of the animals treated with 50 mg/kg. All doses of UFAS caused increases in the number of cells (specifically macrophages and neutrophils) in the BAL after 24h and 1 week. Histopathological analysis revealed acute inflammation after 24h, which was dose-dependent in severity and characterized by e.g. neutrophil infiltration and thickening of the alveolar walls. After 1 and 4 weeks, there was chronic inflammation, with macrophage infiltration and type II cell hyperplasia, and some fibrosis. By 14 weeks, the lung injury score had returned to control levels. Levels of messenger RNA and protein for a number of cytokines/chemokines were elevated in response to the UFAS after 24h and 1 week (IL-1β, IL-6, IL-8, TNFα, MCP-1 and MIP-2).

The authors conclude that instillation of UFAS induced transient but severe lung inflammation, accompanied by up-regulation of pro-inflammatory cytokines and chemokines. They suggest that the transient nature of the inflammatory response in response to ultrafine particles is due to the translocation of the particles from the site of deposition; the fate and consequences of this are yet to be conclusively investigated.

Two papers have investigated the effects of oral administration of nanoparticles in mice. Cha and Myung (2007) reported non-specific haemorrhage, lymphocyte infiltration and medullary congestion in mice after oral administration of either micron-sized or nanoparticles of zinc (300 nm), iron (100 nm) and silicon (10-20, 40-50, 90-110 nm), suggesting no differences in toxicity between the different sizes of particle [18]. The authors noted decreases in mitochondrial activity and DNA content, and increases in glutathione production in response to the particles in cells grown *in vitro*. Meng et al (2007) have compared the effects when mice were fed nano- and micron-scale copper (23.5 nm versus 17 μm) or copper ions [19]. The nano-copper induced swelling of the stomach after 24h, and accumulation of copper in the kidneys after 24 and 72h; copper ions similarly accumulated in the kidneys after 24h, but by 72h, the levels in the kidneys had decreased almost to control levels. Biochemical analysis revealed similar changes in serum and urine copper levels in response to nano-copper or copper ions. Hoshino et al (2007) have investigated the toxicity of quantum dots (QD) both *in vivo* and *vitro*, and suggest that the biological effects of the QD may result from the capping materials rather than the core constituents [20]. The final paper described a new dynamic system for continuously generating a mass concentration as high as 2μg/L of nanoparticles (with a median diameter of 40-60 nm) for nasal delivery, by using an inline tube furnace for flash vaporizing micron-sized particles fed from an aerosol generator [21].

Two papers have investigated the effects of ambient ultrafine particles in animals. In brief, Nemmar and Inuwa (2008) reported that intravenous administration of ultrafine diesel exhaust particles induced both pulmonary and systemic changes in rats [22], and Inoue et al (2007) demonstrated that pulmonary exposure to carbon nanoparticles can increase lung hyper-responsiveness, especially in the presence of antigen [23].

2.3 *In vitro* studies

There were 40 publications reporting the potential effects of nanoparticles in cultured cell systems *in vitro*, three of these having been already discussed in the context of dispersion techniques for nanoparticles (Section 2.2). Eight papers have considered carbon nanotubes, three describing experiments in animal cells and five in human cells; four publications have examined uptake of CNTs into human cells *in vitro*.

Building on previous work (noted in Issue 3, July 2007), Porter et al (2007) have reported results of transmission electron and confocal microscopies for imaging CNTs in human cells, as described in Section 1.4 above [24]. They noted that the CNTs entered both the cytoplasm and nuclei of cells, leading to dose-dependent cell death. Two papers have examined uptake of CNTs by human HeLa cells. Yehia et al (2007) demonstrated (i) dispersion of CNTs in cell culture medium supplemented with serum using a range of techniques (e.g. atomic force microscopy, absorption and Raman spectroscopies), and (ii) time- and temperature-dependent uptake by cells using confocal and microRaman spectroscopies [25]. Transmission electron microscopy showed that the CNTs were in intracellular vacuoles, but there were no differences from controls in terms of cell morphology, growth rates or superoxide levels in mitochondria, suggesting that the CNTs were not cytotoxic to the HeLa cells. Similar results were obtained by Chin et al (2007), who have shown that coating SWCNTs with an amphipathic helical peptide that they termed "nano-1", enhances cellular uptake of the CNTs, without affecting the rate of growth or death of the cells [26]. CNTs are also being explored for delivery of other agents into cells; the technique of "nanospearing" using CNTs has been shown not to cause cell activation or death, nor to change cell signalling in primary in *ex vivo* B lymphocytes [27].

In previous issues of NanoAlert, the potential for nanoparticles of titanium dioxide, silica and carbon (fullerenes) to induce genotoxicity has been considered (and a letter and new data relating to one of these studies has been published [28]). In the most recent health effects searches, six further papers were identified that explored the genotoxicity of nanoparticles and the interactions of CNTs with DNA. In the Ames bacterial reverse mutation test, water-soluble FePt nanoparticles capped with tetramethylammonium hydroxide were weakly positive in one *Salmonella typhimurium* strain (TA100, without metabolic activation), but negative in all the other tests (*S. typhimurium* strains TA98, 1535, 1537, *Escherchia coli* WP2uvrA/pKM101) [29]. The photo-clastogenicity of different titanium dioxide nanoparticles was tested in Chinese hamster ovary (CHO) cells, in the absence or presence of ultra-violet light [30]. At concentrations up to 5 mg/ml (if no cytotoxicity was observed) or at concentrations that induced 50% cytotoxicity, none of the TiO_2 nanoparticles induced chromosomal aberrations. Similarly, Jin et al (2007) reported no cytotoxic or genotoxic effects of silica nanoparticles *in vitro* [31]. These reports contrast with previously published work from Wang and coworkers (reported in Issues 2, May 2007 and 3, August 2007) that suggested genotoxicity of TiO_2 and SiO_2 nanoparticles in human lymphoblastoid cells. Genotoxicity has been reported by other groups: different sizes of cobalt ferrite nanoparticles (6 nm versus 10 or 120 μm) were evaluated in the micronucleus test using human peripheral lymphocytes over a dose range of 5-100 μM [32]. Both genotoxicity (increased micronuclei and decreased cytokinesis blocked proliferation index) and cytotoxicity were observed at lower doses (by mass) of the nanoparticles compared to the micron-sized particles (5 μM versus 50 μM). Zhu et al (2007) have shown that in mouse embryonic stem cells, MWCNTs caused apoptosis, and increased the DNA mutation frequency and the expression of several proteins involved in DNA repair [33]. Peng et al (2007) have shown that SWCNTs can accelerate S1 nuclease cleavage of human DNA [34].

Many papers have been published on the potential tissue engineering applications of nanomaterials; discussion of these reports is outside the scope of this bulletin, except where

they give useful insight into the potential health effects of the nanoparticles. In the latest searches, two contrasting papers on CNTs were identified. The first reported that MWCNTs containing minimal levels of catalytic metals supported long-term growth and migration of different types of cells, including skin fibroblasts, Schwann cells and neurons [35]. In contrast, the second paper reported that single-, double- and multi-walled CNTs reduced the viability of primary osteoblasts, and inhibited osteoblast and adipocyte differentiation, with or without being taken up into the cells [36].

Several groups of authors have chosen to use the human epithelial cell line, HeLa, as a model system for studying potential nanotoxicity. Adili et al (2008) have extended work on the cytotoxicity and genotoxicity of silica nanoparticles to examine the toxicity of silica nanowires [37]. They have used both HeLa and HEp-2, a human epithelial laryngeal cell line, and reported that whilst low concentrations were not cytotoxic, concentrations of 190 μg/ml and above induced significant death of both cell lines. Cell death primarily involved necrosis rather than apoptosis. Three other groups have used HeLa cells to examine uptake of nanoparticles (including superparamagnetic iron oxide, gold or poly(n-butylcyanoacrylate) nanoparticles) into cells, following translocation with techniques such as confocal laser scanning microscopy and inductively coupled plasma optical emission spectroscopy [38-40].

Many experiments either *in vivo* or *vitro* on the potential human health effects of nanoparticles have used mass as the dose descriptor, although work highlighted in previous issues of NanoAlert has suggested that surface area may be more appropriate. A study identified in the latest search has examined the pro-inflammatory effects of different sized particles (e.g. titanium dioxide, carbon black) in the lung epithelial cell line, A549, and provides further evidence that surface area may be the best dose-metric [41]. This cell line has also been used to study the effects of the zeta potential of cerium oxide nanoparticles on protein adsorption and cellular uptake; Patil et al (2007) have reported that positive zeta potential enhances BSA adsorption whilst negatively charged particles are preferentially taken up into the cells [42]. Gold nanoparticles can induce dose-dependent apoptotic cell death of A549 cells, but do not affect other cell lines such as BHK21 (baby hamster kidney) or HepG2 (human hepatocellular liver) cells [43]. Apoptosis of the human hepatoma cell line, Bel 7402, can be induced by cerium-doped titanium dioxide nanoparticles after photo-excitation [44].

Movement of fluorescent QDs into and within cells can be readily followed microscopically. Uptake of QDs into the capsule and cortex of the eye lens has been studied, the authors reporting that the nanoparticles did not penetrate through the intact lens capsule [45]. Nabiev et al (2007) report that in human blood-derived macrophages, non-functionalized QDs use cellular active transport systems to target specific intracellular locations, including the nucleus [46]. Similar conclusions have been reached by Ruan et al (2007), who showed that Tat-peptide conjugated QDs bind to negatively charged plasma membranes and are internalised by macropinocytosis. They are then trapped in vesicles that are actively transported within the cell by molecular motors to the perinuclear microtubule organising centre (MTOC) [47]. Uptake of QDs bound to DNA has also been demonstrated [48].

Of the papers that have used *in vitro* systems to analyse the potential human health effects of engineered nanoparticles, a further 15 papers which have not yet been mentioned in this bulletin were identified in the searches.

Two papers have investigated some of the different types of effects that nanoparticles might have on the respiratory tract. Yacobi et al (2007) have demonstrated that some nanoparticles (specifically ultrafine ambient particles, positively charged QDs and SWCNTs) can decrease the transmonolayer resistance of primary rat alveolar epithelial cells when added to the apical surface of the cells, whilst polystyrene nanoparticles or negatively charged QDs did not [49]. Bakashi et al (2008) present data suggesting that gold

nanoparticles can interact with and potentially sequester pulmonary surfactant phospholipids, which could affect surfactant function [50].

The effects of Degussa P25 TiO_2 have been investigated in rodent brain cultures. In mouse microglial cells (BV2), TiO_2 induced a rapid and prolonged release of ROS, with up-regulation of inflammatory, apoptotic and cell cycling pathways, and down-regulation of energy metabolism [51]. Whilst there was no cytotoxicity of P25 in purified neuronal cultures, despite increases in ATP and caspase activity, neuronal apoptosis was seen in primary co-cultures of different types of cells from rat striatum. These results suggest that P25 may damage neurons through microglial production of ROS.

Xiong et al (2007) have noted differences in the toxicity of nano- and micro-particles of silver in the mouse adipose cell line, L929 [52]: nanoparticles displayed cytotoxicity at concentrations of 50 μg/ml and above, whilst significant toxicity of micro-particles was observed only at ≥ 250 μg/ml.

The remaining papers have focussed on methodology, and some have therefore already been mentioned in the first section of this bulletin (section 1.4). Jiang et al (2008) have explored the relationship between the physicochemical properties of TiO_2 nanoparticles and their capacity to induce ROS [53]. They found that for a fixed total surface area, ROS-generation per unit surface area varied with particle size according to a S-shaped curve, rising between 10 and 30 nm to a plateau with the highest ROS generation occurring in response to particles of 30 nm. The hierarchy of the influence of crystal phase on ROS generation was amorphous > anatase > anatase/rutile mixtures > rutile.

Methods for quantification of nanoparticles in solution and in biological tissues are also discussed in Section 1.4, e.g. [54-56]. Of relevance to investigations of the health effects of nanoparticles is the observation by Isaacson et al (2007) that C60 fullerenes can adsorb to test vials, leading to reductions in concentrations by as much as 50% over 6 hours [54].

The cell viability of human H4 neuroglioma cells treated with metal oxide nanoparticles has been quantified using high content image analysis, classifying the cells' nuclei into bright, dark or background categories [57]. The final three papers in this area have focussed on visualising gold nanoparticles in biological samples using e.g. quantitative scanning transmission electron microscopy [58-60].

2.4 Computational modelling

Two papers were retrieved by the searches that focussed on mathematical modelling of deposition of particles in the respiratory tract. In the first paper, a computational fluid and particle dynamics model was used to investigate the effects of different types of airway obstructions [61]. Blockages, constriction or sidewall tumours significantly affected deposition patterns. Sidewall and central tumours can increase deposition of larger particles, whilst only sidewall tumours decrease deposition of nanoparticles. Deposition in constricted airways is greater than in healthy airways.

Wang and Friedlander (2007) propose a method for using data on nanoparticle size distributions obtained from a differential mobility analyser (DMA) to calculate deposition in the respiratory tract [62].

2.5 Reviews

A large number of reviews were identified in the searches (83), an increase again on each of the previous bulletins, and these will only be summarised very briefly here. Considering this large and rapidly growing area, the technical structure of the nanotechnology literature has

been investigated [63], and the Nanoparticle Information Library, a mechanism for sharing and linking data, described [64].

Three reviews have been published on the uses of nanoparticles, focussing on QDs [65; 66] and water treatment [67]. Fifteen reviews have considered nanotechnology generally and its implications [68-83].

The potential health effects of nanoparticles have been the subject of eight reviews [84-91], and three meeting reports [92-94]. Issues surrounding the use of nanoparticles in skin preparations is discussed by Draelos (2007) [95]. Of the potential mechanisms of action of nanoparticles in living systems, Zabirnyk et al (2007) have reviewed the role of autophagy in cellular uptake of nanoparticles [96], and the key role of oxidative stress has been explored in four reviews [97-100]. Furthermore, a workshop of leading experts on nanotoxicology has concluded that *in vitro* cellular methods have an important role to play in screening for toxicity by oxidative stress [101]. Muhlfield et al (2008) have focussed on the methodological differences between studies of combustion-generated and engineered nanoparticles [102].

One of the conclusions of an expert workshop on nanoparticle hazard assessment was that a standard set of particles needs to be available for validation and tests benchmarked [103]. The American National Institute of Standards and Technology (NIST) has recently announced its first reference standards for nanoparticles: citrate-stabilised nanosized gold particles in a colloidal suspension, which have been extensively characterised, and are targeted for biomedical research [104].

Approaches for hazard and risk assessment of nanomaterials have been reviewed [105-107], Moore focussing on nanomedicine and risk [108; 109]. Principles to guide environmental health and safety decisions in nanotechnology research have been published [110]. One interesting paper has considered how the risk frames of scientists differ according to whether they are involved in developing new nanotechnologies or assessing their likely health effects [111], two other reviews comparing the views of scientists and the general public on the potential risks posed by nanomaterials [112; 113].

Regulatory approaches have been the subject of seven reviews [114-119]. Roco (2008) has proposed that a global approach to governance of emerging technologies such as nanotechnology is required [120].

Approaches for discussing issues around nanotechnology with the public are considered in six reviews [121-126], and Wilkinson et al (2007) have discussed how nanotechnology has been portrayed by the British press [127]. How individuals and society handle new technology is considered by Mordini (2007) [128]. Ten reviews have considered the societal and ethical implications of nanotechnology [129-138].

There have been three reports on funding programs for nanotechnology-related issues [139-141].

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