*Task force report*

# A work group report on ultrafine particles (American Academy of Allergy, Asthma & Immunology): Why ambient ultrafine and engineered nanoparticles should receive special attention for possible adverse health outcomes in human subjects

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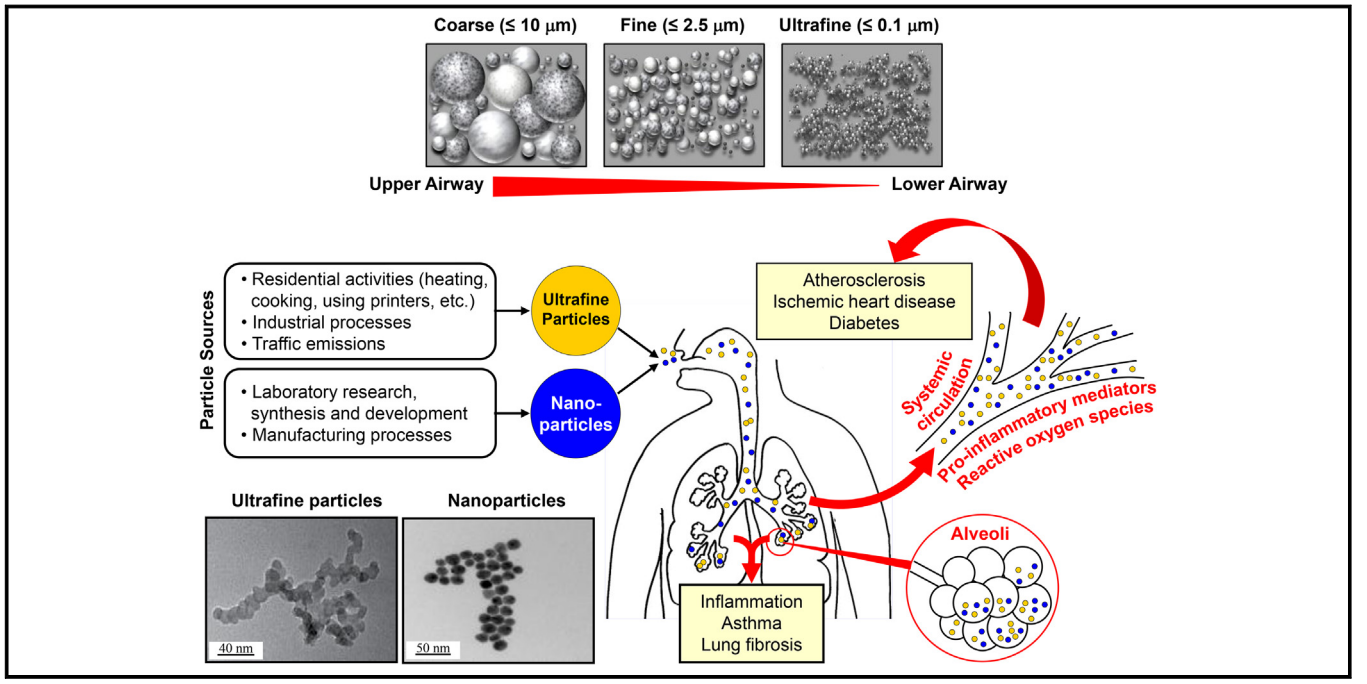
## GRAPHICAL ABSTRACT

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Supported by grants NSF DBI-1266377 (to A.N.), NIEHS U19ES019528 (to A.N.), and NIEHS P30 ES01247 (to S.G.).

Disclosure of potential conflict of interest: S. Georas received research support from the National Institutes of Health (NIH). A. Nel receives research support from the NIH. The rest of the authors declare that they have no relevant conflicts of interest. The views expressed in this article are those of the authors and do not necessarily reflect the official policy of the Department of Defense, Department of the Army, US Army Medical Department. or US Federal Government.

Received for publication September 20, 2015; revised January 30, 2016; accepted for publication February 24, 2016.

Available online April 6, 2016.

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0091-6749/$36.00



http://dx.doi.org/10.1016/j.jaci.2016.02.023

**Ultrafine particles (UFPs) are airborne particulates of less than 100 nm in aerodynamic diameter. Examples of UFPs are diesel exhaust particles, products of cooking, heating, and wood burning in indoor environments, and, more recently, products generated through the use of nanotechnology. Studies have shown that ambient UFPs have detrimental effects on both the cardiovascular and respiratory systems, including a higher incidence of atherosclerosis and exacerbation rate of asthma. UFPs have been found to alter *in vitro* and *in vivo* responses of the immune system to allergens and can also play a role in allergen sensitization. The inflammatory properties of UFPs can be mediated by a number of different mechanisms, including the ability to produce reactive oxygen species, leading to the generation of proinflammatory cytokines and airway inflammation. In addition, because of their small size, UFPs also have unique distribution characteristics in the respiratory tree and circulation and might be able to alter cellular function in ways that circumvent normal signaling pathways. Additionally, UFPs can penetrate intracellularly and potentially cause DNA damage. The recent advances in nanotechnology, although opening up new opportunities for the advancement of technology and medicine, could also lead to unforeseen adverse health effects in exposed human subjects. Further research is needed to clarify the safety of nanoscale particles, as well as the elucidation of the possible beneficial use of these particulates to treat disease. (J Allergy Clin Immunol 2016;138:386-96.)**

***Key words:*** *Ambient ultrafine particles, engineered nanoparticles, particle deposition and distribution, allergic inflammation, asthma, lung inflammation, oxidative stress, effect on human health*

*Abbreviations used*

AgNP: Silver nanoparticle
CNT: Carbon nanotube
DC: Dendritic cell
EC: Elemental carbon
ENM: Engineered nanomaterial
MWCNT: Multiwall carbon nanotube
OC: Organic carbon
OVA: Ovalbumin
PAH: Polycyclic aromatic hydrocarbon
$PM_{2.5}$: Particulate matter with an aerodynamic diameter of less than 2.5 μm
$PM_{10}$: Particulate matter with an aerodynamic diameter of less than 10 μm
ROS: Reactive oxygen species
$TiO_2$: Titanium dioxide
UFP: Ultrafine particle
ZnO: Zinc oxide

Compared with our understanding of the health effects of particulate matter with an aerodynamic diameter of less than 10 μm ($PM_{10}$, coarse PM) and less than 2.5 μm ($PM_{2.5}$, fine PM), there is a considerable knowledge gap about the effect of particles of less than 100 nm on human health. Increasing evidence from air pollution and nanosafety research suggests these submicron-scale particles have physicochemical properties significantly different from those of larger PM and therefore might exert adverse health effects, including promoting asthma exacerbation and allergic sensitization to common allergens, through different mechanisms (Table I).[1,2] Currently, these particles are classified into 2 major categories based on their sources. Ultrafine particles (UFPs) refer to the particles that are incidentally generated in the environment, often as byproducts of fossil fuel combustion, condensation of semivolatile substances, or industrial emissions, whereas nanoparticles are manufactured through controlled engineering processes.[1] Although there are many differences in the physicochemical composition of UFPs and nanoparticles, one common feature is their extremely small size; this allows these particles to have unique characteristics that can cause harmful health effects to human subjects (Box 1 and Table II).[1]

In 2013, the Health Effects Institute Review Panel concluded, based on the database available at that time, that there was no evidence that the adverse health effects of UFPs were dramatically different from those of $PM_{2.5}$. However, epidemiologic and clinical trial studies published in 2014 and 2015 question this conclusion (see below for further discussion).[3-9] Moreover, experimental evidence suggests that UFPs might be more dangerous than $PM_{10}$ and $PM_{2.5}$ because of their chemical composition, small size, large surface area/mass ratio, capability of generating reactive oxygen species (ROS), high retention rate, and deep penetration in the respiratory system.[10,11]

Several key facts indicate a critical need to address the adverse health effects of ambient UFPs. First, although $PM_{10}$ and $PM_{2.5}$ can be removed easily through phagocytosis, the extremely small size of UFPs enables them to evade such host defense and deposit in the lung with a high rate of retention. Thus, for the same volume of air inhaled, the actual dose and regional effects of UFPs in the lung might be significantly greater than that of $PM_{2.5}$. Moreover, the size of UFPs allows them to translocate to other organs through the systemic circulation, leading to toxicological mechanisms that are very different from those of $PM_{2.5}$.

Second, the large surface area enables UFPs to carry large quantities of adsorbed hazardous materials on a per-mass basis, including organic chemicals and metals that can generate ROS and oxidative stress. Oxidant injury plays an important role in UFP-induced adverse health effects, including exacerbation and promotion of asthma, chronic obstructive pulmonary disease, and atherosclerosis.[11-14]

Third, unlike $PM_{2.5}$, UFPs are not homogeneously distributed in the atmosphere but rather localized in hot spots of exposure (eg, near roads with busy traffic). This has resulted in a lack of extensive UFP monitoring networks and limited epidemiologic studies, a situation that is unlikely to change until regulatory agencies decide to track these particles as criteria pollutants.

Fourth, the composition of semivolatile organic compounds on the UFP surface can vary dynamically depending on the source and molecular size, challenging efforts to draw simple conclusions about their health effects.

Fifth, although the health effects of $PM_{10}$ and $PM_{2.5}$ are determined based on PM mass, the "weightless" nature of UFPs requires other exposure metrics (ie, particle number and surface area). Unfortunately, epidemiologic studies using these metrics are currently limited.

Finally, although improved engine and fuel technologies have significantly reduced the emission of particulate soot, UFPs can still be formed from vapor condensation and they can be even

**TABLE I.** Comparison of $PM_{10}$, $PM_{2.5}$, and UFPs

| Characteristics | $PM_{10}$ | $PM_{2.5}$ | UFPs |
|---|---|---|---|
| Aerodynamic diameter (μm) | 2.5-10 | 2.5-0.1 | <0.1 |
| Deposition in alveolar space | No | No | Yes |
| Surface area/mass ratio | + | ++ | +++ |
| OC content | + | ++ | +++ |
| EC content | +++ | ++ | + |
| Metal content | +++ | ++ | + |
| Exposure metrics*† | Mass | Mass | Particle number or surface area |
| Central monitoring sites*† | Yes | Yes | None |
| National Ambient Air Quality Standards set by the US Environmental Protection Agency | 150 μg/m$^3$ (24 h [not to be exceeded more than once per year on average over a 3-y period]) | 35 μg/m$^3$ (24 h [98th percentile, averaged over 3 y]) | None |

*Submicron particles have relatively little mass and are affected to a greater degree by forces other than gravity (eg, thermal, radiation, and electrical forces and particle concentration), and therefore they are not efficiently collected by traditional particulate samplers that rely on gravitational or inertial forces for particle collection.
†Instruments to measure airborne UFPs operate on the principles of thermophoresis, diffusion charging, or condensation, with results reported in units of particle number concentration, particle volume concentration, or particle surface area per volume of air sampled rather than by mass concentration, as in the case of $PM_{10}$ and $PM_{2.5}$.

**TABLE II.** Comparison of ENMs and ambient UFPs

| Particle type | ENMs | UFPs |
|---|---|---|
| Sources | Engineered (controlled synthesis) | Incidental (combustion) |
| Morphology | Regular (sphere, tube, cube, rod, wire, plate) | Irregular |
| Homogeneity | Yes | No |
| Organic chemical content | Low | High |
| Metal impurity | Varies | High |
| ROS generation | Varies | Yes |
| Exposure route | Inhalation, skin, ingestion, injection | Inhalation |
| Adverse health effects | Unknown | Yes |

smaller than the emission particles. Moreover, changes in the size and structure of the soot particles, due to engine modifications, can create more oxygen-containing reactive functional groups (eg, OH) on the particle surface. Upon particle uptake by cells the presence of these functional groups can lead to the generation of ROS.[15-18]

In contrast to UFPs, nanoparticles are intentionally created with the specific size, shape, surface characteristics, and functionality required for their applications (Table II). Nanotechnology, especially the commercial production and use of engineered nanomaterials (ENMs), is a rapidly developing industry that increasingly affects our lives because of potential exposure to more than 1300 nanotechnology-based consumer products that include at least 1 nanocomponent (Table III).[1,19-29] Therefore the extensive use and environmental/occupational exposure to ENMs have raised significant concerns regarding their safety profiles, especially for ENMs in powder form, which can be inhaled during production, transfer, packaging, and processing.

Although currently there is no definitive evidence to link nanoparticle exposure to any human disease, experimental data indicate that several types of ENMs can be potentially hazardous.[1] The physicochemical characteristics of nanoparticles that can have health implications include particle size, shape, aspect ratio, composition, charge, surface reactivity, solubility, and ability to generate ROS. Similar to UFPs, the nanoscale size can enhance nanoparticle translocation and deposition by interfering with their clearance. These features have the potential to induce cytotoxicity and inflammation and activate an injury response pathway that includes calcium influx, mitochondrial depolarization, and plasma membrane damage.[30,31]

The objective of this article is to provide an up-to-date report on the effect of UFPs on human health and potential nanomaterial hazards. We will summarize the known health effects of UFPs from cellular, animal, and human research data and discuss the potential mechanisms and exposure routes involved in the disease process, focusing on the proinflammatory effects of UFPs in the respiratory and immune systems. We will also review the adverse effects of ENMs based on their unique physicochemical properties.

## UFPs

### Sources and generation

Ambient UFPs originate from natural and anthropogenic activities and processes (Table I).[32-35] Combustion-derived UFPs characteristically have an elemental carbon (EC) or organic carbon (OC) core carrying trace metals, sulfate, ammonium, and volatile and semivolatile components.[32,34,36,37] Other components of combustion-derived UFPs will depend on fuel type, burn conditions, and atmospheric conditions. There has been less research describing the composition of noncombustion sources of UFPs, but environmental factors and human activities likely influence the composition of airborne UFPs.[32,38]

Because of the ubiquitous nature of their sources, the presence of UFPs in outdoor and indoor air is not a recent or unusual occurrence. Monitoring particles in the ultrafine size range has focused on specific sources (roadways, combustion, and appliances) and has required sampling equipment that addresses the unique behavior of UFPs. However, there is currently no standardized UFP measurement method or reporting, and there are no federal standards for UFP levels (Table I).

### Exposure assessment and environmental levels

Exposure assessment studies have used different particle metrics and provided important but limited characterization of UFP levels and types in ambient air and, recently, in residential and office locations.

**TABLE III.** Nanomaterials used in commercial products and their potential exposure route

| Type of nanoparticles | Products | Exposure routes | References |
|---|---|---|---|
| Fumed silica | Food, pharmaceutics, rubber, plastics, paints, desiccants, cosmetics | Lung, gastrointestinal tract | 22, 23 |
| Silver | Filter, inks, food package, clothing, surgical masks, cosmetics, sprays | Lung, gastrointestinal tract | 24, 25 |
| CNTs | Coating, film, microelectronics, composite materials, energy storage, biotechnology | Lung, skin | 26 |
| Graphene and graphene oxide | Water purification, coating, battery electrode, medicine, transistors | Lung, skin | 27 |
| $TiO_2$ | Sunscreen, food | Skin, gastrointestinal tract | 28 |
| Molybdenum disulfide | Lubricant spray, petroleum refining | Lung | 29 |

**TABLE IV.** Sources of UFPs and their background concentrations in cities and upwind of roadways

| | |
|---|---|
| Natural sources[34] | |
| Biological agents (viruses, microbes, and fungal parts), combustions, geological processes (volcanic eruptions), and atmospheric transformations (gas to nuclei mode and condensate aerosols) | |
| Anthropogenic sources[34] | |
| High temperature processes (welding, smelting), combustion (power generation, mobile sources, residential and commercial heating, cooking), and industrial processes | |
| Background concentrations in cities and upwind of roadways | |
| Range | $1 \times 10^3$ to $5 \times 10^4$ p/cc[33,34,36,43-48] |
| Peak concentration* | $8 \times 10^4$ to $3.5 \times 10^5$ p/cc[46,48] |
| Factors affecting UFP levels | Season,[44,47] relative humidity,[43,49] traffic volume,[36,49,51] vehicle type,[44,46,50] and traffic flow pattern[44,51] |

*p/cc*, Particles/$cm^3$.
*Within 20 meters of the roadway.

## Ambient

Ambient levels of airborne UFPs are challenging to characterize geographically or over time because concentrations decrease sharply downwind from sources and UFPs shift in size from nucleation to accumulation mode with time and distance from their emission source through agglomeration and condensation. For combustion sources, the fuel, combustion conditions, and pollution controls will alter the particle numbers and size distribution. Occupational exposures will be particularly high during high-temperature operations (eg, welding and smelting), high-speed manufacturing, and combustion processes, but we currently have limited information about UFP exposure in these settings. The introduction of catalytic converters on cars and trucks to reduce tailpipe emissions of hydrocarbons and carbon monoxide had the unintended consequence of shifting the bulk of the particle size distribution of exhaust PM to smaller diameters of 20 to 30 nm.[39] Particle mass decreases with catalytic conversion, but the number of particles in the UFP range increases and includes traces of the catalyst used.[39,40] Because fuels have had to conform to lower sulfur content requirements, UFP levels in exhaust emissions have decreased for vehicles using low- and ultralow-sulfur fuels.[32,41] However, UFPs that are formed during vapor condensation can still be quite significant.[42]

Most studies of ambient UFPs have focused on urban areas and roadways. Background UFP concentrations in cities and upwind of roadways are summarized in Table IV.[33,34,36,43-51] Higher concentrations were associated with lower humidity,[43,49] greater proportion of diesel vehicles,[36,44,46,49-51] winter months,[44,47] and when traffic accelerates after stopping.[44,51] Not surprisingly, UFP concentrations decrease with distance from the highway.[39,46]

## Residential/office

Many common indoor sources in residential and office settings generate UFPs, and UFP concentrations increase during specific indoor activities (Table V).[34,52-57] Although the spectrum of consumer products generating UFPs is broad, exposure and risk assessments are not available for most products. Most of our understanding of in-home or in-office exposures to UFPs comes from extrapolating from studies on incidental UFP levels and emission sources and from UFP emission testing that is performed for products marketed outside the United States. Afshari et al[52] conducted chamber studies to quantify UFP emissions from common household activities; their findings, as well as those of others, are summarized in Table V.[34] Currently, our knowledge about the fate of these particles in ambient air or after inhalation is limited.

Office printers have recently been recognized to generate substantial amounts of indoor UFPs. In fact, several European countries have set emission limits for office printers with categories that include volatile organic compounds, formaldehyde, dust, and ozone. As of 2013, the European "Blue Angel" program (http://www.blauer-engel.de/en) included a detailed testing methodology for particles (7-300 nm) and prescribed an emission limit of $3.5 \times 10^{11}$ particles/$cm^3$ per 10-minute print run. Horner and Steady[57] presented a summary of test results based on an initial compilation of more than 35 different printers from several manufacturers. Despite controlled test conditions and relatively constant particle losses to the chamber, the variation in emission levels from the printers was substantial (Table V). Future studies investigating the fate and potential adverse effects of inhaling printer-derived UFPs requires consideration.

# SOURCES AND EXPOSURE ASSESSMENT OF ENGINEERED NANOPARTICLES

With the emergence of nanotechnology, workplace exposures can occur throughout the lifecycle of ENMs from laboratory development through production, sales, installation, use, disposal, or recycling. Occupational exposure assessments overall have lagged behind the rapid expansion of nanotechnology. Currently,

**TABLE V.** UFP emissions from common household and office activities[52,55,57]

| Household activity | Peak UFP concentration (p/cc) |
|---|---|
| Burning pure wax candles | $24 \times 10^4$ |
| Burning 3 cigarettes for 10 min | $21 \times 10^4$ |
| Frying meat in oil in a Teflon pan on an electric stove for 45 min | $15 \times 10^4$ |
| Spraying 20 g of a pure citrus air freshener | $3 \times 10^4$ |
| Vacuuming for 50 min | $2.1 \times 10^4$ |
| Operating a propane camping stove | $7.9 \times 10^4$ |
| Operating an electric radiator | $22 \times 10^4$ |
| Operating an electric stove | $11 \times 10^4$ |
| Operating an electric air heater | $12 \times 10^4$ |
| Dry ironing cotton material | $0.055 \times 10^4$ |
| Operating a vented gas clothes dryer | $10 \times 10^4$ ($6 \times 10^{12}$/drying cycle) |
| **Office activity** | **UFP concentration (p/cc)** |
| Printing (10-min print run)* | $10^8$-$10^{12}$*<br>$10^6$-$10^{10}$† |

*p/cc*, Particles/cm$^3$.
*Total UFP emissions normalized to a 10-minute print run over an hour.
†In a 30.6-m$^3$ office with an air-change rate of 0.68.

only limited data are available on the concentration of air-borne nanomaterials in occupational settings, such as factories or laboratories. For example, in a silver nanoparticle (AgNP) manufacturing facility, airborne AgNP levels of 5 to 289 μg/m$^3$ were detected in the injection room.[58] These measurements overlap with the recommended threshold limit value of 100 μg/m$^3$ for AgNP inhalation by the American Conference of Industrial Hygienists.[59] As for carbon nanotubes (CNTs), Han et al[60] reported peak multiwall carbon nanotube (MWCNT)–containing airborne dust levels being as high as 400 μg/m$^3$ in a production laboratory. Although numerous publications have described the challenges and knowledge gaps about the safety issues with ENMs, including nanoparticles,[61-66] a 2008 survey of 40 companies in Europe working with nanomaterials found that most did not perform risk assessment. Moreover, for those that did, they did not consider use, waste disposal, or unintentional releases.[67] A few studies looking at workplace breathing zone concentrations in ENM manufacturers relied on various exposure metrics, such as gravimetric-based respirable or inhalable PM mass or EC, as a more specific marker for nanotubes or fibers,[68] making characterization of occupational exposures across the nanomaterial lifecycle difficult. Federal agencies in the United States, such as the US Environmental Protection Agency, have produced risk assessments for certain common nanomaterials (eg, silver and titanium dioxide [$TiO_2$] nanoparticles), but more research is needed in this area.

## BIOLOGICAL EFFECTS

### Particle deposition, retention, and distribution in the lung and beyond

UFPs and nanoparticles are in the respirable size range and have a physicochemical composition that enables their penetration into the airways, parenchyma, and alveolar airspace in the lung. The extremely small size and large surface area per unit mass of UFPs and nanoparticles are 2 of the major determinants for their potential adverse health effects during particle transport, deposition, and cellular perturbation. In general, deposition of UFPs or nanoparticles in the lung is accomplished almost exclusively by means of diffusion, during which the thermodynamic diameter (and not the aerodynamic diameter) is mainly responsible for efficient deposition in the alveolar airspace (Box 1). The submicron size of UFPs enables them to travel to and deposit in the alveolar region with much higher efficiency because of their strong diffusion capability.[69] In addition, the small size allows UFPs to evade their clearance from the area, leading to long-term particle retention. Kawanaka et al[70] found that UFPs contributed as much as 23% to 30% of the alveolar deposition of polycyclic aromatic hydrocarbons (PAHs) coming from roadside sources, whereas the contribution of UFPs to the total PM mass was only 2.3%; this also suggests that the large surface area of inhaled UFPs allows them to deliver a significantly greater amount of hazardous chemicals to the region, where they can cause subacute and chronic inflammation (Box 1). The surface characteristics of nanoscale particles facilitates the formation of a protein or lipid corona in biological media because of the binding of proteins or detergents, which might alter their cellular uptake and induced biological responses.[71] A sizeable number of UFPs can be deposited in the alveolar airspace, where pulmonary surfactant aides their retention on the lung epithelium.[72] In the case of poorly soluble iridium-192 nanoparticles, 70% to 80% of nanoparticles are translocated rapidly to the interstitium and hence do not remain in the alveolar airspace.[73]

### Link between UFP-induced oxidative stress and inflammation

Experimental evidence from studies on traffic-related UFPs indicates that ROS produced by OC and PAHs on the particle surface plays a key role in the injurious effects of UFPs. Redox-active organic chemicals (eg, PAHs and quinones) are the major contributors to UFP-generated ROS.[11,74,75] PAHs can be converted to quinones by means of biotransformation through reactions involving enzymes, such as cytochrome P450 1A1, epoxide hydrolase, and dihydrodiol dehydrogenase. One-electron reductions of redox-cycling quinones by NADPH cytochrome P450 reductase form semiquinones, which can be recycled back to the original quinones with concomitant generation of superoxide and other types of ROS (Fig 1).[11,75]

The key regulator to protect cells against the damaging effects of ROS is nuclear factor (erythroid-derived 2)–like 2, a transcription factor that mediates the majority of antioxidant and detoxification enzymes.[76] ROS accumulation as a result of either overproduction or inadequate antioxidant defense leads to oxidative stress.[12,76] Several proinflammatory signaling pathways (eg, mitogen-activated protein kinase [MAPK] and nuclear factor κB [NF-κB]) are redox sensitive.[14,77,78] Therefore failure of cells to restore redox homeostasis can activate these pathways and induce airway inflammation (Fig 1). Interestingly, younger age appears to enhance susceptibility to the oxidant effects of UFP exposure. For example, inhalation of combustion-derived, flame-generated ultrafine soot particles caused more severe glutathione depletion and weakened induction of detoxification enzymes in neonate rats compared with that seen in adult animals.[79]

One controlled human exposure study concluded that particle size fraction (coarse, fine, and UFP) was not significantly associated with cardiopulmonary health outcomes.[80] However, this lack of size fraction–dependent effects was likely due to the use of different dosimetric metrics; that is, coarse and fine

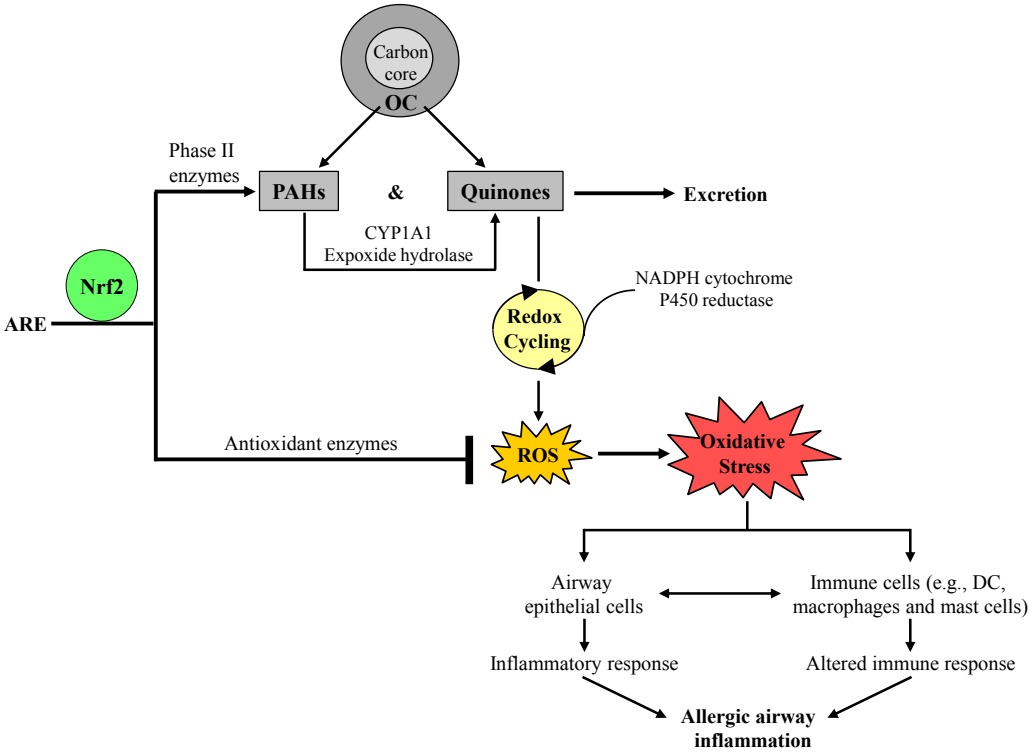


**FIG 1.** Generation of oxidative stress by ambient UFPs and its role in allergic airway inflammation. UFPs carry a large amount of OC, including PAHs and quinones. Once inside the cell, PAHs can be converted to quinones through metabolism catalyzed by CYP1A1 and epoxide hydrolase. Quinones on the UFP surface undergo redox cycling between semiquinones and original quinones through 1-electron reduction by NADPH cytochrome P450 reductase, resulting in ROS generation. Nuclear factor (erythroid-derived 2)–like 2 *(Nrf2)* defends cells against oxidative injuries by binding to the antioxidant response element *(ARE)* together with other transcription factors in the promoters of antioxidant and phase II enzymes, leading to activation of effective protective mechanisms. When the Nrf2-mediated pathway is functional, activated antioxidant and phase II enzyme metabolize UFP-associated chemicals and remove excessive ROS. However, if antioxidant defense fails, ROS accumulation will escalate to cellular oxidative stress, which can induce the inflammatory response and alter cellular functions in the respiratory (eg, airway epithelial cells) and immune (eg, DCs, macrophages, and mast cells) system. The resulting allergic airway inflammation can be further amplified by interactions between airway epithelial and immune cells.

PM exposure was based on mass, and UFP exposure was based on particle number.[80] On the basis of mass concentration, Li et al[81] demonstrated that ambient UFPs had higher PAH content and greater oxidant potential and were much more prone to introducing cellular injury compared with $PM_{10}$ and $PM_{2.5}$, which were simultaneously collected at the same site.[81] Other studies also reported stronger pro-oxidative and proinflammatory effects of UFPs. For instance, a study comparing different sizes of PM from urban and rural areas revealed that regardless of the collection site, the finest PM fractions were stronger in inducing the biomarkers of PAH exposure, oxidative stress, and inflammation in human airway epithelial cells.[82] Using ultrafine carbon black and ferric sulfate as a model UFP from combustion sources, Weissenberg et al[83] showed that particle-induced intracellular, rather than extracellular, oxidative stress was required for Akt and extracellular-signal regulated kinase 1/2 activation.

In addition to the direct involvement of PM-induced oxidative stress, there are other mechanisms responsible for the adverse effects of UFPs. Ambient UFP-induced increases in oxidized glutathione levels can lead to modifications of nitric oxide synthase and decreased nitric oxide production by human endothelial cells.[84] Ultrafine carbon particles can also downregulate cytochrome P450 1B1 expression in bronchial epithelial cells, monocytes, and sputum macrophages from healthy nonsmokers and patients with chronic obstructive pulmonary disease.[85] In the case of traffic-related UFPs, this might lead to increased availability of organic compounds in the lung. Finally, the extremely small size alone has been found to be more potent in interfering with the immune response.[86,87] For example, polystyrene particles of all sizes (coarse, fine, and ultrafine) could enhance ovalbumin (OVA)–induced allergic airway inflammation (ie, eosinophil influx in the lung and OVA-specific IgE production); however, the strongest effect was observed in animals exposed to UFPs.[87]

## Engineered nanoparticles

Similar to UFPs, the size of ENMs ranges from 1 to 100 nm in at least 1 dimension (Box 1).[1] However, they are inherently different from UFPs in many aspects (Table II). Evidence from extensive cellular and animal studies suggests that the hazardous potential of ENMs are determined by their physicochemical properties, including morphology, size, charge, dissolution, aspect ratio, surface coating and reactivity, redox-active properties, and aggregation.[88] Although nanoparticles can form agglomerates in the respiratory tract or in biological fluids, some nanoparticle fractions can remain and still exhibit "nano"

properties, even after several days, and potentially exert toxic effects in the lung. Ryman-Rasmussen et al[89] showed that 14 days after inhalation exposure, MWCNTs were still present as single tubes in the subpleural region in mice, along with subpleural fibrosis. Wang et al[90] demonstrated that citrate-coated 110-nm AgNPs remained as singlet particles in the mouse lung 21 days after exposure and were associated with chronic lung inflammation. The dosimetry for cellular and animal studies has been calculated based on real-life exposures to AgNPs and MWCNTs in manufacturing facilities. These calculations are developed based on the premise that the same surface area dose (mass/surface area) for the lungs of human subjects and animals will generate similar responses. For example, lung exposure dose (mass/surface area) for animal experiments (0.1-2 mg/kg) using nanosilver is comparable with the monthly lung deposition level in a human worker potentially exposed to 289 $\mu g/m^3$ AgNPs in the injection room. Similarly, the *in vitro* dose range (approximately 12.5-100 $\mu g/mL$) is also comparable with that used in the animal experiment based on surface area dose calculations.[90]

To date, many studies have linked nanoparticle physicochemical properties to their toxicological outcomes. $TiO_2$ nanoparticles, the most abundantly produced nanomaterials that can be found in many commercial products, can cause oxidative stress–mediated acute lung inflammation.[91,92] Oberdorster[93] showed that on a mass-dose basis, ultrafine $TiO_2$ is more toxic than fine $TiO_2$ particles. However, when the particle doses were expressed as particle surface area, the responses of ultrafine and fine $TiO_2$ particles fell on the same dose-response curve, suggesting that surface area is an important property for ENM's toxic potential.[93] The crystal structure (eg, anatase vs rutile form) and photoactivation properties of $TiO_2$ nanoparticles also play important roles in their capability of generating ROS and inducing cytotoxicity.[94,95] Zinc oxide (ZnO) nanoparticles have received significant attention because of their use in sunscreens, electronics, optics, and photonics.[96] Pulmonary exposure to ZnO nanoparticles generated as a byproduct of welding could lead to transient acute lung inflammation, a disease called metal fume fever.[97,98] Xia et al[99] showed that the toxicity of ZnO was dependent on particle dissolution and shedding of toxic zinc ions.

CNTs are nanomaterials with a long aspect ratio that have wide applications.[100,101] Studies have shown that their dispersal state, hydrophobicity, and purity could affect the profibrogenic cellular responses, correlating with the extent of pulmonary fibrosis.[102,103] Other ENMs with long aspect ratios also had similar effects. Ji et al[104] demonstrated that at lengths of 200 nm or greater and aspect ratios of 22 or greater, cerium dioxide nanorods induced a progressive proinflammatory response and cytotoxicity. The relatively low "critical" length and aspect ratio were associated with small nanorod/nanowire diameters (6-10 nm), which facilitate the formation of stacking bundles that pierce the lysosomal membrane, causing release of cathepsin B, NLRP3 inflammasome activation, and production of the proinflammatory cytokine IL-1β.[104] Additional research is needed to understand the interactions occurring at the nano-bio interface between ENMs and biological systems.

## UFPs and nanoparticles in immune responses and models of allergic inflammation and asthma

Many animal model studies have documented the ability of inhaled UFPs and nanoparticles to act as proallergic adjuvants, boosting the allergic immune response to inhaled allergens.[105-107] Because different UFPs and dosing regimens were used, it is currently not possible to construct a unifying model for the enhancing effect of inhaled UFPs on allergic inflammation. Ochs and Weibel[108] estimate that UFPs could encounter 40 different cell types as they journey through the respiratory tract. However, it is likely that the major cell types coming into contact with UFPs are macrophages, epithelial cells, dendritic cells (DCs), and endothelial cells at the epithelial, interstitial, and subinterstitial layers, respectively. There are primarily 3 pathways for the fate of UFPs after deposition in the lung: (1) phagocytic clearance by alveolar/airway macrophages through the mucociliary escalator, (2) uptake by lung-resident DCs and transport to draining lymph nodes,[109] or (3) translocation across the epithelial layer into the bloodstream, pleural space, or distant organs.[89,110] The pro-oxidant property of UFPs plays an important role in this effect. Intranasally instilled ambient UFPs with a high OC/PAH content and strong oxidant potential is a potent adjuvant for allergic sensitization to OVA in mice, leading to pronounced allergic inflammation in the lung and nose.[111] Moreover, inhalation of pro-oxidant UFPs during OVA challenge further exacerbated this inflammation in previously sensitized animals.[112] Thus ambient exposure to UFPs can be considered a risk factor for both the development and exacerbation of asthma. Several studies used laboratory-generated UFPs to represent a certain component of ambient UFPs or the carbon core of traffic-derived PM. Using EC-UFPs, Alessandrini et al[113] demonstrated that the adjuvant activity of inhaled EC-UFPs on allergic lung inflammation was accompanied by local lipid peroxidation and NF-κB activation. Exposure of sensitized mice to EC-UFPs before OVA challenge also led to the goblet cell metaplasia of Clara cells and overproduction of mucus and Clara cell protein.[114] These changes, as well as the adjuvant activity of EC-UFPs, could be suppressed by antioxidant N-acetyl cysteine.[113,114] In addition to their capability of upregulating proinflammatory cytokines and chemokines through oxidative stress, UFPs also alter the balance between proinflammatory and anti-inflammatory lipid mediators. Exposure of OVA-sensitized mice to ultrafine carbon particles before OVA challenge enhanced allergic inflammation and lipid peroxidation in the lung and skewed lipid mediator balance toward a proinflammatory response with a significant increase in leukotriene $B_4$ levels.[115]

Similar results have also been observed for nanoparticles and ENMs. In rats some nanoparticles can interact and stimulate mast cells to secrete histamine, thereby modifying allergic responses in atopic models.[116] Inhalation of gold or $TiO_2$ nanoparticles enhanced lung inflammation and airways hyperreactivity in a mouse model of isocyanate-induced asthma.[117] These effects might be due to direct activation of lung DC subsets by inhaled nanoparticles.[118] Coexposure to OVA and CNTs synergistically enhanced airway fibrosis in mice, suggesting a possible role for ENMs in airway remodeling.[119] A recent study concluded that intravenously administered CNTs and graphene nanosheets induce $T_H2$-immune responses through the IL-33/ST2 axis because responses were partially attenuated in ST2-deficient mice.[120] In the case of nanoparticles, immune effects are influenced by particle size and shape. Intratracheal administration of agglomerated CNTs results in granuloma formation,[121] whereas dispersed CNTs (eg, coated by surfactant) results in more diffuse fibrosis.[110] Interestingly, CNTs still accumulate in lung lymph nodes almost 1 year after aerosol exposure.[122] Consequently, it seems reasonable to conclude

that pulmonary defense mechanisms are not able to handle the challenges posed by these new engineered nanomaterials and that more research of the immunologic consequences of these biopersistant materials is urgently needed.

Although these studies suggest that inhaled UFPs and nanoparticles will potentiate allergic lung inflammation, other observations paint a more nuanced picture. For example, Rossi et al[123] showed that exposure to $TiO_2$ nanoparticles over 4 weeks dramatically attenuated OVA-induced inflammation and airways hyperreactivity. Additionally, certain fullerene-derived nanoparticles can suppress OVA-induced lung inflammation, probably by inhibiting mast cell activation.[124] Inhaled nanoparticles can induce local and systemic immunosuppression. For CNTs, this involves suppression of mitogen-driven antibody production and T-cell proliferation in the spleen in a TGF-β– and COX2-dependent manner.[125] Repeated inhalation of CNTs suppressed the ability of macrophages to phagocytose and clear *Listeria monocytogenes*, which translated to enhanced lung inflammation.[126] Similar results were observed in a mouse model of *Pseudomonas aeruginosa* infection, although pathogen clearance was not affected.[41] These studies highlight the potential of UFPs and nanoparticles to suppress the immune response to infectious pathogens. Consequently, more research is needed to understand how UFPs and nanoparticle composition and exposure conditions influence proinflammatory versus anti-inflammatory and potentially immunosuppressive effects. Future studies also need to consider the potential for swallowed UFPs and nanoparticles to affect the gut microbiome, providing increasing evidence that perturbations in intestinal microbes and their metabolism have a profound effect on asthma and allergies.[127-129]

## EFFECT OF AMBIENT UFPs AND NANOPARTICLES ON HUMAN HEALTH

The adverse cardiopulmonary effects of UFPs have been demonstrated in epidemiologic association studies and an increasing number of controlled exposure human studies.

### Epidemiologic association studies

An early study by Peters et al[130] reported that decreased peak expiratory flow and increased respiratory symptoms in asthmatic subjects were associated with exposure to ambient fine and UF particles. However, the effects of the 5-day mean UFP number was larger than that of the mass of the fine particles, and the effect of UFP numbers on peak expiratory flow was stronger than that of $PM_{10}$. More recently, a case-control study from Chile found that increased outpatient visits caused by respiratory illness were significantly correlated with increased levels of UFPs generated from residential wood burning.[131] More evidence looking at the association between UFPs and allergic diseases came from children's studies. A time-stratified, case-crossover study involving 74 children showed that the largest increase in the relative odds of pediatric asthma-related visits was associated with the 4-day mean concentration of ambient UFPs but not with the accumulation mode of PM, black carbon, and sulfur.[7] In addition, Song et al[132] reported that after a short-term exposure to ambient UFPs, children with eczema had increased urinary levels of 8-hydroxyl-2-deoxyguanosine, a major byproduct of oxidative DNA damage, compared with those without eczema. This increase was associated with the level of UFPs and the particles' PAH content. The deleterious cardiovascular effects of UFPs are continuously being reported by studies involving human subjects. Exposure to UFPs is found to be associated with altered heart rate, heart rate variability, changes in microvascular function, and systemic inflammation. Two studies from Denmark demonstrated that exposure to UFPs away from home was significantly inversely correlated with microvascular function and positively associated with systemic inflammation.[6,8] There was no association between these changes and $PM_{10}$ and $PM_{2.5}$. Decreased lung function (ie, $FEV_1$ and forced vital capacity) and increased levels of type 2 diabetes marker (HbA1c) were associated with levels of indoor UFPs but not $PM_{2.5}$.[6,8] A recently published 6-year (2001-2007) cohort study including more than 100,000 women in California reported that the mortality caused by ischemic heart disease was significantly associated with UFPs, their EC and metal contents, and mobile sources. Although a similar association was also found between ischemic heart disease mortality and $PM_{2.5}$, statistical analysis showed that UFP mass and its constituents had a better fit and a lower *P* value than those of $PM_{2.5}$.[9] Whether the adverse cardiovascular effects of UFPs are related to particles' capability to penetrate into the systemic circulation is unclear.

### Controlled human exposures

Chalupa et al[133] reported an inverse relationship between carbon UFP lung deposition and particle size; particle deposition was further increased in asthmatic patients. EC-UFPs could interfere with the distribution of blood leukocytes and the expression of adhesion molecules in both healthy and asthmatic subjects, which might contribute to increased leukocyte retention in the alveolar bed.[134] Inhalation exposure to concentrated UFPs collected in an area with busy traffic in Los Angeles, California had acute adverse cardiopulmonary effects, including decreased arterial oxygen saturation and $FEV_1$ in both healthy and asthmatic subjects.[135] Exposure to concentrated ambient UFPs is also associated with increased production of fibrin degradation products (D-dimer) and IL-8 in bronchoalveolar lavage fluid from healthy subjects, suggesting mild prothrombotic and proinflammatory effects of these particles.[136] The potential long-term effect of inhaled ultrafine carbon particles on the course of inflammation in asthmatic patients was investigated in a double-blind, randomized, crossover clinical pilot study. Using 2 different exposure protocols, Schaumann et al[4] reported that although UFP exposure had no acute effect on allergen-induced inflammation, the subgroup of subjects who inhaled UFPs during the first exposure exhibited a surprising and significant increase in lung inflammation after either filtered air exposure or subsequent allergen challenge 28 days later. The mechanisms for this apparent long-lasting effect of UFPs are unclear.

UFPs can also affect persons with diabetes or metabolic syndrome. A single 2-hour inhalation of EC-UFPs interfered with heart rate and heart rate variability in diabetic subjects, which could last for hours.[3] A randomized crossover study by Devlin et al[5] demonstrated that ambient UFPs affected cardiac repolarization and heart rate variability and induced markers of vascular inflammation and fibrinolysis in patients who had metabolic syndrome and also carried the glutathione-S-transferase Mu1-null allele. Because these changes were mainly associated with particle numbers, it was concluded that UFPs were responsible for these effects. This suggests that defects in antioxidant

**Box 1.** Unique features of UFPs and nanoparticles

- UFPs: incidentally generated in the environment; aerodynamic diameter <0.1 micrometer
- Nanoparticles: manufactured through controlled engineering processes; at least 1 dimension <0.1 micrometer
- Both particles can effectively deposit in the alveolar space through diffusion
- Both have high surface area/mass ratio
- Large surface area allows UFPs to carry a relatively large load of hazardous cargo
- Both physical and chemical properties determine the health effects of UFPs and nanoparticles
- UFPs and select engineered nanoparticles can induce oxidative stress, airway inflammation, and toxicity
- Particles can be transported by lung DCs to draining lymph nodes or translocate to distant organs through the bloodstream and can have adverse systemic health effects in many organs

defenses, whether genetic or acquired, can be considered a risk factor for adverse health effects of inhaled UFPs. Future studies specifically defining susceptible cohorts of subjects are needed and will not only enhance our understanding of pathobiological mechanisms but also lay the groundwork for rational preventative strategies.

## CONCLUSION

Although recent progress has been made in understanding the adverse effects of ambient UFPs and nanoparticles and their potential mechanisms of action, there is still a critical knowledge gap in clearly identifying the effect of exposure to these nanoscale pollutants on human health. Because of their extremely small size, UFPs and ENMs have unique physicochemical properties that might affect their exposure, deposition and translocation in the body, and capability to cause different health issues. Increasing evidence strongly suggests that UFPs and nanoparticles can cause adverse health outcomes in human subjects, including those with asthma, likely through a number of similar mechanisms that have been demonstrated by experimental studies. Thus it is imperative to further strengthen our research in the health effects of nanoscale pollutants so that preventive strategies and regulatory guidelines can be developed to reduce exposure and protect human health.

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