# Particulate matter and the airway epithelium: the special case of the underground?

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ABSTRACT Airborne particulate matter (PM) is a leading driver of premature mortality and cardiopulmonary morbidity, associated with exacerbations of asthma and chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and lung cancer. The airway epithelium, as the principal site of PM deposition, is critical to the effects of, and initial response to, PM. A key mechanism by which PM exerts its effects is the generation of reactive oxygen species (ROS), inducing antioxidant and inflammatory responses in exposed epithelial cells. However, much of what is known about the effects of PM is based on research using particulates from urban air. PM from underground railways is compositionally highly distinct from urban PM, being rich in metals associated with wheel, rail and brake wear and electrical arcing and component wear, which endows underground PM with potent ROS-generating capacity. In addition, underground PM appears to be more inflammogenic than urban PM in epithelial cells, but there is a lack of research into effects on exposed individuals, especially those with underlying health conditions. This review summarises current knowledge about the effects of PM on the airway epithelium, how the effects of underground PM may be different to urban PM and the potential health consequences and mitigation strategies for commuters and workers in underground railways.

## Introduction

Exposure to airborne particulate matter (PM), which encompasses solid particles or liquid droplets suspended in the air, is associated with almost 9 million deaths per annum worldwide [1, 2]. Adverse respiratory outcomes associated with PM exposure include exacerbations of asthma and chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and lung cancer [3–6]. PM is normally classified according to its aerodynamic diameter, most commonly as $PM_{10}$ with a diameter <10 μm ($PM_{10-2.5}$, defined as coarse PM), $PM_{2.5}$ (fine; diameter <2.5 μm) and $PM_{0.1}$ (ultrafine; 0.1 μm). Particles at the larger end of this scale are generally derived from crustal and abrasion sources, such as soil erosion, weathering, sea spray and road wear, whereas the smallest particles generally derive from combustion or other high-temperature processes. Coarse PM predominantly deposits in the upper airways, trapped by

hairs and mucus, whereas fine PM may reach as far as the terminal bronchioles and alveoli. Ultrafine PM can enter the alveoli, may translocate across the gas–blood barrier, and persist for several months post-inhalation [7]. Regulation of ambient PM is by mass concentration, but this fails to take into account the composition of PM. Much of the current understanding of the effects of PM, from the population level down to the cellular and molecular level, is based upon studies of ambient PM, as found in urban air. Extrapolation of these findings to PM from alternative sources assumes that source-related composition, which may vary considerably [8], does not play a role in the effects [9]. One such alternative source is underground railways.

## Underground railways

Underground railways are heavily used mass transit systems in many of the world's major cities, with 53.8 billion underground railway journeys being made worldwide in 2017 [10]. In underground railway systems, the airborne concentration of PM is often many times greater than above ground (table 1). Studies of PM fluxes and composition have shown that the predominant sources of PM mass in the underground are trains, from shearing of the wheels and rails, wearing of electrical rail or overhead wire and current collector and electrical arcing. These processes generate predominantly coarse and fine PM rich in metallic elements including iron, manganese, chromium (from steel rails/wheels), barium (from brakes) and copper (from electrical components), among others [11, 18], although high-temperature processes such as friction and current arcing can also generate ultrafine PM [24, 25]. In addition, depending on station location, PM enters from outside, predominantly in the ultrafine fraction and probably from road vehicle exhaust, contributing to particle number more than particle mass [26]. PM deposited on surfaces in the underground is resuspended and circulated by the piston action of trains, especially where ventilation systems are lacking, with on-platform airborne PM concentrations generally being higher than those in carriages [19], and higher in stations which are deeper and further from the tunnel entrance [15, 27].

## PM in the airways

The major site of deposition of inhaled PM is the bronchial epithelium, a pseudostratified epithelial layer which provides a chemically, immunologically and mechanically protective barrier against environmental insults [28, 29]. The epithelium is covered with a mucous layer, containing mucins, highly glycosylated proteins, in which particles are trapped before ciliary clearance. In contrast, particles reaching the lower airways tend to be cleared by macrophage-mediated phagocytosis [30]. Nonetheless, particles or their

TABLE 1 Particulate matter with a 50% cut-off aerodynamic diameter of 10 µm ($PM_{10}$) and 2.5 µm ($PM_{2.5}$) concentrations in underground railway systems featured in this review

| City | Underground | | Ambient | | Reference |
|---|---|---|---|---|---|
| | $PM_{10}$ µg·m$^{-3}$ | $PM_{2.5}$ µg·m$^{-3}$ | $PM_{10}$ µg·m$^{-3}$ | $PM_{2.5}$ µg·m$^{-3}$ | |
| **Athens** | | 68 | 41 | 20 | [11] |
| **Barcelona** | | 58 | 24 | 14 | [11] |
| **London** | 1000–1500 | 300–420 | 23 | 12 | [12] |
| **London** | 133 | 73 | 23 | 12 | [13] |
| **Los Angeles** | 44 | 33 | 25[#] | 12 | [14] |
| **Milan** | 71–283 | | 36 | 27 | [15] |
| **Montreal** | 97[¶] | 36[¶] | 15[#] | 8 | [16] |
| **Porto** | | 84 | 11[#] | 5 | [11] |
| **Paris** | 361 (RER), 68 (Metro[¶]) | | 28 | 16 | [17] |
| **Prague** | 215 | 94 | 23 | 17 | [18] |
| **Shanghai** | | 32–57 | 59 | 45 | [19] |
| **Stockholm** | 232 | 71 | 20 | 5 | [20] |
| **Taipei** | 227 | 85 | 28[#] | 19 | [21] |
| **Toronto** | 304 | 100 | 16[#] | 9 | [16] |
| **Turin** | 23[¶] | 16[¶] | 34 | 25 | [22] |
| **Vancouver** | 56 | 17 | 12[#] | 7 | [16] |

Where multiple studies have been performed on the same system, the most recent study with $PM_{10}$ and $PM_{2.5}$ measurements is cited. Ambient measurements are taken from the World Health Organization (WHO) Ambient (Outdoor) Air Quality Database [23]. [#]: $PM_{10}$ not measured directly, but calculated by WHO from measured $PM_{2.5}$ concentration using country-specific $PM_{10}/PM_{2.5}$ ratio as conversion factor; [¶]: trains run on rubber tyres.

components may reach the underlying cells, and exert effects, key among which is thought to be oxidative stress.

### PM-induced oxidative stress and oxidative damage *in vitro*

Oxidative stress occurs when there is an excess of potentially damaging oxidants, including free radicals and reactive oxygen species (ROS), over cellular antioxidant defences. As a consequence, there is oxidation of cellular components such as nucleic acids, proteins and lipids, leading to tissue injury and infiltration of inflammatory cells [31]. PM can exert oxidative stress through several mechanisms [32]. Certain surface or soluble components of PM, especially transition metals, can generate ROS on account of their ability to act as electron donors [33]. Transition metals are able to exist in multiple oxidation states, and thus donate electrons to molecular oxygen to generate ROS, facilitated by the oxygen-rich environment of the airways, forming superoxide, hydrogen peroxide and hydroxyl radicals, as well as potentially damaging reactive nitrogen and reactive sulphur species through downstream reactions [33, 34].

As well as acting as a source of ROS, PM can elicit increased ROS generation by exposed cells. Exposure to wildfire $PM_{2.5}$ has been seen to increase expression of dual oxidase 1 (DUOX1) in human bronchial epithelial cells [35], with bronchial and alveolar DUOX and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity potentially being a key mediator of the inflammatory effects of PM [36, 37]. Furthermore, PM can induce mitochondrial toxicity with consequent overproduction of ROS by mitochondria, dysregulation of the electron transport chain, loss of mitochondrial membrane potential and impaired oxidative phosphorylation [38, 39]. Further effects on the mitochondria and ATP generation may manifest directly or indirectly through different mechanisms for water-soluble metallic and water-insoluble organic PM components [40, 41], with nuclear factor erythroid 2-related factor 2 (Nrf2) potentially playing an important role in the maintenance of mitochondrial function, as well as more canonically through induction of phase II enzymes [41]. ROS can trigger release of the Nrf2 transcription factor from its cytoplasmic anchor kelch-like ECH-associated protein 1 (KEAP1) through mechanisms which may include direct oxidative attack or non-oxidative mechanisms, followed by binding of Nrf2 to the antioxidant response element, under the control of which are multiple antioxidant and detoxification enzymes including those relating to antioxidant activity (haemoxygenase-1 (HO-1), glutathione peroxidase), glutathione synthesis and (re)cycling (*e.g.* glutathione reductase, glutathione-S-transferase), NADPH regeneration (*e.g.* glucose-6-phosphate dehydrogenase) and xenobiotic metabolism (*e.g.* transaldolase, NAD(P)H-quinone oxidoreductase-1) [42–44].

A consequence of the richness in transition metals of underground PM is its ability to potently deplete antioxidants such as ascorbate or reduced glutathione, and generate free radicals independently of cells [45, 46]. This generation of ROS has been seen in primary bronchial epithelial cells exposed to underground PM, in a manner suggesting that ROS-generating potency increases as PM size decreases [24]. Comparison of underground PM with other PM types suggests that it is a more potent generator of ROS than PM from other sources, including urban PM, road wear, diesel and wood burning in A549 type 2 alveolar epithelial cells [47], and compared to similar PM sources in RAW264.7 murine macrophages, accompanied by increased lipid peroxidation [48]. Similarly, increased concentrations of oxidised biomolecules have been observed with underground PM appearing more potent than other PM types in inducing oxidative plasmid scission in a cell-free assay [12] and DNA damage in A549 cells [47, 49]. This oxidative damage to DNA exerted by underground PM can be mitigated by the iron chelator/redox inactivator desferrioxamine [50], while induction of the antioxidant enzyme HO-1 stimulated by underground PM is susceptible to desferrioxamine and the ROS scavenger N-acetylcysteine [51]. These data suggest that underground PM is able to generate ROS by itself, and in exposed cells, more potently than PM from above-ground sources on a PM mass basis, in a metal content-related manner, although conclusions are mixed as to whether this is a property principally of iron, the most abundant metal in underground PM, or other metals, especially those originating from the braking system.

### Inflammatory and barrier responses to PM *in vitro*

When exposed cells are unable to rectify oxidative stress through clearance of particles or increased antioxidant generation, inflammation ensues [52]. This is primarily coordinated through activation of mitogen activated protein kinases (MAPKs) with downstream phosphorylation of inhibitor of κB (IκB) and thus activation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) signalling, with increased expression of a battery of inflammatory mediators including interleukin (IL)-1β, IL-6 and IL-8 as well as matrix metallopeptidase-9 and cyclooxygenase-2 [53]. Involvement of microRNAs in the effects of PM has been demonstrated, with PM inducing sustained activation of NF-κB *via* phosphoinositide 3-kinase/Akt-mediated suppression of miR-331 [54], disinhibiting expression of inhibitor of κβ kinase-β (IκK-β), consequently increasing phosphorylation of IκB [54].
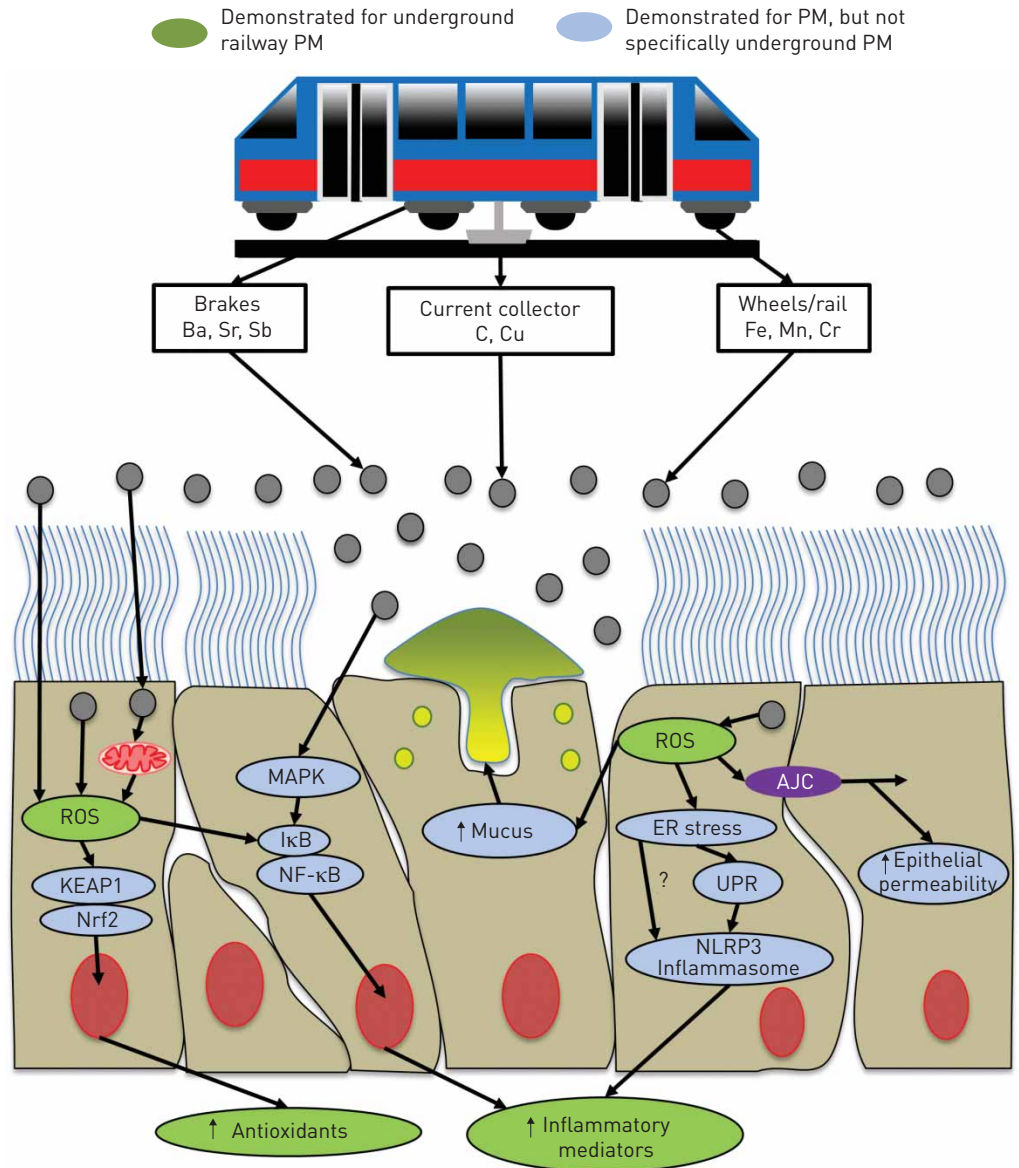
FIGURE 1 The effects on the bronchial epithelium of underground railway particulate matter (PM). PM in underground railways is derived principally from train-related sources, including wheel-on-rail and brake-on-wheel interactions, as well from wear and current arcing involving the current collector, with each source producing compositionally distinct metal-rich PM. Although there are likely to be element-specific effects on cells, a key mechanism through which PM exerts it effects is *via* generation of reactive oxygen species (ROS), which may occur extracellularly, or intracellularly following entry of PM into the cell. ROS may be generated directly by the particle or *via* mitochondrial dysregulation and activation of endogenous ROS-generating enzymes. Through oxidation of KEAP1, which sequesters it in the cytoplasm and targets it for degradation, the transcription factor Nrf2 is allowed to translocate to the nucleus, where it binds the antioxidant response element, activating transcription of a variety of antioxidant-related enzymes. Through ROS-dependent and ROS-independent mechanisms involving mitogen-activated protein kinase, nuclear factor (NF)-κB is released from its cytoplasmic anchor inhibitor of κB (IκB), translocating to the nucleus to activate inflammatory mediator expression, while concentrations of active forms of inflammatory mediators interleukin (IL)-1β and IL-18 are increased by ROS-mediated activation of the NLRP3 inflammasome *via* endoplasmic reticulum (ER) stress and the unfolded protein response (UPR), although a UPR-independent mechanism has been demonstrated, albeit not currently for PM. In addition, PM-derived ROS are able to upregulate expression of the mucin MUC5AC, resulting in increased mucus secretion to improve the physical epithelial barrier, while PM are able to impair the ionic and macromolecular tightness of the barrier by inducing dissociation of adherens and tight junction proteins away from the apical junction complex (AJC). Ba: barium; Sr: strontium; Sb: antimony; C: carbon; Cu: copper; Fe: iron; Mn: manganese; Cr: chromium.

Additionally, there may be involvement of toll-like receptors (TLRs), principally TLR4 and TLR2, in the cytokine response to PM in multiple cell types [55–57], signalling through the myeloid differentiation

primary response protein MyD88 resulting in NF-κB translocation to the nucleus and induction of cytokines such as IL-6 and IL-8 [28, 58]. PM-associated lipopolysaccharide, a component of Gram-negative bacterial cell walls, may activate TLR4 [59], while TLR2 recognises other PM-associated microbial components including lipoteichoic acid and proteoglycans, but may also be responsive to metals or endogenous damage-associated molecular patterns [60]. ROS may induce endoplasmic reticulum (ER) stress and activate the unfolded protein response (UPR) [61, 62]. This may result in outcomes including arrest of protein translation, increased inflammatory mediator production through NF-κB, and apoptosis, which are co-ordinated through different arms of the UPR and may vary depending on the stimulus and cell type [63]. For example, there is evidence for UPR activating the intracellular danger-sensing nucleotide-binding domain and leucine-rich repeat protein-3 (NLRP3) inflammasome in PM-mediated neutrophilic airway inflammation [64], which appears to be principally involved with the innate immune response to PM and which increases activation of the cytokines IL-1β and IL-18 [65, 66]. However, it has also been demonstrated that ER stress-induced NLRP3 activation, while still requiring ROS, may occur independently of UPR, and proceed instead *via* a mitochondria-dependent pathway [67]. Given the aforementioned effects of PM on mitochondrial metabolism, it would be of interest to determine whether these effects on the NLRP3 inflammasome occur through common mechanisms.

PM can affect the integrity of the airway epithelial barrier. Mice exposed to ambient $PM_{2.5}$ collected during haze episodes in Hong Kong and China showed suppressed levels of E-cadherin alongside increased concentrations of interferon (IFN)-γ, IL-2, IL-4, IL-6 and IL-10 in the bronchoalveolar lavage fluid (BALF) [68], while combustion-generated PM induced epithelial-to-mesenchymal transition in bronchial epithelial cells *in vitro* and in a murine model, including decreased E-cadherin expression and loss of epithelial cell morphology [69]. Similarly, human and rat alveolar epithelial cells exposed to $PM_{10}$ and diesel exhaust particles exhibit reduced membrane occludin and dissociation from the cytoskeletal linker zonula occludens-1 [70]. In addition, ultrafine PM may enter human bronchial epithelial cells [50, 65, 71], potentially triggering autophagy [71].

Increased susceptibility of the asthmatic airways to PM may arise from impaired barrier function of the asthmatic airway epithelium, such as impaired junction formation with decreased tightness against ionic and macromolecular passage [72, 73], and increased PM-stimulated release of proinflammatory cytokines and airway remodelling factors [73, 74]. In addition, PM may exacerbate mucus hypersecretion, through upregulation of bronchial epithelial expression of MUC5AC and the epidermal growth factor receptor ligand amphiregulin [76]. Furthermore, asthma is associated with a background of pre-existing oxidative stress as well as increased susceptibility to ROS-associated damage [77, 78]. Indeed, genetic polymorphisms in enzymes involved in cycling of the antioxidant glutathione have been associated with asthma [79], as have decreased levels of airway superoxide dismutase and catalase [80, 81].

Studies have noted underground PM concentration-dependent increases in release of proinflammatory cytokines from A549 and primary bronchial epithelial cells, with underground PM generally being shown to be more potent than urban PM and other PM types in epithelial cells [50]. However, this is notably not the case for macrophage exposure, where underground PM tends to elicit less of an inflammatory cytokine response than urban PM [49, 82, 83]. This disparity may be due to the relatively high concentration of lipopolysaccharide (LPS) in urban PM compared to underground PM [82], to which macrophages are relatively more responsive than epithelial cells since the latter poorly/do not express CD14 or MD-2 involved in LPS-TLR4 signalling [83–85]. It may also be due to liberation of urban PM-specific PM components in the acidic phagosome, which has been observed with gold nanoparticles [87]. Unlike urban PM, the proinflammatory activity of underground PM seems to be predominantly confined to the insoluble fraction of the particulate [50], probably in the metal fraction, given that this inflammogenicity is abrogated by iron chelation. Furthermore, this activity is less pronounced for underground PM with a much lower metal content, such as may be found in underground systems where rubber pneumatic tyres are in use [17]. Indeed, the iron content of underground PM has been shown to be mainly in the form of insoluble iron oxide [88]. A recent report by the UK Committee on the Medical Effects of Air Pollution (COMEAP) on inhalable dust in the London Underground suggested that the insoluble nature of iron in underground PM at normal physiological pH may result in overestimation of the risk of exposure to underground PM given the diminished bioavailability of insoluble metal compared to soluble metal [89], although metal solubility may increase in the acidic environment of the lysosome, with implications for toxicity [90]. However, conversely, insoluble metallic PM may have a prolonged persistence and be more likely to enter distal organs intact [7, 91]. Similarly, the relatively less inflammogenic nature of underground PM compared to urban PM suggests that, mass-for-mass, underground PM may represent less of a health risk, although this conflicts with the increased level of oxidative stress exerted by underground PM. The contribution of underground sources to overall PM concentration is weighted towards the coarse and fine fractions, meaning that the portion of the toxic burden carried by the ultrafine

fraction may be relatively less than above ground [13, 26], although this is not a uniform finding and may depend on underground network-specific factors [25].

### *In vivo* exposure studies

Studies evaluating the composition of underground PM compared to PM found in overground light rail and road journeys suggest that, while underground PM may have relatively low concentrations of the carcinogenic polyaromatic hydrocarbons associated with diesel combustion, this may be outweighed by the presence of metals in underground PM, and the sheer airborne concentration of underground PM [14, 92]. Indeed, even a relatively short commute in an underground railway may contribute a large proportion of an individual's daily exposure to PM and airborne metals [16]. Therefore, it is perhaps surprising that studies of the effects of acute and chronic exposure to underground railway air have found little evidence of excess risk [93]. Exposure of volunteers in the Stockholm underground for 2 h found no change in lung function parameters, BALF cell counts or cytokine concentrations in healthy or mildly asthmatic volunteers, although there was increased self-reporting of lower and upper airways symptoms in the different groups [20, 94]. In addition, the study found increased concentration of circulating coagulation markers and BALF oxylipins in the healthy group only, suggesting that disease-specific differences may not necessarily manifest at the site of the pathology, although the clinical significance of this is unclear [20, 94]. A similar lack of obvious effect has been noted in Stockholm underground workers over the course of an 8-h shift, albeit with an increase in circulating coagulation markers, as with the aforementioned study [95, 96]. Similarly, 5-h exposure of volunteers at multiple sites across the Netherlands, including an underground railway station, found that exhaled nitric oxide fraction was not obviously associated with underground exposure [97], nor was nasal lavage inflammatory cytokine concentration [98] or coagulation markers in contrast to the Stockholm studies [99, 100], although nasal lactoferrin expression was associated with underground railway PM metals [98], as were circulating white blood cell, neutrophil and monocyte counts [101]. Studies of chronic workplace exposure have shown a similar lack of effect: Stockholm underground drivers were not noted to be at increased risk of lung cancer [102] or myocardial infarction [103]. However, these studies were performed in only two underground systems, using generally healthy young adult and middle-aged volunteers. Much more work is required before conclusions can be drawn about the effects of underground railway PM on incidence and exacerbation of respiratory diseases, differential effects on those with pre-existing respiratory disease and chronic effects.

### Protection against PM

Given the clearly elevated PM mass concentrations in underground stations, there have been several proposals for their reduction [104]. Those focusing on reducing PM generation include the use of pneumatic tyres rather than metal wheels [17, 22], and the use of nonferrous materials to decrease the potential ROS-generating capacity of PM [105], although the use of pneumatic tyres may pose a different risk, acting as a source of inhalable microplastics [106, 107]. Those focusing on reducing exposure to PM include the installation of filters to attract and sequester magnetic PM [88, 108], and the washing of tunnel walls to diminish resuspension of PM by train passage [21]. Full-height platform edge doors, originally intended to prevent passenger access to the tracks when there is no stationary train in the station, have been suggested as being perhaps the most effective mechanism to decrease PM exposure for passengers on platforms [109], while there is some evidence that PM concentration varies along the platform, implying that exposure might be modified by passenger location [19, 110]. Face masks may reduce inhalation of PM, and have been shown to have beneficial effects in highly polluted urban settings [111, 112], but "real-life" filtration of PM may be less than expected, with poor facial fit a particular problem [113], and there are significant ethical questions regarding the use of masks by the public and public-facing staff.

### Conclusions

There is an expanding body of evidence for the effects of PM on the airways (figure 1), and the mechanisms by which such outcomes occur, but there is a need to consider PM as a class of chemically and toxicologically heterogeneous toxicants, rather than simply as a single homogeneous entity. Given that underground railways generally have concentrations of airborne PM several times higher than above ground, and that physicochemical and *in vitro* data suggest that underground PM is a potent inducer of oxidative stress in airway epithelial cells, it is noteworthy that there is a lack of evidence for effect *in vivo*. There is a clear need for further studies of the effects of underground PM *in vivo*, especially focusing on demographic groups other than those predominantly represented in underground staff, such as severe asthmatics and the elderly, and *in vitro* and *in vivo* studies to determine effects of PM beyond the range of antioxidant and inflammatory markers usually evaluated in such studies.

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