Title page

The biological effects upon the cardiovascular system consequent to exposure to particulates of less than 500 nm in size.

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Key terms
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Air pollution.
Acute coronary syndromes
Oxidant stress
Pathophysiology

Abstract

Context

Ultrafine particulate matter contribution to cardiovascular disease is not known and not regulated. PM up to 500 nm are abundant in urban air and alveolar deposition is significant.

Objective

Effects beyond the alveolar barrier within the body or *in vitro* tissues exposed to particles <500 nm.

Methods and results

Databases: MEDLINE; Ovid-MEDLINE PREM; Web of Science; PubMed (SciGlobe). 127 articles. Results in tables:'subject type exposed', 'exposure type', 'technique'.

Conclusion

Heart rate, vasoactivity, atherosclerotic advancement, oxidative stress, coagulability, inflammatory changes are affected. Production of reactive oxygen species is a useful target to limit outcomes associated with UFP exposure.

Non-standard Abbreviations and Acronyms

8-OHdG- 8-hydroxy-2'deoxyguanosine
ALT- alanine aminotransferase
AST- aspartate aminotransferase
AMP- accumulation mode particle
CAD- coronary artery disease
CAP- concentrated ambient particle
CD- cellular differentiation
CMD- count mode diameter
COX- cyclooxygenase
CRP- C -reactive protein
DCeE- diesel and cerium spiked exhaust
EC_{50}- concentration of substance required to achieve half the difference between baseline and maximum response in the independent variable
EDHF- endothelium-derived hyperpolarizing factor
E_{max}- maximum possible effect of exposure substance
GSH/GSSH- glutathione/glutathione disulphide (oxidized form)
GP2b3a- glycoprotein 2b3a
HRT- heart rate turbulence
HRV- heart rate variability
KO- gene knockout
LDLR- low density lipoprotein receptor
L-NMMA- nitric oxide synthase
LCA- left carotid artery inhibitor
MCP-1- monocyte chemotactic protein-1
NAC- N-acetyl cysteine
PCDW- platelet component distribution width
QTc- corrected QT interval
RAS- renin-angiotensin system
rMSSD- mean square root of the sum of squared differences between adjacent normal-to-normal heart beat intervals
SBP- systolic blood pressure
SDNN- standard deviation of normal-normal heart beat intervals
TAT- thrombin-antithrombin
TCPP- meso-tetra-(p-carboxyphenyl) porphyrin
TpTe- time from the peak to the end of a T-wave

Introduction

Airborne particulate matter (PM) is the solid component of air pollution and contributes to excess mortality from pulmonary and cardiac causes in urban environments. The biological mechanisms by which particulate matter acts on the cardiovascular system are poorly defined in the current medical literature. In their recent update, the American Heart Association has stated that the overall evidence is consistent with a causal relationship between $PM_{2.5}$ exposure and cardiovascular morbidity and mortality (Brook et al., 2010). Smaller particles comprised of low toxicity, low solubility materials such as carbon black and titanium dioxide have been found to have greater toxicity than larger, respirable particles made of the same material and the upper cut-off size for this increased toxicity is around 200 nm, although the cut-off may not be sharp (Donaldson et al., 2000). The term ultrafine particles (UFP) does not have a universally agreed definition but is widely accepted as describing particles of less than 100 nm in diameter. The greater proportion of UFP in urban air derive from combustion sources, notably traffic related ((HEI), 2010).
For PM_{10}, the latest UK Government legislation (2010) has set 40 $\mu g/m^3$ as the yearly average limit, and 50 $\mu g/m^3$ as the daily limit, which is only allowed to be exceeded 35 times in one year ((DEFRA), 2010). Statutory limits surrounding $PM_{2.5}$ are more complex and standards for UFP are not in existence, but ambient UFP typically exists in concentrations <20 $\mu g/m^3$ in urban centres (Ning et al., 2007, Moore et al., 2007, Pakkanen et al., 2001). The authors of the Six Cities study demonstrated an overall cardiac mortality increase of 26% between the most and least polluted cities for $PM_{2.5}$ in their cohort study (Dockery et al., 1993). The APHEA-2 and NMPAS studies found a 0.69% (Katsouyanni et al., 2001) and 0.31% (Samet et al., 2000) increase, respectively, in daily cardiovascular mortality per 10 $\mu g/m^3$ increase in PM_{10}. The adverse effects of $PM_{2.5}$ in the epidemiological literature are likely to be driven by combustion derived nano-particles (CDNP) and for CDNP three properties appear important: surface area, organics and metals (Duffin et al., 2007). Ambient concentration of UFP does not correlate well with the larger fractions (Mills et al., 2011, Hoek et al., 2010, Pekkanen and Kulmala, 2004) and the effects of UFP on cardiovascular health are independent from larger particle fractions due to differential and diverse toxicities (Nel, 2005). Although the percentage alveolar deposition of inhaled nano-particles peaks around 50 nm there is an appreciable percentage deposition in the size range 100 nm to 500 nm (Carvalho et al., 2011, Oberdorster, 2001). Further to this, measurements of total particle number (TPN) by size demonstrate that, in urban air, the TPN in the size range 100 nm to 500 nm is not insignificant compared to TPN in the size range 20 nm to 100 nm (Beddows et al., 2009, Avino et al., 2013, Harrison et al., 2000). Therefore to assess the cardiovascular public health risk presented by CDNP in urban air, it is appropriate to review biological effects for NPs up to a 500 nm size rather than just for the somewhat arbitrarily defined UFP. It is important that the mechanisms underlying this excess cardiac mortality are understood. Such understanding could assist in identifying suitable biomarkers of effect which could then be used in epidemiological studies to delineate the truly affected and those at increased cardiovascular risk but prior to diagnosis of disease. This is of particular relevance given that individual exposure measurements are impractical and expensive. It could further provide evidence of associations between specific physical and/or chemical constituents of particulate matter and effects which could be addressed by primary prevention policies.
Many different mechanisms have been proposed to elucidate the excess mortality. Early *in vitro* (Veronesi et al., 1999, Jimenez et al., 2002) and experimental animal trials (Sun et al., 2005, Costa and Dreher, 1997, Tousoulis et al., 2012, Oberdürster, 2000) have detected significant changes in multiple biomarkers for acceleration of atherosclerosis ((HEI), 2002, (DoH), 2005). Further studies, including human volunteer trials, followed with varying results, and several mechanisms were proposed to link UFP with atherosclerotic deaths. The most notable include; suppression of heart rate variability (HRV), reduced coronary flow, oxidative stress leading to endothelial dysfunction, platelet activation and acute phase protein

response which all potentially contribute to cardiovascular mortality (Pope et al., 2002, Delfino et al., 2011, Brook, 2008). Currently, the mechanisms leading to atherosclerotic deaths have not yet been defined clearly and uncertainty remains surrounding causal relationships. An American Heart Association writing group, the Health Effects Institute and UK Department of Health all recognize determining causal pathways for future preventive strategies as an essential area of future research(Brook et al., 2004, (HEI), 2002, (DoH), 2005).
This review will systematically examine the effector mechanisms linking inhalation of ultrafine particulate matter to increased incidence of atherosclerotic deaths in human and animal subjects.

Methods

Devising search terms

To construct the search strategy, a method was adapted from the German Institute for Quality and Efficiency in Health Care (Hausner et al., 2012). The authors initially used basic search terms (ultrafine, nanoparticle, particulate matter, heart, cardiovascular system and vascular) to return a preliminary set of 42 papers related to the review topic. From these 42 papers, a 2/3 (test group) 1/3 (validation group) split was made using 2:1 restricted randomization on random allocation software (Gerard, 2008). 28 were randomized to the test group and 14 to the validation group. The 2/3 test group was used to construct a search strategy by retrieving “particulate matter” search terms and “biological mechanism” search terms from the article abstracts. These two groups of search terms were combined using the OVID interface to search the Medline database in an attempt to return the test group. See table 1 for the constructed search. All 28 test references were returned, proving methodological effectiveness of combining the extracted “particulate matter” search terms, with the “biological mechanism” search terms. The author conducting the search was blinded against the validation group. The search was validated by searching for the 14 previously unused references. All 14 were returned using the same search strategy. See figure 1 for a diagrammatic representation of the search construction.

Article search methods.

1. An electronic search of the MEDLINE database (1946-present) via Ovid, and Ovid-MEDLINE(R) In-Process & Other Non-Indexed Citations (PREM) as well as Web of Science database via Web of Knowledge was performed on 24/01/13. “Particulate matter” search terms were combined with “biological mechanism” search terms using the operator AND. Results were limited to post 1980 as relevant epidemiological work started around 1980 (Dockery et al., 1993, Samet et al., 2000, Katsouyanni et al., 2001).

2. PubMed was searched via the SciGlobe search engine. SciGlobe is a novel search engine which discerns the intent of the author to link search terms and uses modern linguistic technology to return focused publications from few keywords. The search terms “ultrafine and cardiovascular”, “air pollution and cardiovascular” and “nanoparticle and cardiovascular” were used to find articles where SciGlobe identified relationships existing between the two. The database was searched on 29/01/13.

Selection Criteria

All articles returned from manual and electronic search strategies were assessed for inclusion or exclusion against the criteria in table 2. Included articles featured both human and animal studies.

Inclusion assessment

Abstracts were extracted to EndNote, de-duplicated and viewed on-line. These articles were assessed for relevance based on the inclusion/ exclusion criteria and relevant articles were obtained in full. Two authors independently reviewed articles against the selection criteria for inclusion or exclusion. To make evident the transparency of the search method, all search returns and subsequent exclusions are presented as a QUORUM flow chart in figure 2.

Study Quality assessment

The study design which best controls for any known or unknown confounding of the exposure-response relationship is that of controlled exposure of humans or animals. These data have the obvious advantage over *in vitro* studies in that the response is measured which most closely resembles the response of real interest i.e. response in a human subject. Other study designs are subject to a greater potential for confounding variables obscuring true relationships of exposure and effect.

Results

Findings of the critical appraisal are presented in tabular format for the different categories of study design reviewed:
Table 3.a - Randomized controlled trials in humans (Inhalation of nanoparticles)
Table 3.b - Randomized controlled trials in animals (Inhalation of nanoparticles)
Table 4 - Human longitudinal cohort studies
Table 5.a - Controlled animal experiments (instillation of suspended nanoparticles)
Table 5.b - Controlled animal experiments (injection of nanoparticles into circulation)
Table 6.a - Controlled *In vitro* experiment (human tissues)
Table 6.b - Controlled *In vitro* experiment (animal tissues)
For each included article; subjects, exposure type and technique, outcomes and results, critical appraisal of the article, and conclusions are highlighted within the tables.
A summary of the detailed appraisals across the different study designs is presented below under the headings, “Vasoactivity”, “Heart Rate Blood Pressure and Autonomic Modulation”, “Thrombosis”, “Plaque Advancement”, “Acute Phase Protein and Cytokine Release”, and “Oxidative Stress”.

Vasoactivity

Acetylcholine and bradykinin are endothelium dependent vasodilators (Mills et al., 2011, Mikkelsen et al., 2011). The best evidence for endothelium dependent vasoactivity exists as a cross-over trial using 16 human inhalation exposures of carbon black (CB) at 70 $\mu g/m^3$ which showed no change for acetylcholine or bradykinin dependent arteriolar dilation (Mills et al., 2011). A 10 µg pulmonary deposition of TiO_2 nanoparticles attenuated endothelium dependent vasodilation in rats, with prostanoid dysfunction being a probable contributor (LeBlanc et al., 2010). Controlled inhalation exposure to an identical TiO_2 dose also found impaired reaction of arterioles to endogenous prostanoids (Knuckles et al., 2012). One strong experiment used inhalation exposure of nickel nanoparticles, over a time period designed to reflect occupational exposure and found the effect of acetylcholine attenuated (Cuevas, 2010). The strongest evidence using Ca^{2+} donation to mediate relaxation shows that TiO_2 nanoparticles attenuate this effect (LeBlanc et al., 2009, Nurkiewicz et al., 2008). Nitric oxide (NO) responsiveness is an endothelium-independent mechanism (Nurkiewicz et al., 2009). The strongest available evidence, human controlled inhalation at levels similar to those experienced in heavy traffic, reported normal NO function for CB, but impairment after ambient UFP exposure at 5 times the CB concentration (348 $\mu g/m^3$) (Mills et al., 2011). TiO_2 exposure through inhalation at doses relevant to occupational exposure showed no effect on NO dependent vasodilation (LeBlanc et al., 2009, Nurkiewicz et al., 2009), but did show a reduction in endothelial NO production (Nurkiewicz et al., 2009).
Two strong studies used controlled inhalation of TiO_2 nanoparticles in rodents (Knuckles et al., 2012) and CB in humans (Shah et al., 2008). In both, reactive hyperaemia blunted,

indicating an arterial dilation failure. Impaired tonic sympathetic influence was also seen (Knuckles et al., 2012).
Reduced coronary flow after infusion of UFP occurred in all isolated heart perfusion models (Hwang et al., 2008, Tong et al., 2010, Wold et al., 2006, Simkhovich et al., 2007, Cho et al., 2009). Infarct size was extended post-exposure to UFP and carbon nanotube exposure in an isolated heart perfusion model (Cho et al., 2009). In two cohorts of stable CAD patients, ST-depression correlated with ambient UFP levels (Delfino et al., 2011, Pekkanen et al., 2002). β-blockers may have been protective for ST-depression (Pekkanen et al., 2002).
The potent vasoconstrictor endothelin-2 was raised approximately 20% in rats after direct inhalation of UFP from a roadside for 3 days (Elder et al., 2004a).

Targets for future intervention
In 45 subjects, 72 exercise induced ST-segment depressions >0.1 mV over 6 months were recorded in a risk assessment study of UFP exposure. Those patients using β-blocker medication conferred a weaker association between ST-segment depression and UFP exposure compared with non β-blocker medicated patients. No heart rate difference existed between groups suggesting that β-blockers may exert a protective effect by different mechanisms than the assumed heart rate modulation (Pekkanen et al., 2002).

Summary
Strong evidence exists to indicate endothelium-dependent and independent pathways, including altered prostanoid and hormone expression, mediate UFP induced vasoconstriction. Autonomic impairment may also occur. Coronary circulation is reduced post-UFP exposure. Ischaemic effects may be limited by β-blocker use.

Heart rate, blood pressure and autonomic modulation
Altered autonomic function and increased cardiac demand are associated with ischaemic deaths, as is increased blood pressure (Avino et al., 2013). The strongest evidence for nanoparticle-induced heart rate change exists as controlled inhalation exposure to CB. Heart rate was increased after >24 hr exposure to concentrations of 15.6 mg/m^3 (Niwa et al., 2008), 180 µg/m^3 (Harder et al., 2005) and 172 µg/m^3 (Upadhyay et al., 2008) in rats, but no change was seen in humans at 50 µg/m^3 for 2 hours, nor for SBP, but when SBP and HR changes were considered as an arithmetical product, a statistically significant increase existed between exposed and control males (Shah et al., 2008).
Ambient exposure over 10 weeks in a cohort of myocardial infarction survivors found a small but significant increase in SBP (0.9 mmHg)(Rich et al., 2012). Controlled experimentation in rodents found that SBP was increased after exposure concentration and duration >180µg/m^3 and> 24 hours (Niwa et al., 2008, Upadhyay et al., 2008), but not below these (Harder et al., 2005). The greatest exposure, 15.6mg/m^3 over 4 weeks, increased SBP by 30mmHg at 14 and 28 days in rodents (Niwa et al., 2008).
Reduced heart rate variation (HRV), a marker of autonomic imbalance, is causally implicated in coronary disease and sudden cardiac death(Xhyheri et al., 2012). Controlled exposure trials in young patients showed HRV increases post exposure to UFP (Zareba et al., 2009, Samet et al., 2009). Respiratory influences on HRV decline with age, and combined with these findings suggest HRV modulation by UFP is dependent on baseline parasympathetic tone (Samet et al., 2009, Demeersman, 1993). Accordingly, all observational studies in human subjects aged >60 years (Folino et al., 2009, Weichenthal et al., 2011, Wu et al., 2012) and in elderly cardiac disease patients (Rich et al., 2012, Timonen et al., 2006) showed HRV reductions. Controlled exposure in cardiac disease patients however, showed no change (Routledge et al., 2006).

Summary
Increases in heart rate and SBP are both plausible in animal models. SBP may be slightly increased after ambient exposure. Reductions in HRV may occur in the elderly and cardiac diseased, post exposure.

Thrombosis
The best available evidence describing thrombosis formation is mostly formed by intra-tracheal instillation or injection of polystyrene nanoparticles, and not via inhalation exposures. Polystyrene particles are charged, like most UFP, but are otherwise unreactive (Nemmar et al., 2002, Cohen et al., 1998). In a photo-chemically induced vascular injury model, instilled 60 nm positively-charged polystyrene particles enhanced thrombus formation *in vivo* at concentrations of 50 -500 µg/kg (Nemmar et al., 2002, Nemmar et al., 2003, Silva et al., 2005, Bihari et al., 2010) and 50 mg/kg(Silva et al., 2005). 400 nm (500 µg/kg) instilled particles enhanced thrombus formation after photochemical injury in one experiment (Nemmar et al., 2004a).These particles partly enhanced platelet function in another instillation experiment which was limited by excessive aggregation caused by use of a very small solute volume (205 µl)(Nemmar et al., 2003). Negatively-charged 60 nm nanoparticles had no thrombotic effect (Nemmar et al., 2003, Silva et al., 2005) and may have had an inhibitory effect (Nemmar et al., 2002, Bihari et al., 2010) when examined in the photochemical injury model. 20 nm negatively-charged particles were associated with microscopic thrombi in mice, indicating a direct effect on thrombotic pathways (Furuyama et al., 2009).

Summary
It is likely that thrombosis is enhanced by nanoparticle or UFP exposure, but it is not known if this occurs at ambient inhalation concentrations. Charge may play an important role.

Plaque advancement
Exposure to ambient UFP for 40 days increased atherosclerotic plaque size in mice compared with controls (Araujo et al., 2008). Oropharyngeal or intra-tracheal instillation of particles also accelerated atherosclerotic advancement in mice (Mikkelsen et al., 2011, Niwa et al., 2007, Cassee et al., 2012).

In one intra-tracheal instillation of CB in $ApoE^{-/-}$ mice, no change in plaque morphology was seen. However, the exposure was limited by possible pollution with larger particles ($PM_{2.5}$). Disproportionately high mortality rates in the exposure group occurred also (Vesterdal et al., 2010).

Targets for future intervention
Cerium oxide is a fuel additive designed to increase fuel combustion efficiency (Sajith et al., 2010). Recently, a vulnerable murine model was exposed to diluted diesel exhaust (DE) of 1.7 mg/m^3 or diesel exhaust of 1.7 mg/m^3 spiked with 9 parts per million ultrafine CeO_2 particles (DeCe), intermittently for 4 weeks (Cassee et al., 2012). DeCe significantly limited plaque complexity compared to the DE group, suggesting a protective effect.

Summary
It is likely that atherosclerotic plaque size is advanced in animals exposed long term to UFP. Cerium oxide nanoparticle spiked diesel fuel may limit the deleterious effects of diesel exhaust.

Acute phase response and cytokine release
IL-6 is a known mediator of atherosclerosis (Huber et al., 1999). Seventeen experiments using a variety of nanoparticles or UFP showed IL-6 expression increased 2 hours-28 days post exposure after *in vitro* and *in vivo* exposures (Nemmar et al., 2011, Park et al., 2009, Niwa et al., 2008, Corbalan et al., 2011, Aung et al., 2011, Delfino et al., 2010, Delfino et al., 2009, Downs et al., 2012, Karoly et al., 2007, Mo et al., 2009a, Mo et al., 2012, Nishimori et al., 2009, Park et al., 2010, Srinivas et al., 2011, Zhu et al., 2011, Nishanth et al., 2011). Of the strongest evidence, a controlled inhalation exposure study using CeO_2 nanoparticles at 641 mg/m^3 showed an increase of IL-6 in rodents (Srinivas et al., 2011), but lower concentrations of CB in rodents and humans did not (0.05-0.150 mg/m^3) (Elder et al., 2004b,

Stewart et al., 2010). All instillations in rodents showed an increase (Nemmar et al., 2011, Park et al., 2009, Niwa et al., 2008, Mo et al., 2012, Park et al., 2010), bar one (Kim et al., 2012).
CRP is an independent causal factor in atherosclerosis (Bisoendial et al., 2007). Human and animal studies using controlled inhalation at low concentrations (50-172 $\mu g/m^3$) (Upadhyay et al., 2008, Rich et al., 2012, Routledge et al., 2006, Stewart et al., 2010), ambient exposure (Rich et al., 2012, Folino et al., 2009), and intra-tracheal instillation (Jia et al., 2012) showed no change in CRP levels after UFP, CB or carbon nanotube exposure. Very high inhalation exposure of 15.6 mg/m^3 CB in rodents compared to other inhalation studies (Upadhyay et al., 2008, Routledge et al., 2006, Stewart et al., 2010) caused an increase at 28 days (Niwa et al., 2008).
TNF-α is an important mediator in atherosclerosis (Yoshizumi et al., 1993). Nine *in vivo* experiments constitute the best available evidence. Three moderate strength studies using nanoparticle instillation exposures in animals found no TNF-α increase (Nemmar et al., 2011, Park et al., 2010). Six similar experiments found significant TNF-α protein or genetic expression with a range of nanoparticle or UFP exposures(Delfino et al., 2010, Downs et al., 2012, Nishimori et al., 2009, Park et al., 2010, Srinivas et al., 2011) . In a longitudinal cohort, for subjects prescribed clopidogrel and statins, correlation of UFP with TNF-α and inflammatory P-selectin was reduced, compared with those non-medicated (Delfino et al., 2009).

Targets for future intervention
In a cohort study, 64 coronary disease patients were followed up weekly for six weeks. Results showed significant correlations between UFP concentration (<250 nm) and inflammatory biomarkers (sTNF-RII and sP-selectin). The association between UFP and sP-selectin was weaker for patients on clopidogrel (Delfino et al., 2009). sP-selectin activates endothelial cells, leukocytes and promotes interaction of these cells with platelets (Blann et al., 2003). A similar more pronounced correlation reduction was seen for sTNF-RII in patients using statins (Delfino et al., 2009).

Summary
IL-6 and TNF-α are likely expressed after UFP or nanoparticle exposure. CRP expression might be increased at high exposure concentrations. Observational work suggests cardiac drugs may provide a protective effect against inflammatory biomarker increases.

Oxidative Stress
Three inhalation exposure trials exist for CB exposure, an important surrogate for ambient UFP. One showed no change in rodents exposed to 114 and 268 nm CB particles by continuous inhalation for 7 hrs at 1 mg/m^3 (Gilmour et al., 2004). Smaller particles (25-36 nm) caused oxidative stress in humans (Huang et al., 2010b) and rodents (Elder et al., 2004b) after reduced exposures (both exposures <150 $\mu g/m^3$ at <6 hours). Weaker evidence from intra-tracheal instillation in mice (Hwang et al., 2010), oral instillation in mice (Sharma et al., 2012) and controlled inhalation exposures (LeBlanc et al., 2010, Nurkiewicz et al., 2009) in rodents also showed an increase oxidative stress after ZnO nanoparticle exposure.

Lipid peroxidation is a result of oxidative stress, can lead to cell membrane break down, and is implicated in atherosclerosis (Mylonas and Kouretas, 1999).The strongest available evidence exists as controlled inhalation of ambient UFP at a busy roadside in rodents over 40 days (Araujo et al., 2008), where an increase in liver peroxidation was seen. Instillation of TiO_2 over 60 days also showed an increase (Cui et al., 2010). A marker of lipid peroxidation, heart malondialdehyde, was not raised post intra-tracheal instillation of CB after six days. Before instillation, very large CB agglomerates in the micrometre range were visualised by dynamic light scattering analysis, implying limited particle surface area (Jia et al., 2012).

Targets for future intervention
Radical scavengers and anti-oxidants administered to cells exposed to UFP and engineered nanoparticles eliminated previously observed inflammatory cytokine expression (Mo et al., 2009a, Wan et al., 2008), cytotoxicity (Mo et al., 2009a), and endothelial function (Vesterdal et al., 2012).

Recently, a vulnerable murine model was exposed to diluted diesel exhaust (DE) of 1.7 mg/m^3 or diesel exhaust of 1.7 mg/m^3 spiked with 9 parts per million ultrafine CeO_2 particles (DeCe), intermittently for 4 weeks (Cassee et al., 2012). Addition of DeCe particles to the fuel reduced particle number by 30% and a reduction of 10% surface area.
Compared to DE, CeO_2 supressed the production of reactive oxygen species in murine macrophages by 44% and induced resistance to cell cytotoxicity by 37% when cells were pre-treated with CeO_2 for 8 hours before exposure to DE ($p<0.01$) (Xia et al., 2008).

Summary
It is highly likely that UFP induces oxidative stress in the cardiovascular system. Oxidative stress is important in moderating downstream outcomes and presents a useful target. Cerium oxide spiked fuels may limit oxidative stress secondary to diesel ultrafine particulate.

Discussion

Studies that use suspended environmental samples for research into their biological effects usually require a preparation step prior to investigation, the purpose of which is to disrupt particle agglomerates into more representative constituent particles. The method most commonly applied is sonication. However, it should be noted that there is considerable lack of standardisation in the application of sonication and few studies accurately report the pre-dosing, *in vitro* or *in vivo* preparation procedure (Berube et al., 1999, Taurozzi et al., 2011). To consider this and related aspects of sample preparation is beyond the scope of this review, however it is important that the concept and the variation in particle preparation is recognized. Future research should therefore consider applying standardised methodologies and reporting mechanisms to sample preparation with special attention being paid to ultrasonic dispersion as differences in the pre-analytical dispersion methods likely lead to variability in results when comparing data between studies.

It is likely that coronary flow is reduced after injection of UFP and nanoparticles into *ex vivo* isolated heart models (Hwang et al., 2008, Tong et al., 2010, Wold et al., 2006, Simkhovich et al., 2007, Cho et al., 2009). Ultimately, myocardial infarction size was increased in these models (Cho et al., 2009). It is unclear if a reduction in coronary flow occurs after ambient exposure to UFP in the same manner as that after injection of UFP or nanoparticles, but the included data suggest that vasoconstriction is accentuated and vasodilation blunted in most major arteries after inhalation of UFP in humans (Mills et al., 2011, Shah et al., 2008). Evidence from animal models (Harder et al., 2005, Niwa et al., 2008, Upadhyay et al., 2008) and limited controlled human experimentation (Rich et al., 2012, Shah et al., 2008) suggest that increases in heart rate and blood pressure are biologically plausible sequelae after UFP exposure. HRV modulation probably occurs post UFP exposure, but risk of confounding from the observational studies which form the bulk of the evidence in older patients, and the impact of age in relation to baseline parasympathetic influence limits confidence in the magnitude and direction of HRV change (Samet et al., 2009, Demeersman, 1993).
Clearly, exposure to 60-400 nm positively charged nanoparticles enhanced thrombosis in artificial thrombus models (Nemmar et al., 2002, Nemmar et al., 2003, Cohen et al., 1998, Silva et al., 2005, Bihari et al., 2010). *In vitro* work suggests a thrombotic effect may exist (Radomski et al., Kendall et al., 2011), but a strong conclusion is limited by poor generalizability from use of surrogate particles in artificially induced thrombus models to inhalation of UFP in the real world.
Most of the included literature examined whether UFP produced excessive reactive oxygen species, overwhelming cellular defences. Strong studies concerning animals exposed to feasible UFP concentrations experienced in ambient air support a role of oxidative stress in UFP mediated toxicity (Araujo et al., 2008, Elder et al., 2004b, Huang et al., 2010b, Cui et al., 2010). Few studies have causally linked oxidative stress to potential secondary effectors such as interleukin release or endothelial dysfunction (Mo et al., 2009a, Mo et al., 2012, Vesterdal et al., 2012, Wan et al., 2008).

In vitro and intra-tracheal exposures tended to show IL-6, and TNF-α increases, but controlled exposures often did not. CRP was only raised in one very high exposure inhalation trial. It is unclear whether this effect is important at ambient exposure levels. Oxidative stress mediates inflammatory, endothelial and vasoactive pathology, and this may be an important target for future prevention strategy. Cerium oxide fuel additives may also limit deleterious processes secondary to UFP exposure. Also relevant for prevention, multiple cardiac drugs conferred a protective effect in medicated patients (Folino et al., 2009, Delfino et al., 2009, Corbalan et al., 2011).

When considering the effect of long term inhalation of UFP on the cardiovascular system, the strongest evidence would include human exposures of a long duration to ambient UFP or

its major constituents at levels considered feasible in an outdoor urban environment. The strongest available evidence exists as short term human volunteer exposures of 2-6 hours (Mills et al., 2011, Shah et al., 2008, Routledge et al., 2006, Zareba et al., 2009, Samet et al., 2009, Stewart et al., 2010, Frampton, 2007, Pietropaoli et al., 2004, Huang et al., 2010a) or longer exposures of 28-40 days in vulnerable murine and rodent models (Araujo et al., 2008, Cassee et al., 2012, Niwa et al., 2008). Controlled inhalation exposures failed to show positive findings for vasoactivity (Mills et al., 2011), inflammatory responses (Elder et al., 2004b, Stewart et al., 2010), oxidative stress (Gilmour et al., 2004) or cellular adhesion molecules (Elder et al., 2007), but *in vitro* experiments found positive results for inflammatory responses (Corbalan et al., 2011, Karoly et al., 2007, Zhu et al., 2011), oxidative stress (Nishanth et al., 2011, Vesterdal et al., 2012, Trpkovic et al., 2010) and cellular adhesion (Li et al., 2007, Li et al., 2010b, Vesterdal et al., 2012). It is possible that for exposure to UFP at ambient concentrations, 1) a larger sample is needed to show small statistically significant changes or 2) the differences between *in vitro* and *in vivo* conditions allow spurious *in vitro* findings.

The ability of UFP to react with the experimental medium or absorb nutrients was a recognized limitation of *in vitro* experimentation (Han et al., 2011, Clift et al., 2010, Guo et al., 2008), as was the risk of particle aggregation *in vitro*. This limitation was also appreciated during intra-tracheal instillation (Gilmour et al., 2004, Bourdon et al., 2012, Khandoga, 2004, Gosens et al., 2010). Attempt has been made to correlate findings from *in vivo* and *in vitro* experiments to enhance comparability(Sayes et al., 2007), but further validation of *in vitro* systems is needed to meaningfully correlate results.

Most studies are credited with using exposures well-defined by particle mass and size to determine UFPs' biological effects. Few included studies additionally analysed the synergistic and anti-synergistic interaction between UFP and ambient co-pollutants, for example bacterial lipopolysaccharide and gaseous pollutants (Mills et al., 2011, Elder et al., 2004a, Elder et al., 2004b). This is important considering excessive lung inflammation from lipopolysaccharide exposure may widen alveolar pores allowing more particles to cross (Bannerman and Goldblum, 1999), or gaseous components may react with nanoparticles forming compounds or varying volatility (Kelly and Fussell, 2012).

An advisory paper by the Health Effects Institute (HEI) describes broad research steps in addressing the limitations of current research. Extending long term animal inhalation designs to humans was recommended ((HEI), 2013). Findings from this review allow more defined recommendations. Further experimental research should investigate:

- The possible protective effect of anti-inflammatory medicines, and particularly anti-oxidant medicines, on UFP mediated cardiovascular disease. The data in this review suggested oxidative stress was an important mediator in several measured outcomes. Recently, effort has been made to limit free radical production caused by concentrated ambient particulate matter inhalation with omega-3 supplementation (Tong et al., 2012).
- Different susceptibilities of systemically inflamed populations, such as COPD patients, to certain mechanisms. Particularly any enhanced inflammatory effect from UFP, seen sub-clinically in the healthy population (Delfino et al., 2009, Delfino et al., 2010, Downs et al., 2012, Karoly et al., 2007).
- Possible direct interaction between UFP and hepatocytes to stimulate the synthesis of coagulation factors which are raised post-exposure to nanoparticles (Khandoga, 2004).
- UFP mediated change in biomarkers specific to vascular constriction; endothelin-1/2, ACE and angiotensin. Building on limited work, controlled experimentation may yield these as useful epidemiological biomarkers, particularly the potent, specific

vasoconstrictors endothelin-1/2 and ACE which both rose 20- 25% after ambient exposure to UFP (Elder et al., 2004a).

- The effect of chronic exposures in animals and humans, particularly to well-defined combustion products or controlled exposures to ambient UFP. A controlled trial combining a concentrated ambient exposure relevant to public health and of 28 days duration serves as an example of rodents exposed chronically to UFP (Araujo et al., 2008).

Conclusion

Review of a large literature set has indicated that ultrafine particles of different compositions do cause toxicity to cardiovascular tissues, but uncertainty remains over which materials are the most toxic. There is limited strong evidence to support individual mechanisms leading to excess cardiac mortality but it seems likely that increased oxidative stress plays a role, and may mediate other effects of impaired vasodilation, inflammation and endothelial activation. Whilst animal studies played a useful role, further research using longer term randomized controlled trials in humans is recommended in strengthening these findings. Several included articles indicate oxidative stress mediates downstream effects of inflammation and endothelial dysfunction, presenting a useful target for prevention of mortality.

Declaration of interest

The authors have no declarations of interest to report.

Sources of funding

None

Table 1. Search terms

Particulate matter terms
pm25/ or pm10/ or ufp/ or ultrafine particle/ or cap/ or concentrated ambient particle/ or nanoparticle/ or particulate matter/ or particle exposure/ or diesel exhaust/ or air pollution/ or particulate emission/ or traffic exhaust/ or vehicle emission/
Biological mechanism terms
thrombosis/ or systemic inflammation/ or crp/ or c?reactive protein/ or fibrinogen/ or fibrinolysis/ or platelet activation/ or platelet aggregation/ or endothelial d?sfunction/ or fibrinolysis/ or oxidative stress/ or nrf2/ or antioxidant/ or vasoconstriction/ or vascular function/ or tissue plasminogen activator/ or plaque/ or endothel$ inflammation/ or myocardial infarction/ or acute phase response/ or atherosclerosis/ or oxidative stress/ or Hdl/ or ldl/ or cholesterol/ or carotid intima medial thickness/ or cimt/ or pmn/ or polymorphonuclear/ or superoxide anion/ or endothelin/ or Endothelial progeinator/ or MTHF/ or NADPH oxidase/ or EOHF/ or Vascular cell adhesion molecule 1/ or lectin-like oxidized low density lipoprotein receptor 1/ or thiobarbituric acid reactive substance/ or homocysteine/ or nitrotyrosine/ or isoprostane/ or il-1/ or il-6/ or il-8/ or crp/ or heart rate varia?/ or HRV/ or autonomic/

Table 2. Selection Criteria

Inclusion criteria	Exclusion criteria
Original research	No biological mechanism investigated.
0-500 nm particles used in exposure	Particles >500 nm.
Outcome consequent to exposure is measured systemically i.e. outcomes detected beyond the alveolar barrier,	Pulmonary outcomes i.e. outcomes occurring before UFPs have crossed the alveolar barrier.
Outcome consequent to exposure measured on systemic tissues such as vascular endothelial cells or lymphocytes.	Outcome consequent to exposure measured on non-systemic tissues such as bronchial epithelial cells.
Outcome measure related to atherosclerotic advancement.	Outcomes measured systemically, but related to non-cardiovascular related outcomes i.e. neurological.

Table 3a. Randomized controlled trials in humans (Inhalation of nanoparticles). The tables are ordered by strength of evidence, and alphabetically within tables. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Where subjects have been randomized, this is referred to as a randomized trial, and a controlled trial if not. Definitions: ↑= increase, ↓= decrease, ─ =no change, expts= experiments, hrs= hours, mins= minutes.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Frampton et al 2006 Randomized crossover trial (Frampton et al., 2006).	56 non-smokers 18-40 years old. 16 with asthma.	Mouthpiece inhalation exposure. UPDOSE. 12 subjects exposed to 10 µg/m^3, 25 µg/m^3 CB or air for 2 hrs. 15 min exercise/half hour to achieve 20 L/min ventilation UPASTHMA. 16 mild asthmatic subjects exposed to 10 µg/m^3 CB or air for 2 hrs with the exercise. UP50. 16 subjects exposed to 50 µg/m^3 CB or air for 2 hrs with the exercise.	UPREST. 12 subjects exposed to 10 µg/m^3 CB (25 nm) or air for 2 hrs	CD3+, CD11a, CD18, CD25, CD49d, CD54 CD62 Numbers of monocytes, basophils, eosinophils.	Reduced CD54 on monocytes p=0.0012. Expression of CD54 on PMNs decreased p=0.031. Reduction in CD62L showed exposure–sex interaction, rising in females and dropping in males for monocytes p=0.0006. Expression of CD62L on PMNs increased in males only p=0.01. UFP blunted increase in CD18 on monocytes p=0.0002. CD11a expression on PMNs increased p=0.037. CD25 expression on CD3+ T cells increased in females. Exposure increased CD25 expression on T cells p=0.001. Monocyte numbers in Females decreased p=0.035; exposure–sex interaction, p=0.002. A decrease in female blood basophils in females was also seen p=0.015. In asthmatics, UFPs reduced CD11b on monocytes and eosinophils p=0.029 p=0.015. Basophils and eosinophils decreased, p=0.02. UFP exposure did not change platelet counts.	Within subject controls, randomized to exposure order. Multiple experimental protocols which showed consistent effects to support data. Exposure concentrations reflect real world conditions.	Carryover effect occurred in some patients.	Peripheral blood monocytes, basophils eosinophils and cellular adhesion molecules are reduced on some monocytes and PMNs when exposed to CB UFP.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Huang et al 2010 Randomized cross-over trial (Huang et al., 2010b).	6 healthy 19-32 year olds.	Mouthpiece inhalation exposure	CB 25 nm at 50 µg/m^3 exposure for 2 hours with 15 min exercise intermittently (n=3). Control-filtered air (n=3) Tests conducted 0 and 24 post exposure.	Inflammation-related gene expression. Vascular growth-related pathways. Oxidative stress gene expression. Genetic expression of Ingenuity signalling and toxicological pathways	Many genes up-regulated across the following areas. Superoxide release and metabolism; Peroxidase activity, oxidoreductase activity, Other genes involved in oxidative stress, Inflammation genes, Apoptosis inducers, cell division relevant genes.	Standardized ventilation. Validated procedure.	Small subject numbers.	Inhalation of ultrafine carbon up-regulated oxidative stress and inflammation relevant genes.
Mills et al 2011. Randomized double blind cross-over trial (Mills et al., 2011).	16 healthy male subjects between 18 and 32 years of age exposed on 4 separate occasions 2 weeks apart to filtered air, carbon nanoparticles, diesel exhaust nanoparticles and exhaust gases	Whole body exposure.	70 µg/m3 carbon nanoparticles mean of 37 nm. Diesel exhaust mean of 67 nm at 348 µg/m^3. 2 hours of exposure. Assessments carried out 6 hours later.	Blood pressure, vasoactivity (endothelium dependent and independent mechanisms) Fibrinolytic function.	Blood pressure ↑ from the control of 133 mmHg to 145 mmHg with UFP exposure. They also attenuated vasodilatation to bradykinin, sodium nitroprusside and acetylcholine. Carbon nanoparticles ─ effect. Fibronolytic function was unaffected by any exposure.	Centrifuged for 30 mins. Within subject controls. Exposure concentration similar to real world conditions.	No assessments carried out immediately after exposure. The attenuation of dilation in arteries is assessed, not vaso-constriction.	Diesel nanoparticles reduce vasoreactivity by both endothelium dependent and independent mechanisms and raise blood pressure. Carbon nanoparticles have no such effect and fibrinolytic function is unaffected.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Pietropaoli et al 2004. Randomized cross-over trial (Pietropaoli et al., 2004).	16 non-smokers 18-40 years old.	Mouthpiece inhalation exposure.	50 µg/m3 CB or air for 2 hrs with intermittent exercise.	Carbon monoxide diffusing capacity (vascular function).	At 21 hrs, carbon monoxide diffusing capacity ↓ in CB group.	Double blinded. Particle size determined immediately prior to inhalation. Gender equal across groups, and order of presentation randomized separately by gender.	Diffusion capacity also affected by haemoglobin reactivity, and gas diffusion.	While carbon monoxide diffusing capacity is affected by other factors than vascular reactivity, a transient effect was seen.
Routledge et al 2006 Randomized controlled trial (Routledge et al., 2006).	20 human volunteers with stable CAD and 20 healthy patients.	Sealed cylinder around volunteer's head.	10-300 nm mode=20-30 nm at 50 µg/m^3 for 1 hour. 5 exposed to air, 5 exposed to CB, 5 exposed to SO_2, and 5 to both pollutants for both groups.	HRV and baroreflex sensitivity. Platelet count and aggregation. White cell count. CRP, fibrinogen and D-dimer.	No change in cardiac vagal control, blood counts or systemic inflammatory response.	Double blinded concentrations used equivalent to 1 hour exposure of high urban pollution. Mode of experimental UFP comparable to roadside mode of UFP	Patients medicated throughout trial, including aspirin. Mixed population. Study powered to detect a marker of HRV, not ischaemic mechanisms. CRP was higher in the CAD group vs healthy group at baseline $p<0.01$.	Low exposure concentrations used in small numbers of high risk medicated human subjects did not alter autonomic or coagulatory function.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Samet et al 2009 RCT (Samet et al., 2009).	19 healthy volunteers 18-35 years old.	Whole body exposure chamber.	20 l/min ventilation of 1.2 x 10^5 UFP particles/m^3 between 20 and 250 nm over 2 hours. Exposed to filtered UFP (n=10) and air (n=9).Tests conducted before, at 0 and 18 hours post exposure.	Coagulation makers. Blood cholesterol and triglyceride. QT duration and variance, HRV.	D-dimer levels increased 0 and 18 hours post exposure. Triglycerides and VLDL reduced 0 hours post exposure. Total cholesterol/HDL ratio increased at 18 hours. Mean QT duration reduced at 18 hours. QT variance increased 0 hours post exposure. Low and high frequency indices of HRV increased.	Randomized, concealed experiment and participants unable to distinguish between exposures in preliminary tests.	Short exposure time. Low risk group.	Fibrin degradation increased post exposure, suggesting its increased production. Transient beneficial changes in lipids occurred. Heart rate control and repolarization affected by UFPs in a way which suggests vagal tone increased.
Shah et al 2008 Randomized cross-over trial (Shah et al., 2008).	16 healthy human volunteers.	Mouthpiece exposure system.	Inhaled air or 50 μg/m^3 carbon UFP for 2 hr, with inter-mittent exercising. (mean particle size 25 nm) Measurements at 0, 3.5 hr, 21 hr, and 45 hrs.	Peak forearm blood flow after ischemia. HR, BP. Nitrite and nitrate levels.	UFP had no effect on pre-ischaemia flow or resistance or post ischaemic total flow. Post ischaemia, peak forearm blood flow blunted by UFP. Mean blood pressure divided by forearm blood flow↓ with air, but not UFP. HR, BP─.HR x BP↑ in males, but not females. Venous nitrate levels↓, nitrite─.	Double blinded. No infection history. Realistic particle concentration	Mouthpiece alters breathing patterns. Small sample number.	UFP reduces reactive hyperaemia peak flow in healthy non-smokers. NO may not be important in reactive hyperaemia.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Stewart et al 2010 Randomized cross-over trial (Stewart et al., 2010).	19 non-smokers 30-60 years old with Type 2 diabetes.	Mouthpiece inhalation exposure.	50 μg/m^3 CB UFPs of median size 32 nm and filtered air for 2 hours. 3 weeks between inhalations. Measurements made 3.5 hrs post exposure.	Platelet expression of CD40L, CD62P and leukocyte expression of CD62P+. sCD40L, vWF CRP, IL-6 levels.	Compared with control, CD40L↑ at 3.5 hours. CD40L showed significant UFP-time interaction. Platelet CD62P levels correlated with CD40L. Leukocytes expressed CD62P+. vWF expression─. sCD40L ↓. IL-6, CRP ─. All markers showed sex interaction and CD62P+, age interaction. Older males more at risk. There were no significant UFP effects on soluble C-reactive protein, E-selectin, L-selectin, CD62P, intercellular cell adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), serum amyloid A (SAA), or the coagulation markers (D-dimer, Factors VII and IX).	Single particle type, but concentration reflects real world daily exposures. Within subject controls. Double blinded.	Diabetics restricted to narrow age range, no cardiovascular disease nor any lipid or platelet active drugs. Not representative of most diabetics.	Short term exposure to CB for 2 hours transiently activates blood platelets, possibly leukocytes and vascular endothelium.
Vinzents et al 2005 Randomized cross-over trial (Vinzents et al., 2005).	10 healthy males and 5 females. 25 years of age.	Cycling through rush hour traffic on pre-defined routes. Other indoor particulate matter exposures were recorded.	UFP exposure recorded on 5 days over 4 months. mean cycling time 93 mins per day during rush hours. Day 6-similar exercise in laboratory.	Oxidative DNA damage in mononuclear cells.	Regression co-efficient of a mixed effect model of level of DNA damage with subjects as random factor were estimated as 0.37 x10^{-3} to 1.77 x 10^{-3} for indoor exposure, and 1.50 x 10^{-3} to 2.42 x 10^{-3} for outdoor exposure. R_2=50.3.	Portable measuring device validated against stationary device. Study method validated in inter-laboratory trials. Physiological exposure.	Low risk participants undergoing strenuous exercise, not reflecting the at risk population.	Oxidative stress markers are raised in mononuclear cells post exposure to UFP.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Weichenthal et al 2011 Randomized cross-over trial (Weichenthal et al., 2011).	42 healthy adults.	Ambient particulate exposure. Cycled on high and low traffic routes.	>100 nm ambient UFP, high traffic route, low traffic route.	LF, HF, LF:HF ratio, SDNN, rMSSD, pNN50	HF↓ with UFP at 2 and 4 hours. PNN50↓ at 2 hours. No other changes.	Personal exposure. Realistic exposure.	5 day between trial cross-over periods. Other controlled trials show >5 day effects after UFP exposure. Few positive findings with multiple comparison testing.	Decreased parasympathetic modulation of the heart occurs post UFP exposure.
Zareba et al 2009 Randomized cross-over trial (Zareba et al., 2009).	24 healthy 18-40yrs subjects.	Mouthpiece inhalation exposure.	25 nm CB exposure. Period 1. 10 μg/m^3 UFP or air. Period 2. 10 μg/m^3, 25 μg/m^3 or filtered air. Exercise took place with each exposure.	HRV=RR, SDNN, rMSSD, Low and high and low/high frequency. Repolarization= Qtc, T-wave amplitude, PCA, ST level.	SDNN↑ Low and high and low/high frequency↑ rMSSD no change. Qtc↓ T=wave amplitude↑	Exercise increased ventilation to standardized rate.	Young, healthy subjects. Both very low doses used.	Mild increase in para-sympathetic tone at lower but not higher dose.

Table 3.b.Controlled animal experiments Inhalation of nanoparticles. The table is ordered alphabetically. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Where animals have been randomized to groups, this is included in column 7 under study strengths, and the trial is referred to as a controlled trial. Subject numbers reported in this table are taken from the subject numbers used only for included experiments. Definitions: ↑= increase, ↓= decrease, ─ =no change, hrs= hours, expts=experiments, mins= minutes.

Author, study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Amatullah et al 2012 Controlled trial (Amatullah et al., 2012).	14 Female BALB/c mice 6-8 weeks old.	Nasal exposure	Ambient UFP av. 401µg/m3 of <200nm 4hr exposure or air at 2L/min.	Heart rate. SDNN.	Heart rate↓ less than control post exposure. SDNN↓	Ambient roadside UFP concentrated. Nasal inhalation limits dermal effects of particles, which can occur at high concentrations.	HR for coarse and fine UFP groups dropped significantly from baseline, indicating a systematic cardiovascular change by use of exposure chambers.	Heart rate variability decreases with ambient UFP exposure compared to air. Heart rate might increase.
Araujo et al 2008 Controlled trial(Araujo et al., 2008).	51 ApoE-/- male mice. 4 weeks old on normal diet.	Ambient UFP inhalation 300m from a freeway.	17 mice exposed to air, 17 to UFP matter and 17 to $PM_{2.5}$. 1-180nm at 2-6 x ambient levels of UFP. 75 hours over 40 days of exposure. Tests conducted 1-2 days later.	Atherosclerotic change. HDL protectiveness via monocytic infiltration. Oxidative stress was assessed.	UFP exposure resulted in a 55% increase in atherosclerotic lesion than control and 25% against $PM_{2.5}$. UFP increased plaque monocytic infiltration compared to control. MDA, Nrf2, catalase, GST-Ya, NQO-1, SOD2 and ATF4 gene mRNA all increased over control.	Large study population with a long exposure time. All animals accounted for. Exposure represented real particulate matter.	Controls exposed to ambient PM when being transported each day. Particle concentration was 2-6 x higher than normal exposure. Did not measure UFP in circulation.	Atherosclerotic advancement is caused by UFP exposure. Anti-inflammatory HDL is reduced and anti-oxidant mRNA expression is increased.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Cassee et al 2012 Controlled trial (Cassee et al., 2012).	11 week old 20 male and 20 female ApoE-/- mice	Nasal exposure	DE control group, and DE exposure group, a DCeE control group and a DCeE exposure group.1741µg/kg DCeE. 1740µg/kg for DE. Both had a mean size of 82.5nm. Exposure of 5 days/week for 28 days. (n=10 per group)	Atherosclerotic change. Pro-inflammatory cytokines. Haematological cell changes.	More adjoining plaques occurred at high DE compared to control. DCeE reduced total particulate number by 30%. No significant changes in atherosclerosis were seen compared to control. No difference in response to 3 day and 17 day murine examinations. Hepatic pro-inflammatory cytokines were reduced with DCeE exposure compared to DE alone. No haematological cell changes occurred.	Realistic diesel exposure concentration over a long study period. Tightly controlled exposure concentrations. Nasal inhalation limits dermal translocation of particles.	Exhaust exposure was regulated by exhaust mass, so the engine running conditions changed throughout the experiment. See Li 2010 for an example of the effect.	Atherosclerosis was markedly different between DE and control. DCeE reduced the size and number of atherosclerotic lesions compared to DE but not significantly. Cerium reduces both particle number and surface area by agglomeration.
Cuevas et al 2010 controlled trial(Cuevas, 2010).	Male C57bl/6 mice 12 weeks old.	Whole body exposure chamber.	40nm nickel. 5hr/day for 1,3 or 5 days to 100,150 or 900µg/m3. Test conducted 24 hours later.	Vasoconstriction (phenylepherine). Ach dependent vasorelaxation.	In the carotid artery, Ach dependent vasorelaxation was impaired and phenylepherine dependent constriction was accentuated.	designed to represent occupational exposures	No assessment of NO dependent mechanisms.	vasorelaxation is blunted. Vasoconstriction is accentuated.
Elder et al 2004 Controlled trial(Elder et al., 2004a).	Male F344 rats 21 months old.	Controlled exposure to ambient exposure	6hr/day over 1 or 3 days. 37-106 µg/m3. Tests conducted 18hrs post exposure. Filtered air control	ICAM-1 on blood cells. Blood fibrinogen. Blood endothelin-2.	Only results for UFP vs. control. No increase in ICAM-1. Fibrinogen increased. Endothelin-2 increased.	Physiological exposure to real-world concentrations for a long duration.	Artificial temperature in an otherwise real-life environment.	Vasodilatation and coagulability are impaired in compromised rats exposed to ambient UFP.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Elder et al 2004 controlled trial (Elder et al., 2004b).	MaleF344 rats 23 months old and spontaneously hypertensive rats 11-14 months old.	Whole body exposure chamber.	36nm CB at 150µg/m3 for 6 hours was the exposure. Exposure groups. 1) Intraperitoneal (IP) injected saline +inhaled air. 2) IP injected saline +inhaled particles. 3) IP injected bacterial lipopolysaccharide (LPS) +inhaled air. 4) IP injected LPS +inhaled particles. Tests conducted at 24 hours.	Oxidative stress (DCF). Acute phase response and coagulability.	Only results for UFP vs control (protocol 2). PMN oxidative stress increased in old F-344 rats only. Fibrinogen increased in F3-44 rats. Thrombin-antithrombin (TAT) complexes reduced. Fibrinogen decreased in SH rats. TAT complexes increased. No change in cell number, viscosity, IL-6.	High number of subjects used. Infection/inflammation simulated with LPS. Vulnerable old rats reflect at risk human groups.	No measurements at 0-24 hours post exposure.	CB nanoparticles can enhance inflammatory effects and alter coagulation protein levels
Gilmour et al 2004 Controlled trial (Gilmour et al., 2004).	12 week old male Wister rats.	Whole body exposure chamber.	Rats exposed to 114nm or 268nm CB or air for 7 hours at 1mgm3. Assessment made at 0, 16 and 48 hours post exposure.	BAL cell and mRNA. Blood coagulation and leukocyte levels. Oxidative stress (DCFH)	Leukocytes increased by 130% at 0hrs and 135% at 48 hours $p<0.05$, no change at 16hrs. No change in Factor VII, fibrinogen or vWF. No systemic oxidative stress imparted.	Well characterized particles by chemical make-up, charge and density. Inhalation used as delivery method.	UFPs agglomerated reducing surface area and transfer across the alveolar barrier. Short exposure time. Baseline recordings not reported.	CB exposure causes show a leukocyte increase *in vivo*.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Harder et al 2005 Controlled trial (Harder et al., 2005).	Male Wister rats 12-15 weeks old.	Whole body exposure chamber.	38nm CB 180µg/m3 6 mice as control, 6 in exposure group. 24hr exposure to CB. Tests conducted at 24 hours.	Mean blood pressure. Plasma fibrinogen. Coagulation markers.	Fibrinogen blood─. Factor 7a ─. Mean blood pressure (BP)─. Heart rate↑. No cardiac inflammation or cardiomyopathy. Blood coagulation and clot lysis factors─.	Immediate analysis after a long exposure. Useful particle type. Expriment reflects real-world exposure concentrati on.	Small subject numbers.	Blood coagulability is not altered with CB exposure. The autonomic system affected post NP exposure..
Khandoga et al 2010 Controlled trial (Khandoga et al., 2010).	Female C57B1/6 mice 5-7 weeks old.	Whole body exposure chamber.	CB 440µg/m3 48nm mean. 24 hour exposure or filtered air. Sacrificed 2 or 8 hr post exposure. Systemic measures at 2 hours into exposure.	Platelet adherence and platelet accumulation in arterioles and venules. Sinusoidal damage. Fibrinogen. Cytokines. Blood cell number. Platelet activation. *In vitro* platelet activation by CB	Platelet adherence and platelet accumulation in sinusoids in arterioles and venules. No platelet rolling. No leukocyte adherence. No sinusoidal damage. Fibrinogen deposited in liver and heart. No cytokine change. No increase in blood cell number. Platelet size and platelet component distribution width increased +markers of platelet activation. CD62P expression increased *in vitro*.	Physiologic exposure type. Many aspects of thrombogen esis measured. Attempted to mimic earlier intra-arterial exposure with same amount particles potentially trans locating from lung to circulation	High exposure concentration used.	Inhaled nano-sized CB enhances thrombogenesis in healthy murine microcirculation. This may not be associated with an inflammatory reaction.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Knuckles et al 2012 Controlled trial (Knuckles et al., 2012).	Sprague-Dawley rats 7-9 weeks old.	Whole body exposure chamber.	138nm TiO_2 particles at 5.7mg/m3 for 4 hrs over 1 day. Tests conducted at 24 hrs.	Hyperaemia. Arteriole responsiveness to sympathetic stimulation post phentolamine and L-NMMA treatment. Thromboxane and prostacyclin mimetics. Phentolamine blockade.	Nanoparticle exposure ↓ vasodilation with NOS and COX inhibitors. L-NMMA and Phentolamine ↑ sensitivity to α-adrenergic blockade. Thromboxane led to ↑ constriction and prostacyclin to ↓ dilation with nanoparticle exposure. Exposure caused persistent blunting of dilation when sympathetic effect removed.	Agglomeration effect restricted by use of Venturi vacuum pump.	Multiple baseline imbalances.	Nano TiO_2 exposure decreased NO influence on sympathetic arteriolar constriction, arteriolar response to metabolic stimuli and sensitivity to COX products. α-adrenergic receptor sensitivity increased.
LeBlanc et al 2009 Controlled trial(LeBlanc et al., 2009).	Sprague-Dawley rats 10-12 weeks old.	Whole body exposure chamber.	TiO_2 100nm. 240 min at 6 mg/m3 or filtered air for same time. Ex-vivo Coronary resistance vessels used for experiments post exposure.	Myogenic responsiveness (arteriolar diameter at different internal pressures). Vasodilation to shear stress. Ach, $Ca^{2+}$and SNP (NO) dependent vasodilation.	No difference in myogenic responsiveness. Shear stress ─ . Both groups showed similar flow/shear stress relationships. Shear stress individually; TiO_2 relaxed less (slight constriction) vs. control and relaxed less per flow rate. Ach, Ca^{2+} dependent vasodilation ↓ with TiO_2. NO donation─.	Previously validated method. Multiple comparison statistical adjustment made.	Vessels isolated from hormonal and nervous input. Very high exposure dose used.	TiO_2 exposure causes coronary arterioles to demonstrate increased tone, and reduced vasoreactivity to shear stress, ACh, and Ca^{2+}.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
LeBlanc et al 2010 controlled trial (LeBlanc et al., 2010).	83 Sprague-Dawley rats. 10-12 weeks old.	Whole body exposure chamber.	100nm TiO2 at 6mg/m3. n=33 control. N=50 nanoparticle. Ex-vivo Coronary resistance vessels used for experiments post exposure.	Ach vasorelaxation. Indomethacin (prostaglandin) and L-NMMA vasorelaxation. Arachidonic acid metabolism. Thromboxane A2. Oxidative stress (ethidium bromide fluorescence).	ACH vasorelaxation impaired with TiO2, restored with ROS scavenging. Indomethacin and L-NMMA response blunted with UFP. Arachidonic acid caused vasoconstriction in exposed group vs. dilation in control. Thromboxane no effect. Increased oxidative stress in arteriole wall.	Validated exposure concentration. Occupational relevance.	Vessels isolated from hormonal and nervous input.	Coronary arterioles- ACH relaxation dependent on PG and NO. TiO2 drives vasoconstrictor production of arachidonic acid. Response to thromboxane unaltered. Oxidative stress increased.
Liberda et al 2010 Controlled trial (Liberda et al., 2010).	C57BL/6 mice 10-12 weeks old	Whole body exposure.	Nickel 10hr/2 days 1237µg/m3. 15hr/3 days 725µg/m3, 25hrs/5day at 86 µg/m3.	VEGF and human stromal cell-derived factor (SDF1-α). Bone marrow EPC numbers. Tube formation and chemotaxis.	Function impaired no change in VEGF and SDF-1α. Bone marrow EPC ↓. Tube formation and chemotaxis↓	Physiologic exposure mechanism.	1/2 day gap between exposure ending and sacrifice.	Blood cells important for endothelial repair are reduced in numbers in the bone marrow, suggesting vascular recruitment.
Niwa et al 2008 Controlled trial (Niwa et al., 2008).	Sprauge-Dawley rats. 6 weeks old.	Whole body exposure chamber.	120nm CB at 15.6mg/3 for 6 hours/day, 5 days/week for 4 weeks. Randomised to carbon black (n=25) or filtered air (n=25)Tests conducted at 1- 30 days	MCP-1, IL-6,CRP, 8-OHdG, blood cell number, heart rate, systolic blood pressure, CB presence in tissues.	MCP-1, IL-6, CRP all ↑ in CB group at 28 days. No other biochemical changes. Heart rate↑ at day 14 and SBP at days 14 and 28. No CB found in liver, spleen or aortic tissue.	Randomization of mice to groups. Physiological exposure of long duration	Very high exposure dose.	No liver injury was seen. Inflammatory markers, heart rate and blood pressure rose after a very high CB exposure.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Nurkiewicz et al 2008 Controlled trial(Nurkiewicz et al., 2008).	Male Sprauge-Dawley rats. 6-7 weeks old.	Whole body exposure chamber.	One group exposed to TiO2 at 100nm, depositing between 3.7-37.6 µg/rat. Another group to 710nm, depositing between 8.26-89.80µg/rat. One control group. Experiments conducted 24 hrs later.	Endothelium dependent arteriolar dilation (Ca2+ dependent endothelial NO formation)	Systemic arteriolar dilation impaired 24 hours post exposure at doses 10, 19 and 38µg. Vasoactivity impairment dependent on time-concentration product of nanoparticle exposure.	Several steps taken to avoid agglomeration. Multiple arterioles sampled per rat better represents within arteriole heterogeneity and increases sample size..	Age difference existed between the ultrafine TiO2 group and all other groups $p<0.05$. No randomisation reported.	Ultrafine particles have a greater blunting effect on vasodilation, even causing vasoconstriction. The effect is dependent on alteration of the concentration-time product of exposure, not its individual parts.
Nurkiewicz et al 2009 Controlled trial (Nurkiewicz et al., 2009).	Male sprague-Dawley rats.	Whole body exposure chamber.	100nm TiO2 at 1.5–16 mg/m3 for 4-12 hours to produce 5 doses. 67µg $PM_{2.5}$ group, 20µg nano-TiO2 and control group.	Nitric oxide levels and oxidative stress measurements (ethidium bromide fluorescence). Nitrotyrosine staining. Nitric oxide production. Radical scavenging	Sodium nitroprusside had equal effect on arteriolar dilatation in all exposures. Ca2+ ionophore limited arteriole dilation to 23% vs. original diameter. Ethidium bromide fluorescence 0.09 AU greater vs. control. Nitrotyrosine expression 2200µm2/section greater vs. control. Nitrotyrosine/vWF expression 8x higher vs. control. Nitric oxide production reduced at 4 and 6µg. Nitric oxide production and Ca2+ ionophore dependent vasodilation increased 4x by radical scavenger.	Externally validated method. Dosing equivalent to high doses reported in industry exposure. Validated use of time-concentration product for dosing. Demonstrated NO uncoupling dependent on ROS.	UV stimulation of particles in practice was not reflected in a sealed chamber. Background electrical noise affected NO probes.	Inhalation of TiO2 nanoparticles attenuated endothelium-dependent arteriolar dilation but vascular smooth muscle sensitivity NO unaffected. Oxidative/ nitrosative stress was caused. NO production reduced. Both effects involve radical production.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Srinivas et al 2011 Controlled trial(Srinivas et al., 2011).	72 healthy Wister rats, 8 weeks old.	Nasal inhalation	36 (equal Male/female ratio) control rats. 36 (equal Male/female ratio) exposed to CeO2 (55nm mean) at 641mg/m3. 4 hour exposure. Tests conducted at 24, 48 hours and 14 days.	Liver function. Cholesterol. IL-1β, TNF- α and IL-6.	Liver function–. Cholesterol↑ in female rats at 14 days. IL-1β, TNF- α, IL-6↑ max 24hrs, high for 14 days.	Large study population with a long exposure time. Long follow-up time. Nasal inhalation limits dermal effects of particles, which can occur at high concentrations.	Few outcomes of relevance in this study. Very high exposure concentration.	Systemic inflammation is imparted by inhalation of cerium oxide nanoparticles
Upadhyay et al 2008 Controlled trial(Upadhyay et al., 2008).	Spontaneously hypertensive rats. 6-8 months old.	Whole body exposure chamber.	8 rats exposed to a CB of 31nm at 172µg/m3 for 24 hours. 8 were controls. Assessment was made 1-5 days later.	Blood pressure and heart rate. Cardiac histopathology. Acute phase response. Haematological change. RAS involvement.	Blood pressure ↑ by 6mmHg vs. control, returning to baseline 4 days post exposure. Heart rate ↑ 17bpm 1 day post exposure, and returned to baseline by day 4. No histological change in the heart. No CRP, haptoglobin or fibrinogen. Neutrophil and leukocyte blood concentrations ↑ 1 day post exposure. No significant change occurred in renin-angiotensin system measurements.	Measured mediators of hypertension changes (Renin-angiotensin system) changes. Useful surrogate for ambient UFP.	Within animal control for cardio physiologic analysis.	Nano-sized CB raises blood pressure and heart rate after inhalation without any acute phase changes.

Table 4. Human longitudinal studies. The tables are ordered alphabetically. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Definitions: ↑= increase, ↓= decrease, ─ = no change, hrs= hours, mins= minutes.

Author, study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Delfino et al 2009. Longitudinal cohort (Delfino et al., 2009).	64 human population subjects. Non-smokers, previous CAD, >65 years of age.	6 weeks exposure in warm weather, and 6 in colder weather.	≤250nm sized particles at 10.27 µg/m3 in warm, µg/m3 in cold season. 1,3 and 5 day lags measured.	IL-6, TNF-α, sTNF-receptor 2, CRP, sP-selectin. Erythrocyte antioxidant enzymes, glutathione peroxidase-1 and copper-zinc SOD.	IL-6 and sTNF-receptor 2 were correlated with ultrafine particles. sP-selectin was not. The two antioxidant enzymes were inversely associated with ultrafine particles on days 1 and 3. Statins were associated with a reduced sTNF-receptor 2 effect and clopidogrel with reduced sP-selectin.	Reported measures at subject level. Physiological and real time exposure. Removed data associated with reported illness.	Observational data does not allow individual air components to be attributed to disease. Confounding and bias risk. Subjective reporting of home data.	Quasi-ultrafine particles are associated with increased systemic inflammation, platelet activation and a reduction in antioxidant enzymes activity. protective effect of clopidogrel and statin were noted.
Delfino et al 2011. Longitudinal cohort (Delfino et al., 2011).	66 human population subjects. Non-smokers, previous CAD, >65 years old.	6 weeks exposure in warm weather, and 6 in colder weather.	≤250nm sized particles (quasi ultrafine) at 9.77 µg/m3 average over whole trial period.	Association of ≥1mm ST depression with quasi-ultrafine particles.	Relative rate of ST-segment depression was positive over 2-day averages particulate matter 1.57, and for 1-day averages, Relative rate = 1.17.	Comprehensive exclusion of all who could have demonstrated spurious ST changes.	28/66 dropped out. Confounding risk of observational data. Subjective reporting of home data.	Quasi ultrafine particles are associated with ST depression.
Delfino et al 2010. Longitudinal cohort (Delfino et al., 2010).	Human population. Non-smokers, Previous CAD, >60 years of age.	6 weeks exposure in warm weather, and 6 in colder weather.	≤250nm sized particles (quasi ultrafine) at 9.51 µg/m3 in warm, 8.65 µg/m3 in cold season.	Association of IL-6, TNF-α with quasi-ultrafine particles.	Polycyclic aromatic hydrocarbons and hopane parts of ultrafine PM associated with both biomarkers.	Within subject control. *A priori* adjustment for infection.	PAH could correlate with an unmeasured species.	In observational data, inflammatory markers correlate with polycyclic aromatic hydrocarbon and hopane levels of quasi-ultrafine particles.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Folino et al 2009 Longitudinal cohort (Folino et al., 2009).	39post-MI patients of mean age 60 years.	Ambient exposure	Three 24hr assessments in spring-winter. ≤250nm NPs.	SDNN average NN, RMSSD, pH, LTB4, endothelial NO, PTX3, CRP, CC16, IL-8	SDNN and SDANN negatively correlated with $PM_{0.25}$. RMSSD no change. No significant results for patients on β-blockade. Only PTX3 showed a negative correlation, CRP, CC16, IL-8 no change	Personal exposure and Holter device monitors. Medication use adjusted for.	B-blocker use. Short lag time.	P0.25 correlates with HRV changes. No convincing inflammatory response was seen.
Rich et al 2012 Longitudinal cohort (Rich et al., 2012).	76 non-smokers with previous MI but without other sever cardiac disease.	Ambient exposure	Particle categories; 10–100 nm(UFP), 100–500nm(AMP) and ≤ 2.5 μm ($PM_{2.5}$).	rMSSD, SDNN , TpTe Mean normal-normal beat time, QTc, HRT, deceleration capacity, blood pressures, CRP, white blood cell count.	IQR increases in AMP in previous 6 and 24 hr reduced rMSSD, TpTe; fibrinogen; HRT in the previous 24-47 hours. Each IQR increase in UFP 24–47 hr decreased SDNN and rMSSD and increased fibrinogen. Diastolic blood pressure associated with each IQR increase in UFP, AMP, and $PM_{2.5}$ at almost all lags. TpTe, HRT, and fibrinogen changes associated with UFP or $PM_{2.5}$ reduced when controlling for AMP, except for HRT, which was larger. CRP, fibrinogen, systolic bp, Mean normal-normal beat time, QTc, blood cell count had no associations	Patients were not limited in drug regimens or relevant co-morbidities.	PM monitoring sites potentially up to 21km from patients homes.	Subclinical modulation of autonomic balance occurs with exposure to UFP and AMP. Systemic inflammation does not.
Rückerl et al 2007 Longitudinal cohort (Rückerl et al., 2007).	57 >50 years olds with Current CHD.	6 months exposure. Bi-monthly measurements.	10-100nm (UFP) and 100-1000nm (AP).	Plasma sCD40L. Blood cell count	sCD40L ↑, and leukocytes dropped at 0 days for UFP and AP. Platelet counts ↓ with UFP at 0 days, 3days and 5 day average.	Validated method. Low attrition rate.	Confounding by other inflammatory causes.	Platelet activation and reduction in number occurred rapidly. leukocyte numbers reduced.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Timonen et al 2006 longitudinal cohort (Timonen et al., 2006).	131 elderly subjects with CAD	Ambient exposure.	<100nm ambient particles. 6 month follow up. 17,000 particles/cm3 in two centres, and 21,000 in the third.	SDNN, HF, LF/HF	Results for overall pooled effect only. SDNN, HF─. LF/HF↓ as particle count rose and 1,2,3 day lag and 5 day average.	Large, high risk population.	Diagnosis self-reported by patient. Lag time of 0-5 days only.	At a 2 day lag, for every 10,000 particle/cm3 increase there was a 13.5% drop in LF/HF ratio.
Wu et al 2012 Longitudinal cohort (Wu et al., 2012).	17 postmen on delivery routes with no CAD, travelling by motorcycle.	Ambient particulate exposure.	5-6 days ambient exposure to $PM_{0.25}$. HRV measurements made during exposure, and cardio-ankle vascular index, after.	Cardio-ankle vascular index (arterial stiffness index). LF, HF, LF:HF ratio, SDNN, rMSSD.	No significant changes fpr $PM_{0.25}$ throughout. Manganese↑ cardio-ankle vascular index. Calcium ↓rMSSD. Nickel, zinc and chromium↓ LF:HF ratio.	Personal exposure and ambient city levels measured. Realistic particle exposure.	Few outcomes from multiple comparison testing. No lag effects measured.	Some $PM_{0.25}$ components increase arterial stiffness, and alter cardiac autonomic function.

Table 5.a. Controlled animal experiment (instillation of suspended nanoparticles). The table is alphabetically. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Where animals have been randomized to groups, this is included in column 7 under study strengths, and the trial is referred to as a controlled trial. Subject numbers reported in this table are taken from the subject numbers used only for included experiments. Definitions: ↑= increase, ↓= decrease, ─ no change, hrs= hours, expts= experiments, mins= minutes.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Bourdon et al 2012 Controlled trial (Bourdon et al., 2012).	Female C57B1/6 mice 5-6 weeks old.	Intra-tracheal instillation.	Single dose of 0, 0.018, 0.054 and 0.162mg 14 nm CB. Tests conducted at 1, 3 and 28 days post exposure. n=6 per group.	Hepatic inflammatory and acute phase gene expression. Lipid profile.	Saa3 experienced a 4 fold increased; Orm3, a 2 fold increase; Saa1, a 1.5 fold increase at 1 day. CRP gene raised 1 fold at 28 days. HMG-CoA reductase genes all increased approximately 2 fold at day 1, and 2/9 genes at 28 days. Serum amyloid A rose at 1 and 28 days, HDL at 3 and 28, LDL at 28 days.	Equivalent to human exposure in working environment.	Non physiological exposure. Agglomeration not prevented. Broncho-alveolar lavage (BAL) fluid could form protein corona around nanoparticles, limiting surface exposure.	Acute phase genes, and HMG-CoA reductase genes, including the rate limiting step gene, *Hmgcr*, were up-regulated. The acute phase pathway activates the HMG-CoA reductase pathway.
Chang et al 2007 Controlled trial (Chang et al., 2007).	60 spontaneously hypertensive rats. 11 weeks old.	Intra-tracheal instillation.	14 nm CB at 415 µg/animal and 830 µg/animal. Low dose group (n=4) High dose group (n=4). Measures made for 2 weeks 10 days post instillation.	Normal-to-normal intervals (NN), natural logarithm transformation of SDNN and RMSSD.	Low and high dose ↓ ANN and ↑ LnSDNN.	Particles sonicated for 30 minutes pre-instillation.	No measurements made for HRV between 1-10 days post exposure.	CB alters HRV similarly at high and low doses.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Cho et al 2009 Controlled trial (Cho et al., 2009).	Female CD-1 mice and hearts isolated from CD-1 female mice. 197 mice for blood/tissue experiments, 40 mice for cardiac injury experiment.	Oropharyngeal instillation and bolus injection into heart model.	Ambient UFP ≤100nm exposure near road and far from road. Administered as 25 or 100µg bolus for biomarker tests, and 100µg for cardiac injury experiment. Biomarkers tested at 4 and 18hrs. Cardiac injury tested at 18hrs.	Cell numbers, platelets, haematocrit, CRP, LDH, liver function markers. Coronary flow Left ventricular end diastolic pressure, ventricular contraction. Cardiac-ischaemia-reperfusion injury.	Cell numbers, platelets, haematocrit, CRP, LDH, liver function markers ─. Coronary flow↓ (near road only) left ventricular diastolic pressure and ventricular contraction─. Myocardial infarction size greater after UFP infusion and left ventricular diastolic pressure slower to recover (far road group only).	Ambient PM collected over 2 weeks, for reasonable representation of 'average' exposure. Nanoparticles sonicated and centrifuged for 30 minutes.	Non physiological exposure method. Cell isolated from *in vivo* cellular and hormonal effects.	Ambient exposure worsens cardiac injury after an ischaemic event.
Courtois et al 2008 Controlled trial (Courtois et al., 2008).	Adult male Wister rats 12-14 weeks old.	Intra-tracheal instillation	5mg of TiO2 13, 15 and 21 nm. $PM_{2.5}$ comparator and control. Tests conducted at 12 hours	Nitric oxide response	Nitric oxide dependent vasodilation not affected by Ti02. $PM_{2.5}$ did impair responsiveness.	Similar sized particles (I.e., not large range of particle sizes around mean diameter) strengthen the conclusion of specific nanoparticle effects.	Particle agglomeration not determined.	Ti02 does not alter nitric oxide dependent vasoreactivity. Possibly due to low reactivity, not size.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Cui et al 2010 Controlled trial (Cui et al., 2010).	80 ICR mice exposed to TiO2. Age not reported.	Intra-gastric administration	Control group and 5, 10, 50 mg/kg 7nm TiO2 NPs administered every 48hrs for 60 days. Tests conducted at 60 days.	Lipid peroxidation, ROS, mRNA expression SOD, CAT, GSH-Px, MT, HSP70, CYPA1, P53, GST and TF.	MDA↑, NO↑, H2O2↑, O2-↑, all anti-oxidant genes↓. All detoxification genes↓ except CYPA1↑.	Nanoparticles were sonicated for 15-20 minutes and vibrated for 2-3 prior to instillation.	Exposure type not wholly relevant. Assumes nanoparticles cross the alveolar barrier in great amounts.	On exposure to TiO2, the liver suffered ROS damage and cell membrane peroxidation, Particles impaired antioxidant and defence mechanisms.
Cui et al 2012 Controlled trial (Cui et al., 2012).	40 ICR female mice. Age not reported.	Intra-gastric administration	Control group (n=20) and TiO2 10 mg/kg (n=20). 90 days of exposure. Experiments conducted at 90 days.	NP deposition. Histopathology. Lymphocytes, neutrophils and white blood cell number. ALT, AST, ALP, LDH, total cholesterol and triglycerides. Genetic regulation.	NP deposited in liver. Inflammatory cell infiltration, vein congestion, nuclear vacancy and oedema and apoptosis observed in hepatic tissue. Lymphocytes, neutrophils and white blood cell number↓. ALT, AST, ALP, LDH, total cholesterol and triglycerides↑. Apoptosis, oxidative stress, inflammation, response to stress, metabolism and other genes up/down-regulated.	Randomized to groups. Genetic changes with Illumina BeadChip confirmed with RT-PCR	False positive findings for the 1142 genes. Very high dose used. None of the genetic markers followed up to detect protein changes.	Histopathology suggests an inflammatory response. Liver function is impaired and genetic changes signal a stress response.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Folkmann et al 2012 Controlled trial (Folkmann et al., 2012).	72 obese and 72 lean female Zucker rats (metabolic syndrome)	Gastrointestinal exposure	1)32 lean, 32 obese rats exposed to single dose 0, 0.064, 0.064 or 6.4mg/kg CB. Tests conducted at 24hrs. 2)24 obese,24 lean rats exposed to weekly doses for ten weeks at 0, 0.064 or 0.64mg/kg CB. Tests conducted at 24 hrs. 3) 16 lean, 16 obese rats exposed to 0 or 0.64mg/kg CB, weekly for 10 weeks. Tests conducted at 13 weeks. CB mode size 86nm	Endothelium dependent (acetylcholine), endothelium independent (nitric oxide), voltage dependent calcium channel (felodopine) vaso-relaxation. Receptor dependent vasoconstriction (phenylepherine). mRNA expression in aortic tissue.	Protocols 1 and 3 showed no change. 2) CB 0.064 mg/kg doubled EC50, 0.064 mg/kg quadrupled EC50 vs. control $p<0.05$ for both obese and lean. Emax also lower $p<0.05$. No nitric oxide, felodipine or phenylepherine related changes occurred. 1) Nos2, CP-1, chrm-3, Nos3 genes-no change. Hmox1 increased at all doses in obese vs. lean $p<0.05$. 2) Hmox-1 no change. 3) Increased Hmox-1 in obese vs. lean $p<0.05$. Ocln increased in all obese, reduced in all lean at 0.064 and 0.06mg/kg $p<0.05$.	Subjects randomized. Highly susceptible animal model. Particles sonicated. Buffered washouts of neurotransmitters before tests on aorta segments.	Non-physiological delivery of nanoparticles. Very high exposure doses.	Repeated exposure to CB nanoparticles caused endothelium dependent vaso-relaxation to be impaired in lean rats and further impairment in obese rats. This effect was not apparent 13 weeks later. Single doses had no effect. No inflammatory genes up-regulated.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Furuyama et al 2009 Controlled trial (Furuyama et al., 2009).	Male BALB/c mice. 6 weeks old.	Intra-tracheal instillation.	50µg 20nm, 200nm gold and fluorescein-labelled carboxy-polystyrene particles. Both negatively charged.	Particle deposition in organs (of relevance to this review)	20 and 200nm gold particles deposited in heart and liver. Polystyrene 20nm particles detected in the liver, on vascular endothelium and on ventricular endothelium where the 20nm particles associated with small thrombi.	Particles shown to directly interact with thrombi, regardless of possible distant effect from lung inflammation.	No sonication of particles, but serum used to limit aggregation.	Nanoparticles deposit in extra pulmonary organs after crossing the alveolar barrier and may cause thrombi formation.
Gilmour et al 2007 Controlled trial (Gilmour et al., 2007).	BALB/c female mice 8-10weeks old.	Oropharyngeal aspiration	50µl saline containing 50 or 100µg UFP from four locations. Tests conducted 18hrs post instillation.	Creatine kinase and other haematological measurements.	No change.	Wide variety of PM used.	Non-physiological exposure, where particles may not have reached the lung. Very large range of results between mice suggesting inconsistent dosing.	Ambient UFP may not be toxic when instilled oropharyngeally.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Hwang et al 2010 Controlled trial (Hwang et al., 2010).	20 C57BL/6 mice 3 weeks of age.	Intra-tracheal instillation	Exposure to TiO2 <100nm (n=10) at 20mg/kg/ml (0.5mg/mouse) and saline control (n=10). Instillations were 2 x week, for 12 weeks.	Oxidative damage in peripheral mononuclear cells.	Oxidative damage in peripheral mononuclear cells↑.	Relevant material to industrial exposures. Particles autoclaved prior to exposure. Mouse matches human autonomic balance better than other strains.	Not well defined in particle size. Not sonicated. Few useful outcomes.	Oxidative stress raised post exposure to UFP in mononuclear cells.
Jia et al 2012 Controlled trial (Jia et al., 2012).	Male C57BL/6 mice 10-11 weeks old.	Intra-tracheal instillation	CB 19nm at 0.05, 0.15 and 0.6 mg/kg 3x over 6 days. Cage control vehicle control and 3 exposure groups. N=10/ group. Tests conducted 24hrs post exposure.	MDA in heart muscle, cardiac troponin I, CRP and white blood cell count in plasma. SDDN, rMSSD, low and high frequency domain and their ratio (LF, HF).	MDA─, cardiac troponin I ↑. CRP and white blood cell count ─. SDNN and rMSSD↓ for 0.15, 0.6mg/kg. LF─, HF↓ for 0.6mg/kg. LF/HF ratio ─.	20 min sonication time. Assessors blinded. Mouse matches human autonomic balance better than other strains.	No measurements made at 0-24 hours post exposure.	Cardiac parasympathetic function is impaired without cardiac injury in response to ultrafine CB.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Jun et al 2011 Controlled trial(Jun et al., 2011).	Platelets extracted from human blood in healthy male donors and male Sprague-Dawley rats.	*In vitro* incubation. IV bolus and intra-tracheal instillation	Silver 10-100nm at doses 0,10,100,200 and 250µg/ml for aggregation experiment. *In vivo* IV dose 0, 0.05, 0.1 mg/kg. Intra-tracheal instillation 0-250µg/ml.	Platelet aggregation. Phospholipid (phosphotadyl serine) expression. Thrombin generation. Calcium (flow cytometry) and serotonin release, CD62P expression. *In vivo* thrombus formation (weight). *Ex vivo* platelet aggregation, phospholipid expression.	*IN VITRO* platelet aggregation ↑ in dose dependent manner from 50µg/ml and was markedly increased with sub-threshold/minute doses of thrombin to almost 100% at 250µg/ml. Phospholipid exposure ↑ in a dose dependent manner from 200µg/ml, reaching almost 100% expression at 250µg/ml. Thrombin generation↑ at 250µg/ml and in a dose dependent manner from 200µg/ml with thrombin incubation. Calcium release ↑ (250µg/ml), enhanced with thrombin and aggregation effect blunted by use of calcium chelator. Serotonin release ↑ at 250µg/ml and enhanced with thrombin. CD62P↑ at 100, 250 µg/ml and enhanced with thrombin. *IN VIVO* thrombus formation↑ at 0.1mg/kg. *EX VIVO* platelet aggregation ↑from 50µg/ml and 100µg/ml with thrombin in dose dependent manner. Phospholipid expression↑ at 250µg/ml with and without thrombin.	Sonication and vigorous vortexing of nanoparticles pre-exposure. Spurious nanoparticle effect on platelet aggregation avoided by cell counting. Very comprehensive investigation of coagulability in several models.	Non-physiological exposure methods. In the *in vitro* experiment the cells were isolated from *in vivo* cellular and hormonal effects.	Platelet aggregation and coagulability is enhanced by silver nanoparticles. A hyper coagulable state might pre dispose to enhanced negative effects.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Inoue et al 2006 Controlled trial(Inoue et al., 2006).	33 ICR mice 6 weeks old	Intra-tracheal instillation	14 and 56nm at 4mg/kg. LPS at2.5mg/kg via intratracheal instillation. 29-33 mice divided into 1) control group 2)2.5mg/kg LPS 3,4) 4mg/kg carbon black nanoparticles 14 and 56nm. 5,6) the nanoparticle groups with LPS .Assessments made 24 hours later.	Activated protein C, prothrombin time, Activated Partial Thromboplastin Time and fibrinogen	LPS +14nm particle increased fibrinogen levels compared to LPS alone. P<0.01. No other results were significant for nanoparticles.	Strain of mice highly responsive to LPS possibly accentuating LPS and nanoparticle effect above significance	24 hour delay between exposure and assessment may miss transient changes as observed in other included trials.	14nm particles are more toxic than 56nm. LPS accentuates the prothrombotic effect of 14nm nanoparticles. PS does not adhere to nanoparticles (unpublished data)

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Kim et al 2012 Controlled trial (Kim et al., 2012).	Male Sprauge-Dawley rats, 5 weeks old.	Intra-tracheal instillation.	40nm and 202nm CB 0, 1, 3, or 10 mg/kg bolus. Tests conducted at 24hrs and 1 week.	Platelet Aggregation (platelet function analyzer-100). Plasma Coagulation (*In vitro* and *In vivo*). Blood cell count. IL-6 snd TNF-α. Homocysteine and cysteine. GSH. Heart rate and wave features. SDNN, RMSSD, LF, HF, LF/HF. Deposition of CB	PFA-100 closure time↓ at 1 week with 40nm only. Prothrombin time ─ and Activated Partial Thromboplastin Time↑ for both at 1 week at 3mg/kg *in vivo*, *in vitro* ─. Blood cell count─ IL-6 snd TNF-α─. 202nm ↑ homocysteine at 3mg/kg at 1 week only. Cysteine and GSH─. CB deposition in liver ─	*In vitro* testing of clotting time used to prove clotting time alterations concerned with impaired production, not function of clotting factors.	Mild agglomeration seen. No measurements made between 24hrs and 1 week.	CB of 40nm produces a hypercoagulable state. CB of 202nm raises homocysteine and possibly atherosclerotic risk. Mild impairment of clotting factor production is seen probably due to impaired production.
Li et al. 2010 Controlled trial (Li et al., 2010c).	Kunming mice 7 weeks old.	Intra-tracheal instillation	TiO2 3nm instilled once weekly, for 4 weeks to accumulate 13.3mg/kg. Tests conducted 24hrs post exposure.	Histopathological examination	protein exudate in liver, liver cell necrosis, exudation blood cells in liver	Sonicated particles prior to use.	Possible contamination of TiO2 sample by larger particles.	*In vivo*, hepatic cells are stressed by 3nm TiO2 exposure.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Mikkelsen et al 2011 Controlled trial (Mikkelsen et al., 2011).	42 ApoE-/- mice. Human umbilical vein endothelial cells.	Intra-tracheal instillation.	4 groups of 10-11 ApoE-/- mice. Control. Fine TiO2, photocatalytic and nano TiO2 bolus doses at 0.5mg/kg. Vasoreactivity- All 4 groups received 2 doses 24 hours apart. Assessment was at 26 hours. Plaque progression- nanoparticle and control group received weekly doses for 4 weeks. Tests conducted at 9 weeks.	*IN VIVO* Endothelium dependent: (ACh, calcitonin-gene related peptide) and independent (nitroglycerin and felodipine) vasoactivity. Plaque progression. *IN VITRO* HUVEC NO release on particle exposure.	*IN VIVO* No vasoactive effect. Nanoparticles increased plaque area. 5.5% vs. 4.1% control. p=0.018. *IN VITRO* Moderate dose increased NO release in HUVEC for nanoTiO2. NOS inhibitor reduced this effect.	Centrifuged for 30 minutes.	Per-protocol analysis. BAL fluid vehicle could cause protein corona on the nanoparticle surface	Exposure to nanoparticles progresses atherosclerosis. There is no effect on vasodilation.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Mo et al 2008 Controlled trial (Mo et al., 2008).	Male Wister rats 7-8 weeks of age. Neutrophils from rats.	*In vitro* and intra-tracheal instillation.	Nickel, cobalt and TiO2 at 20nm 0-10µg/ml *in vitro* experiments . 12hr culture. *Ex vivo* used one dose of 0.1, 0.5 or 1mg in 1ml saline. 12 hour exposure. 3 days post exposure, neutrophils isolated and cultured for 12, 24 and 48 hrs.	*IN VITRO* LDH, TNF-α, MIP-2 and NO release. *EX VIVO* TNF-α, MIP-2 and NO release.	*IN VITRO* LDH release at 10µg/ml. Neutrophil TNF-α, MIP-2 and NO ↑with nickel and coblt exposure, not TiO2. *EX VIVO* TNF-α, MIP-2 and NO ↑ with nickel and cobalt exposure. TiO2 ↑ TNF-α .	Particles centrifuged for 30 minutes prior to use. *Ex vivo* experiments used alongside *in vitro* exposures, to describe biological plausibility and real effect.	Non physiological exposure type. Confirmation of *in vitro* results *in vivo* might have been more useful than isolated neutrophils. Cells isolated from *in vivo* cellular and hormonal effects.	Exposure to 3 different nanoparticles types caused neutrophil release of TNF-α, MIP-2 and NO. TiO2 was less potent.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Mo et al 2009 Controlled trial (Mo et al., 2009b)	C57BL/6J and gp91phox knock-out mice. Pulmonary vascular endothelial cells from male C57BL/6J and gp91phox KO mice.	*In vitro* incubation and intra-tracheal instillation.	Ambient UFP <160nm at 0, 10, 20, 50, 100 µg/ml for 12 hours in ROS study. And 200µg/ml for 12-72hrs for cytotoxicity studies.	*IN VITRO* Cytotoxicity, ROS generation (DCF florescence). P67phox knockout (essential regulatory component of NADPH oxidase). gp91phox knock out to confirm. p47phox, 67phox and Rac 1 translocation. p38 and ERK1/2 MAPK activation. IL-6 expression. *IN VIVO* ROS generation.	*IN VITRO* In wild type murine cells, cytotoxicity occurred at 100 and 200µg/ml. DCF florescence from 10µg/ml ↑ dose response with UFP. Inhibited with catalase, DPI and NAC. P67 KO ameliorated UFP effect. Effect confirmed with gp91phox knock out cells and confirmed *in vivo*. IL-6 gene ↑ and dependent on NADPH and P38 (endothelial cell). 47phox, p67phox and Rac 1 were all translocated to the plasma membrane (endothelial cell). p38 and ERK1/2 MAPKs activated and NADPH dependent. *IN VIVO* ROS generation confirmed *in vivo* in C57BL/6J mice, and not gp91phox knockout mice, demonstrating NADPH dependence. Effect attenuated by NAC, CAT and DPI.	Mediating biomarkers were demonstrated by inhibition studies. ROS demonstrated in both exposure types. Realistic particle exposure.	Limited outcomes examined *in vivo*.	UFP exposure caused endothelial cell ROS generation. NADPH oxidase produces this ROS. MAP kinases activation due to oxidative stress up-regulates IL-6.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Author and study type	Subject type
Nemmar et al 2002 Controlled trial (Nemmar et al., 2002).	Male and female hamsters between 100-110g.	Intra-tracheal instillation and intra-venous injection.	60nm un-modified polystyrene nano-particles at 50, 500, 5000 µg/kg, amine modified polystyrene at 50, 100, 500 µg/kg and carbo-xylate modified nano-particles at 5, 50 500µg/kg. All groups had 4-5 hamsters /exposure concen-tration. For intra-tracheal instillation, 6 each were used / exposure concen-tration. Assess-ments made at 30 min and at 1 hr.	Arterial and venous thrombus formation in ultraviolet light and free-radical mediated endothelial injury.	Intravenous instillation of unmodified particles ─ at any concentration. Carboxylate-modified particles inhibit thrombus formation at 100 and 500 µg/kg. Amine-modified particles ↑ thrombus formation at 50 and 500 µg/kg. Intra-tracheal instillation at 5mg/kg of amine modified particles ↑ thrombus formation compared to control.	Particle induced thrombus histologically matched to control thrombus. Sonication and then Immediate vortexing of particles before instillation to prevent aggregation. Intra-tracheal instillation verified observations.	Less physiologic approaches used. Not true pollutant particles used.	Intravenous instillation of positively charged amine particles are prothrombotic at 50 µg/kg. Negatively charged caboxylate particles inhibit thrombus formation and unmodified polystyrene particles have no effect. Intra-tracheal instillation of positively charged particles causes a thrombotic effect.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Nemmar et al 2003 Controlled trial(Nemmar et al., 2003).	Male and female hamsters between 100-110g.	Intra-tracheal instillation.	60nm unmodified, amine, or carboxylated 400-nm amine-modified polystyrene particles. All at 500 µg/animal, except 60nm amine, at 5, 50 and 500 µg/animal. Measurements made at 1 hour.	Thrombus measured by ultraviolet light and free-radical mediated endothelial injury technique. Lung micro-circulation histology. Closure time in the Platelet Function Analyser (PFA).	Aminated particle↑ thrombus size in dose response. Larger amine particle did not. In circulatory thrombi, platelet marker G28E5 ↑in lung. 60 and 400nm aminated particles shortened PFA closure time.	Prior sonication used to de-aggregate particles.	Non-physiological exposure type. Polystyrene nanoparticles are not found in ambient UFP.	Aminated particles enhance peripheral thrombosis in rats.
Nemmar et al 2004 Controlled trial(Nemmar et al., 2004b).	Male and female hamsters between 100-110g.	Intra-tracheal instillation	500µg per animal of 400nm aminated polystyrene particles. 24 hours post exposure.	Venous thrombus formation in ultraviolet light and free-radical mediated endothelial injury. Plasma histamine and plasma vWF.	Thrombus size ↑ with 400nm particles compared to control. Plasma histamine ↑ from 21.6nmol/l to 37.6nmol/l in 400nm particles compared to control and no difference in plasma vWF.	Lysis of blood cells demonstrated lower histamine levels after *in vivo* exposure to nanoparticles, proving the histamine is released from blood cells, not pulmonary inflammation.	Large nanoparticles. No steps taken to observe them within circulation. Effect may not be nanoparticle mediated. (Conhaim et al suggests only 2% of lung pores are 400nm in size).	400nm particles promote thrombus formation and histamine release from basophils. As no vWF was detected, thrombosis may not depend on endothelial activation.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Author and study type	Subject type
Niwa et al 2007 Controlled trial (Niwa et al., 2007).	Male LDLR/KO mice. 6 weeks old.	Intra-tracheal dispersion.	CB as 10mg over 10 weeks with a particle size of 121nm, (n=10) with half of these on a high cholesterol diet. Exposure to air (n=10), half on a high cholesterol diet. A group were exposed to CB after 3 days of high cholesterol diet.	Histological analysis of the aorta. Microscopic analysis of aorta liver, kidney and spleen. Lipid levels. CRP.	Group on high cholesterol diet and CB exposure had a higher percentage of atherosclerotic change and CRP levels compared to air and cholesterol diet group. CB not detected by microscopic analysis.	Study time period and diet optimized to detect an effect. Mice randomized to each group.	Exposure mice smaller at baseline vs. control group P values not available. Statistical adjustment not reported in multiple comparison tests. Infrequent exposures.	Atherosclerosis is advanced by CB exposure. CRP levels are also increased.
Park et al 2009 Controlled trial (Park et al., 2009).	ICR mice. Gender, age not reported.	Intra-tracheal instillation.	21nm TiO2 administered at 5, 20 and 50 mg/kg. (n=10-12/ group) Tests conducted at 1, 3, 7 and 14 days.	Lymphocyte phenotypes, genetic expression, serum biochemistry and cytokines. Mortality.	Lymphoctye phenotypes were not reported with confidence intervals or significance. 38 genes up-regulated, some markers of inflammation.IL-1 IL-6, IL- 12 and TNF-α, IL- 3-5, IL-10, IFN gamma, IL-2 was increased. No rats died during exposure.	Long period for assessments post exposure. No rat deaths despite high exposure levels.	No randomization . Non-physiological exposure. Many particles were agglomerated at ~200nm.	Inflammatory genes were up-regulated after TiO2 exposure.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Park et al 2009 Controlled trial (Park and Park, 2009).	ICR mice. Gender, age not reported. RAW 264.7 murine macrophages.	Intraperitoneal exposure.	12nm silica nanoparticles at 50, 100, and 250mg/kg for splenocyte proliferation test. Other tests used a 50mg/kg dose. Tests conducted at 12, 24, 48 and 72 hours post exposure.	*IN VIVO* Splenocyte proliferation, lymphocyte distribution, Il-1β, TNF-α, macrophage activation. NO production. *IN VITRO* ROS generation (DCF). GSH oxidation. NO production. Cytotoxicity.	*IN VIVO* Splenocytes proliferated to 180% compared to the control group at 50mg/kg but viable cell numbers ↓ past 100mg/kg. NK and T-cells both ↑ but B-cell levels↓ compared to control. Both pro-inflammatory cytokines ↑ at 12, 24, 28 and 72 hours. Cytotoxic effect shown in activated macrophages at 24, 48 and 72 hours. NO level ↑ above control at 24, 48 and 72 hours. *IN VITRO* ROS ↑ in concentration dependent manner. GSH ↓. NO ↑ in concentration dependent manner. Cytotoxicity ↑ in conc dependent manner.	Sonicated nanoparticles to prevent aggregation.	No randomisation. Non physiological method of delivery. Silica may not all have been absorbed across the peritoneum.	Splenocytes exhibit a dose dependent proliferation. The nanoparticles affected cellular immunity. Level of secondary messenger of inflammation (NO) increased. Levels of pro inflammatory cytokines were also increased
Park et al 2010 Controlled Trial(Park et al., 2010).	Male and female ICR. 6 weeks old	Oral instillation.	22, 42, 71, and 323 nm silver nanoparticles, 1 mg/kg for 14 days (5 mice per group), or, 0.5 or 1.0mg/kg for 28 days (6 mice per group).	TGF-β. Nk B cell and CD8+ distribution. ALT, AST, ALP, protein, albumin, creatinine. IL-1, TNF-α, and IL-6, Th1-type cytokines (IL-12 and IFN-γ), Th2-type cytokines (IL-4, IL-5, IL-10).	22-71nm particles ↑ TGF-β levels, NK +B cell and CD8+ T-cell numbers. ALT, ALP and AST↑. IL-1, 4, 6, 10, 12 and IgE↑ TNF-α, IL-5, IFN-γ─.	Long exposure duration.	No sonication of particles reported. Exposure type not wholly relevant to inhalation exposure.	Repeated doses of silver nanoparticles induce cytotoxicity and inflammatory responses.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Sharma et al 2012 Controlled trial (Sharma et al., 2012).	75 Swiss albino mice. 6 weeks old.	Oral instillation.	30nm ZnO. Group 1- vehicle control. 2) ZnO nanoparticles at 300mg/kg body weight. 3) 50mg/kg. 14 days exposure.	ALT, ALP, bilirubin, blood urea nitrogen, creatinine levels. Lipid peroxidation. Necrosis and apoptosis. Oxidative stress (Fpg-modified Comet assay).	ALT and ALP↑. Bilirubin, blood urea nitrogen, creatinine ─ for 300mg/kg vs. control. Lipid peroxidation↑ at 300mg/kg only. A 14 day exposure at 300mg/kg showed liver cell necrosis and apoptosis. Oxidative stress in DNA increased in 300mg/kg group in AU, not 50mg/kg. (Comet assay).	Nanoparticles sonicated for 10 minutes prior to use.	Non physiological exposure type. High doses administered.	ZnO nanoparticles induced oxidative stress and apoptosis in liver cells.
Silva et al 2005 Controlled trial (Silva et al., 2005).	7 male Fisher rats weighing 250-300g. (Population number estimated from results)	Intra-tracheal and intravenous exposure of nanoparticles with a free-radical mediated endothelial injury (RB).	Ultrafine aminated polystyrene particles at 0.02, 0.5, 50 mg/kg. 60nm carboxy-lated nanoparticles at 0.1 and 50 mg/kg for one hour exposure.	Thrombus formation after intra tracheal and intravenous instillation.	Thrombus formation was achieved faster for aminated nanoparticles compared to carboxylated, which experienced no significant change. Aminated UFP at 0.02, 0.5 and 50mg/kg were all significantly prothrombitic compared to vehicle alone. IV administered nanoparticles at 0.5mg/kg induced similar effects with Rose-Bengal dye to accentuate thrombosis, but longer illumination time needed.	Each animal was its own control. Prior sonication and vortex of particles reduced aggregation. Demonstrated similar effects when particles were administered either side of the alveolar barrier.	Polystyrene particles are a proxy for true pollutants. Small number of subjects.	Positively charged polystyrene particles are prothrombotic whilst negatively charged particles are not. Large doses are not as prothrombotic, possibly due to agglomeration. Administering particles either side of the alveolar barrier causes similar thrombi.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Tong et al 2010 Controlled trial (Tong et al., 2010).	Female CD-1 mice 12 mice for inflammatory study, 10 for cardiac perfusion.	Oropharyngeal instillation. Direct infusion into perfused heart circulation.	100µg of <100nm UFP. Measurements made 24hrs post-exposure.	Plasma blood count and biochemistry. Haemodynamics, cardiac perfusion. Recovery of ischaemic heart and necrotic area.	No change in blood cell count or biochemistry. HR and flow rate decreased. No other haemodynamic change. LVDP post ischaemia reduced with UFP. Infarct size increased.	Infarct size measured, not just perfusion changes.	Non-physiological exposure. Cardiac perfusion measurements made at 24 hrs, potentially missing changes 0-24hrs.	UFP exacerbated cardiac ischaemia and reperfusion injury.
Vesterdal et al 2009 Controlled trial(Vesterdal et al., 2009).	ApoE-/- mice 11-13 and 40-42 weeks old.	Intraperitoneal exposure.	Old and young female mice exposed to 0.05 and 0.5mg/kg 0.7nm CB (n=9-11 per 4 groups). Old male mice exposed to 0.5mg/kg (n=5).	Emax of endothelium dependent vaso-relaxation. EC50 value of SNP induced vaso-relaxation. Receptor dependent vasoconstriction (% increase)	Emax values ↓ at both doses in young females 58.7 and 60.0% versus 70.8% control, and in old female mice at low dose 37.8% compared to the control group (60.4%) . High dose females and males did not reach significance. EC50 value of old age mice ─. In young female mice a 16.5 nM EC50 value existed (Control 10.1 nM). The EC50 for mice treated with the lower dose─. No receptor dependent vasoconstriction tests reached significance.	Pre-validated doses. Stratified by gender and age.	Non-physiological delivery of nanoparticles. Sonication was ineffective. Low subject number in male group. Pre-existing poor vasomotor function in male mice. Atherosclerotic plaque impairment of vasoactivity.	Endothelium dependent and independent vasorelaxation is impaired by CB. Receptor mediated relaxation is not. Younger mice were affected more than older mice.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Vesterdal et al 2010 Controlled trial (Vesterdal et al., 2010).	Female ApoE-/- mice. Young (13 weeks) group and old group.(49 weeks) .	Intra-tracheal instillation	14nm CB particles at 0.05, 0.5, 0.9 and 2.7 mg/kg for the young group. Assessment 24 hours later. Group 2 0, 0.9mg/kg assessed 2 hours post exposure. Old group at 0 or 0.5 mg/kg assessed 5 weeks after a 2 weekly exposure.	Endothelium in/dependent and receptor mediated vasoreactivity . Plaque progression. Endothelial activation	0.5mg/kg exhibited acetylcholine -dependent relaxation. Sodium nitroprusside ─. 0.05 and 0.5 mg/kg of carbon black exhibited phenylepherine-induced vasoconstriction 24 hours later. Plaque size was similar for nanoparticles and control. Endothelial activation─.	Well characterized particles, used in other outcomes. Lowest dose used reflected normal exposure to carbon black. Blinded experiment.	BAL fluid vehicle could cause protein corona reacting with the nanoparticle surface. Nanoparticles polluted with $PM_{2.5}$. Inflammation from an intra-tracheal dose may increase permeability for second doses. Several mince died post exposure.	Endothelial NO availability is reduced by carbon nanoparticles causing vasomotor impairment.

Table 5.b. Controlled animal experiments (Injection of nanoparticles into circulation). The table is ordered alphabetically. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Where animals have been randomized to groups, this is included in column 7 under study strengths, and the trial is referred to as a controlled trial. Subject numbers reported in this table are taken from the subject numbers used only for included experiments. Definitions: ↑= increase, ↓= decrease, ─ =no change, hrs= hours, expts= experiments, mins= minutes.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Bihari et al 2010 Controlled trial (Bihari et al., 2010).	C57BL mice. Whole blood from C57BL mice. Age not reported.	Intravenous injection and *in vitro* incubation.	TiO2 40nm at 1 mg/kg. 60nm Amine- and carboxyl-polystyrene nanoparticles at 0.5 mg/kg. Negative control. Positive control-epinephrine 12.5 μM. Thrombosis experiments conducted 10 mins after infusion.	Platelet CD26P expression. Platelet–granulocyte complexes. Platelet-platelet aggregation. Platelet, leukocyte and erythrocyte counts, haematocrit, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration of erythrocytes. Mesenteric thrombosis formation. Microvascular thrombosis.	*IN VITRO* Platelet CD26P and Platelet–granulocyte complexes ─ for TiO2 and carboxyl-particles, SWCNT and aminated particles↑. Platelet-platelet aggregation ─ for aminated particles. *IN VIVO* No differences in platelet, leukocyte and erythrocyte counts, haematocrit, haemoglobin concentration, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration of erythrocytes. Mesenteric thrombus formation time↑ with carboxylated particles, ↓ with aminated particles, TiO2. TiO2, polystyrene particles─ on thrombus cessation time.	Positive control used for proof of experimental feasibility. Wide range of particles used. Multiple arteries used in identical experimental conditions.	It is assumed that nanoparticles can pass via the lung into the circulation in large quantities.	Aminated particles increase platelet activity and enhance thrombus formation.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Cho et al 2009 Controlled trial(Cho et al., 2009).	Female CD-1 mice. Hearts isolated from CD-1 mice.197 for biomarker experiments, 40 mice for cardiac injury.	Oropharyngeal instillation and bolus injection into heart model.	Ambient UFP ≤100nm near road and far from road. Administered as 25 or 100µg bolus for biomarker tests, and 100µg for cardiac injury. Biomarkers tested at 4 and 18hrs. Cardiac injury at 18hrs.	Cell numbers, platelets, haematocrit, CRP, LDH, liver function markers. Coronary flow, Left ventricular end diastolic pressure, ventricular contraction. Cardiac-ischaemia-reperfusion injury.	Cell numbers, platelets, haematocrit, CRP, LDH, liver function markers ─. Coronary flow↓ (near road only) left ventricular diastolic pressure and ventricular contraction─. Myocardial infarction size greater after UFP infusion and left ventricular diastolic pressure slower to recover (far road group only).	Ambient PM collected over 2 weeks, for reasonable representation of 'average' exposure. Nano-particles sonicated and centrifuged for 30 minutes.	Non physiological exposure method. Cell isolated from *in vivo* cellular and hormonal effects.	Ambient exposure worsens cardiac injury after an ischaemic event.
Downs et al 2012 Controlled trial (Downs et al., 2012).	Adult male Wister rats. Age not reported.	IV injection.	Gold 2, 20 and 200nm at 6.00 × 10^−5 1.12 × 10^−4 1.41 × 10^−3 g/ml respectfully. Silica 15, and 55nm Quartz 400nm at particle numbers 1.75 × 10^17 4.14 × 10^15 1.68 × 10^12 respectfully.	DNA damage, Liver injury, peroxidation and oxidation. Inflammatory markers.	Small DNA damage was made at 15nm 50mg/kg. 15nm silica increase micro nucleated reticulocytes at 50mg/kg. 55nm increased at 125mg/kg. Liver peroxidation non-significant. 8-oxoGua ELISA not raised. 50mg/kg of 15nm silica doses raised IL-6 and TNF-α. Quartz also raised TNF-alpha at 100mg/kg Gold was deposited in the liver, but did not reach any significant end points.	Neutral gold used as a controlled exposure alongside vehicle. Wide array of observations determined for liver injury.	92nm particles were intended as 2nm particles. Wide size range of silica nanoparticles. Low gold doses greatly limited the surface area available. Aminotransferases not measured. Quartz control not well defined nanoparticles, not included in this data.	Raised inflammatory markers and liver injury resulted from several silica sizes and concentration, possibly from a secondary effect rather than direct ROS production.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Hwang et al 2008 Controlled trial(Hwang et al., 2008).	Spontaneously hypertensive rats and Wister rats. 13-14 weeks old used for Langendorff isolated heart perfusion models.	Perfusion of isolated heart	Diesel exhaust UFP <100nm from12.5mg of diesel exhaust. Control used. (av.n=11 per group) UFP and control within each rat type.	LVDP, dp/dimin, dp/dt max (ventricular contractility), Coronary flow.	LVDP and Coronary flow decreased in WKY rats. LVDP, dp/dimin, dp/dt max, Coronary flow all reduced in SHR. No significant difference between the two rats at any measurement.	Age matched between rat types. Similar inhalation exposure to real-world human exposure.	Heart isolated from hormones and nerves. Perfusion pressure low.	Myocardial contraction impaired in SHR and WKY. Hypertension does not worsen the response to nanoparticle infusion.
Jun et al 2011 Controlled trial (Jun et al., 2011).	Platelets extracted from human blood in healthy male donors and male Sprague-Dawley rats.	*In vitro* incubation. IV bolus and intra-tracheal instillation	Silver 10-100nm at doses 0, 10, 100, 200 and 250µg/ml for aggregatory experiment. *In vivo* IV dose 0, 0.05, 0.1 mg/kg. Intra-tracheal instillation 0-250µg/ml.	Platelet aggregation. Phospholipid (phosphotadylserine) expression. Thrombin generation. Calcium (flow cytometry) and serotonin release, CD62P expression. *In vivo* thrombus formation (weight). *Ex vivo* platelet aggregation, phospholipid expression.	*IN VIVO* thrombus formation ↑at 0.1mg/kg. *EX VIVO* platelet aggregation ↑from 50µg/ml and 100µg/ml with thrombin in dose dependent manner. Phospholipid expression↑ at 250µg/ml with and without thrombin.	Sonication and vigorous vortexing of nanoparticles pre-exposure. Spurious nanoparticle effect on platelet aggregation avoided by cell counting. Very comprehensive investigation of coagulability in several models.	Non-physiological exposure methods. In the *in vitro* experiment the cells were isolated from *in vivo* cellular and hormonal effects.	Platelet aggregation and co-agulability is enhanced by silver nanoparticles. A hyper coagulable state might pre dispose to enhanced negative effects.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Khandoga et al 2004 Controlled trial (Khandoga, 2004).	Female C57B1/6 mice 5-7 weeks old.	CB suspension administered via polyethylene catheter into left carotid artery as a bolus.	14nm CB 1x10^7 and 5x10^7 nanoparticles for 2 hours. n=6 buffered control. n=6 high dose. n=6 low dose. n=6 GP2b3a blocker, (tirofiban). Tests conducted at 2 hours.	Platelet-endothelial and leukocyte-endothelial interactions. Endothelial activation. Sinusoidal perfusion, fibrinogen deposition. All in hepatic circulation.	Adherent platelet numbers ↑ 3 fold in sinusoids and venules for both UFP groups. GP2b3a blockade inhibited this effect. No platelet rolling. Fibrin deposition in both coronary and hepatic vasculature, and vWF for hepatic vasculature. Leukocyte interactions and sinusoidal perfusion was not affected by UFPs.	Infusion represents the same range of systemically absorbed particles with 24hr exposure in a human	Partial particle aggregation in the suspension was recorded. Experimental design assumes UFP passes the alveolar barrier in large concentrations.	UFP causes platelet accumulation on the hepatic post arterial endothelium associated with fibrin and vWF deposition. Lack of p-selectin suggests no inflammatory character.
Nishimori et al 2009 Controlled trial (Nishimori et al., 2009).	BALB/c mice	Intravenous injection.	Silica 76nm, 0.3 and 1µg. 70nm was delivered at 25mg/ml, and then varying doses to measure dose response.	Liver, spleen, kidney injury. ALT levels, inflammatory markers.	Hepatocyte death, ALT ↑ when Kupfer cells inhibited. When sinusoidal membrane disrupted, ALT levels —. Hepatocytes histologically denatured and ↑ collagen deposition, hydroxyproline contents ↑. No other organ changes. ALT levels, IL-6, TNF-α ↑ at 20mg/ml. ALT ↑with chronic administration.	Particles not aggregated upon injection.	Non physiological exposure type. Injection with 70nm particles was often lethal.	Sinusoidal cells may not act as a barrier against nanoparticles. Nanoparticles cause liver injury and continued administration causes fibrosis, possibly altering coagulation protein production.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Radomski et al 2005 Controlled trial (Radomski et al.).	Platelets separated from blood from healthy human volunteers. Wistar–Kyoto rats. Age not reported.	*In vitro* incubation and IV injection.	Mixed carbon nanoparticles, purified carbon fullerenes at 50µg/ml *in vivo* for thrombosis experiment, 200µg/ml *in vivo*. 5-500µg/ml *in vitro*.	*IN VITRO* Platelet aggregation. ATP release. Degranulation. GP2b3a expression. P-selectin expression Gp1b expression Aggregation inhibition by prostacyclin and S-nitroso-glutathione,(endogenous inhibitors of haemostasis) and aspirin (GP2b3a inhibitor. MMP-9/MMP-2 ratio. PKC dependence. *IN VIVO* Platelet aggregation.	*IN VITRO* Mixed carbon NPs ↑ platelet aggregation. Carbon fullerenes–. Mixed carbon NPs caused ATP release and platelet degranulation. Mixed carbon NPs ↑GP2b3a expression. P-selectin↑ for mixed carbon NPs. GP1b↓ for mixed carbon NPs. Mixed carbon NPs ↓ aggregation with prostacyclin and S-nitroso-glutathione, not with aspirin. MMP-9/MMP-2 ratio↑ in carbon fullerenes only. PKC dependence– for all particles. *IN VIVO*. Mixed carbon nanoparticles ↑ platelet aggregation at 50µg/ml. Carbon fullerenes–.	Nano-particles sonicated for 2 minutes. vortexed prior to testing. Investigated aggregation through several methods, and confirmed *in vivo*.	Limited *in vivo* experiments. IV injection assumes that all particles pass the alveolar barrier.	Carbon nanoparticles and materials activate platelet and enhance vascular thrombosis, but might do through different mechanisms.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Silva et al 2005 Controlled trial (Silva et al., 2005).	7 male Fisher rats weighing 250-300g. (Population number estimated from results)	Intra-tracheal and intravenous exposure of nanoparticles with a free-radical mediated endothelial injury (RB).	Aminated polystyrene particles at 0.02, 0.5, 50 mg/kg. 60nm Carboxylated nanoparticles at 0.1 and 50 mg/kg for one hour exposure.	Thrombus formation after intra tracheal and intravenous instillation.	Thrombus formation was achieved faster for aminated nanoparticles compared to carboxylated, which experienced no significant change. Aminated UFP at 0.02, 0.5 and 50mg/kg were all significantly prothrombitic compared to vehicle alone. IV administered nanoparticles at 0.5mg/kg induced similar effects with Rose-Bengal dye to accentuate thrombosis, but longer illumination time needed.	Each animal was its own control. Prior sonication and vortex of particles reduced aggregation. Demonstrated similar effects when particles administered either side of the alveolar barrier.	Polystyrene particles are a proxy for true pollutants. Small number of subjects.	Positively charged polystyrene particles are prothrombotic whilst negatively charged particles are not. Administering particles either side of the alveolar barrier causes similar thrombi.
Simkhovich et al 2007 Controlled trial (Simkhovich et al., 2007).	Langendorff heart models from 4month old and 26 months from female Fisher 334 rats.	Direct infusion into perfused heart circulation.	9 young and 10 old hearts in control group, 15 young and 13 old hearts exposed to diesel UFP <100nm. Tests at 0, 10, 20, 30mins.	Developed pressure. Left ventricular end diastolic pressure, positive and negative left ventricular pressure (+ve dP/dt and -ve dP/dt). Coronary flow.	In young and old, developed pressure ↓ by 33 and 35%. LVEDP ↓in young hearts only. +ve dP/dt ↓ in young hearts at 20, 30mins and in old hearts at all times. -ve dP/dt↓ for both hearts at all times. Coronary flow ↓ at 20, 30mins in young and all times in old heart. For all measurements there was highly significant group x age effect.	Relevant for true human exposure over one day.	Non-physiologic exposure. Heart removed from hormonal and vagal input.	Young and old hearts are equally affected by UFP. Cardiac haemodynamic performance is impaired.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Stampfl et al 2011(Stampfl et al., 2011).	Langendorff heart model (guinea pig).	Direct infusion into perfused heart circulation.	14nm CB (4-8x 10^9) 120nm CB (8 x 10^9, and TiO2 (2-4 x 10^9) 50nm SiO2 7nm (1 x 10^9) particles.	Heart rate. Noradrenaline (NA) concentration changes.	At 1 hour, HR↑ by 5% (CB14) and 6% (TiO2) 10%(CB120); at 2 hours and double dose, 15% (CB), 13% (CB120) and 13% (TiO2). SiO2 ↑ 7% during 2nd hour (lowest concentration). NA↑69% for CB14 and Ti02.	Suitable heart model for electrophysiological studies.	Limited reporting of P-values did not allow inclusion of some results. Heart rate results are considered with vagal input removed.	HR is increased by exposure to UFP when isolated. Noradrenaline release is increased.
Tong et al 2010 Controlled trial (Tong et al., 2010).	Female CD-1 mice 12 mice for inflammatory and, 10 for perfusion study.	Oropharyngeal instillation. Direct infusion into perfused heart circulation.	100µg of <100nm UFP. Measurements made 24hrs post-exposure.	Plasma blood count and biochemistry. Haemodynamics, cardiac perfusion. Recovery of ischaemic heart and necrotic area.	No change in blood cell count or biochemistry. HR and flow rate decreased. No other haemodynamic change. LVDP post ischaemia reduced with UFP. Infarct size increased.	Infarct size measured, not just perfusion changes.	Non-physiological exposure. Heart rate results are considered with vagal input removed. Cardiac perfusion measurements made at 24 hrs only.	UFP exacerbated cardiac ischaemia and reperfusion injury.
Wang et al 2009 Controlled trial (Wang et al., 2009).	40 Sprague-Dawley rats 180-200g.	Intra articular injection	46nm TiO2 at 0, 0.2, 2 and 20mg/ml (n=10/group). Tests conducted 7 days later.	AST, ALT, alkaline phosphatase, LDH. Heart and hepatocyte damage.	AST /ALT ratio and LDH↑ at 2 and 20mg/ml. Swollen vascular endothelium in heart. Fatty degeneration of hepatocytes, inflammatory response, dose dependently.	Nanoparticles sonicated for 50 minutes. Random assignment to groups.	Highly aggregated. Cardiovascular outcomes were secondary endpoints. Not a useful exposure method.	Both the liver and heart are damaged by translocation of particles to these organs.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Wold et al 2006 Controlled trial (Wold et al., 2006).	Female Sprague-Dawley rats, and isolated Langendorff-perfusion model from rats.	Intravenous infusion.	Diesel exhaust ≤100nm at 50µg/ml (n=3). Ambient UFP ≤150nm (n=4) and control (n=3) tests conducted at 0, 5, 30, 60mins. Industrial diesel ≤100nm at 50µg/ml, (n=6), its soluble component (n=5), control (n=8).	*IN VIVO* ventricular premature beats. Heart rate and blood pressure. Ejection fraction. *EX VIVO* left ventricular end diastolic pressure. Left ventricular contractility. Coronary flow.	*IN VIVO* Diesel exhaust caused ventricular premature beats in 2/3 rats. Ambient UFP caused severe hypotension, heart block and death in one rat. No haemodynamic changes. Ejection fraction ↑for ambient UFP only. *EX VIVO* Left ventricular end diastolic pressure↑ for diesel exhaust. ventricular contractility ↓ for diesel exhaust. Left ventricular systolic pressure, contractility and coronary flow↓ for industrial diesel c.f. baseline. For soluble fraction, left systolic pressure and coronary flow↓ c.f. baseline.	Particles were sonicated for 30mins prior to use. Particles used are highly relevant to ambient exposure to UFP.	Intervention groups had lower blood pressure, lower heart rate and lower ejection fractions compared to control at baseline. Industrial diesel exhaust outcome changes statistically compared to own baseline, not control changes. Concentrations estimated from PM-UFP amount.	Injected nanoparticles can reduce cardiac function, but soluble components may not have an effect.

Table 6.a. **Controlled *In vitro* experiment- human tissues.** The table is ordered alphabetically. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Definitions: ↑= increase, ↓= decrease, ― = no change, hrs= hours, expts= experiments, mins= minutes.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Aung et al. 2011 Controlled trial (Aung et al., 2011).	Human aortic endothelial cells.	*In vitro* incubation.	0.056, 0.1, 0.18, 0.32, 0.56nm ambient UFP at 10µg/ml for all studies except immunofluorescence (50 µg/ml).	Genetic expression.	Stress (SAPK/JNK), transcription factors (ATF-3), and inflammatory responses (E-selectin, PTGS2, MIP-2, MCP-1) All NPs increased secretion of IL-6 and MCP-1 .	Particle type represents ambient UFP well. Cell type useful endothelial representation.	Limited relevant outcomes. Cell isolated from *in vivo* cellular and hormonal effects.	Oxidative stress and inflammatory responses up-regulated in aortic cells exposed to UFP.
Chen et al 2011 Controlled trial (Chen et al., 2011).	Human fibrinogen	*In vitro* incubation.	Gold 10nm particle. Concentrations of 0.775nM and 2.48nM used.	Fibrinogen-particle binding.	Fibrinogen-particle binding↑ in dose and time dependent manner.	Result confirmed by separate methods (UV absorbance spectrum, dynamic light scattering and visualisation)	Experiment media was un physiological (Buffered saline). Gold is a poor surrogate nanoparticle due to its low reactivity and absence in ambient PM.	Gold nanoparticles can form complexes with fibrinogen.
Corbolan et al 2011(Corbalan et al., 2011).	Human umbilical vein endothelial cells.	*In vitro* incubation.	Cells were exposed to 11nm silica at 10 µg/mL for 1 hr, 53, 148 and 496nmsilica at 50 µg/mL for 3 hours.	NO and ONOO− production. NF-κB binding activity and downstream genetic activation. Cytokine release.	NO↑ and ONOO−↑. NO /ONOO−↓. NF-κB binding activity↑. ICAM1, VCAM1, SELE, MMP9, COX2, F3, IL6, and IL8↑. Il-6 and 8↑	Sonication for 2 minutes and vortexing of particles before use. Positive control, calcium ionophore used.	Corona formation with bovine serum. Cell isolated from *in vivo* cellular and hormonal effects.	In endothelial cells exposed to silica NP's, an unfavourable shift of NO /ONOO− ratio occurred, suggesting oxidative damage. Many inflammatory genes up-regulated.

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Corbolan et al 2012 controlled trial (Corbalan et al., 2012).	Blood from healthy human volunteers.	*In vitro* incubation.	11 nm, 53 nm, 118 nm, and 496 nm silica NPs at 1-200 µg/ml. Negative control.	P-Selectin and GPIIb/IIIa activation. Platelet aggregation. Concentration of NO and ONOO−. TXA2 (ASA), ADP (apyrase), and MMP2 (phenanthroline) pathway inhibitors used to determine pathways involved in aggregation.	P-selectin↑ GPIIb/IIIa and platelet aggregation ↑. NO↑ and ONOO−↑. NO /ONOO−↓ MMP2 and ADP pathways involved in agglomeration of platelets.	Nanoparticles sonicated for 2 minutes prior to use.	Inhibition tests could have shown if inflammatory expressions were oxidative stress dependent.	In human platelets exposed to silica NP's, an unfavourable shift of NO /ONOO−occurred suggesting oxidative damage and aggregation markers were raised, alongside increased aggregation.
Deng et al 2011 Controlled trial (Deng et al., 2011).	plasma samples from 8 healthy human volunteers	*In vitro* incubation	Negatively charged poly (acrylic acid)-coated gold particles of 5, 10 and 20nm.	Fibrinogen-particle binding. Binding of fibrinogen to Mac-1-receptor-positive THP-1 cell. NF-kB activation. IL-8, TNF-α secretion. Particle charge effects.	Fibrinogen-particle binding↑. Mac-1 receptor binding↑. Fibrinogen/NP complexes ↑NF-kB. IL-8, TNF-α↑ neutral charge inhibited binding to fibrinogen. Other particles all bound fibrinogen with the smaller ones promoted fibrinogen-THP-1 cells more. This process was partly MAC-1 dependent.	Many outcomes assessed to determine conclusion. Relevant plasma type.	Cell isolated from *in vivo* cellular and hormonal effects.	Negatively charged nanoparticles cause fibrinogen unfolding and activate the MAC-1 pathway. Inflammatory mediators are released.

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Forestier et al 2012 controlled trial(Forestier et al., 2012).	Blood from healthy human volun-teers.	*In vitro* incubation	14nm CB 0.1 µg/ml	I-1b, IL-8, thrombin. Coagulation-prothrombin time, aPTT, fibrinogen levels and d-dimers. Platelet plug formation speed. Collagen-induced platelet aggregation.	I-1b−, IL-8−. Thrombin formation↑ in a platelet dependent fashion. Coagulation markers−. Plug formation speed−.Platelet aggregation↑	Piloted to ensure capture of all changes. Nanoparticles sonicated for 10 minutes prior to exposure.	More outcomes existed for DEP than for UFCB. Cell isolated from *in vivo* cellular and hormonal effects.	Coagulation accentuated after UFCB exposure.
Foucard et al 2010 Controlled trial (Foucaud et al., 2010).	human mono-cytic cell line THP-1	*In vitro* incubation	14 and 260nm CB 0-500µg/mg	Cell-free (14 and 260nm) and within 4-HNE production (14nm). HO-1 and HSP70, GSH and GSSG. 4-HNE treatments	Cell-free oxidative stress for 14nm↑ 260nm↓. 14nm only 4-HNE production↑ HO-1─, HSP70↑, GSH↓, and GSSG↑. 4-HNE treatment caused HO-1↑ and glutathione↓.	Positive control used (4-HNE) to elicit inflammatory response.	Spontaneous oxidation of fluorescent dye when measuring oxidative stress. Cell isolated from *in vivo* cellular and hormonal effects.	Exposure to UFCB elicits a oxidative stress response. CB causes 4-HNE production The 4-HNE produced elicits a cellular stress response.
Gojova et al 2007 Controlled trial (Gojova et al., 2007).	Human aortic endotheli al cells	*In vitro* Incubation	Fe2O3 5 and 45nm. Y2O3 20-60nm. ZnO 100-200nm Control used. 1 and 10 µg/mL for mRNA studies. 1, 10 and 0 µg/mL for protein levels.	HAEC, ICAM-1, IL-8, and MCP-1 mRNA expression and blood levels.	Fe2O3 exposure= ICAM-1 ─ IL-8─,MCP-1─ mRNA levels. Y2O3 and ZnO exposure= ICAM-1↑, IL-8↑, and MCP-1↑ mRNA levels. ZnO ld_{50} 4hrs. Fe2O3 and Y2O3 did not. Fe2O3= ICAM-1 ─, MCP-1─ protein serum levels. Y2O3 caused I C A M - 1 ─, MCP-1↑ protein serum levels. ICAM-1 ↑, MCP-1↑ protein serum levels with ZnO.	Nanoparticles sonicated for 5 minutes prior to incubation. Wide range of nanoparticle exposures and concentrations . Useful cell type.	Positive control may have been useful considering the large number of negative results.	Nanoparticles elicit an inflammatory response in aortic endothelial cells *in vitro*.

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Gonçalves et al 2010 Controlled trial (Goncalves et al., 2010).	Neutrophils isolated from healthy human volunteer.	*In vitro* incubation..	TiO2 nanoparticles at 0, 2 10, 20, 50 or 100 µg/ml.	Cell morphology. TiO2-induced phosphorylation. P38- and ERK 1/2- MAPK activation. Neutrophil apoptosis. GRO-α and IL-8 production.	Neutrophil morphology suggests activation on TiO2 exposure. TiO2-induced tyrosine phosphorylation of several proteins. ERK1/2 MAPK↑ P38 MAPK↑. TiO2 significantly inhibited PMN apoptosis. IL-8 and GRO-α production↑.	Wide range of concentrations used. Relevant cell type used.	MAPK pathway not shown to causally influence downstream cell reactions. Cell isolated from *in vivo* cellular and hormonal effects.	Neutrophils activated by *in vitro* exposure to TiO2.
Jun et al 2011 Controlled trial (Jun et al., 2011).	Platelets extracted from human blood in healthy male donors and male Sprague-Dawley rats.	*In vitro* incubation. IV bolus and intra-tracheal instillation.	Silver 10-100nm at doses 0,10,100,200 and 250µg/ml for aggregation experiment. *In vivo* IV dose 0, 0.05, 0.1 mg/kg. Intra-tracheal instillation 0-250µg/ml.	Platelet aggregation. Phospholipid (phosphotadylserine) expression. Thrombin generation. Calcium (flow cytometry) and serotonin release, CD62P expression. *In vivo* thrombus formation (weight). *Ex vivo* platelet aggregation, phospholipid expression.	*IN VITRO* platelet aggregation ↑ in dose dependent manner from 50µg/ml and was markedly increased with sub-threshold/minute doses of thrombin to almost 100% at 250µg/ml. Phospholipid exposure ↑ in a dose dependent manner from 200µg/ml, reaching almost 100% expression at 250µg/ml. Thrombin generation↑ at 250µg/ml and in a dose dependent manner from 200µg/ml with thrombin incubation. Calcium release ↑ (250µg/ml), enhanced with thrombin and aggregation effect blunted by use of calcium chelator. Serotonin release ↑ at 250µg/ml and enhanced with thrombin. CD62P↑ at 100, 250 µg/ml and enhanced with thrombin.	Sonication and vigorous vortexing of nanoparticles pre-exposure. Spurious nanoparticle effect on platelet aggregation avoided by cell counting. Very comprehensive investigation of coagulability in several models.	Non-physiological exposure methods. In the *in vitro* experiment the cells were isolated from *in vivo* cellular and hormonal effects.	Platelet aggregation and coagulability is enhanced by silver nanoparticles. A hyper coagulable state might predispose to enhanced negative effects.

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Karoly et al 2007 Controlled trial (Karoly et al., 2007).	Human pulmonary artery endothelial cells.	*In vitro* incubation.	0-100 µg/mL UFP or vehicle for 4 hours. n=4 per group	Genetic expression. Quantitative polymerase chain reaction with UFP, insoluble and soluble fractions. Cytokine expression. Anti-Tissue-Factor blocking agent experiment.	320 genes↑ 106 genes↓ coagulation, Tissue Factor↑ polymerase chain reaction= F3↑ cytokine signalling eg MCP-1, IL-8, CXCL1-3 ↑. Wnt signalling↑. MAPK signalling↑. Water-soluble UFP fractions induced F3, F2RL2, and heme-oxygenase 1 expression. IL-6 and IL-8 levels↑. Tissue factor blocking agent attenuated IL-6 and IL-8 release.	Ambient UFP used. Tissue factor shown to mediate IL-6, 8 releases.	Huge amount of outcomes. Cell isolated from *in vivo* cellular and hormonal effects.	Genetic expression of clotting factors is enhanced post UFP exposure *in vitro*.
Kennedy et al 2009 Controlled trial(Kennedy et al., 2009).	Human aortic endothelial cells.	*In vitro* incubation	0.001–50 µg/ml of Fe2O3 5-90nm, Y2O3 20 to 60 nm, CeO2, ZnO rods 100 to 200 nm x 20 to 70 nm .	ICAM-1, IL-8,, MCP-1 mRNA levels. ICAM-1 and MCP-1 protein expression. Nrf2 translocation. ROS production (DHE stain). GCLC, HMOX1, and NOX4 mRNA expression	mRNA = Y2O3 ↑ ICAM-1, IL-8, MCP-1 at 50 µg/ml and ICAM-1 at 10µg/ml. ZnO ↑ ICAM-1 and IL-8at 50µg/mL, and ICAM-1 at 10µg/ml, CeO2↑ IL-8 at 50µg/ml all at 4 hrs. At 1, 2 an 8 hrs at 10µg/ml, ZNO↑ ICAM-1, IL-8, MCP-1. MCP-1 protein i↑ at 10, 50Iµg/ml for ZNO, ICAM-1 ↑ at 50µg/ml for ZnO only. Nrf2 translocation ─ for all NPs. ROS↑ 10 and 50 µg/ml ZnO, 50 µg/ml Y2O3, Fe2O3─. HMOX-1↑ at 10µg/ml ZnO.,Y2O3, Fe2O3─	Nanoparticles were sonicated for 5 minutes prior to use.	Cell isolated from *in vivo* cellular and hormonal effects.	Various nanoparticles induce reactive oxygen species formation, and an inflammatory response.

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Kendall et al 2011 Controlled trial (Kendall et al., 2011).	Fibrinogen exposure	*In vitro* incubation	Amino, carboxyl, hydroxylate and sulphate Polystyrene, 100, 200 and 500nm. Hydrophillic silica 12nm. CB 76nm.	Particle aggregation in saline.	All particles had similar aggregation speeds in fibrinogen except for aminated polystyrene particles, due to bridge flocculation from particle adsorption to NP surface.	Nanoparticles sonicated before use. Control inspected for contamination.	Saline is not wholly relevant to *in vivo* effects in real life.	Nanoparticle interaction with fibrinogen alters Nanoparticle surface available for cellular effects. Aminated particles may remain in the body
Kim et al 2012 Controlled trial (Kim and Choi, 2012).	U937 Monocyte cells.	*In vitro* incubation.	Silver 5nm particles at doses 0-0.8µg/ml.	Cytotoxicity (Time taken for 50% cell death). IL-8 production.	50% cytotoxicity occurred at 0.36 µg/ml IL-8 production ↑ at 0.5 µg/ml.	Endotoxin tested for in silver nanoparticles to limit particle pollution.	Single NP dose used for IL-8 and the main experimental outcome was not of interest. No sonication used to de-aggregate particles.	IL-8 is release from macrophages exposed to modest doses of silver NPs.
Li et al 2009 Controlled trial (Li et al., 2009).	Human aortic artery endothelial cells	*In vitro* incubation.	Diesel UFP 12.5, 25, 50 µg/ml or control. Incubated for 1 hour.	Superoxide production and protein cabonylation. HO-1 and tissue factor expression. JNK activation, its inhibition and silencing.	Intracellular and mitochondrial superoxide production ↑. Protein carbonylation↑. Heme oxygenase-1 and tissue factor mRNA expression↑, O_2^- scavengers attenuated this effect. JNK activated/phosphorylated. Inhibition of JNK attenuated superoxide production and genetic expression. JNK silencing enhanced this effect.	30 min sonication of UFP prior to use.	Cells isolated from *in vivo* cellular and hormonal effects.	Diesel UFP induces oxidative stress in aortic endothelial cells via JNK activation.

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Li et al 2010 Controlled trial (Li et al., 2010a).	Human aortic endothelial cells (HAEC) and Monocytic THP-1 cells.	*In vitro* incubation.	Diesel UFP1 (Idling engine) UFP 2 (city driving conditions) 10-18, 18-32, 32- 56, 56-100, 100-180 at 50 µg/ml or control.	Superoxide production. HO-1, OKL38 and TF mRNA . IL-8, MCP-1, VCAM genes. Dose response HO-1 and VCAM genes. Monocytic binding. NF-kB activation.	(HAEC) superoxide↑ for UFP1+2. HO-1, OKL38 and TF genes↑ for 1+2. UFP1 VCAM↓, MCP-1, IL-8─. UFP2 VCAM, MCP-1, IL-8↑. Dose response UFP1= HO-1↑ and VCAM gene ↓. UFP 2= HO-1gene↑, VCAM gene↑. UFP1↑ monocytic binding. UFP2 greatly↑ binding. UFP1↓ UFP2↑ NFkB. Inhibition of NFkB abrogated IL-8, MCP-1, VCAM expressions and partially- monocyte binding.	Use of different exhaust types to quantify how city driving specifically contributes to ambient UFP.	Cells isolated from *in vivo* cellular and hormonal effects.	Both UFP types cause monocytic binding. UFP2 was more potent and partially dependent of NF-kB signalling.
McGuinnes et al 2010 Controlled trial (McGuinnes et al., 2011).	Whole blood and isolated platelets from healthy human volunteers.	*In vitro* incubation.	Polystyrene uncharged particle 88nm, carboxylated 79nm and aminated 75nm or control. Incubated for 20 minutes at 260 µg/ml.	Platelet aggregation. Polymorphonuclear neutrophilic leucocyte-platelet aggregates. Combined CD14 + CD42b positive events. Expression of CD62P and PAC-1 in whole blood and on platelets. Annexin V binding. Haemoglobin release.	Positively charged particles associate with platelets in platelet-platelet or monocyte-platelet aggregates. Both charged particles caused agglomeration of platelets. Uncharged no effect. Polymorphonuclear neutrophilic leucocyte-platelet aggregates no change for any particle type. CD14 + CD42b positive events for both charged particles. Carboxylated particles showed increased expression of CD62P and PAC-1 in whole blood and platelets. Both charged particles induced Annexin V binding. Aminated particles only caused Heamoglobin release, but even small plasma concentrations diminished this effect.	Sonicated for 10 minutes prior to incubation. Relevant cell types used.	Short incubation time. Cells isolated from *in vivo* cellular and hormonal effects.	Oppositely charged particles had similar platelet-activating properties, but through different mechanisms.

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Mayer et al 2009 Controlled trial(Mayer et al., 2009).	Whole human blood samples.	*In vitro* incubation.	Carboxylated polystyrene particles between 33-212nm. Amide polystyrene particles, 300nm. 0.05 mg/ml, 0.5 mg/ml, 2 mg/ml and 2.5 mg/ml for 10-120 minutes.	LDH release. Complement activation. Coagulation. Thrombocyte activation. Granulocyte activation. Haemolysis.	LDH release ─. C3a-. D-dimer-. Prothrombin-. CD42b and CD62P-. Granulocyte activation- for amide, ↑ for carboxylated particles 33-160nm. Hb release↑ in all particles.	Sonicated for 15 minutes prior to use. Positive controls used for proving experiment plausibility.	Charge neutralized by protein solution. Short incubation time. Cells isolated from *in vivo* cellular effects. Neutral particles would have been useful to delineate between 'charge' and 'size' effect.	Negatively and positively-charged polystyrene particles induce cellular damage and possibly granulocyte activation for negatively-charged particles only.
Metassan et al 2010 controlled trial (Metassan et al., 2010).	Purified human fibrinogen and human thrombin.	*In vitro* incubation.	Ambient UFP ≤220nm at 17, 34 and 60µg/ml and 380 µg/ml for plasma experiment.	FIBRINOGEN Fibrin clot pore size. Fibrin polymerisation (rate of polymerization and maximum optical density). Visualised fibrin network structure. IN PLASMA fibrin clot pore size. ROS generator added to fibrinogen. ROS scavenger.	FIBRINOGEN PM↑ clot porosity at 17 and 34 µg/ml. 68µg/m caused faster clot formation with formation of altered fibrin fibres, optical density increased for 34 and 68µg/ml. Fibrin network decreased in density, 68µg/ml increased network heterogeneity with fibrin clustering. A decrease in fibre density was seen. Fibre diameter not altered. IN PLASMA 380µg/ml ↑ increased fibrin clot pore size. ROS produced similar findings to PM, but additionally decrease fibre diameter. ROS scavenger attenuated effect of PM on clot.	Nanoparticles centrifuged for 30 minutes prior to use.	Plasma fibrinogen not additionally tested with identical PM due to PM shortage.	Fibre network heterogeneity and fibrin clustering may explain the increased porosity of the nanoparticle bathed fibrin clots. To change pore size in plasma, much higher concentrations were needed.

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Napierska et al 2009 Controlled trial (Napierska et al., 2009).	The human endothelial cells (EAHY926 cell line).	*In vitro* incubation.	Silica 16, 19, 60, 104, 335nm and Ludox® Silica 14 and 15nm. Cell cytotoxicity and viability studies conducted over 24 hours.	Cell viability and cytotoxicity (TC50).	Cell death ↑ in a dose dependent and inverse- size dependent manner for both Silica types. Cytotoxicity (TC50) ↓ as size↓. Cell death was predominantly by necrosis, peaking at silica 14nm, with apoptosis peaking at 16nm.	Wide range of exposure sizes.	Outcomes may not have relevance for real life exposures where much lower concentrations would be experienced. Cells isolated from *in vivo* cellular effects.	Cell death occurred via necrosis, and apoptosis. Cell death was dose and inverse-size dependent.
Napierska et al 2012 Controlled trial (Napierska et al., 2012).	The human endothelial cells (EAHY926 cell line).	*In vitro* incubation.	60 and 16nm silica particles. Iron doped silica (FeSi) 16nm. Incubated for 30 minutes for ROS experiment, 4 hours for GSH oxidation and 24hrs for cytotoxicity.	Cell free ROS. GSSG/GSH ratio. Cytotoxicity (TC50). Lipid peroxidation. HO-1 mRNA.	ROS= FeSi↑ Silica 60nm↓ 16nm─. FeSi= cell glutathione ↓. GSSG/GSH and MDA and HAE↑, pure silica─. Cytoxicity ↑ in a concentration dependent and inverse particle size manner. Ho-1 expression for all particles ↑. NADPH oxidase-1 and glutathione reductase↑ for FeSi only. Antioxidant treatment prevented the HO-1 increase but not the glutathione↓ or glutathione ratio↑.	Relevant cell line used. Relevant UFP particle and engineered nanoparticles used.	The authors consider whether SFe nanoparticles are also able to quench the fluorescence signal. Cells isolated from *in vivo* cellular and hormonal effects.	Iron doped silica nanoparticles are considerably more toxic and up-regulate oxidative stress markers more than pure silica.

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Peters et al 2007 Controlled trial (Peters et al., 2007).	Human dermal micro vascular endothelial cells.	*In vitro* incubation.	Cobalt 28nm, nickel 62nm at 0, 0.5, 5, 10, 25 and 50µg/ml.	Cytotoxicity. ROS production and GSH production. IL-8 protein release. ICAM-1 expression. MCP- 1 release.	Concentration dependent drop reduction of cell number within 24hrs. CO ↑ ROS and GSH in dose dependent fashion, Ni ─. CO↑ IL-8 at 50µg/ml, NI ↑ in dose dependent fashion. ICAM-1 ↑ at 50µg/ml Co, Ni ─. Co↑ MCP-1 in a dose dependent manner, Ni─.	H2O2 added to NI-ROS experiment to confirm Nickel's lack of effect.	Cell type from a vascular bed which may not be physiologically similar to larger arteries.	Co increased oxidative stress levels, and exhibits an inflammatory response. Ni might elicit inflammation.
Radomski et al 2005 Controlled trial (Radomski et al.).	Platelets separated from blood from healthy human volunteers. Wistar–Kyoto rats. Age not reported.	*In vitro* incubation and IV injection.	Mixed carbon nanoparticles, purified carbon fullerenes at 50µg/ml *in vivo* for thrombosis experiment, 200µg/ml *in vivo*. 5-500µg/ml *in vitro*.	*IN VITRO* Platelet aggregation. ATP release. Degranulation. GP2b3a expression. P-selectin expression Gp1b expression Aggregation inhibition by prostacyclin and S-nitroso-glutathione,(endogenous inhibitors of haemostasis) and aspirin (GP2b3a inhibitor. MMP-9/MMP-2 ratio. PKC dependence. *IN VIVO* Platelet aggregation.	*IN VITRO* Mixed carbon NPs ↑ platelet aggregation. Carbon fullerenes─. Mixed carbon NPs caused ATP release and platelet degranulation. Mixed carbon NPs ↑GP2b3a expression. P-selectin↑ for mixed carbon NPs. GP1b↓ for mixed carbon NPs. Mixed carbon NPs ↓ aggregation with prostacyclin and S-nitroso-glutathione, not with aspirin. MMP-9/MMP-2 ratio↑ in carbon fullerenes only. PKC dependence─ for all particles. *IN VIVO*. Mixed carbon nanoparticles ↑ platelet aggregation at 50µg/ml. Carbon fullerenes─.	Nanoparticles sonicated for 2 minutes. vortexed prior to testing. Investigated aggregation through several methods, and confirmed *in vivo*.	Limited *in vivo* experiments. IV injection assumes that all particles pass the alveolar barrier.	Carbon nanoparticles and materials activate platelet and enhance vascular thrombosis, but might do through different mechanisms.

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Sharma et al 2011 Controlled trial (Sharma et al., 2011).	Human liver cell HepG2.	*In vitro* incubation.	30nm ZnO. 12 and 24 h at 14 and 20 µg/ml for cytotoxicity and 6hrs for DNA damage experiment.	Cytotoxicity. DNA damage (Olive tail moment and %DNA in comet assay). ROS production (DCF)	Cytotoxicity ↑ in a dose and time dependent manner. DNA damage↑. ROS↑.	DNA damage identified through two mechanisms.	Non physiological medium.	ZnO causes cyto- and genotoxicity, possibly via ROS production.
Sharma et al 2012 (Sharma et al., 2012).	Human liver cell HepG2.	*In vitro* incubation.	30nm ZnO at 0.8, 2, 8, 14-20µg/ml. (300 mg/kg) for 14 consecutive days.	Cytotoxicity (50% cell death). ROS production (DCF). Lipid peroxidation. Oxidative DNA damage (Olive tail moment and %DNA tail) and NAC inhibition. Apoptosis. Bax, Bcl2 , procaspase-9. P53 and Nf-kB. MAPK (ERK1/2, JNK, p38 kinase) activation. NAC inhibition. Cell death pathway.	Cytotoxicity ↑ in a dose and time dependent manner. (CC50 14.5µg/ml). ROS↑ 31% at 20µg/ml at 6hrs. Lipid peroxidation↑ at 20µg/ml. DNA damage↑ at 6 and 14hrs at 20µg/ml in Comet assay, Fpg modified Comet assay showed significantly higher values suggesting DNA damage; NAC was protective in dose dependent manner. Apoptosis ↑ at 14 and 20µg/ml. Bax↑ Bcl2↓ procaspase 9↓ P53 and Nf-kB─. JNK and p38 activation↑ proteins─. Both activations NAC inhibited. ERK1/2 activation─. Cell viability unaffected after NP exposure with NAC, Vitamin C. Viability↓ with vitamin E, JNK and p38 inhibitor.	Sonication 30mins. Inhibition tests allowed mediators to be ascertained. Relevant cell type.	Cells isolated from *in vivo* cellular and hormonal effects.	After ZnO exposure, cytotoxicity is seen after ROS generation. NAC inhibited this effect.

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Trpkovic et al 2010 Controlled trial (Trpkovic et al., 2010).	Blood from healthy human volunteers.	*In vitro* incubation.	CB 55, 100 and 125nm 0.25-10µg/ml for 1, 6, 18hrs hour for haemolytic studies, and 1 for ROS measurement.	Erythroctye haemolysis. Oxidative stress (fluorescence marker).	CB55↑haemolysis in time and dose dependent manner. Albumin reduced haemolytic activity. Oxidative stress (AU) ↑ and inhibited by NAC (antioxidant) No change for larger particles in any outcome.	Relevant tissue type.	Cells isolated from *in vivo* cellular and hormonal effects. Plasma restored to erythrocytes impeded the haemolytic effect observed with NPs.	CB raises erythrocyte oxidative stress in-vitro. Haemolysis caused *in vitro* is reversed by albumin.
Vesterdal et al 2012 Controlled trial (Vesterdal et al., 2012).	Human umbilical vein endothelial cells and aortic segments from C57BL/6 mice.	In-vitro incubation.	CB 295nm at 10-100µg/ml in ex-vivo vasomotor experiment. CB 85nm 0, 0.1, 10, 50 and 100µg/ml for cell experiments.	ROS production in HUVEC (DCFH assay and fluorescence microscopy) NO production. VCAM-1 and ICAM-1 expression. LDH% max. Cell cytotoxicity and proliferation. Endothelium dependent /independent vasorelaxation-Emax (Ach/SNP). Receptor dependent vasoconstriction Emax. (PE).	HUVEC- DCFH assay- acellular ROS↑ 10, 50, 100µg/ml. Cellular 0.1, 10, 50µg/ml↑. Microscopy-additional cellular ROS increase at 100µg/ml. UFP stimulated NO─. In presence of DPI, NO↓. 10µg/ml increased insulin-stimulated NO production, 100µg/ml decreased it. VCAM-1 and ICAM-1↑at 100µg/ml. LDH ↑ at 100µg/ml. Cell viability and proliferation─ at 0-100µg/ml. Vasomotor function- Ach-10µg/ml NPs↑, 100µg/ml NPs↓ Emax. SNP Emax- ↓. PE Emax-↓. Vessel diameter ↓with 10µg/ml exposure.	Nanoparticles sonicated for 1 hour before use. NO dependent vasoactivity tested in cellular NO release and NO dependent vasodilation in aortic rings.	Assumes direct action on cells by nanoparticles. Aortic rings removed from nervous and hormonal input.	CB nanoparticles increase ROS production, up-regulation of immune cell recruitment and a vasoconstrictive effect. Cell membranes are damaged. NO production may arise from NADPH and NOS.

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Wan et al 2008 Controlled trial(Wan et al., 2008).	U937 monocytes.	*In vitro* incubation.	TiO2 20nm, Cobalt 20nm at 0, 0.625, 1.25, 2.5 and 5µg/ml.	Cytotoxicity. Oxidative stress (DCF fluorescence). MMP-2, 9 mRNA expressions. TIMP-1,2 expression . AP-1/ PTK pathway inhibition (curcumin/ genistein, herbimycin A)	No significant cytotoxic effects for doses used. Oxidative stress; TiO2─, Cobalt↑ all doses, in dose dependent fashion. Effect supressed by ROS scavenger and inhibitor (NAC and CAT). MMP-2,9 mRNA expression and activity↑ at 2.5µg/ml + for Cobalt, not TiO2 These effects were supressed by NAC and CAT. TIMP-1─. TIMP-2 dose response↓ to Cobalt at 5µg/ml, TiO2─ (Effect prevented by CAT and NAC). AP-1 and PTK inhibitors ↓ MMP-2, 9.	Nanoparticles sonicated for 30minutes pror to use. Inhibitors of ROS used to demonstrate mediation in TIMP increases.	Assumes direct action on cells by nanoparticles. Cells removed from nervous and hormonal input.	ROS are increased post exposure. MMP-s up-regulated, activity increased and their inhibitors down-regulated.
Wilson et al 2002 Controlled trial (Wilson et al., 2002).	Human monocyte (Mono Mac 6). J774 mouse macrophage cell line.	*In vitro* incubation.	260nm and 14nm CB at 10-130µg/ml for 10 minutes for both ROS experiments and 24 hrs in the murine macrophage cell line J774.	Cell free ROS. Intracellular ROS (CuSO4, FeCl3 and FeSO4 incubation also). GSH. TNF-α expression.	14nm CB ↑260nm─cell-free oxidative stress. For 14nm CB, oxidative stress ↑ and further↑ with metal salts, 260nm─. Cellular oxidative stress↑ for 14nm ─ for 260nm. GSH and ATP↓ with 14nm CB and further↓ with FeSO4, CB─. For 14nm CB TNF-α↑	Multiple cell types used.	Cells removed from nervous and hormonal input.	14nm CB particles increase oxidative stress and TNF-α release. Iron salts accentuate oxidative stress cell free and intracellularly.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Yamawaki et al 2006 Controlled trial (Yamawaki and Iwai, 2006).	Human umbilical vein endothelial cells.	*In vitro* incubation.	CB 248nm mean (1, 10 and 100 µg/ml)	LDH release. Cell proliferation. PCNA expression (expressed in S phase of cell cycle). MCP-1 protein expression. VCAM-1 protein expression. Connexin37 expression. eNOS expression. Inflammatory gene expression.	LDH ↑ in a dose dependent manner. Proliferation inhibited at 10 and 100 µg/ml. PCNA ↓at 100µg/ml. MCP-1 expression↑. VCAM-1─. Connexin expression ↓. eNOS expression↓. Inflammatory genes; ICAM1, IL-8, HO-1, VCAM-1, PTGS2 (prostaglandin-endoperoxide synthase 2), SELE (selectin E), CCL2 (chemokine (C-C motif), MCP-1 ↑.	Relevant cell line used. Relevant UFP particle used.	Cells removed from nervous and hormonal input. Large nanoparticles used, which may not cross the alveolar barrier.	Antiproliferative effects, Pro-inflammatory, eNOS expression and inhibition of gap junctions seen after HUVEC exposure to CB.
Ye et al 2010 Controlled trial (Ye et al., 2010a).	Human hepatic cells.	*In vitro* incubation.	0.2, 0.4, 0.6 mg/ml SiO_2 of sizes 21, 48 and 86 nm. 12, 24, 36 and 48hr exposures	Cell cytotoxicity. LDH release. ROS, lipid peroxidation and GSH levels. Annexin V-FITC/PI double-staining and DNA ladder assay. p53, Bax and Bcl-2 expression	Cell viability decreased in a dose dependent fashion with ↑concentration and ↓size. LDH↑ with concentration for 21nm particle only. 21 nm SiO_2 dose dependent ↑ in lipid peroxidation, ROS and ↓ GSH. No change for 48 and 86nm particles. Assay showed 21nm particles cause apoptosis by dose-dependent manner. p53, Bax and ↑ by 21nm particles. Bcl-2─	Relevant cell line used. Relevant UFP particle used.	Did not examine potential mediating effects of oxidative stress on apoptotic changes. Cells removed from nervous and hormonal input.	21nm particles are cytotoxic. Oxidative stress is mediated by ROS. Apoptotic pathways are up-regulated by 21nm SiO_2.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Zhu et al 2011 Controlled trial (Zhu et al., 2011).	Human aortic endothelial cells from a 38-year old female donor and U937 monocytic cells from a 38-year old donor.	*In vitro* incubation.	Fe_2O_3 22nm. Fe_3O_4 43nm. Both at 2, 20, 100 µg/mL for 1-400min exposure.	Cell cytotoxicity. ROS generation (DCF). U937 cells adhesion to HAECs. NOS activity and NO release. ICAM-1 and IL-8 protein expression	Both iron oxides ↑ cellular cytotoxicity. Both↑ ROS production 20-100µg/ml at 6hrs. Both HAEC-U937 adhesion↑ at 20µg/mL after 4 hours. Both ↑NOS activity and NO release at 2µg/mL. ICAM-1 and IL-8↑with both nanoparticles.	30 min sonication of nanoparticles prior to use. Useful nanoparticle material used.	No ROS-cytotoxicity/up regulation/il-8 expression dependency shown. Cells removed from nervous and hormonal input.	Iron oxides generate monocyte adhesion. This might be due to ROS and NO production up-regulating ICAM-1 and IL-8 expression.

Table 6.b. **Controlled *In vitro* experiment- animal tissues.** The table is ordered by strength of evidence. All positive outcomes are reported positive at a p=0.05 significance level regardless of the included study's definition of significance. Where subject age or gender is reported by the study it is included in column two and the age at the start of the study is used. Definitions: ↑= increase, ↓= decrease, ― = no change, hrs= hours, expts= experiments, mins= minutes.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Choi et al 2008 Controlled trial (Choi et al., 2009).	RAW 264.7 macrophages.	*In vitro* incubation.	Photo luminescent silicon 3nm particles at 1-200µg/ml.	NO, Il-6 and TNF-α production.	Il-6 and TNF-α ↓. NO−.	Very small nanoparticles used.	Cell deaths at higher doses. Cell isolated from *in vivo* cellular and hormonal effects.	Unexpected observation of reduction in inflammatory mediators at higher doses.
Heng et al 2011 Controlled trial (Heng et al., 2011).	RAW 264.7 murine macrophage line.	*In vitro* incubation	ZnO sphere 113nm and ZnO sheet 420nm at 5, 10, 15, 20µg/ml.	Cytotoxicity (Concentration applied for 50% cell death). TNF-α production.	50% cytotoxicity for 113nm was 11.7µg/ml, 420nm was 12.3µg/ml. 114nm and 420nm↑ TNF-α in a dose dependent fashion, 114nm more so.	Nanoparticles sonicated (duration not reported).	Cell isolated from *in vivo* cellular and hormonal effects.	In macrophages ZnO NP exposure increases inflammatory mediator release.
Li et al 2003 Controlled trial (Li et al., 2003).	Murine RAW 264.7 macrophage cell line	*In vitro* incubation.	Ambient UFP < 150nm at 8-12µg/ml and 50µg/ml for HO-1 experiment.	ROS (DTT assay) HO-1 expression. GSG/GSSH. Mitochondrial damage.	ROS↑. HO-1↑. GSG/GSSH↓. Extensive disruption of mitochondrial cristae.	Positive controls used to demonstrate plausibility of cell experiments. Good reference material for ambient UFP.	Positive controls used to demonstrate plausibility of cell experiments.	UFPs generate oxidative stress *in vitro*.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Mo et al 2008 Controlled trial (Mo et al., 2008).	Male Wister rats 7-8 weeks of age. Neutrophils from rats.	*In vitro* and intra-tracheal instillation.	Nickel, cobalt and TiO2 at 20nm 0-10µg/ml for *in vitro* test. 12hr culture. *Ex vivo* used one dose of 0.1, 0.5 or 1mg in 1ml saline. 12 hour exposure. 3 days post exposure, neutrophils isolated and cultured for 12, 24 and 48 hrs.	*IN VITRO* LDH, TNF-α, MIP-2 and NO release. *EX VIVO* TNF-α, MIP-2 and NO release.	*IN VITRO* LDH release at 10µg/ml. Neutrophil TNF-α, MIP-2 and NO ↑with nickel and coblt exposure, not TiO2. *EX VIVO* TNF-α, MIP-2 and NO ↑ with nickel and cobalt exposure. TiO2 ↑ TNF-α.	Particles centrifuged for 30 mins prior to use. *Ex vivo* experiments used alongside *in vitro* exposures, to describe biological plausibility and real effect.	Non-physiological exposure type. Confirmation of *in vitro* results *in vivo* might have been more useful than isolated neutrophils. Cells isolated from *in vivo* cellular and hormonal effects.	Exposure to 3 different nanoparticles types caused neutrophil release of TNF-α, MIP-2 and NO. TiO2 was less potent.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Mo et al 2009 Controlled trial (Mo et al., 2009a).	C57BL/6J and gp91phox knock-out mice. Mouse pulmonary microvascular endothelial cells from male C57BL/6J and gp91phox knock-out mice.	*In vitro* incubation and intra-tracheal instillation.	Ambient UFP <160nm at 0, 10, 20, 50, 100 µg/ml for 12 hours in ROS study. And 200µg/ml for 12-72hrs for cytotoxicity studies.	*IN VITRO* Cytotoxicity, ROS generation (DCF florescence). P67phox knockout (essential regulatory component of NADPH oxidase). gp91phox knock out to confirm. p47phox, 67phox and Rac 1 translocation. p38 and ERK1/2 MAPK activation. IL-6 expression. *IN VIVO* ROS generation.	*IN VITRO* In wild type murine cells, cytotoxicity occurred at 100 and 200µg/ml. DCF fluorescence from 10µg/ml ↑ dose response with UFP. Inhibited with catalase, DPI and NAC. P67 KO ameliorated UFP effect. Effect confirmed with gp91phox knock out cells and confirmed *in vivo*. IL-6 gene ↑ and dependent on NADPH and P38 (endothelial cell). 47phox, p67phox and Rac 1 were all translocated to the plasma membrane (endothelial cell). p38 and ERK1/2 MAPKs activated and NADPH dependent. *IN VIVO* ROS generation confirmed *in vivo* in C57BL/6J mice, and not gp91phox knock-out mice, demonstrating NADPH dependence. Effect attenuated by NAC, CAT and DPI.	Mediating biomarkers were demonstrated by inhibition studies. ROS demonstrated in both exposure types. Realistic particle exposure.	Limited outcomes examined *in vivo*.	UFP exposure caused endothelial cell ROS generation. NADPH oxidase produces this ROS. MAP kinases activation due to oxidative stress up-regulates IL-6.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Mo et al 2012 Controlled trial(Mo et al., 2012).	Mouse pulmonary microvascular endothelial cells from male C57BL/6J and gp91phox knock-out mice.	*In vitro* incubation.	Ambient UFP <160nm at 0, 10, 20, 50 µg/ml for ROS study for 2 hours and 100-200µg/ml for cytotoxicity studies over 24 hours exposure.	Cytotoxicity, ROS generation (DCF fluorescence). P38 and ERK1/2 MAPK activation. Erg-1 regulation and MAPK dependence. IL-6 expression.	In wild type murine cells, cytotoxicity occurred at 100 and 200 µg/ml, DCF fluorescence from 20µg/ml ↑ dose response with UFP. P38 and ERK1/2 MAPKs activated in wild type, not gp91 KO. ↑ erg-1 expression at mRNA and protein level. UFPs did not induce Egr-1 in gp91phox KO mice. Inhibition of p38 and ERK1/2 MAPKs attenuated UFP-induced Egr-1 up-regulation. IL-6 expression ↑ with UFP exposure, in mRNA and protein form in wild type mice, not gp91 KO. This was a P38 and erg-1 dependent effect.	Mediating biomarkers were demonstrated by inhibition studies. Realistic particle exposure.	Cells isolated from *in vivo* cellular and hormonal effects.	Cells exposed to UFP generated ROS, activated MAPK-1 and increased IL-6 expression. Erg-1 was also up-regulated. NAPH oxidase is a key mediator in ROS driven biomarker expression.
Nishanth et al 2011 Controlled trial (Nishanth et al., 2011).	RAW 264.7 murine macrophages.	*In vitro* incubation.	Silver 15, 40nm, Aluminium 20, 50nm, CB 20, 40nm. Gold 20,40nm. Carbon coated silver 25, 45nm at 0-150µg/ml for 0-72 hours.	ROS production (DCFH fluorescence). Nf-kB activation. COX-2, TNF-α mRNA and protein expression. IL-6 release.	ROS= 5µg/ml silver ↑. Aluminium↑ CB↑ Carbon coated silver ↑ all doses, Gold ↓. Nf-kB↑ with all except Au–. After exposure, Silver, aluminium, CB, carbon coated silver ↑COX-2 and TNF-α mRNA, and protein. Au only at 24hrs (mRNA) and 48 (protein). IL-6 protein↑ for all except Au.	Positive control (LPS) considered and investigated.	Wide range of size and economically useful particles.	ROS release is increased post exposure to NPs, gold is not as reactive as the other particles.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Radomski et al 2005 Controlled trial (Radomski et al.).	Platelets separated from blood from healthy human volunteers. Wistar–Kyoto rats. Age not reported.	*In vitro* incubation and IV injection.	Mixed carbon nanoparticles, purified carbon fullerenes at 50µg/ml *in vivo* for thrombosis experiment, 200µg/ml *in vivo*. 5-500µg/ml *in vitro*.	*IN VITRO* Platelet aggregation. ATP release. Degranulation. GP2b3a expression. P-selectin expression Gp1b expression Aggregation inhibition by prostacyclin and S-nitroso-glutathione,(endogenous inhibitors of haemostasis) and aspirin (GP2b3a inhibitor. MMP-9/MMP-2 ratio. PKC dependence. *IN VIVO* Platelet aggregation.	*IN VITRO* Mixed carbon NPs ↑ platelet aggregation. Carbon fullerenes─. Mixed carbon NPs caused ATP release and platelet degranulation. Mixed carbon NPs ↑GP2b3a expression. P-selectin↑ for mixed carbon NPs. GP1b↓ for mixed carbon NPs. Mixed carbon NPs ↓ aggregation with prostacyclin and S-nitroso-glutathione, not with aspirin. MMP-9/MMP-2 ratio↑ in carbon fullerenes only. PKC dependence─ for all particles. *IN VIVO*. Mixed carbon nanoparticles ↑ platelet aggregation at 50µg/ml. Carbon fullerenes─.	Nanoparticles sonicated for 2 minutes. vortexed prior to testing. Investigated aggregation through several methods, and confirmed *in vivo*.	Limited *in vivo* experiments. IV injection assumes that all particles pass the alveolar barrier.	Carbon nanoparticles and materials activate platelet and enhance vascular thrombosis, but might do through different mechanisms.
Rosas-Hernandez et al 2009. Controlled trial (Rosas-Hernandez et al., 2009).	Sprague-Dawley rat coronary endothelial cells and rat aortic rings.	*Ex vivo* and *in vitro* incubation.	Silver, 45nm particles at 0-100µg/ml (Incubations 2min-2hrs) *in vitro*, and 5 µg/ml *ex vivo*.	*IN VITRO* cell proliferation. NO production. *EX VIVO* Vasoconstriction with/without phenylepherine. Ach effect.	*IN VITRO* cell proliferation inhibited at low doses and LDH released, ↑ at doses 50-100µg/ml. 10 µg/ml + stimulated NO production. Proliferation was NO dependent, shown by L-NAME blocking (shows eNOS dependent) 10µg/ml +. *EX VIVO* At 5µg/ml dose, vasoconstriction ↑ with/without phenylepherine and effect↓ when endothelium was removed. UFP inhibited Ach mediated vasorelaxation. ─ at high dose (45µg/ml).	Brief sonication used to disaggregate particles. Air bolus used to follow the intra-tracheal instillation. Vasoactivity tests in two different models.	NO dependent effects *in vitro* not similarly demonstrated *in vivo*. Non physiological exposure type.	A low concentration of silver was vasoconstrictive, a high concentration was vasodilatory. Vasoconstriction inhibited Ach induced, NO dependent vasorelaxation. Vasodilation partially abolished by L-NAME.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Xia et al 2008 Controlled trial (Xia et al., 2008).	RAW 274.7 cells.	*In vitro* incubation.	TiO2 284nm, ZnO 36nm, CeO2 323nm. For 0-20hrs and 50µg/ml.	Cytotoxicity. ROS generation (DCF-H2O2 production and MitoSOX Red=O2-production). HO-1 protein expression. Nrf2, NQO-1mRNAexpression. JNK activation.. IL-8 and TNF-α protein expression.Ca2+ release. CeO2 protective effect.	TiO2 ↑ cytotoxicity. O2-and H2O2↑for ZnO only. HO-1 protein ↑ for ZnO only. Nrf2 and NQO-1 mRNA↑ with ZnO. JNK↑ with ZnO. IL-8 and TNF-α↑ with ZnO. Ca2+↑ with ZnO. CeO2 reduced cell death count and oxidative stress (MitoSOX Red) when administered to cells with DEP vs. DEP alone.	Wide range of industrial nanoparticles used.	Cells removed from nervous and hormonal input. Murine macrophages used. Assumes direct effect upon cells.	All 3 tiers of oxidative stress defence were overcome by ZnO, but not the other particles. This could be due to size difference between particles, and not Inherent reactivity.
Ye et al 2010b Controlled trial(Ye et al., 2010b).	Rat embryonic ventricular myocardial cell line H9c2(2-1).	*In vitro* incubation.	21 and 49nm SiO2 particles at 0.1 to 0.3 mg/ml. (1.6mg/ml for cytotoxicity). Incubated 12-48hr.	Cytotoxicity (50% cell death). Cell viability. LDH levels. Intracellular ROS, GSH. MDA levels. Cell cycle flow cytometry. p53, p21 and Bax protein levels.	21nm= 0.32 and 49= 1.29 mg/ml for 50% cell death. Cell viability↓ for 49nm and more rapidly at 21nm, both time and dose dependent. 0.3mg/ml for 24 hrs↑ LDH. Only 0.3mg/ml ↑ROS, GSH↓ and MDA↑. Cell cycle arrest in G1, S phase% of cells↓. P53 and P21↑at both doses at 21hr and P53 at high dose+ 48hrs, Bax─.	Pre-determined dosing from pilot work. Relevant cell type used.	Cells removed from nervous and hormonal input. High doses used.	SiO2 particles have a toxic effect on H9c2(2-1) cells, with oxidative stress being a component of this. p53 and p21 proteins are likely involved in the G1 phase arrest.

Author and study type	Subject type	Exposure technique	Exposure type	Outcomes	Results	Study strengths	Study weaknesses	Conclusion
Yu et al 2010 Controlled trial (Yu et al., 2010).	Pulmonary micro vascular endothelial cells from male C57BL/6J mice.	*In vitro* incubation.	TiO2 28nm, CuO 42nm 0-10 µg/ml for cell viability. 0.625-2.5µg/ml for other experiments.	Cell viability. ROS generation (DCF). P38 MAPK activation. PAI-1 mRNA and protein expression. (NAC, CAT, DPI inhibition/scavenger of ROS, mRNA and protein expression) P38 inhibition of PAI-1 expression.	Cell viability, TioO2─, CuO toxic from 5-10 µg/ml in a dose responsive manner. EC50 approx. 7.5µg/ml. ROS↑ at 0.625µg/ml CuO for 12hrs in a dose responsive fashion, TiO2─. NAC, CAT, DPI all attenuated ROS production. P38 MAPK↑ CuO 0.625-2.5µg/ml after 3 hrs, and 2.5µg/ml at 1-12 hours, TiO2─. NAC, CAT, DPI↓ P38 MAPK. CuO 1-3hr 2.5µg/ml↑ PAI-1 mRNA and 12hr protein. NAC, CAT, DPI↓ these effects. P38 inhibition↓PAI-1 expression.	Multiple assay types used. (AlamarBlue™and MTS). Tested ROS pathway in several ways.	Agglomerated to 280 Ti and CuO 220nm. Cells removed from nervous and hormonal input.	CuO is more toxic than TiO2. During oxidative stress, PAI-1 is up-regulated, and is dependent on the p38 MAPK pathway.
Zhang et al 2011 Controlled trial (Zhang et al., 2011).	Murine RAW 274.7 macrophages	*In vitro* incubation.	Gold 97nm from 0-100µg/ml measurements made at 24- 48hrs.	Cytotoxicity. TNF-α and IL-6 protein release. NO release. ROS production (DCF).	No change in cell viability or LDH release occurred. IL-6 and TNF-α─. NO─. ROS production─	Nanoparticles inspected for LPS. LPS and H2O2 used as positive control. Assay absorbance adjusted for nanoparticle interference.	Cells removed from nervous and hormonal input. Not a useful nanoparticle material. High agglomeration rates.	Gold particles are unreactive when exposed to macrophages at varying doses *in vitro*.

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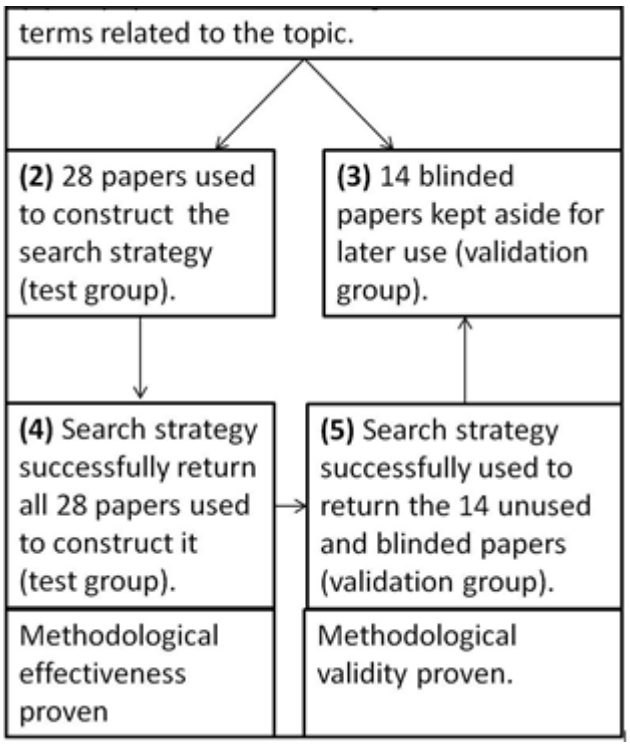
terms related to the topic.
(2) 28 papers used to construct the search strategy (test group).
(3) 14 blinded papers kept aside for later use (validation group).
(4) Search strategy successfully return all 28 papers used to construct it (test group).
(5) Search strategy successfully used to return the 14 unused and blinded papers (validation group).
Methodological effectiveness proven
Methodological validity proven.

Figure 1

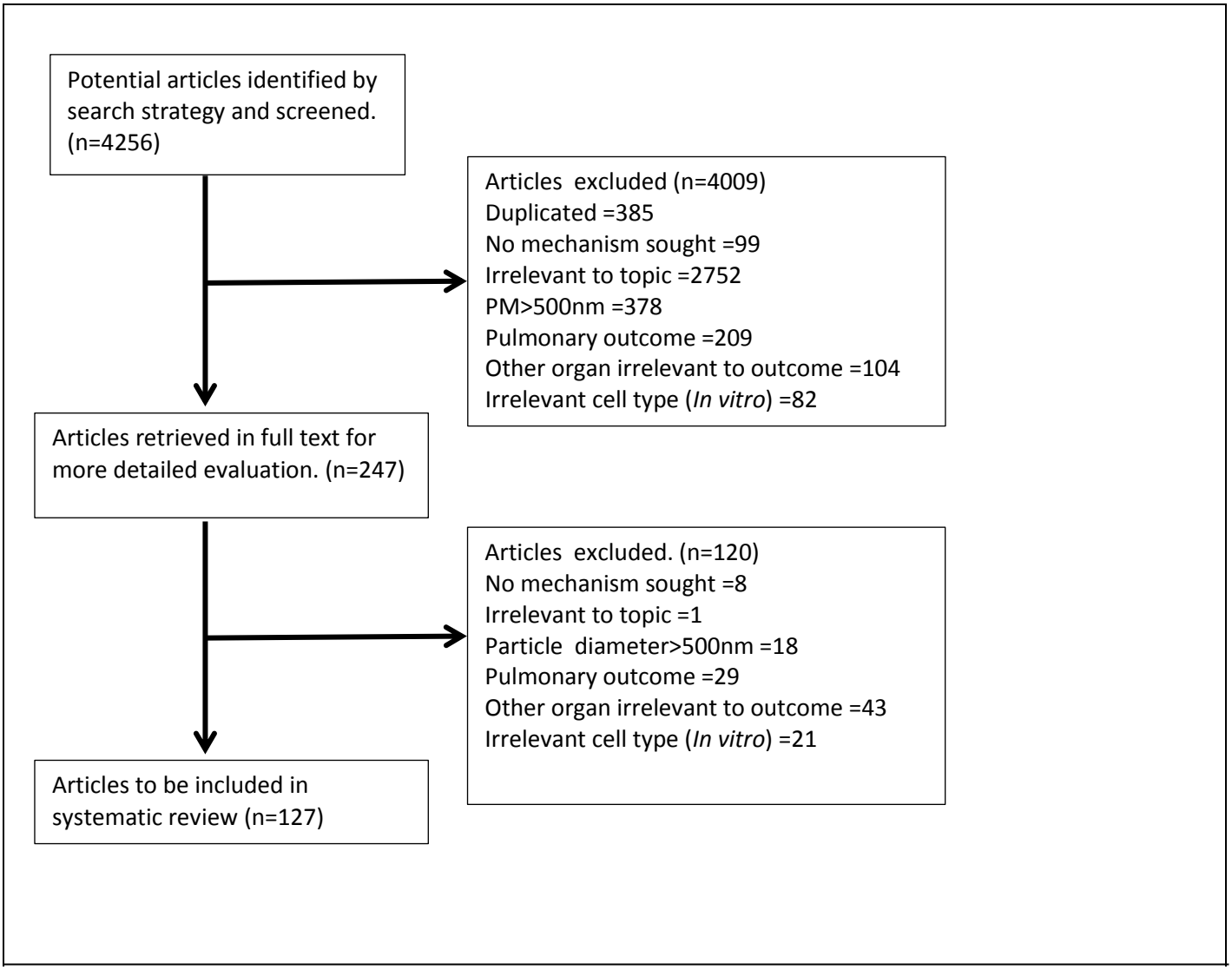
Potential articles identified by search strategy and screened. (n=4256)
Articles excluded (n=4009)
Duplicated =385
No mechanism sought =99
Irrelevant to topic =2752
PM>500nm =378
Pulmonary outcome =209
Other organ irrelevant to outcome =104
Irrelevant cell type (*In vitro*) =82
Articles retrieved in full text for more detailed evaluation. (n=247)
Articles excluded. (n=120)
No mechanism sought =8
Irrelevant to topic =1
Particle diameter>500nm =18
Pulmonary outcome =29
Other organ irrelevant to outcome =43
Irrelevant cell type (*In vitro*) =21
Articles to be included in systematic review (n=127)

Figure 2

Figure captions

Figure 1. Method adapted from the German Institute for Quality and Efficiency in Health Care.

Figure 2. QUORUM Flow chart