Health & Safety Executive NanoAlert Service

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Bulletin Contents:

1. BACKGROUND TO THE SERVICE

1.1 Introduction: This bulletin service is designed to provide an overview of publications of studies that have examined the exposure and potential health effects of nanomaterials. The focus of the service is human exposure and health effects, particularly in the occupational setting. Inevitably there will be some overlap between studies of exposure of other groups (i.e. consumers). However, as the nanotechnology literature is expanding rapidly, the literature search results have been screened to ensure that the studies listed are relevant to HSE and its responsibility to manage health and safety in the workplace.

This is not a review service, but will provide an overview of the evidence in peer reviewed scientific papers. The bulletins will summarise the range of papers, which have been published in two areas of interest:

- Measurement, characterisation and control of exposure to nanoparticles
- Potential for toxic effects of nanoparticles in humans

1.2 Summary of literature published in 2000-2006: This is the first HSE NanoAlert bulletin and contains relevant publications from the previous six years. Each subsequent bulletin will summarise publications from the previous four-month period. In this bulletin, the databases used in the searches, and criteria employed to screen publications and select those that are most relevant will be explained. In future bulletins, this strategy will be available on a website and via an electronic link.

1.3 Description of the search strategy: The terminology used in the nanomaterials field has rapidly become very complex. To ensure that a comprehensive search of the literature was conducted, HSE Information Service staff, who are experienced in interrogating databases, were asked to develop an appropriate search strategy. Following discussions between HSL NanoAlert and HSE Information Service staff, a comprehensive list of subject terms (Appendix 5.1) was developed and employed in specific combinations in selected databases (physical and chemical material sciences; biological and health sciences; occupational health and safety literature; public access / government databases). Sample abstracts of references were examined and the search terms refined to exclude irrelevant literature, since the initial searches had generated an unmanageably large number of hits (e.g. 46,935). A final set of search terms was identified and references for each topic area retrieved (totalling ~1300 in the area of measurement, characterisation and control of exposure and >500 for the potential toxic effects of nanoparticles for humans).

1.4 Sifting of publications: The abstracts of all the references retrieved (>1800) were screened, using specific criteria (Appendix 5.2) and focusing on *engineered* nanoparticles, to shortlist the most relevant publications. These references were then examined in more detail. The bulletin is an overview of all of the relevant references with more detailed summaries of some key studies, followed by a bibliography listing the pertinent literature. In some circumstances, the bibliographies will not represent the entirety of the literature identified. For example, as the number of nanomaterial publications increase, the summary of toxicity studies may list only those that are directly relevant to human health, but publications considering the health effects of nanomaterials in other organisms will be retained in bibliographic databases. Readers wishing to explore other areas of the literature

should contact HSL staff (Section 4), who can interrogate the databases to identify the appropriate references.

1.5 Criteria for screening the search results and identifying relevant papers: The criteria used to screen the search results were developed following discussion between HSL and HSE staff with expertise in these subjects. They are listed in Appendix 5.2, but in summary, these weighted those studies that provide evidence of occupational exposure, human exposure, development of measurement techniques or methodology, controls to reduce exposure, human health effect data, arising from epidemiological or human studies, validated toxicology tests in animals and research employing isolated human or animal tissues or cells. Emphasis was placed on studies of engineered nanoparticles.

1.6 Copyright agreement: Under the conditions of copyright agreement, HSL staff cannot send readers HSL's electronic copies of the papers obtained for preparation of the bulletins. If you wish to obtain full copies, this can be done either via the HSE Information Services or HSL staff who will obtain duplicate copies. HSL staff are also prevented from distributing whole collections of references and their associated abstracts.

2. MEASUREMENT, EXPOSURE AND CONTROL

From the baseline search carried out on published articles dated between 2000 and 2006, 285 papers were selected and the abstracts were reviewed and prioritised according to agreed criteria (see Appendix 5.2, Table 4). Those that considered engineered nanoparticles were assigned a higher priority than those investigating the effects of ambient ultrafines. A breakdown of the number of papers per topics is shown in Figure 1.

- The search identified three studies on workplace exposure or dispersion of nanoparticles. These papers reported:
  - possible leaks in the production line of carbon black (2006).
  - cases of people suffering from allergy and asthma to printer's ink (2005). The causes of these reported illnesses are not clear.
  - epidemiological surveys of major TiO_2 manufacturing sites (2004). It is not clear which of the measurement concentration levels relate to fine or ultrafine particles.
- The search retrieved six papers on assessment and / or sampling of nanoparticles in the workplace. Three abstracts from the same authors reported workplaces and simulation measurements on the handling of carbon nanotubes. The last three remaining papers focussed on airborne measurements in workplaces of carbon black and TiO_2 nanoparticles.
- Less than 20 papers reported assessment and sampling of submicron by-products (e.g. welding fumes, beryllium, diesel exhaust particles) in workplaces.
- The search identified 32 papers on development of instruments and methods to measure exposure to nanoparticles / ultrafines. Twenty-three papers reported development of instruments or techniques, intercomparison exercises, anomalous responses and performance of current instruments. These papers mainly focussed on current instruments or methods for measurement of airborne nanoparticles or ultrafines as described in Figure 1. Two of these papers looked at sampling strategies. Eight papers reported development of non-conventional instruments such as mass spectrometers to measure or characterise nanoparticles in workplaces including mass spectrometers.
- The search retrieved very few papers looking at the effectiveness of control measures to reduce exposure to nanoparticles. The eleven papers on assessment of control techniques reported studies in the environmental waste industry rather than in nanoparticles manufacturing. The eleven papers on respiratory protective equipment / personal protective equipment included two papers on evaluation of asbestos fibres and viruses / bacteria rather than on nanoparticles.
- The search identified approximately 90 papers on the use of instruments / methods to assess exposure of airborne environmental ultrafine particles (e.g. diesel particles). These papers reported environmental measurements mainly by conventional techniques to monitor mass, number and surface area of ultrafines as well as characterisation methods such as electron microscopy and mass spectrometry. For a number of years, the scientific community has developed methodologies to measure ultrafines in the environment, which are informing the development of techniques for measurement of nanoparticles in workplaces.

- Interestingly, a significant number of reviews or general articles (53) have been published on measurement techniques to assess exposure to nanoparticles in workplaces (8), or control measures (6), and in particular on regulations (15) and risk assessment (24).

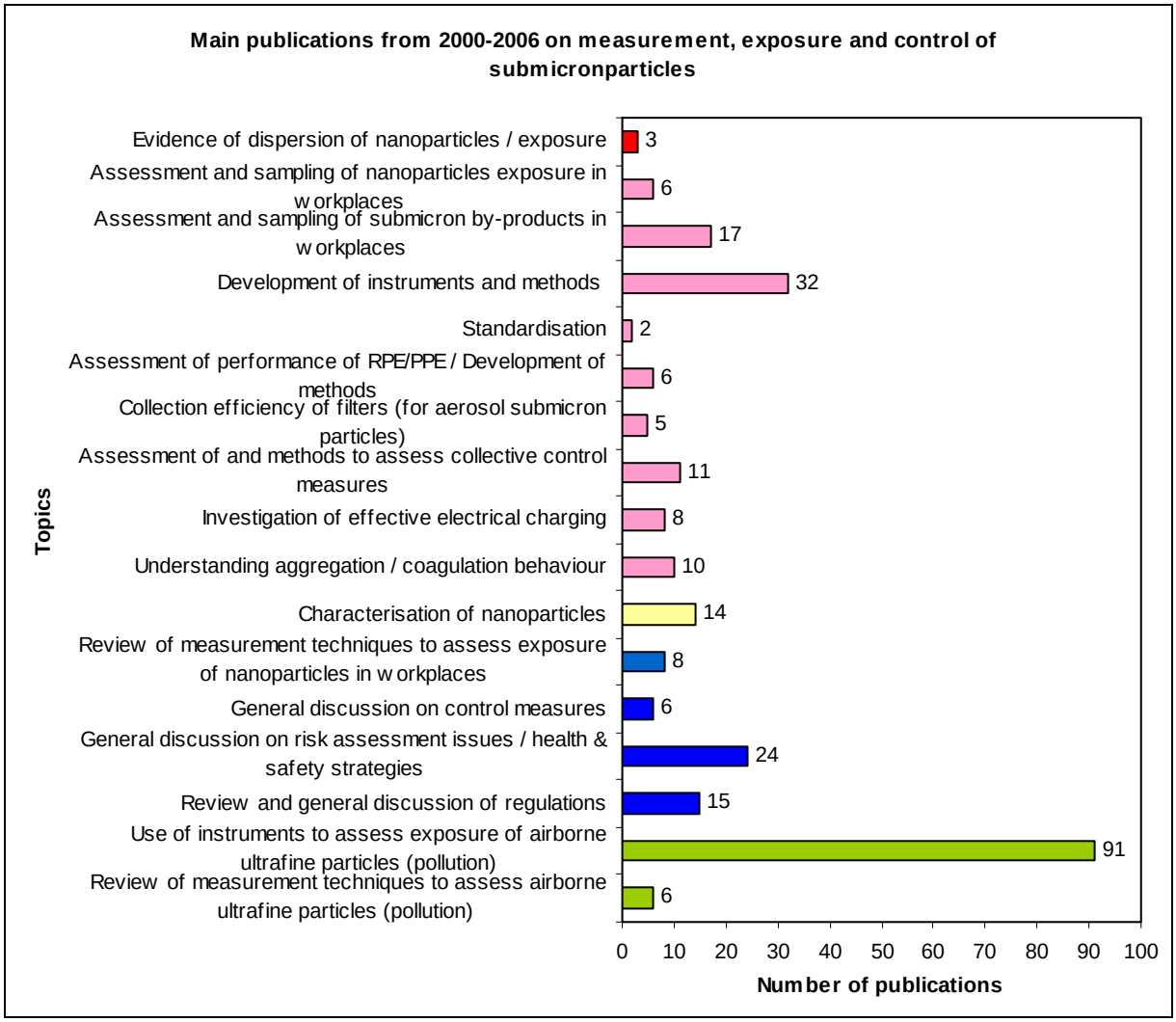

Figure 1: Breakdown of the number of papers per topic (measurement, exposure and control)

2.1 Measuring and monitoring of airborne nanoparticles

Until it has been agreed which are the most appropriate metrics 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. However, this may result in the use of instruments that are not compatible with the established personal sampling procedures that are used to assess compliance with exposure limits, or for epidemiological studies.

In recent years, a number of articles on the measurement of mass, number, surface area concentrations of ambient ultrafine aerosol particles from combustion or hot processes have been published. Very few studies have evaluated these instruments for the measurement of

engineered nanoparticles in the workplace. Most of these instruments are too large to be used for personal monitoring and/or their inlet efficiency does not meet the required criteria.

Static measuring instruments and 'off-line' analysis of air samples have been used to assess occupational exposures [1]. A list of instruments and techniques that could be used for monitoring exposure to nanoparticles and nanotubes in the workplace is shown in Table 1.

Metric	**Devices**	**Remarks**
Mass directly	Size selective static sampler	The only devices offering a cut point around 100 nm are cascade impactors (Berner-type low pressure impactors, or Micro-orifice impactors). Allows gravimetric and chemical analysis of samples on stages below 100 nm
	TEOM® (Tapered Element Oscillating Microbalance)	Sensitive real-time monitors such as the TEOM may be useable to measure nanoaerosol mass concentration on-line, with a suitable size selective inlet.
Mass by calculation	ELPI (Electrical Low pressure Impactor)	Real time size-selective (aerodynamic diameter) detection of active surface-area concentration giving aerosol size distribution. Mass concentration of aerosols can be calculated, only if particle charge and density are assumed or known. Size-selected samples may be further analyzed off-line (as above).
	SMPS (Scanning Mobility Particle Sizer)	Real time size-selective (mobility diameter) detection of number concentration, giving aerosol size distribution. Mass concentration of aerosols can be calculated, only if particle shape and density are known or assumed.
Number directly	CPC (Condensation particle counter)	CPCs provide real time number concentration measurements between their particle diameter detection limits. Without a nanoparticle pre-separator, they are not specific to the nanometre size range. P-Trak has diffusion screen to limit top size to 1 µm.
	SMPS	Real time size-selective (mobility diameter) detection of number concentration, giving a number-based size distribution.
	Electron Microscopy	Off-line analysis of electron microscope samples can provide information on size-specific aerosol number concentrations.
Number by calculation	ELPI	Real time size-selective (aerodynamic diameter) detection of active surface-area concentration, giving aerosol size distribution. Data may be interpreted in terms of number concentration. Size-selected samples may be further analyzed off-line.
Surface-area directly	Diffusion Charger	Real-time measurement of aerosol active surface-area. Active surface-area does not scale directly with geometric surface-area above 100 nm. Note that not all commercially available diffusion chargers have a response that scales with particle active surface-area below 100 nm. Diffusion chargers are only specific to nanoparticles if used with an appropriate inlet pre-separator.

	ELPI	Real time size-selective (aerodynamic diameter) detection of active surface-area concentration. Active surface-area does not scale directly with geometric surface-area above 100 nm.
	Electron Microscopy	Off-line analysis of electron microscope samples can provide information on particle surface-area with respect to size. TEM analysis provides direct information on the projected area of collected particles, which may be related to geometric area for some particle shapes.
Surface area by calculation	SMPS	Real time size-selective (mobility diameter) detection of number concentration. Data may be interpreted in terms of aerosol surface-area under certain circumstances. For instance, the mobility diameter of open agglomerates has been shown to correlate well with projected surface area.
	SMPS and ELPI used in parallel	Differences in measured aerodynamic and mobility can be used to infer particle fractal dimension, which can be further used to estimate surface-area.

Table 1. Instruments and techniques for monitoring nanoaerosol exposure (source of data: Dave Mark, *Occupational exposure to Nanoparticles and Nanotubes*, to be published in Environmental Science and Technology Issue No. 24 "Nanotechnology: Consequences for Human Health & the Environment".)

In addition to concentration levels, the physical and chemical characteristics of the engineered nanoparticles are important parameters for discrimination against natural ultrafine particles or those produced from combustion (e.g. diesel particles). Physical and chemical characterisation techniques usually require the collection of a sample for off-line analysis [2] (Table 2).

In recent years, a number of articles have been published on the use of instruments outlined in Table 2 for chemical analysis of ultrafine particles generated from combustion or hot processes.

Instrument	**Parameters**	**Collection for off-analysis**	**Remarks**
TEM (Transmission Electron Microscopy)	Size, shape, surface area, chemical composition	• Small pore size membrane filter • Direct sampling onto TEM grid coated with a carbon film using thermal precipitator or electrostatic precipitator [3], [4]	Shape and size distribution can be obtained. With TEM, EDS (X-Ray energy dispersive spectroscopy) and EELS (energy loss spectroscopy) allows the investigation of chemical composition of individual nanoparticles. TEM images allow the determination of the fractal dimension of agglomerate structures [5]. Resolution below 1nm for high resolution microscopes (HRTEM)

STEM (Scanning Transmission Electron Microscopy)	Size, shape, surface area, chemical composition	• Small pore size membrane filter Direct sampling onto TEM grid coated with a carbon film using thermal precipitator or electrostatic precipitator	Shape and size distribution can be obtained. With STEM, and EDS allows the measurement of the elemental composition of the nanoparticles and the visual mapping of the elements of nanoparticles collected on filters or carbon grids. EELS (energy loss spectroscopy) also available. STEM images allow the determination of the fractal dimension of agglomerate structures [5]. Resolution below 1nm for high resolution microscopes (HRSTEM)
FEG-SEM (field-emission gun - scanning electron microscopy)	Size, shape, surface area, chemical composition	• Small pore size membrane filter	Shape and size distribution can be obtained. With FEG-SEM, EDS (X-Ray energy dispersive spectroscopy) allows the investigation of the chemical composition of nanoparticles ((individual and mapping analysis available). High resolution SEM images allow the determination of the fractal dimension of agglomerate structures [6]. Resolution of 1-2 nm.
Mass spectrometry (e.g. ATOFMS, TDCIMS)	Chemical composition	• On-line analysis: mass spectrometer coupled to a size selection device and particle counter • Off-line analysis: particles collected for laboratory analysis (e.g. by a cascade impactor)	• On-line analysis: Information (usually qualitative) of the chemical composition can be obtained. • Off-line analysis: Information (usually qualitative and semi-quantitative) of the chemical composition can be obtained. For the analysis of ultrafine particles, university researchers are the main users of this type of instruments.

Table 2. Physical and chemical characterisation techniques for analysis of primary and agglomeration of nanoparticles.

Evaluation of instruments

It is important that the performance of instruments, used in workplaces for assessing exposure to airborne engineered nanoparticles, are systematically investigated either against generated test aerosols (with nanoparticles of different particle sizes, morphologies and agglomeration states) or by inter-comparison exercises. A few papers have been published on the evaluation of these types of instruments [7], [8], [9].

Anomalous responses or discrepancies in differential mobility analysers have been reported with ultrafine fibrous carbon aerosol [10], agglomerates [11], [12] or nanoparticles below 10 nm in size [13].

The following paper presents an evaluation of different methods to measure engineered nanoparticle aerosols generated in laboratory conditions:

Comparing aerosol surface-area measurements of monodisperse ultrafine silver agglomerates by mobility analysis, transmission electron microscopy and diffusion charging. Ku BK; Maynard AD (2005. [7]

This paper evaluates and compares three methods: the scanning mobility particle sizer (SMPS), transmission electron microscopy (TEM) and diffusion charging (DC) for estimating aerosol surface area.

Test aerosols were monodisperse silver particles that ranged in morphology from spherical particles to agglomerated particles with corresponding fractal dimensions from 1.58 to 1.94. The aerosols were generated in a specially constructed test facility utilising two horizontal tube furnaces in series. Particle formation was primarily by homogenous nucleation. Particles of a certain size i.e. mobility diameter, were subsequently selected by a differential mobility analyser (DMA) so that they could be then sampled by the three methods.

For agglomerated silver particles when the agglomerate was smaller than 100 nm the response of the diffusion charging device was found to be proportional to the mobility diameter squared and this was independent of morphology. For particle sizes between 80 and 200 nm the response of the diffusion charging device varied as the mobility diameter to the power of 1.5.

From the TEM analysis, the projected surface area of the agglomerates agreed well with the surface area estimated from the mobility diameters measured by SMPS for particles smaller than 100 nm in size. The surface area of monodisperse particles measured by DC and SMPS was comparable to the geometric surface area below 100 nm but in the size range 100 to 200 nm these methods underestimated the geometric surface area. **All three methods SMPS, TEM and DC gave measurements of surface area that were in good agreement with each other for monodisperse aerosol particles smaller than 100 nm.**

It should be borne in mind that the silver aerosols tested here are essentially monodisperse and may be unlike any of those found in the field which are more likely to be highly polydisperse. The authors, however, concede that more work needs to be carried out with the diffusion charging device in order to experimentally and comprehensively validate the response of this instrument to polydisperse aerosols.

Some of the findings given in this paper are broadly consistent with those from other sources using aerosols more relevant to workplace conditions [8].

The effective electrical charging of nanoparticles has a direct bearing on the size of the smallest nanoparticle that can be detected or captured. Until recently this has usually been carried out using a radioactive bipolar chargers but other more efficient methods / devices have also been investigated. These alternative devices could be used as direct substitutes for radioactive sources in current instruments, and are discussed in the following three papers:

Ion beam charging of aerosol nanoparticles. Seto T, Orii T, Sakurai H, Hirasawa M, Kwon SB (2005). [14]

In order to achieve high efficiency charging of airborne nanoparticles under low pressure conditions when using a low pressure differential mobility analyser (LP-DMA), the authors have developed a method of charging using a positive Helium ion beam which they claim can experimentally produce a charging probability of more than 60% for particles of between 10 and 40 nanometres in electrical mobility diameter at a reduced pressure of between 350 and 650 Pascal. For the charging of neutral particles, they compare the performance of their ion beam aerosol charger to that of an alpha emitting radioactive source.

Unipolar charging of nanometre aerosol particles in a corona ioniser. Hernandez-Sierra A, Alguacil FJ, Alonso M (2003). [15]

This paper describes a unipolar corona ioniser which has been evaluated for charging particles below 10 nm particle equivalent mobility diameter at different air flow rates, different corona polarity and voltages and at different positions of the corona electrode tip. The authors claim that the efficiency of this device is almost an order of magnitude greater than that attainable with conventional radioactive bipolar chargers for 10 nm particles and consistent with the findings of other workers who have developed similar devices.

Unipolar charging of nanosized aerosol particles using soft X-ray photoionisation. Han B, Shimada M, Choi M, Okuyama K (2003). [16]

The development of a unipolar charging device based on soft X-ray photoionisation is described and its potential in efficiently charging aerosolised nanoparticles is investigated by comparison with the performance of an Americium 241 radioactive charger. Also in this paper theoretical calculations based on the unipolar diffusion charging theory are carried out in order to compare with those actually obtained, and the X-ray photionisation unipolar charger is used in conjunction with a differential mobility analyser (DMA) to obtain a high yield of monodisperse nanoparticles from a polydisperse aerosol. The findings indicate that the ion production rate of the X-ray unipolar charger is at least five and a half times greater than that of conventional radioactive chargers and that the ion concentration is roughly three times higher. It was concluded that the performance of the X-ray unipolar charger was in good agreement with theoretical calculations, was more capable of charging particles sized between 10 and 40 nm in diameter than an Americium 241 radioactive charger and therefore had a potential for charging high number concentrations of a nanoparticles for use in nanoparticles investigations.

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. This includes: water based or mixing type **condensation particle counters**; nano, long, radial or adjustable column **differential mobility analysers**, a fast or nano **scanning mobility particle sizer.**

New instruments are also continuously being developed and there are small portable instruments for measuring particle number concentrations (TSI P-Trak), particle surface area concentrations (EcoChem DC2000CE) and health-related surface area concentrations (TSI Aerotrak 9000). Whilst these instruments are not yet truly personal, they are compact enough to be carried from location to location in the workplace and to be sited close to the worker at each location.

Development of instruments for measuring mass concentration of nanoparticles:

Development of a near-continuous monitor for measurement of the sub-150 nm PM mass concentration. Chakrabarti B, Singh M, Sioutas C (2004). [17]

A potential new device is reported for measuring mass concentrations of particles < 150 nm fraction of ambient aerosols using well-known beta attenuation detection of particles selected by an impactor pre-separator cutting at 150 nm. Results agree well with mass concentration data from a cascade impactor with collection stages down to 56 nm (MOUDI). The new device has a minimum integration period of 2 hrs for ambient aerosols and so may not be useful for monitoring rapidly changing processes. In addition, the use of a C_{14} radioactive source for beta particles makes it of limited use in workplaces.

Development of small instruments for measuring number size distribution of nanoparticles:

Measurement of ultrafine aerosol size distributions by a combination of diffusion screen separators and condensation particle counters. Feldpausch P, Fiebig M, Fritzsche L, Petzold A (2006). [18]

A new technique has been reported, concerning a combination of particle size selection by diffusion screens followed by counting by CPC. Studies have been carried out to investigate the dependence of particle penetration through the screens on a number of important factors (number of screens, flowrate and pressure drop) and the results were found to agree with theory. The emphasis of this work was for measuring particle size distributions in the upper atmosphere, but the principle of operation can be developed further for use in workplaces. One company is currently developing a workplace instrument for measuring number concentrations as a function of particle size using similar techniques. **If successful, it will have significant advantages over the SMPS, such as portability, cost and no requirement for a radioactive source for particle charging.**

Few papers reported development of non-conventional instruments to measure or characterise nanoparticles in workplaces including mass spectrometers and the following paper on a nanoaerosol mass spectrometer had been identified to be of interest for potential measurement of airborne nanoparticles:

Chemical characterisation of individual, airborne sub-10nm particles and molecules. Wang S, Zordan CA, Johnston MV (2006). [19]

This paper describes a nanoaerosol mass spectrometer (NAMS) for real-time characterisation of individual airborne nanoparticles. This instrument has been tested with sucrose particles produced from an electrospray. The authors have shown a detection efficiency of 10^{-4} for particles of 9.5 nm diameter, which allows characterisation of particles with concentrations in excess of about 10^5 particles /cm^3. The detection efficiency was defined as the fraction of particles entering the inlet.

2.2 Exposure data

Workplace exposure

Inhalation toxicology and epidemiology studies on submicron particles suggested that the monitoring of exposure against mass concentration alone is not sufficient and it is necessary to measure the level of particles in terms of surface area and number concentrations. Recent studies have usually included measurement of all three metrics.

Very few assessments of exposure level to engineered nanoparticles in the workplace have been carried out and they have mainly focussed on existing manufacturing processes such as carbon black, TiO_2 or silica fumes. In addition, a number of aerosol measurements on ultrafine unwanted by-products in workplaces such as welding fumes, beryllium, diesel exhaust particles have been reported.

The main studies on workplace concentrations of ultrafine or nanoparticles carried out since 2000 include measurements of:

- Riediger et al (2001): Welding fumes, metal fumes, carbon black, silica fumes and particulate diesel motor emissions [20].

- Moehlmann (2005): Offices, silicon melting, metal grinding, plasma flame cutting, bakeries, and airport aprons [21]. This measurement program was started in 1998, by the Institute for Occupational Safety and Health of the German Berufsgenossenschaften (BGIA) and the Institute for Hazardous Materials Research (IGF). The authors have published a summary table of the airborne ultrafine particle levels and have shown that the concentration ranged from a few thousand to 108 particles /cm^3 by measuring particles between 10 and 500nm (which included agglomerates).
- Wake (2001): Fine carbon black, fine nickel powder, high specific surface area precious metal blacks, titanium dioxide, metal / metal oxide fumes from thermal spraying and coating / zinc / zinc oxide from metal processing and refining, steel foundry fumes, welding fumes, solder fumes [22]. In general, the outdoors submicron concentrations were similar or higher than the level of ultrafine particles in the vicinity of the persons operating the processes, including in the bagging plants. However, occasionally higher concentrations were seen during the bagging of fluffy and pelletised carbon.

Outdoor ambient (or reference place) measurements were usually acquired for comparison with indoor submicron particle levels associated with workplace processes. The high outdoor concentrations of ultrafine particles may sometimes account for increased levels of nanoparticles observed in workplaces. Currently, the instruments monitoring mass, number or surface area concentrations are not capable of measuring in real-time the level of engineered nanoparticles and discriminating them from by-product aerosol entering the workplace from outside.

Particle characteristics in the reactor and pelletizing areas of carbon black production. Kuhlbusch TAJ, Fissan H (2006). [23]

This paper describes the physical and chemical characteristics of airborne particles that were measured in the reactor and palletising areas at three carbon black production plants. The measurements were carried out to assess process related sources of particles released into the atmosphere of certain work areas, particularly around the reactor and in pelletising areas where exposure to the airborne ultrafine, PM1, PM2.5 and PM10 carbon black fractions was expected to be highest.

Instruments used in this study were the aerodynamic particle sizer (APS) to measure in the 0.3 to 15 micron size region and the scanning mobility particle sizer (SMPS) to measure in the 15 to 734 nm size regions. Two tapered element oscillating microbalances (TEOM) were used to measure the online PM10 mass concentration of the carbon black aerosol.

No elevated ultrafine particle number concentrations in relation to ambient levels were determined in the work areas of one plant. Intermittent elevated ultrafine particle concentrations were observed in the pelletising and reactor areas of a second plant but these could be related to nearby traffic emissions. The ultrafine particle number concentrations at the second plant were comparable to those associated with urban traffic sites. In a third plant the ultrafine particle concentrations were elevated in the pelletiser and reactor areas. In this plant the reactor area was the only enclosed reactor area investigated and the source of the ultrafine emission was more likely to be due to grease and oil fumes released during maintenance operations. The elevated numbers of ultrafine particles in the pelletising area of the third plant, are thought to be related to leaks in the production line.

More than 700 cases of people suffering from allergy and asthma to printer's ink have been reported in Germany by the «Interessengemeinschaft Tonergeschae digter», a self-help group of people reporting detrimental health effects from exposure to toner [24]. The composition of toners can include heavy metals and volatile organic compounds (VOC), which may become airborne as nanoparticles during printing.

Agglomeration / nanopowder behaviour

The behaviour of submicron particles is mainly governed by diffusional forces. Brownian motion becomes important and the primary particles, through collision, tend to agglomerate and therefore grow in size.

Although the dustiness behaviour of nanoparticles is an important property, few studies if any, have explored this. For materials where nanoparticles do not become readily airborne under normal handling procedures, the associated risk from inhalation will be considerably reduced.

Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. Maynard AD; Baron PA; Foley M; Shvedova AA; Kisin ER; Castranova V (2004) [25].

This paper investigates the potential exposure route of single walled carbon nanotubes (SWCNTs) and the propensity of two types of SWCNTs (laser ablation SWCNTs and high-pressure carbon monoxide (HiPCO) process SWCNTs) to become airborne. Laboratory and field measurements have been carried out using a scanning mobility particle sizer (SMPS) configured with a nano differential mobility analyser (DMA), a SMPS configured with a long DMA and a condensation particle counter and an aerodynamic particle sizer

The laboratory measurements qualitatively assessed the propensity of these two types of SWCNTs to become airborne using a two-component shaker fluidized bed. For both materials, relatively gentle agitation did not generate significant levels of airborne particles below 1µm. More vigorous agitation led to the generation of particles smaller than 100nm in diameter. Laser ablation SWCNT led to particles around 200nm diameter being released initially. The aerosol concentration of particles below 500nm decreased rapidly over time. Energetic agitation of HiPCO SWCNTs produced airborne particles following a bimodal distribution with modes below 50nm and between 100nm and 1µm. The aerosol was stable over 15 minutes.

Field measurements have shown no evidence of a significant increase in particle number or mass concentration during the handling of SWCNTs (transferring SWCNTs powder from production vessel to storage bucket and then tipping into a second bucket). Increased particle number concentration during clean up have been measured on three of the sites. In two of these cases, a vacuum cleaner (a device fitted with a low efficiency filter and a device incorrectly fitted with a HEPA filter) was used in the enclosure. It is unclear whether the increase was due to the vacuum cleaner itself or to the use of it.

Cotton gloves deposits of SWCNTs, worn by workers during field handing of materials, were estimated at between 0.2 mg and 6mg per hand. It is likely that cotton gloves would have collected more materials than latex gloves or bare hands.

Higher air and glove SWCNT concentrations were measured for the HiPCO material. The authors explained that these higher airborne concentrations may have been due to HiPCO SWCNTs having a lower density compared with the laser ablation SWCNTs and becoming more easily airborne as large clumps of materials.

Development and validation of a simple numerical model for estimating workplace aerosol size distribution evolution through coagulation, settling, and diffusion. Maynard AD; Zimmer AT (2003) [26].

A numerical model has been developed to predict how the size distributions of nanoaerosols change with time from emission in the workplace. Mechanisms considered included coagulation, settling and diffusion. Reasonable agreement was achieved with experimental

data when assuming spherical particles and coalescence on coagulation and the formation of fractal-like particles. **The model may be of use to those involved in deciding the systems required for exposure control, but will require knowledge of the initial characteristics of the particles emitted from the source.**

Producers of nanoparticles are currently developing manufacturing processes that prevent aggregation of particles. As an example, Li-Dan and Kaner-Richard B (2006) [27] have found that highly dispersible polyaniline nanofibres can be synthesised from a conventional reaction at an elevated temperature without mechanical agitation. They claim that this work is valuable for future development in synthesising well-controlled nanoparticles, which will not aggregate in the production process.

Exposure limits

In the UK, the workplace exposure limits (WELs) for airborne dust are expressed in mass concentration. At a concentration in air equal or greater than 10mg/m^3 (TWA inhalable fraction) or 4mg/m^3 (TWA respirable fraction), airborne dust is considered to be a hazardous substance to health [28]. No specific occupational exposure limit at work has been implemented in the UK for newly engineered nanoparticles.

NIOSH proposes two different exposure limits (time weighted average calculated on the basis of a 10 hour-day and a 40 hour-week): 1.5 mg/m^3 for fine TiO_2 and 0.1 mg/m^3 for ultrafine TiO_2 (with diameter less than 100 nm), to reflect differences in surface area and potency [29].

2.3 Control Measures

General control measures

Few strategies on the use of collective and personal control measures have been published. Guidance on the safe handling of nanoparticles and other safety approaches as well as current knowledge on control measures (engineering work practices, personal protective clothing, respiratory protective equipment) can be found on the National institute of Occupational Hygiene (NIOSH) website [30]. This complements the existing HSE guidance () [31]. In the book “Nanotechnology – Environment implications and solutions”, Theodore and Kunz presented data generated with high efficiency control devices (e.g. baghouses, electrostatic precipitators, and venture scrubbers). The authors claim that these devices can collect particles in the submicron size with 100% efficiency [32].

Very few peer-reviewed papers on the effectiveness of engineering or personal control measures to reduce exposure from engineered nanoparticle aerosols have been published.

Use of ventilation/filtration for controlling ultrafine particles in indoor spaces:

Effect of central fans and in-duct filters on deposition rates of ultrafine and fine particles in an occupied townhouse. Wallace LA, Emmerich SJ, Howard-Reed C (2004). [33]

A paper reporting control of ultrafine particle levels in homes may be of interest in the workplace. The authors concluded that to minimise the levels of airborne nanoparticles from both indoor and outdoor sources, it is better to use re-circulating ventilation with filtration by electrostatic precipitator rather than systems designed to make the room leak tight.

Capturing invisible dust. Sullivan RA (2001). [34]

This paper describes the use of an electrostatic filter, which has been produced by a process involving triboelectrification, for the removal of particles in the 100 nm to 1 µm size range.

The process of producing unipolar microscopic charge by triboelectrification during the carding of two dissimilar textile fibres during filter production was first carried out 20 years ago by HSL during the development of the filter material subsequently patented, called "Technostat". It is interesting to note that only now has the significance of its efficiency in the removal of very small particles been recognised.

The mobility of nanoparticles, even if they carry a low electrical charge, will be extremely high. The exact amount of charge carried, therefore, will be very important for instruments that classify by electrical mobility (DMA) or capture the nanoparticles using electrostatic forces, electrostatic filters and electrostatic precipitators for example.

Particle collection efficiency of electrostatic precipitators:

Particle collection efficiency and particle re-entrainment of an electrostatic precipitator in a sewage sludge incineration plant. Ferge T, Maguhn J, Felber H, Zimmermann R (2004). [35]

This paper looks at the use of electrostatic precipitators for the remediation of man made ultrafine dust sources particularly in sewage sludge incineration plants. It discusses how the operating conditions of the electrostatic precipitator can be changed in order to reduce the re-entrainment of captured fine particles into the atmosphere. The efficiency of an electrostatic precipitator, operating at different "rapping cycles" was studied at a municipal sewage sludge incineration plant by comparing upstream and downstream particle concentrations simultaneously with aerodynamic particle sizers (APS) and an electrical low pressure impactor (ELPI).

Electrostatic precipitation of ultrafine particles enhanced by simultaneous diffusional deposition on wire screens. Alonso M. and Alguacil FJ (2002). [36]

This paper describes how a laboratory scale electrostatic precipitator has been modified so that the earth collector comprises wire screens, set perpendicular to the gas flow, which simultaneously capture particles by electrostatic and diffusional deposition. The former mechanism is effective in capturing the larger particles and the latter, because the mesh screen elements are likened to the action of fibres, is suggested to be effective at capturing the very small particles. Preliminary results indicate that particles down to a few nanometres can be collected with number efficiencies greater than 99 %. How this has been determined, however, is not clear.

Respiratory protective equipment (RPE)

Few articles on filtration efficiency of submicron nanoparticles have been published. Filtration of nanoparticles may be significantly different from that of standard filter penetration test aerosol. Moreover, contradictory results on the collection efficiency of filters for nanoparticles have been published in the literature [37], [38]. Very few data on the efficiency of RPE to reduce exposure from nanoparticles are available.

Filtration efficiency of aerosol particles below 20 nanometers. Heim M, Mullins BJ, Wild M, Meyer J and Kasper G (2005). [37]

Single fibre collection efficiency for very small particles (<20nm) is reviewed in terms of theory and available experimental evidence. Decreases in collection efficiency with

decreasing size in this range are attributed to “thermal rebound”. This paper describes work to improve the quantity and quality of experimental data on single fibre collection efficiency in the sub-20nm size range, including effects of particle charge.

Particles were generated by atomising / evaporation-condensation of salt and DMA classifier, with or with out charge neutralization. Detection was by CPC. Test filters were stainless steel (fibrous pad), nickel or polypropylene (woven mesh).

The lower particle size detection efficiency of the various CPCs used was measured as a function of particle size.

Extensive testing over the size range 2.5 - 20nm did not exhibit any onset of thermal bounce in reducing particle collection efficiency, and no reduction in efficiency was noted with decreasing particle size. Charge on fibres was observed to enhance collection.

Work is planned to extend the size range studied to below 2.5nm.

Manikin-based performance evaluation of N95 filtering-facepiece respirators challenged with nanoparticles. Balazy A, Toivola M, Reponen T, Podgo A, Zimmer A and Grinshpun SA (2006). [39]

This paper reviews the mechanisms for fine particle filtration, the origin of the generally accepted “most penetrating particle size” of ~0.3µm, and how this may be affected by nanoparticle challenge. It then describes experimental measurement of the nanoparticle filtration efficiency of two models of N95 filtering facepieces (incorporating electrostatic filter material), when sealed to a dummy head at two constant flow rates (30 and 85 l/min). Generation of particles was by atomisation of salt solution, and measurement / detection was by an MSP Corp. 1000XP Wide Range Particle Spectrometer (operating range 10nm to 10µm). Only the data up to 600nm were considered.

Most penetrating size was found to be between 40 and 50nm, with peak absolute penetration increasing with flow rate. In this study, there was no evidence of an increase in penetration of these filters at lower sizes down to the limit of size resolution.

For both N95 models, penetration exceeded the nominal 5% maximum for this class of respirator for particles close to the most penetrating size (30 - 80 nm) at 85 l/min. Particle charge significantly affects capture efficiency of filters, neutral particles being the most penetrating.

2.4 Characterisation of nanomaterials

Characterisation of bulk nanomaterials

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

Few papers have been published on the characterization of nanoparticles in their bulk form, in fluids (biological or water / solvent) or for toxicological evaluation. The following review highlights strategies for the characterisation of nanoparticles for toxicological evaluation:

Research strategies for safety evaluation of nanomaterials. Part VI. Characterization of nanoscale particles for toxicological evaluation. Powers KW; Brown SC; Krishna-VB; Wasdo SC; Moudgil BM; Roberts SM (2006)**.** [40]

This paper discusses characterisation techniques of nanomaterials for toxicological evaluation and makes recommendations.

Complete characterisation should include the measurement of:

- Particle size distribution (in terms of number, volume and surface area) and shape information of the powder 'as received', of the material 'as-dosed' or 'as-exposed' and at the point of interaction with the organism.
- State of dispersion and changes in particle size distribution to that of a fully dispersed system. State of dispersion or agglomerate size of the particles in the biological environment.
- Physical and chemical properties (including elemental composition, density, crystal structure, chemical reactivity, solubility, conductivity, melting point, hardness, optical properties) of the powder 'as received' and of the particles in the biological environment.
- Surface area and porosity of the powder 'as received', of the particles 'as dosed' and in the biological environment (these properties can change as a result of biomolecule adsorption or agglomeration).
- Surface chemistry (such as surface composition and structure, surface energy/wettability, surface charge, surface reactivity or zeta potential, presence and chemical nature of the adsorbed species) of the powder 'as received' and as close to the physiological conditions as practical.

The authors propose five general rules:

- The sample of particles measured should be representative of the bulk material. The errors will be more significant for broader size distribution if the sample is not representative.
- Particles should be dispersed to a maximum state achievable for the measurement of primary particle size and shape.
- The most appropriate instruments and techniques should be used.
- Enough particles should be measured.
- The particles should be characterised as close to the point of application as possible.

Standards for instrument evaluation

Two standardisation papers have been identified including an abstract recommending European calibration standard for nanoparticle counting instruments [41]. The availability of fully characterised standards for the evaluation of instruments is an important issue if users want to obtain correct and reproducible results from their instruments.

2.5 Bibliography of key papers

Measuring and monitoring of airborne nanoparticles exposure

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3. HEALTH EFFECTS

The health effects search terms retrieved much of the large body of literature on the health effects of ambient ultrafine particles and diesel emissions. The studies included investigations of the levels of particles at diverse locations, including the London underground [1], cities [2] or ice-rinks [3], or of the effects in rodents exposed to particles in road traffic [4]. One study measured personal exposure and oxidative DNA damage in venous blood cells in relation to air pollutants in Copenhagen, and noted that short-term high intensity exposure to traffic pollution was associated with elevated levels of damage [5]. Other studies have examined effects in vulnerable populations e.g. investigating lung deposition in the elderly [6], and the synergistic effects between ambient particles and other agents such as ozone and bacterial toxin [7]. There have also been reviews summarising these data e.g. [8; 9].

Of these studies, only one satisfied the priority criterion of having evaluated a potential human biomarker of exposure to particles [10], albeit not with engineered nanoparticles:

Daily variation in fine and ultrafine particulate air pollution and urinary concentrations of lung Clara cell protein CC16. Timonen et al, (2004) [10]

Ambient air particulate pollution has been associated with respiratory mortality and morbidity. This study assessed the association between urinary concentrations of lung Clara cell protein CC16, a marker for lung damage, and variations in fine and ultrafine particulate air pollution in three European cities. Whilst daily variation in ultrafine particle levels was not associated with CC16, the biomarker concentration seemed to increase with increasing concentrations of ambient $PM_{2.5}$ in Helsinki. The authors suggest that exposure to particulate air pollution may lead to increased epithelial barrier permeability in lungs. The differences in association between centres was not understood, but may provide insights into factors affecting the response to particulate air pollution (i.e. subject characteristics) and composition of particles.

3.1 Human studies and epidemiology

The large literature on the potential human health effects of ambient ultrafine particles has been subject to peer review, and therefore, references in this area were excluded and priority given to those articles focussing on **engineered nanoparticles**. The number of papers in the different topic areas is shown in Figure 2.

Three studies have considered the potential for engineered nanoparticles to induce effects directly in humans, and are summarised below. The first represents the only report of volunteers exposed to nanoparticles that was identified in the searches:

Comparing inhaled ultrafine versus fine zinc oxide particles in healthy adults. Beckett et al (2005) [11]

Zinc is generated in large quantities by industrial processes such as welding or cutting galvanised steel. Freshly generated zinc oxide can cause an inflammatory response known as fume fever. The current US OSHA permissible exposure limit for zinc oxide is 5.0 mg/m^3 inspired air, fume or respirable dust, as an 8h time-weighted average over a 40h working week. In this study, 12 subjects were exposed to clean air, **0.5 mg/m^3** ultrafine (40 nm) or fine (291 nm) zinc oxide for 2h at rest on 3 consecutive days, and were studied for 24h after each exposure. The total deposition fraction for the nanosized particles was 75%, considerably higher than the larger particles, and higher than some models would predict. No statistically significant effects were seen; the authors state that the data in the cases

where p<0.05 did not support a true exposure effect, and appeared to be due to chance alone (owing to the large number of statistical comparisons made).

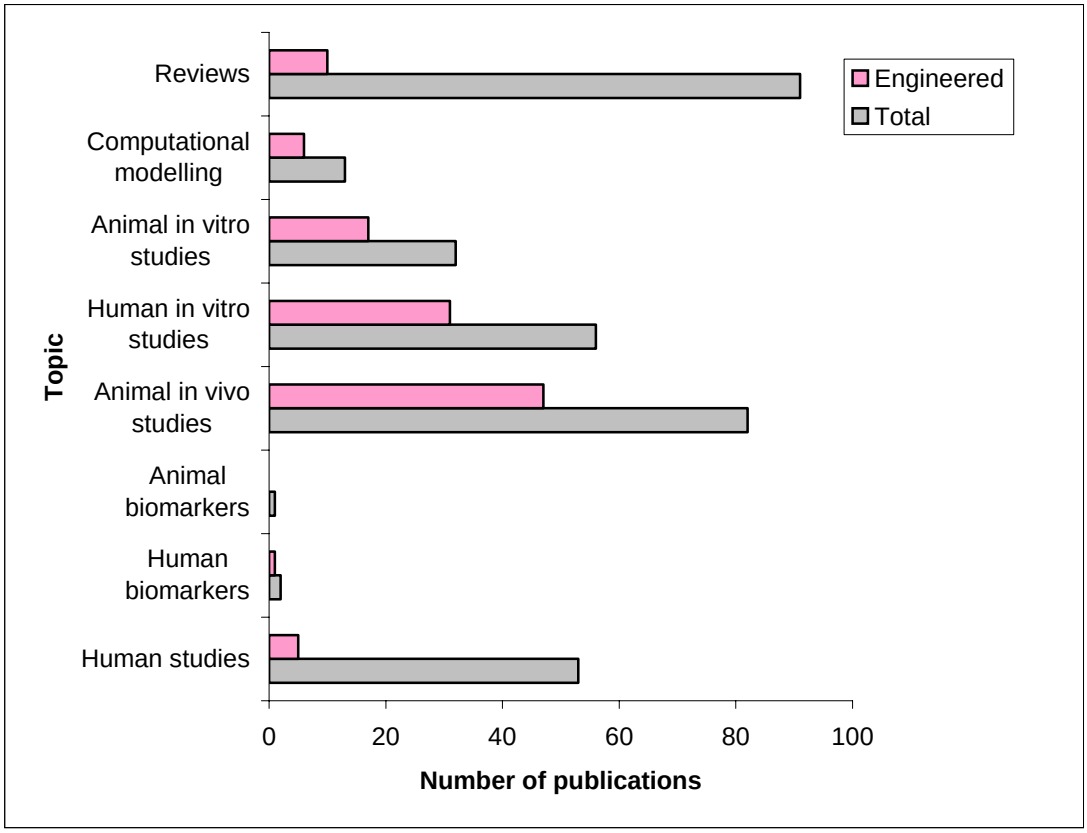

Figure 2: Breakdown per topic of the numbers of papers in 2000-2006 on the human health effects of nanoparticles. The numbers reflect the *total* number of papers, and the number that consider *engineered* nanoparticles. (These numbers include some duplicate references.)

In previous studies (cited by [11]), the effects on human subjects of inhaling fine zinc oxide for 2h included subtle changes in oral temperature, peripheral blood, bronchoalveolar lavage (BAL) cells and cytokines. These effects were only observed however at doses higher than used by [11] (2.5 and 5.0 mg/m^3), and furthermore, they noted that breathing 25 μg/m^3 ultrafine carbon particles during exercise was associated with reduced blood cell adhesion molecular expression, reduced monocyte numbers and activation of T lymphocytes in women.

The second paper describes animal and epidemiological experiments with titanium dioxide, but it should be noted that the size of the particles in the epidemiological studies is not stated. Since workers may have been exposed to particles of variable sizes, the relevance of these findings is unclear.

Titanium dioxide: inhalation toxicology and epidemiology. Hext et al (2005) [12]

Titanium dioxide (TiO_2) is manufactured worldwide in large quantities for use in a wide range of applications and it has generally been considered to be toxicologically inert. However, a number of studies have reported lung tumours in rats (not hamsters or mice) after a lifetime of exposure to very high concentrations of pigment grade TiO_2. Hext et al report both animal and human investigations: in a 90-day study, mice, rats and hamsters were exposed by inhalation to either pigment-grade TiO_2 at 0, 10, 50 or 250 mg/m^3 or ultrafine TiO_2 at 0, 0.5, 2

or 10 mg/m^3 for 6 hours/day, 5 days/week. The authors reported clear species differences. Rats and mice displayed similar lung burdens and clearance rates, whereas clearance rates in the hamster were greater. At high lung burdens, rats showed a marked progression in histopathological lesions, which were not observed in mice or hamsters.

A European multi-centre epidemiological study was based on 27,522 TiO_2 exposed workers employed in 11 manufacturing plants in 6 countries (N.B. *size of particles not specified*). Of the 2,652 deaths observed in the follow-on period, evidence of a carcinogenic risk linked to employment in the industry was not found, although a statistically significant increase in the standardised mortality ratio for lung cancer deaths was noted. In the US multi-centre study based on 5,713 TiO_2 exposed workers, increased risks of lung cancer or other significant adverse health effects were not observed.

The authors conclude that the rat may be oversensitive to high lung burdens of pigment grade and ultrafine TiO_2 compared with mice or hamsters and therefore, there appears to be no clear link between human occupational exposure to TiO_2 and lung cancer risk.

Earlier animal studies have also drawn similar conclusions (e.g. [13]).

The third paper considers risk assessment approaches for assessing human health effects of nanoparticles:

Lung Dosimetry and Risk Assessment of Nanoparticles: Evaluating and Extending Current Models in Rats and Humans. Kuempel et al, (2006) [14]

Whilst human data assessing health risks following occupational exposures to nanomaterials are limited, quantitative data are available from rodent studies. In this study, the authors illustrate the quantitative risk assessment (QRA) steps for estimating human equivalent exposures using dose-response data from rats following chronic inhalation of poorly soluble fine or ultrafine particles (fine or ultrafine TiO_2; ultrafine carbon black (CB) or ultrafine diesel exhaust particulate (DEP)) and inter-species allometric scaling or lung dosimetry model methods. Based on the analysis of the relationship between lung cancer response and various particle-related dose metrics, critical doses corresponding to the benchmark dose (BMD) and 95% lower confidence limit of the BMD (i.e. the BMDL) at a 10% excess risk of lung cancer, were estimated in rats using the multistage model, with linear extrapolation to a 0.1% excess risk. The critical doses (BMD and BMDL) were extrapolated to humans by adjusting for species differences in lung mass or surface area. Working-life time average exposure concentrations associated with the estimated human internal doses were determined using two different human lung dosimetry models. Based on these approaches, estimates of the working lifetime airborne concentrations associated with 0.1% excess risk of lung cancer in humans were found to be 0.07 to 0.3 mg/m^3 for ultrafine TiO_2, CB or DEP and 0.7 to 1.3 mg/m^3 for fine TiO_2. **However, the authors conclude that further research and studies are needed to parameterise and validate the illustrated modelling approaches for predicting human health risks posed by nanomaterials**.

The bulk of the other papers on engineered nanoparticles were in the categories of animal *in vivo* studies (44% of papers) or investigations using human (31%) and animal (17%) *in vitro* systems (as summarised in Figure 2).

3.2 Animal *in vivo* studies

Animal *in vivo* studies have suggested an association between inhalation of ultrafine particles and lung inflammation and damage (reviewed by e.g. [9]). Many of the studies in

this area have considered ambient pollutants and carbon particles, although more studies are now being done on engineered particles.

Eighteen of the animal studies retrieved in the searches reported inflammatory effects in animal lungs following exposure to nanoparticles via the inhalation route. Seven of the papers used nanosized carbon black particles, and reported various inflammatory endpoints, typically increased numbers of cells and proteins in bronchoalveolar lavage fluid from the animals following exposure [15-21]. Two of these papers compared fine and ultrafine forms of the carbon black particles, reporting that only the smaller particles were pro-inflammatory [18; 19]. Four studies compared the effects of other nanoparticles in fine and ultrafine form, all noting that the nanoparticles were more pro-inflammatory than the fine particles: titanium dioxide (TiO_2) [19], cobalt [22], silica [23] and nickel [24] in a follow-on study from their evaluation of cobalt:

Comparative toxicity of standard nickel and ultrafine nickel in lung after intratracheal instillation. Zhang et al. (2003) [24]

Ultrafine nickel (20 nm) has a number of industrial applications. The present occupational exposure limits are based on mass, and do not distinguish between particles of different sizes. This study compared the toxicity of standard (5 μm) and ultrafine nickel (20 nm) following intratracheal injection of 0.1, 0.5, 1.0 or 5.0 mg into rats. Rats were killed after 1-30 days and inflammation assessed by analysis of the BAL, in terms of dose-response and time effects. The nanosized nickel induced a much more severe pulmonary inflammation than the standard sized metal, given on *equal mass basis*. All of the parameters investigated (lung weight, total number of cells in BAL, number of neutrophils, LDH and protein in BAL and release of TNFα) were increased with nano-sized more than micron nickel. **The authors conclude that ultrafine nickel is much more toxic to the rat lung than standard nickel; they hypothesise that the mechanism arises from the nanoparticles' greater surface area, and ability to induce free radicals, TNFα and nitric oxide.**

Even within the nanoscale size range, although 14 nm carbon black particles (not 56 nm) only induce slight lung inflammation and pulmonary oedema when administered to mice, they can aggravate other inflammatory responses e.g. to bacterial lipopolysaccharide [20; 21]. The increased inflammation of ultrafine carbon black particles compared to fine has been shown not to be due to the transition metals associated with the particles [15]. Nanoparticles of cadmium oxide can induce changes in the synthesis of enzymes involved in lung surfactant lipid generation [25].

In addition to investigating the toxicity of the same chemical in different sizes, three studies have compared the effects of inhalation of two or more different nanoparticles on rodent lung inflammation [17; 19; 26]. Renwick and colleagues [19] confirmed that the inflammation and epithelial damage in the rat lung are greater with ultrafine than fine particles, agreeing with previous conclusions, and they also showed that carbon black is more toxic than TiO_2. The study of Dick et al [17], summarised below, has gone further to analyse the effects of surface reactivity for four different nanoparticles of similar size:

The role of free radicals in the toxic and inflammatory effects of four different ultrafine particle types. Dick et al. (2003) [17]

The aim of this study was to investigate the properties which contribute most to the toxicity and inflammatory effects by comparing four different nanoparticles: cobalt (Co, 20 nm), titanium dioxide (TiO_2, 20 nm), nickel (Ni, 20 nm), all three of which have the same surface area, and carbon black (CB, 14 nm), with a surface area five to six times greater; it was noted that all of these nanoparticles existed as aggregates in the samples used. Following intratracheal instillation rats were killed 4 or 18 hours later, and markers of inflammation and epithelial damage assessed in BAL. **The authors conclude that the different particles, instilled on an *equal mass basis*, induced different amounts of inflammation and lung**

injury. The effects seemed to correlate with the ability of the nanoparticles to generate free radicals and oxidative damage, and hence with their surface reactivity rather than size or surface area, although other factors must play a part too, since Ni induced formation of free radicals but was less pro-inflammatory than Co or CB.

The first study to highlight the importance of surface area as a metric for toxicology was that of Oberdorster et al [27] in which they reported that the pulmonary inflammatory response to intratracheal instillation of TiO_2 in rats and mice was greater with ultrafine TiO_2 (20nm) than fine (250nm), and that the effects correlated better with the surface area than the mass of the particles. More recently, these observations have been confirmed and extended by Stoeger et al [28]:

Instillation of six different ultrafine carbon particles indicates a surface area threshold dose for acute lung inflammation in mice. Stoeger et al (2006) [28]

Six different particles (10-50 nm, surface areas 30-800 m^2/g) were instilled into mice on a mass basis: PrintexG, Printex90, two flame soot particles with different organic contents (SootL, SootH), ultrafine carbon particles and diesel exhaust particles (DEP). The inflammatory effects observed in the BAL were ranked: carbon nanoparticles > SootL $\geq$ SootH > Printex90 > PrintexG > DEP. When the inflammatory effects were related to the particle characteristics, the most obvious dose response was for particle surface area. Additionally, the existence of a threshold for the particle surface area was noted at an instilled dose of approximately 20 cm^2, below which no acute proinflammatory responses were detected.

Such observations are particularly pertinent since two studies noted that inflammation in the rodent lung was only observed at the highest mass doses of titanium dioxide [13] and cadmium oxide [29]. Furthermore, the surface area of nanoparticles influences the acute pulmonary inflammatory response in rats more than hydrophobicity [30].

Despite the growing opinion that fine particles are less toxic than ultrafines, one study has shown that nanosized silicon dioxide induces less severe fibrogenesis than microsized particles [31]. Another paper reports that the adverse health effects of fine and ultrafine cadmium oxide are similar following exposure of rats by inhalation for 1-6 days, and that the nanosized cadmium oxide (40 nm) only induces significant lung effects at high doses ($550g/m^3$ compared to $70g/m^3$) [29].

One study was noted in the searches which considered not pulmonary toxicity, but the potential embryotoxicity of nanoparticles [32]. The effects of mixed size polystyrene particles in thawed mouse two cell embryos were investigated after 72 hours. No effects on embryo development were found, either at the two cells or blastocyst stage, although the particles were internalised by the embryos.

3.3 Translocation of nanoparticles

The potential for inhaled particles to access the central nervous system via the olfactory nerves has been highlighted by studies going back to the 1940s. Oberdorster's group has carried out several more recent studies and shown that carbon nanoparticles [33], and manganese oxide (30 nm) [34] can accumulate in the olfactory bulb following inhalation, and the manganese nanoparticles induce inflammatory changes in the brain. These results were previously published in abstract form for the Society for Toxicology meeting [35]. The authors argue that despite the differences between the human and rodent nasal anatomy, these results are relevant to human nanoparticle exposures.

Six other studies have investigated the distribution and translocation of nanoparticles in rodents following inhalation exposure: the results do not give a completely consistent picture of the fate of inhaled particles, and suggest they may vary with specific nanoparticle characteristics. Systemic translocation has been reported with cadmium oxide or iridium either in nano- or micron sized forms, with some translocation to extrapulmonary organs such as the liver [29; 36], although Takenaka et al noted that translocation only occurred at the high doses that also induced significant amounts of lung damage. This group has also studied the distribution of ultrafine silver particles after inhalation in the rat, and found that although retained in the lung, the silver enters systemic pathways and can be detected in secondary organs (kidney, brain and heart) [37]. However two other studies have reported more limited or no translocation of nanoparticles to secondary organs: surprisingly one report is by Takenaka et al [38], in which they calculated that there was no significant clearance of silver nanoparticles from the lung, although the particles were seen in macrophages and the alveolar wall. The second study by Oberdorster's group [39] found that elemental carbon nanoparticles accumulate in the lung following inhalation, but were not detectable in extra-pulmonary organs other than the liver.

One study was retrieved in the searches that considered penetration of nanoparticles through intact skin. Since it is generally thought that nanoparticles such as TiO_2 do not penetrate intact skin, and this study reports the opposite, it has been summarised below:

Penetration of intact skin by quantum dots with diverse physicochemical properties. Ryman-Rasmussen et al, (2006) [40]

Semiconductor nanocrystals or quantum dots (QD) have great potential for use as diagnostic and imaging agents in biomedicine and as semiconductors in the electronics industry. Dermal exposure presents a potential route of exposure to QD for producers and consumers, and so the permeability of skin to commercially available QD of two core/shell sizes and shapes, and three different surface coatings was evaluated. The results indicated that QD of different sizes, shapes and surface coating could penetrate porcine skin at an occupationally relevant dose within the time frame of an average working day. **The authors reported these findings to be surprising since they contradict the conventional opinion that the skin presents an impervious barrier to materials and that abrasion or mechanical stressors are required for nanomaterial penetration.**

3.4 Computational models evaluating nanoparticle deposition

Six studies have examined deposition and distribution of nanoparticles following inhalation (e.g. [41]). **Computational models** have also been developed and tested for evaluating nanoparticle depositions in humans:

Comparison of micro- and nano-size particle depositions in a human upper airway model. Zhang et al. (2005) [42]

The size of inhaled particles influences the type of deposition mechanism and in addition to regional and total deposition fractions, local particle deposition profiles are an important parameter for health effect assessment (e.g. they may play a key role in the development of lung cancer). Unlike *in vivo* or *in vitro* tests, validated computational fluid-particle dynamics (CFPD) models can provide non-invasive, accurate and cost effective means of simulating particle deposition. The results of this study indicated that although depositions of both micro and nano sized particles vary measurably in the human upper airways, deposition distributions were more uniform for nanoparticles. The authors hypothesise that uniformly deposited nanoparticles may have greater toxicity when compared with microparticles at a

similar concentration, since the broader deposition area presents more sites to interact with cell membranes and a greater capacity to absorb and transport toxic substances.

3.5 Studies *in vivo* on carbon nanotubes

Many in the nanotoxicology field are particularly concerned about the potential for **carbon nanotubes** (CNTs) to cause health effects since they have both nanosize and fibre properties. A number of papers were identified in the literature searches, which have looked at the effects of carbon nanotubes *in vivo*: five of these studies were full papers, considered below, whilst the remaining six were abstracts for the Society of Toxicology and other conferences in 2005 and 2004 [43-48]. Five of these conference abstracts reported pulmonary inflammation and development of multi-focal granulomatous lesions and fibrosis in response to inhalation of carbon nanotubes in rodents. Li et al [43] reported dose-dependent aortic changes in mice exposed to CNTs (and oxidative changes in human aortic endothelia grown *in vitro*).

Three of the full papers considered pulmonary effects of CNTs; the study of Shvedova et al [49] builds on the two earlier studies:

Comparative pulmonary toxicity assessment of single-walled carbon nanotubes in rats. Warheit et al. (2004) [50]

Warheit and colleagues compared the acute toxicity of intratracheally instilled single-walled carbon nanotubes (SWCNTs; 1.4 nm diameter by >1μm long) with quartz particles (1-3 μm) and carbonyl iron particles (0.8-3.0 μm) in rats. Only high doses of the quartz particles induced significant sustained pulmonary inflammation, whereas the SWCNTs induced a transient inflammatory response and non-dose-dependent, non-progressive multi-focal granulomas. The formation of the granulomas was not consistent with the lack of sustained inflammatory or toxicity responses, nor with the normal dust-related responses. **The authors suggest that the findings support the hypothesis that carbon nanotubes readily agglomerate, and suggest that workplace exposures are likely to be very low, rendering the results reported of low physiological relevance.**

Pulmonary toxicity of single-walled carbon nanotubes in mice 7 and 90 days after intratracheal instillation. Lam et al. (2004) [51]

Lam and co-workers used three types of CNTs: raw and pure CNTs produced by the methods of high-pressure carbon monoxide conversion, and CNTs from vaporisation from graphite by electric arc. The pure CNTs contained considerably less iron and nickel than the other particles. Each CNT was about 1 nm wide by several μm long. Mice were intratracheally instilled with either a low (0.1 mg) or high dose (0.5 mg) of the CNTs, carbon black or quartz (as controls). All the CNTs induced dose-dependent lung lesions after 7 days, mainly epithelial granulomas, which persisted or worsened after 90 days. The CNTs, regardless of manufacturing process or purity, were more toxic than carbon black and potentially more toxic than quartz. **The authors conclude that on the basis of these results, if workers were chronically exposed to respirable CNT dust at a fraction (by mass) of the current permissible exposure limit set by OSHA (5 mg/m^3) for synthetic graphite, they would develop serious lung lesions, and therefore this PEL for should not be used to protect workers handling CNTs.**

The differences between these studies prompted **Shvedova et al (2005)** [49] to carry out a more detailed analysis: **Unusual inflammatory and fibrogenic pulmonary responses to**

single- walled carbon nanotubes in mice. The CNTs were purified and thoroughly characterised (by TEM), revealing that they contained 0.23% iron, and in suspension, formed a mat of intertwined carbon ropes, which when aerosolised, formed compact aggregates and dispersed structures. Doses up to 50 μg were administered per mouse, and the controls were 40 μg carbon black or crystalline silica. These doses were justified on the basis that a person exposed to the above PEL for twenty 8h days would be equivalent to aspiration of 20 μg SWCNTs by a mouse. After 7 days' exposure, foci of granulomatous inflammation were observed around the SWCNT aggregates, and distant from these, interstitial fibrosis, both of which progressed with time. These effects were not observed with the control particles. There was early inflammation in the lung, with accumulation of neutrophils, followed by lymphocytes and macrophages. The particles also slowed bacterial clearance from the mouse lungs.

These studies therefore suggest that inhalation of carbon nanotubes has the potential to induce inflammation and the formation of granulomas, but they need to be considered in the light of the exposure data from Maynard's group [52], which suggests that levels of airborne CNTs achieved in the workplace may be very low.

Two other studies have investigated the effects of CNTs when implanted subcutaneously in rats [53; 54]. Yokoyama et al noted that the nanotubes were engulfed by macrophages but they did not induce a severe inflammatory response, whilst Sato and colleagues compared the effects of CNTs of two different lengths (220 and 825 nm). The degree of inflammation around the longer CNTs was stronger than that around 220 nm CNTs, and the authors propose that this is because macrophages could envelop the shorter fibres more readily. No severe inflammatory response was observed associated with either size of CNTs.

3.6 *In vitro* studies

Thirteen *in vitro* studies have been carried out to investigate the **toxicity of CNTs**, of which three were abstracts for conferences. For these thirteen studies, the models chosen were as follows: human skin cells (two studies [55; 56]), human skin and epithelial cells (one conference abstract [57]), human epithelial cells (two studies [58; 59] and one conference abstract [60]), human fibroblasts (three studies [61-63]), human or animal macrophages (two studies [64; 65] and one conference abstract [66]) and human T lymphocytes [67].

Multi-walled CNTs (MWCNTs) are taken up by human skin cells *in vitro*, and have pro-inflammatory effects [56]. Single walled CNTs (SWCNTs) induce oxidative stress and toxicity in human keratinocytes [55; 57], as summarised below for the more detailed report by Shvedova et al:

Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. Shvedova et al. (2003) [55]

Shvedova et al showed that SWCNTs (impure, containing 30% iron, no details of size of tubes) induce oxidative stress in human keratinocytes grown *in vitro*; this was an extensive study in that a broad range of endpoints were used to demonstrate induction of oxidative stress, including production of free radicals by electron spin resonance, and depletion of cellular antioxidants and vitamin E. They also showed that the CNTs dose-dependently induced cell death, and altered the morphology of the cells.

The four reports that have considered epithelial cells have used either the human bronchial epithelial cell line BEAS-2B (three studies) or the human kidney cell line HEK293, and shown that SWCNTs induce oxidative stress and toxicity [57; 60], and modulate gene expression [58; 59].

Of the studies carried out in macrophage and monocyte cells, both SWCNTs and MWCNTs were reported to be cytotoxic in murine macrophages [65], and the toxicity of different particles was ranked in alveolar macrophages: SWCNT > MWCNT > quartz > C60 fullerene [64], the authors of the latter paper also noting that higher doses of SWCNTs also impaired phagocytosis. Although there is one conference abstract which reported that SWCNT can induce pro-inflammatory and fibrogenic cytokines in human macrophages [66], only one full paper considered human monocytes or macrophages (considered according to the selection criteria to be of higher priority than studies of animal cells), that of Sato et al:

Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukaemia cell line THP-1 in vitro and subcutaneous tissue rats in vivo. Sato et al. (2005) [53]

Both CNTs (20-40nm wide, and 220 or 825 nm long) induced TNFα dose-dependently in human THP-1 cells, although to a lower level than a microbial lipopeptide. In conjunction with the *in* vivo data mentioned above, the authors conclude that the cellular response (inflammation and engulfment by macrophages) is affected by CNT length, and resultant morphology of the nanotubes.

Three studies have reported that in fibroblasts, the level of purification and modification of SWCNTs alters the cellular toxic response [61-63], such that refined SWCNTs are more toxic than unrefined, whilst sidewall functionalisation reduces toxicity. Furthermore in primary human T lymphocytes, hydrophobic, pristine CNTs are less toxic than oxidised particles [67].

One final study should be noted: namely that in an unspecified cell type, MWCNTs were not cytotoxic [68]. Without detailed evaluation of this publication, the validity of the authors' conclusions cannot be assessed.

***In vitro* studies** have also investigated the potential health effects of other nanoparticles, using a range of different endpoints, which may give useful information about the mechanisms of action of particles. The studies in human cells will be considered first, since these were considered to be of higher priority than those with animal cells.

Most studies of human cells (five of eleven studies retrieved) employed epithelial cells of bronchial or lung origin. In these cells, carbon or carbon black nanoparticles induced a range of inflammatory changes: release of either C-reactive protein [69], macrophage chemoattractants (not seen with nano-TiO_2) [70], or IL-8 [71]. Ultrafine carbon black also caused changes in gene expression (proto-oncogenes and genes involved in apoptosis), not seen with fine particles [72]. One of the most extensive studies in human bronchial epithelial cells was described in a conference abstract and noted that different particles of comparable nano-size (CuO, CeO_2, SiO_2, TiO_2) had different effects on ROS generation, but similar effects on gene expression for all except cell cycle genes where TiO_2 showed a different pattern to the others [73].

Three studies used human macrophages and monocytes, and noted that nanoparticles (carbon black or TiO_2) could impair phagocytosis *in vitro* [74; 75] and induced increases in intracellular calcium [76]

One investigation has compared the toxicity of seven industrially important nanoparticles in human and mouse cells, and compared the effects to other particles of well documented toxicology, which therefore suggested this paper would be of particular interest to HSE:

In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Brunner et al. (2006) [77]

The particles were of similar sizes (6-21 nm) and were classified as slightly soluble (tricalcium phosphate, zinc/iron oxide) or highly insoluble (ceria, titania and zirconia) or reference materials (highly toxic crocidolite asbestos and non-toxic silica particles). DNA

content and MTT assay were used to analyse viability in the human mesothelioma line MSTO-211H and the mouse fibroblast line 3T3. Cytotoxicity was affected by particle solubility, surface chemistry, and cell-type, such that the relative toxicities were:

MSTO-211H: Fe_2O_3 ~ asbestos > ZnO > CeO_2 ~ ZrO_2 ~TiO_2 ~ $Ca_3(PO_4)_2$

3T3: ZnO > asbestos ~ ZrO_2 > $Ca_3(PO_4)_2$ ~ Fe_2O_3 ~ CeO_2 ~TiO_2

The toxicity of iron oxide in MSTO cells and zinc oxide in 3T3 cells was considerably greater than that of non-nano-sized iron ions, demonstrating a nano-scale effect on toxicity. **The authors suggest that these simple assays on cell lines *in vitro* can give useful comparative information on the toxicity of nanoparticles, as an early, "pre-screening" test system.**

The remaining two human *in vitro* studies have evaluated different nanoparticles in different cell types. Hydroxyapatite nanoparticles inhibit the growth of human hepatoma cells and dose-dependently induce apoptosis [78], and titanium nanoparticles induce transcription of stress response chemokines in human osteoblasts [79].

Studies with **animal cells *in vitro*** were of the lowest priority for this bulletin. Of the ten studies not considered elsewhere, one noted that ultrafine TiO_2 induces chromosomal changes (formation of micronuclei) and apoptosis in Syrian hamster embryo cells [80].

Five studies have employed rat or canine alveolar macrophages. Two of these noted that, consistent with other results listed in this bulletin, the surface area of nanoparticles modulates the cellular response, whether this is release of lipid mediators by agglomerated carbon or TiO_2 nanoparticles [81], or the ability to induce oxidative stress by carbon black nanoparticles [82]. In the third study, it was reported that ageing increases the susceptibility of alveolar macrophages to nanoparticles such that cells from older rats released more TNFα than cells from younger rats in response to ultrafine cobalt or nickel [83]. TiO_2 was relatively non-toxic in this study. Two further *in vitro* studies have shown that nanoparticles can induce cytoskeletal changes in primary alveolar macrophages, potentially leading to impairment of phagocytosis [74], a finding supported by a study in a macrophage cell line [75].

Two animal *in vitro* studies have compared the effects of different nanoparticles:

In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Hussain et al (2005) [84]
The acute toxic effects of five different metal / metal oxide nanoparticles were assessed in a rat liver cell line (BRL3A). Silver nanoparticles were highly toxic; molybdenum nanoparticles were moderately toxic, and iron oxide, aluminium, and manganese nanoparticles showed no toxicity at the doses tested, although at high doses, the cells became irregular in shape. The authors propose that cell death induced by silver was likely mediated by oxidative stress.

Effects of nanophase materials (less than or equal to 20 nm) on biological responses. Cheng et al (2004) [85]

Metal nanoparticles were tested in a monodisperse aerosol form on epithelial cells; copper was the most potent at inducing the cytokine IL-8, but the kinetics of induction were different compared with nickel or vanadium, and IL-8 production was increased markedly by acidification of the particles, potentially via oxidative stress. The authors state that this study points to the complexity of cellular responses to nanoparticles.

Functionalisation of gold nanoparticles with cationic side chains (but not anionic side chains) increases cytotoxicity at high doses (although the cell type was not specified) [86].

Finally, the only *in vitro* study using a neuronal cell line reported that nano-sized manganese oxide nanoparticles (40nm) dose-dependently induce depletion of metabolites of the

neurotransmitter dopamine in PC12 cells, in contrast to silver nanoparticles [87].

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5. APPENDICES

5.1 APPENDIX 1 - Search Strategy For HSE Helpdesk

5.1.1 First Search

Initial searches were based on selecting one term from two lists: one NANO* term, and either one health-related term or one exposure-related term:

List 1: NANO* terms:

Nanostructure
Nanoparticle
Nanotube
Nanowire
Nanocrystal
Nanodot
Nanofibre
Nanopowder
Nanocapsule
Nanostructure
Nanometre / nanometer
Nanoaerosol
Nanoagent
Nanoaggregate
Nanoalloy
Nanoarray
Nanoassembly
Nanoassociate
Nanoball
Nanoconjugate
Nanoblob
Nanoblock
Nanobody
Nanobot
Nanocable
Nanocage
Nanocapsule
Nanocarbon
Nanochip
Nanoclay
Nanocluster
Nanocolloid
Nanocomposite
Nanocube
Nanodimension
Nanodisc
Nanodrop(let)
Nanoelement
Nanoemulsion
Nanodetergent
Nanofibril
Nanofilm
Nanofilter
Nanoflake
Nanogram
Nanohazard
Nanohelix
Nanomaterial
Nanometal
Nanoribbon
Nanorice
Nanoscale
Nanosphere
Nanosheet
Nanosize
Nanosubstance
Nanosuspension
Nanotechnology
Nanothread
Nanotoxic*
Nanotubule
Nanovesicle
Nanowhisker
Nanoparticulate
Nanoplatelet
Nanopolymer
Nanorod
Nanorope
Quantum dot
Ultrafine

List 2: Health-related terms:

Biological Monitoring / Biomonitoring / Biomarker
Environmental Monitoring
Exposure
Hazard
Hygiene
Risk / (Health) Risk Assessment
Dose-Response / Concentration
Adverse (Health) Effect
Safety
Toxic* (Toxicity / Toxicology)
Case Study / Case-Control Study/ Epidemiol* / Cohort / Prospective
Occupation* / Industry* / Work*
Ill-Health / Disease
Human / Volunteer
Animal (Primate, Rat, Mouse, Dog, Cat, Rodent, Guinea Pig)
Population / (Sub)-Group
Incidence / Mortal* / Morbid*
Inhal* / Oral / Ingest* / Derm* / Intravenous
Short / Long Term / Acute / Chronic
Systemic / Local
In Vivo/Vitro/Silico / Assay / Model*
Genotoxicity / Genotoxic
Carcinogen / Carcinogenic
Mutagen / Mutagenic
Irritant / Irritancy
Allergy / Allergic
Computational Modelling

List 3: Measurement, exposure and control terms:

Control$4 / Contain$4 / Ventilat$5
Fume NEAR (Cupboard$1 OR Cabinet$1)
Laminar NEAR Flow NEAR Cabinet
Vacuum NEAR Clean$3

Instrument$5 / Detect$3 / Sampl$3 / Monitor$3 / Measure$5 / Analys$4 / Characteri$6

Dust$1 / Release$1 / Dispers$3 / Airborne / Aerosol$1 / Handling

Respirator$1 / Mask$1
Facepiece$1 / Face NEAR Piece1
Breathing NEAR Apparatus$2
Protect$3
RPE OR RPD OR PPE
Glove$1
Clothes OR Clothing
Filter$1 OR Filtrat$3
Efficiency
Penetrat$3
Inhal$5
Derm$7

Expos$3
Hygiene
Risk$1

The HSE Search Service in Sheffield ran the searches using a comprehensive set of databases, and obtained the results:

Hits:

Terms from list 1 and 2 = 46 935
Search refined by the two terms being within 5 words of each other = 5 006
Search further refined by date (since 2000) = 4288

Terms from lists 1 and 3:
a. Search using the near operator, which brings the nano terms within 5 words of the exposure terms: 29,468 hits (24,811 for 2000 onwards and 10,493 for 2005/06).
b. Search from list 1 and 3 in the title and descriptor fields: 43,366 hits (37,007 for 2000 onwards and 14,903 for 2005/06).

The number of results was unexpectedly large. A survey of the titles of the first 50 articles in the searches suggested that many articles were not relevant to the aims of this bulletin and agreed criteria, and therefore the search strategy was refined as follows.

5.1.2 Refined Search Strategies:

The NANO terms list 1 was reduced to 21 terms:

List 1B: NANO* terms:

Nanostructure
Nanoparticle
Nanotube
Nanowire
Nanocrystal
Nanofibre
Nanopowder
Nanocapsule
Nanostructure
Nanoaerosol
Nanoaggregate
Nanocomposite
Nanomaterial
Nanoscale
Nanosphere
Nanosize
Nanotechnology
Nanoparticulate
Nanorod
Nanorope
Ultrafine

The second list of topic specific terms was separated into two lists:

List 2 revised: Health-related terms:

List 2B: General health-related terms:

Monitoring
Exposure
Hazard
Risk
Safety
Toxic*
Health

List 2C: Specific health-related terms:

Human
Volunteer
Worker
Animal
Cell
Hygiene
In silico
Computational
Epidemiol*
Workplace
Occupational
Inhalation
Pulmonary
Oral
Ingestion
Dermal / skin
Systemic
In vitro
Mutagen
Carcinogen
Genotox*
Irritant / irritancy
Allergy / allergic

List 3 revised: Measurement, exposure and control terms:

List 3B: General terms:

Hazard$3
Risk$1
Safety
Toxic$8
Health$1
Work$5
Airborne / Aerosol$1
Aggregat$4 / Agglomerat$3 / Agglomeration$1
Environment$2
Occupation$2 / Hygiene

List 3C: Specific terms:

Respirator$1 / Mask$1
Facepiece$1
Face NEAR Piece$1
Breathing NEAR Apparatus
Respiratory NEAR Protect$3 NEAR Equipment
RPE OR PPE OR RPD
Personal NEAR Protect$3 NEAR Equipment
Respiratory NEAR Protect$3 NEAR Device$1
Protect$3
Glove$1 / Clothing OR Clothes
Filter$1 / Filtrat$3
Penetrat$3
Inhal$5
Dermal / Skin

Dust$1
Release$1 / Dispers$3
Generat$3
Handling
Control$3 / Containment$1 / Ventilat$3
Fume NEAR Cupboard$1
Fume NEAR Cabinet$1
Vacuum NEAR Cleaner$1
Laminar NEAR Flow NEAR Booth$2
Laminar NEAR Flow NEAR Cabinet$1

Instrument$5
Detect$3
Sampling / Sampler$1
Monitor$3 / Measur$5 / Characteri$6
Metrology
Exposed OR Exposure

Results for measurement, exposure and control search strategy

The Sheffield Search team then ran the search, interrogating the number of hits with: Term from list 1B + 3B + 3C (with and without the exclusion of the term "drug$1; thin NEAR film$1; colloid$1")
The search were run using four databases: Chemical Engineering Abstracts (CEAB), Compendex (COMP), Chemical Abstracts (CA) and Analytical Abstracts (ANAB).

The results were:

1. List 1B **AND** List 3B **AND** List 3C with date limit:

CEAB: 1 126 references
COMP: 15 383 references
CA: 2 249 references
ANAB: 403 references
(In the above 'AND' is the boolean operator, words appear anywhere in the record)

2. List 1B **AND** List 3B **AND** List 3C with date limit and with exclusion terms:
CEAB: 880 references
COMP: 12 552 references
CA: 1 845 references
ANAB: 344 references
(In the above 'AND' is the boolean operator, words appear anywhere in the record)

3. List 1B **WITH** List 3B **WITH** List 3C and date:
CEAB: 105 references
COMP: 1 225 references
CA: 538 references
ANAB: 39 references
(In the above '**WITH**' is the boolean operator, words appear anywhere in the record)

4. List 1B **WITH** List 3B **WITH** List 3C and date and with exclusion terms:
CEAB: 91 references
COMP: 999 references
CA: 402 references
ANAB: 33 references
(In the above '**WITH**' is the boolean operator, words appear anywhere in the record)

5. List 1B **AND** List 3B **AND** List 3C **(terms specified in the title and descriptor fields)** and date limit:
CEAB: 60 references
COMP: 1 210 references
CA: 289 references
ANAB: 15 references

6. List 1B **AND** List 3B **AND** List 3C **(terms specified in the title and descriptor fields)** and date limit and with exclusion terms:
CEAB: 49 references
COMP: 973 references
CA: 434 references
ANAB: 14 references

Results for health effects search strategy

The Sheffield Search team then ran the search initially with the databases Medline and Embase, interrogating with:

1. Term from list 1B + 2C + 2C (with and without the exclusion of the term “drug”)
2. Term from list 1B + 2C (with and without the exclusion of the term “drug”)

No synonyms were included for any of the terms. A review search was also done from 2000 (Nano term from List 1 revised in the category of review articles only). The results of this refined strategy are shown in Table 3:

Table 3: Results of different search strategies

List 1	List 2	List 3	Drug?	Where is term?	Boolean operator	Medline	Embase
Yes	Yes	Yes	Yes	Entire document	And	1698	2022
Yes	Yes	Yes	No			960	838
Yes	Yes	Yes	Yes	Entire document	With	220	212
Yes	Yes	Yes	No			106	89
Yes	No	Yes	Yes	Entire document	And	7180	6326
Yes	No	Yes	No			4882	2830
Yes	No	Yes	Yes	Entire document	With	2236	1823
Yes	No	Yes	No			1387	771
Yes	Yes	Yes	Yes	Title, Abstract	And	874	817
Yes	Yes	Yes	No			474	328
Yes	No	Yes	Yes	Title, Abstract	And	4096	3349
Yes	No	Yes	No			2684	1518
Yes	Yes	Yes	Yes	Title, Descriptor	And	412	747
Yes	Yes	Yes	No			202	194
Yes	No	Yes	Yes	Title, Descriptor	And	3899	4056
Yes	No	Yes	No			2298	1447

Yes	Yes	Yes	Yes	Title, Major Descriptor	And	106	87
Yes	Yes	Yes	No			53	23
Yes	No	Yes	Yes	Title, Major Descriptor	And	991	826
Yes	No	Yes	No			600	287
Yes	No	No	Yes/No	REVIEW	And	29 With drug	214 W/o Drug

5.1.3 Final search strategy

Measurement, exposure and control:

Strategic search:

In line with the advice from the Search Service in Sheffield, a final strategy was used, changing the following in List 3B's set of terms:

Hazard$3 was changed to: hazard, hazards, hazardous
Risk$1 was changed to: risk, risks
Toxic$8 was changed to: toxicity, toxicities, toxic, toxics, toxicology, toxicological
Health$1 was changed to: health
Work$5 to: worker, workers, workplace, workplaces
Environment$2 was changed to: environmental

Two terms were also added: dustiness and sampled

The databases were searched for terms from Lists 1B + 3B + 3C:

- in the entire document, using the "with" operator
- in the Title and Descriptor fields, using the "and" operator

Drug$1; thin NEAR film$1; colloid$1 was excluded as terms, and the date limit was applied.

The two sets of results were then combined with the "or" operator, and de-duplicated within each database.

There were 827 hits: Medline 308, Chemical Safety Newsbase 78, Chemical Engineering Abstracts 83, Analytical Abstracts 17, Toxfile 26, Embase 315.

Simple searches:

The Sheffield Search team also ran two simple searches:

- The first search was on OSHROM, which contains the following databases: HSELINE (HSE's database)

NIOSH (National Institute of Occupational Safety and Health - US)
RILOSH (Ryerson International Labour Occupational Safety and Health - Canandian)
OSHLINE (Canadian Centre for Occupational Health and Safety)
CISDOC (International Labour Office)

The terms used was nano* or ultrafine* (quantum dot* retrieved a nil result) and a date limit of 2000 onwards. This resulted in 312 references (6426 before the date limit was applied), which was reduced to 245, sifting out material that was not relevant. References covered both subject areas (health effects and measurement, exposure and control subject areas). There is no de-duplication facility on OSHROM so some references may appear more than once.

- ❖ The second search interrogated Embase database using the 'NANO terms' from the list 1B combined with the word 'review'. This resulted in 228 references. However, most of these references were considered irrelevant for the context of this bulletin.

There was some duplication between databases. These 'hits' were sifted, excluding references that clearly did not meet the modified criteria shown in Table 4.

Health Effects

- In line with the advice from the Search Service in Sheffield, a final strategy was used, adding the term "dose" to List 2B's set of terms.

  Embase, Medline and Toxfile were searched for terms from Lists 1B + 2B + 2C in the entire document, using the "with" operator

  Embase, Medline and Toxfile were searched for terms from Lists 1B + 2B + 2C in the Title and Descriptor fields, using the "and" operator

  Drug was excluded as a term, and the date limit was applied.

  The two sets of results were then combined with the "or" operator, and de-duplicated within each database.

- The same simple searches were used as described above.

There were 502 hits. There is some duplication between databases. These 'hits' were sifted, excluding references that clearly did not meet the criteria agreed in the original Helpdesk Service Protocol (Table 5).

5.2 APPENDIX 5.2 – Search Results

Measurement, exposure and control:

The results are shown in Table 4.

Of the 1300 hits, 285 were selected and the abstracts were requested from Sheffield Information Services. These abstracts were reviewed, and prioritised according to the agreed criteria (Table 4); those that considered engineered nanoparticles were assigned a higher priority than those investigating the effects of ambient ultrafines. Table 4 shows the numbers of papers that met the different criteria. References considered of highest importance were read in full and then summarised. More detailed evaluation of individual papers can be requested.

Table 4: Criteria applied to measurement, exposure and control search results:

Category number	Topics	Priority	Importance	Number of papers	Comments
1	Evidence of dispersion of manufactured nanoparticles in air	High	● High/Medium ■ Medium/Low	● **1** ■ **0**	● One paper (2006) reported possible leaks in the production line of carbon black.
2	Evidence (most likely indirect at present) of human exposure to nanoparticles (occupational > non occupational)	High	● High/Medium ■ Medium/Low	● **1** ■ **0**	● One paper (2005) reported cases of people suffering from allergy and asthma to printer's ink. The causes of these reported illnesses are not clear.
3	Evidence of exposure dose, exposure profile, and exposure history to nanoparticles for humans (occupational > non occupational)	High	● High/Medium ■ Medium/Low	● **0** ■ **1**	■ One paper (2004) reported epidemiological surveys of major TiO_2 manufacturing sites. It is not clear whether the measured concentration levels related to fine or ultrafine particles.
4	Evidence of human exposure to nanoparticles using biological monitoring	High	● High/Medium ■ Medium/Low	● **0** ■ **0**	
6	Evidence of effectiveness / non-effectiveness of control measures in workplaces	High	● High/Medium ■ Medium/Low	● **0** ■ **0**	
7	Assessment and sampling of nanoparticles exposure in workplaces	High	● High/Medium ■ Medium/Low	● **6** ■ **0**	● Three abstracts from same authors reported workplaces and simulation measurements on the handling of carbon nanotubes. The last three remaining papers focussed on airborne measurements in workplaces of carbon black and TiO_2 nanoparticles (one paper had already been categorised in topic area 1).
8	Assessment and sampling of submicron by-products exposure in	High	● High/Medium	● **13**	● These papers reported airborne measurements in workplaces on berrylium ultrafine particles, diesel particles, welding fumes

	workplaces		■ Medium/Low	■ **3**	etc…
9	Development of instruments and methods to measure exposure to nanoparticles / ultrafines (inhalation > dermal > ingestion)	High	● High/Medium ■ Medium/Low × Unclassified	● **24** ■ **0** × **8**	● These 23 papers reported development of instruments or techniques, intercomparison exercises, anamaleous responses and performance of current instruments. These papers were mainly on current instruments or methods for measurement of nanoparticles or ultrafines as described in Table 1. Two of these papers focussed on sampling strategies. × These 8 papers reported development of non-conventional instruments to measure characterise in workplaces including mass spectrometers. We identified one paper as high (a nanoaerosol mass spectrometer).
10	Standardisation	High	● High/Medium ■ Medium/Low	● **2**	One abstract mentioned the necessity of standards for evaluating the performance of nanoparticles instruments.
11	Assessment of performance of PPE / RPE to reduce exposure to nanoparticles / ultrafine – Development of methods to assess RPE / PPE	High	● High/Medium ■ Medium/Low	● **4** ■ **2**	■ One paper on evaluation for asbestos fibres and one on evaluation for viral bacteria
12	Collection efficiency properties of filters for aerosol nanoparticles / ultrafines	Medium / High	● High/medium ■ Medium/Low	● **5** ■ **0**	
13	Assessment of and methods to assess the collective control techniques (other than RPE)	High	● High/Medium ■ Medium/Low	● **7** ■ **4**	● ■ These 11 papers reported assessment of control techniques in the environmental waste industry rather than in the nanoparticles manufacturing.
14	Investigation of effective electrical charging of nanoparticles	High / Medium	● High/Medium ■ Medium /Low	● **8** ■ **0**	
15	Understanding of aggregation / coagulation of nanoparticles / ultrafines behaviour	Medium / Low	● High/medium ■ Medium/Low	● **7** ■ **4**	

16	Development of methods to assess nanoparticles intake exposure using biological monitoring	High	● High/Medium ■ Medium/Low	● **0** ■ **0**	
17	Development of risk assessment tools	Medium	● High/Medium ■ Medium/Low	● **0** ■ **1**	This paper reviews the effectiveness of a software, that predicts the physical and chemical properties based on the chemical structure software (used by the Environmental Protection Agency (USA)). This paper reported that the software cannot be used for nanomaterials at the moment.
18	Characterisation of nanoparticles (bulk, in fluids or for toxicological studies)	Medium	● High/Medium ■ Medium/Low	● **14** ■ **0**	
19	Review* of measurement techniques to assess exposure of nanoparticles in the workplace		⦿ 2006-2005 ⊡ 2004-2003 ✳ 2002-2000	⦿ **5** ⊡ **2** ✳ **1**	
20	Review* of and general discussion on control techniques		⦿ 2006-2005 ⊡ 2004-2003 ✳ 2002-2000	⦿ **2** ⊡ **2** ✳ **2**	
21	General discussion on risk assessment issues and health and safety strategies		⦿ 2006-2005 ⊡ 2004-2003 ✳ 2002-2000	⦿ **21** ⊡ **3** ✳ **0**	
22	Review* of and general discussion on regulations		⦿ 2006-2005 ⊡ 2004-2003 ✳ 2002-2000	⦿ **8** ⊡ **7** ✳ **0**	
23	General discussion on consumer exposure risks	High	● High/Medium ■ Medium/Low	● **2** ■ **0**	
24	Use of instruments / methods to assess exposure of airborne	Low	● High/Medium	● **59**	● ■ These papers reported environmental measurements mainly conventional techniques to monitor mass, number and surface

	environmental ultrafine particles (e.g. diesel particles)		■ Medium/Low	■ **32**	area of ultrafines as well as characterisation methods such as electron microscopy and mass spectrometry.
25	Review* of measurement techniques to assess exposure of airborne environmental ultrafine particles (e.g. diesel particles)		◉ 2006-2005 ⊡ 2004-2003 ✳ 2002-2000	◉ **2** ⊡ **3** ✳ **3**	
26	Generation of methods to obtain a dispersed nanoaerosol	Low	● High/Medium	● **11**	A number of papers on synthesis methodology were not included in this table.
27	Review* of generation methods to obtain a dispersed nanoaerosol		◉ 2006-2005 ⊡ 2004-2003 ✳ 2002-2000	**0** **1** **0**	
					TOTAL of 285

*Review hits may sometimes include book or book chapter

Health Effects:

Table 5: Criteria applied to Health Effects search results

Health Effect / Toxicology	Priority
Evidence (most likely indirect at present) about the health effects of exposure to **manufactured** nanoparticles from **human studies**	High
Evidence of health effects in **humans** related to exposure to nanoparticles using **biomarkers**	High
Evidence about the potential health effects of exposure to manufactured nanoparticles using **animal models**	Medium
Evidence of possible health effects related to exposure to nanoparticles using **biomarkers in animal models**.	Medium
Evidence about the human health effects of exposure to manufactured nanoparticles using **human *in vitro* assays**	Low
Evidence about the potential health effects of exposure to manufactured nanoparticles using **animal *in vitro* models**	Low
Evidence about the likely health effects of exposure to manufactured nanoparticles based upon the use of **computational modelling**	Low

The results are shown in Table 6.

Of the 502 hits, 249 were selected and the abstracts were requested from Sheffield Information Services. These abstracts were reviewed, and prioritised according to the agreed criteria (Table 5); those that considered engineered nanoparticles were assigned a higher priority than those investigating the effects of ambient ultrafines. Table 6 shows the numbers of papers that met the different criteria from the different databases searched, and the total number in each category that considered manufactured nanoparticles, all of which are listed in the bibliography accompanying the health effects bulletin. Key references considered were read in full and then summarised.

More detailed evaluation of individual papers can be requested.

Table 6: Numbers of references classified according to Health Effects Criteria

Database	**Criteria**							
	Human studies	Human biomarkers	Animal studies	Animal biomarkers	Human *in vitro*	Animal *in vitro*	Computational modelling	Reviews
OSH line	19	1	39	0	15	6	7	10
RILOSH	1	0	0	0	0	0	0	10
HSE line	1	0	1	0	2	0	1	14
NIOSH	7	0	8	0	10	3	1	12
Embase 1	4	0	7	0	7	6	0	7
Embase 2	3	0	5	0	7	4	1	4
Toxnet	1	0	2	0	0	1	0	3
Medline	17	1	20	1	15	12	3	31
Total	**53**	**2**	**82**	**1**	**56**	**32**	**13**	**91**
Engineered	5	1	47	0	31	17	6	10