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COMMITTEE ON THE MEDICAL EFFECTS OF AIR POLLUTANTS

Viewpoint on the mechanistic evidence for health effects of particulate matter in the London Underground

1. This paper prepared by Dr Matthew Loxham provides a viewpoint on the mechanistic evidence for health effects of particulate matter (PM) in the London Underground.

2. Note: This is a draft working paper for discussion. It does not reflect the final view of the Committee and should not be cited.

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Viewpoint on the mechanistic evidence for health effects of particulate matter in the London Underground

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Executive Summary

Particulate matter (PM) is the term used to describe airborne solid and liquid particles suspended in the atmosphere. There is a considerable body of evidence associating PM, and in particular fine PM, also known as $PM_{2.5}$, which has a median aerodynamic diameter <2.5 µm, with a range of adverse health effects, following both acute and chronic exposure. Such effects include those manifesting in the respiratory system (asthma exacerbations, lung cancer, chronic obstructive pulmonary disease (COPD) exacerbations) and cardiovascular system (myocardial infarction, ischaemic heart disease, stroke), as well as for type II diabetes, non-lung cancers, and dementia.

Monitoring and regulation of $PM_{2.5}$ is based on the airborne mass concentration of $PM_{2.5}$, generally express as micrograms per cubic metre (µg/m^3). However, it has been suggested that a consideration of this metric alone does not necessarily provide the most accurate information about the likely effects of inhaled $PM_{2.5}$, and that the chemistry and number of particles may also play a role.

Given that the majority of studies of $PM_{2.5}$ focus on ambient/urban $PM_{2.5}$, environments where the airborne PM load is significantly physicochemically distinct from urban environments may warrant special consideration as to the potential health effects of PM exposure. The London Underground, where airborne PM mass concentrations are significantly higher than above ground, on account of the reduced ventilation and high level of PM-generating mechanical activity, and where the physicochemistry of PM is markedly different to that above ground, is one such environment. The large number of people using the London Underground on a regular basis adds to the need for an in-depth analysis of the potential risks to health.

This working paper assesses the evidence for risks to health posed by exposure to PM air pollution in underground railway systems, analysing evidence from *in vivo* and *in vitro* studies performed in underground railway systems around the world. While there is evidence that the effects on some endpoints/markers of toxicity may be more pronounced with PM from underground railways than urban areas, this is not seen across all endpoints, some of which are seen to be affected by urban PM to a greater extent than by underground PM. Furthermore, these differences are generally restricted to *in vitro* studies, whereas *in vivo* studies do not suggest a significantly increased risk to health from exposure to underground PM. However, the drawing of firm conclusions is hampered by a lack of a significant body of work on the subject. There is, however, currently, little evidence to suggest that underground PM poses a significantly increased risk to health compared to ambient PM.

Introduction

Underground railways are heavily used mass transit systems used for several million passenger journeys per day in many of the world's most populous cities. Depending on station depth, proximity to the nearest portal, and air conditioning system, there may be relatively little exchange of air with the outside environment, which may be further modulated by station design [1, 2, 3, 4, 5]. As such, the PM load in underground railway stations has the potential to be (1) significantly greater, on a mass concentration basis, than the outside environment, and (2) influenced principally by source materials and generation methods specific to the underground railway. These processes include wear of wheels and brakes, and arcing of electrical current between the third rail or catenary, and the current collecting apparatus [6, 7]. The physicochemical characteristics of PM in the London underground are documented in another working paper associated with this report [DN: insert hyperlink to working paper on gov.uk website]. Given the potential for underground PM to differ significantly in these properties, the question of how (if at all) underground PM may exert its effects on health merits attention.

The focus of the present working paper is an examination of the evidence for the health effects of PM in underground stations, and PM which may be similar to that found in underground stations, specifically iron-rich PM from iron and steel industries. The working paper is divided into four principal sections, focusing on (1) evidence for effects of exposure to underground PM *in vivo*, (2) evidence from *in vitro* studies, (3) studies which extrapolate *in vitro* findings to evaluate *in vivo* risk, and (4) evidence from *in vivo* studies of exposure to PM not from underground railways, but which may approximate the physicochemistry of underground PM. A final section addresses in brief a collection of studies focusing on various aspects of *in vitro* and *in vivo* effects of underground PM. Note that while "*in vivo*" refers to experiments conducted in any live organism, those referred to in this paper are almost all performed in humans.

Papers were initially selected from a literature review carried out by Prof Mark Nieuwenhuijsen [DN: insert hyperlink to working paper on gov.uk website]. This review was commissioned by Transport for London to provide a rapid assessment of the evidence to assist COMEAP and its Sub-group on decision making. Further references were selected specifically for this working paper, but only those specifically addressing *in vitro* or *in vivo* effects, or papers directly linked to/relevant to such studies were included.

Throughout this working paper, the terms "underground" and "underground railway" are used. This is equivalent to terms such as "subway" and "metro" used elsewhere, with the key distinction being required for the use of the term "underground" is that the station/section in question is subterranean.

(1) Evidence for health effects of exposure to underground air pollution/PM *in vivo*

Klepczynska Nystrom *et al* exposed 20 healthy volunteers to a Stockholm underground environment or a control environment for 2 hours and investigated effects of this exposure on lung function and inflammation in both the lower airways and blood. They found statistically significantly increased levels of fibrinogen in plasma of those exposed in the underground, with increased counts of CD4/CD25/FOXP3 T cells [9]. The increase in CD4 T cells positive for FOXP3, or FOXP3 and CD25 (in blood but not bronchoalveolar lavage fluid [BALF]) indicates an increase in the pool of regulatory T cells (Treg) at 14h following the underground exposure. A key function of Treg cells is to reduce the inflammatory response, which can be achieved through secretion of anti-inflammatory cytokines and effects on other immune cells [10, 11]. As such, the increase in Treg numbers may be a response to the systemic inflammation indicated by increased fibrinogen release. It is unfortunate that levels of the anti-inflammatory cytokine IL-10, released by Tregs, were only assayed in the BALF (where there was no increase in Tregs) and not in the blood (where Tregs were increased), and as such this is unlikely to be of physiological relevance. Similar increases in systemic Tregs but not lung Tregs have been found after $PM_{2.5}$ exposure in mice [12]. Post-exposure blood tests also showed slight but significant increases in other markers of T cell activation. One aspect which was not considered by Klepczynska Nystrom's paper was the ability of Tregs to suppress inflammation, which has been seen to be downregulated by ambient air pollution exposure in children, especially those with asthma [13]. Therefore, while the increase in peripheral blood Treg cells in the present study is a natural consequence of the systemic inflammation indicated by increased fibrinogen concentrations, it is impossible to tell whether there is any effect of underground railway pollution on Treg functioning. There was also a slight and likely physiologically irrelevant, but nonetheless significant, increase in plasma fibrinogen (from 2.2 g/l to 2.3g/l). Fibrinogen undergoes enzymatic cleavage by thrombin to form fibrin, which then forms the "meshwork" for clot formation. Because of this increased tendency towards clot formation, fibrinogen is regarded as a risk factor for a coronary heart disease, stroke, and other vascular disorders [14]. It is notable, therefore, that this study found no difference in levels of plasminogen activator inhibitor-1 (PAI-1), which is also involved in clot formation by inhibiting the activation of an enzyme cascade involved in clot breakdown. This study sampled blood 14h after exposure, which is within the normal time frame for acute response proteins to be increased in the blood, although it is possible that slightly later timepoints may reveal increased levels of such markers where they are not seen at 14h. In addition to C-reactive protein and various inflammatory cytokines (eg IL-6) fibrinogen is one of the most commonly assessed blood-borne markers of the effects of air pollution in humans, specifically as a marker of systemic inflammation, where it is sometimes noted to be correlated with circulating levels of C-reactive protein (reviewed by [15]). The increase in fibrinogen has been suggested to be dependent on the genotype of the individual concerned, at least in the case of gaseous pollutants [16]. The study of Bigert and colleagues [17] included in this literature search, although not this specific section, found increased fibrinogen in underground ticket sellers and PAI-1 in underground train drivers after 2-days of work following a wash-out period of at least 2 days. However, baseline levels were highest in platform workers, who showed no

acute increases in these markers, suggesting that acute changes were not due to particle exposure.

Therefore, while fibrinogen is a widely used biomarker for systemic inflammation as a result of short term exposure to PM, the evidence for a biologically significant effect of underground PM on fibrinogen is insubstantial. Furthermore, this study found no significant increase in the concentrations of any inflammatory cytokines (IL-1β, lL-6, IL-8, and, TNFα) or anti-inflammatory cytokine (IL-10) in bronchoalveolar lavage fluid, a finding which suggests a significant inflammatory response, at least at 14 h post-exposure, was not present. Finally, with regard to cardiovascular disease, a further study by Bigert and colleagues [18] found no significant increased risk of myocardial infarction in underground train drivers, suggestive of a lack of effect on this exposure group following chronic exposure. Nonetheless, direct comparison to the London Underground is difficult given the markedly lower PM load in the Stockholm underground compared to the London Underground. Furthermore, there is an unanswered question of whether these studies were sufficiently powered to uncover an effect, especially given the relatively small number of subway drivers, if one were to exist. Indeed, this study has somewhat large confidence intervals relating to relative risk of myocardial infarction in the study groups.

In Klepczynska Nystrom and colleagues' follow-up paper, mild asthmatics were seen to have increased activated T cells in BALF (identified by the marker CD25) but no increase in Tregs in the blood (unlike their healthy counterparts in the previous study). However, Tregs were increased in BALF (again, unlike their healthy counterparts). In the context of this effect on the lungs, which was not seen in their healthy counterparts, it is notable that asthmatic volunteers, but not the healthy volunteers from the previous study, also reported increased upper airways symptoms, whereas the healthy volunteers reported increased lower airway symptoms. However, the overall conclusions of the authors seem sound – that although there were some changes in markers indicative of systemic inflammation (healthy group) and differences in the response of a group of mild asthmatics (albeit only according to a small number of the many parameters examined), acute exposure to underground air in these cases caused little acute effect.

The lipid mediators studied in BALF following exposure to underground air in the study of Lundstrom *et al* [19] are not commonly used markers in such studies, perhaps on account of the numerous analytes studied, the need for lavage, the requirement for specialised mass spectrometry techniques for their measurement, of their lack of comparability to other studies. The study showed an increased level of nine oxylipins following 2 h exposure to Stockholm underground air in healthy volunteers, which was significantly greater than in asthmatics, where levels tended to remain the same or (in a small number of instances) decrease. These nine oxylipins were prostaglandin E2 (a product of arachidonic acid metabolism by cyclooxygenase), and eight products of biosynthesis from linoleic or α-linolenic acids via 15-lipoxygenase, suggesting a common mechanism of regulation. The authors suggest that, given the bronchoprotective effects of the oxylipins increased in healthy individuals] but not asthmatics following exposure to underground air, there is evidence of a differential effect depending on asthma status. This is true, and also corroborated by the increased BAL cell expression of cyclooxygenase-1 in healthy volunteers only. However, while these results are interesting and certainly suggestive of asthma status-specific effects, the pathways of these metabolites are

significantly complex that it is difficult to draw any robust conclusions regarding their implications for acute responses to underground air exposure. Furthermore, the effects of the observed changes in their concentration is unclear. The lack of research into lipid mediators following pollution exposure is underlined in a recent paper on the effects of biodiesel exhaust [20], where healthy volunteers were exposed to biodiesel exhaust at a PM level not dissimilar to the PM level of the Stockholm underground. They found increased levels of PGE2, 13-HODE, and 12,13-diHOME following biodiesel exposure. Interestingly, while PGE2 and 13-HODE were significantly more responsive to underground air exposure in healthy *vs.* asthmatic volunteers in the Stockholm underground study and 13-HODE was close to significance ($p=0.1$), none of these were significantly increased in the Stockholm underground following exposure *vs.* no exposure, and further examination of the Stockholm underground study shows that the significance threshold for analysis between asthma status was likely attained not simply because of an increase in levels of these oxylipins in healthy lavage fluid, but also because of a decrease in their levels in asthmatics. Indeed, considering the asthmatic and healthy groups separately, only two oxylipins were significantly altered by exposure to underground air (one of these overlapped both groups). This further reinforces the conclusion that the Stockholm study, while suggesting some disease-related differences, does not provide strong evidence *per se* for any significant effect of underground air, but rather evidence of some asthmatic *vs.* non-asthmatic differences.

In a pilot study of workers on the New York underground, Grass and colleagues found that track maintenance and construction workers were exposed to higher concentrations of PM2.5 and airborne Fe, Mn, and Cr compared to other underground workers, although drivers and train flaggers were exposed to the highest concentrations of Fe when expressed as a proportion of PM exposure [21]. Using bus drivers and office workers as control groups, they found little evidence of adverse health effects of working underground using a range of urinary and plasma biomarkers, predominantly metal- and oxidative stress-related. Urinary Mn and 8-hydroxy-2'-deoxyguanosine (8-OHdG, a marker of oxidative DNA damage) concentrations were highest in office workers, while urinary concentrations of the polyaromatic hydrocarbon (PAH) metabolite benzo(a)pyrene diol epoxide (BDPE) were highest in bus drivers. Urinary 8-isoprostane levels were non-significantly higher in underground workers than the control groups, although in the underground group there was a significant correlation with total years of underground work, suggesting a potential effect of cumulative exposure. Plasma concentrations of protein carbonyls (a marker of oxidative damage to proteins) were no different between underground workers and bus drivers, and lower for office workers, although they were correlated with plasma Mn in subway drivers but not the control groups, possibly indicating different drivers of protein oxidation below and above ground. Plasma Mn was similar across all groups, while plasma concentrations of Cr and DNA-protein crosslinks were lower in bus drivers but not office workers, and there was no difference in plasma Pb. As such, this study showed no obvious effect of underground exposures to any of the urinary or plasma biomarkers measured, and nor did raised concentrations of Mn or Cr underground necessarily translate to commensurately raised levels of these elements in the plasma of exposed workers. Conversely, Mehrdad and colleagues found that there was a small but statistically significant increase in urinary concentration of the DNA oxidation biomarker 8-OHdG in underground tunnel workers compared with underground workers who did not

work in the tunnels, after correction for age, BMI, disease, and smoking, although they were unable to correct for alcohol consumption, nor were they able to measure $PM_{2.5}$ or steel dust exposure in either of the two groups [22]. Furthermore, it is not clear whether this difference represents a response to acute exposure over a shift, or accumulation of chronic exposure, since no pre-shift measurements were taken.

The study by Liu *et al* uses heart rate variability (HRV) as an endpoint [23]. HRV is a commonly used endpoint to assess the effects of pollution, representing a risk factor (in itself or as a marker of autonomic imbalance) for a number of adverse cardiovascular outcomes (see eg [24]) and is thought to be caused by effects of pollution on the autonomic nervous system, resulting is disturbed sympathetic and/or parasympathetic tone. This study is by no means the only one to find a decrease in HRV with increasing exposure to $PM_{2.5}$ (for a comprehensive review, see a recent review by [25]), but it is superficially unusual in noting that the decrease in HRV seen by participants using the underground was less than seen for those walking or using the car or bus. However, closer examination of the concentrations of $PM_{2.5}$ to which participants were exposed shows that the underground air was significantly less polluted than environments used for walking or bus exposures, and non-significantly less than car-based exposures environments. This was also true for exposure to PM_{10} and total volatile organic compounds. The finding that the underground was the least PM-loaded environment is highly unusual. Indeed, rare similar findings tend to come from cities where it is the city itself which is unusually polluted, as well as the underground air being unusually clean. One reason for the relatively low PM concentration noted in the Taipei underground here may be the use of an air conditioning system. Therefore, this study cannot be regarded as relevant to understanding exposures in the London Underground although it does imply that when similar PM_{10} and $PM_{2.5}$ mass concentrations are involved, air in the underground is not necessarily more detrimental (in terms of heart rate variability indices) than other modes of transport.

Summary

The papers of Klepczynska Nystrom *et al* [9, 26] suggest a mild effect of exposure to underground PM for 2 h, with differences between healthy and mild asthmatic volunteers. Healthy volunteers showed increased Treg activation in the blood, along with slightly increased levels of other markers of T cell activation, suggesting a mild systemic immune system activation in response to PM, with lower airway irritation, whereas mild asthmatic volunteers showed increased Tregs in BALF, with upper airways symptoms. The increased plasma fibrinogen levels in healthy volunteer plasma are likely another marker of this systemic inflammatory response. One reason for this healthy-asthma difference might be that the presence of mild asthma reduces penetration of the underground PM to the lower airways/alveoli in the mild asthmatics, and thus the effects are restricted to the upper airways, whereas the more patent airways of the healthy volunteers perhaps permit increased penetration of PM to the lower airways, increasing local symptoms and also transmission of inflammatory responses to the circulation through entry of locally released cytokines into the systemic circulation. Although there was no effect on lung function in either group, these observations do suggest that acute exposure may have an effect in the short term. A more important question is whether this effect persists in those exposed continually and for longer periods, and what the consequences of this might be – for example, is there an effect on immune system functioning, and if plasma

fibrinogen is persistently raised in underground workers/regular commuters, is there an increased risk of adverse cardiovascular outcomes? The study of Bigert *et al* [18] suggests that, at least for myocardial infarction, there may be no increased risk, but there is a genuine lack of work examining the long term effects of chronic exposure to underground PM. The observed increased in lipid mediators in Lundstrom's paper [19] is interesting, but is certainly not conclusive, given the small proportion of assayed mediators whose concentration was changed. Lipid mediators and lipid oxidation in general represent an interesting and currently under-investigated avenue for PM research, but this paper is not sufficient to draw any relevant conclusion other than as an illustration that some lipid mediators may change in response to underground air exposure, and that there are asthmatic/healthy differences in response to the underground environment. Similarly to the findings above, the work of Grass and colleagues [21] does not suggest any effect of underground PM exposure consistent across endpoints, while although Mehrdad and colleagues found a small increase in a urinary marker of oxidised DNA, their study has caveats which mean that their data alone cannot be accepted as definitively illustrating an effect on exposed workers. The work of Liu [23], while interesting, is unfortunately of little relevance to the London underground given the unusually "clean" nature of the air in the Taipei underground, although this kind of study, performed in London, would be useful.

(2) *In vitro* studies of the toxicity of underground air pollution

A number of experimental studies (*in vivo* or *in vitro*) on the toxicity of underground air pollution/PM have been conducted. Most of the studies were conducted with particulate matter from the Stockholm underground, but also from underground systems including London Underground.

The paper by Seaton *et al* [27] looks at the characteristics of PM on the London Underground from a size and compositional point of view, with PM collection and monitoring at three deep-level underground stations (Oxford Circus, Holland Park, Hampstead). Noteworthy findings are that the mass concentration of $PM_{2.5}$ underground is much higher than would be seen above ground, but there is not a correspondingly higher level of PM by particle number concentration (PNC). In fact, the number concentration is lower than might be expected in a city street. Overall, the size distribution of underground PM *vs.* urban PM is shifted towards the larger size of the PM spectrum. If composition is disregarded and only size/PNC is considered, this observation suggests that underground pollution may well pose less of a health risk that that above ground, where higher numbers of particulates persist – PNC has been suggested to be more important a dose metric than particle mass [28]. Furthermore, the metal-rich nature of underground PM means that it is likely denser than urban PM, and thus would have a lower PNC, even if the size distribution and mass concentrations were identical. However, Seaton and colleagues' work also showed that, *in vitro*, A549 cells (a type 2 alveolar epithelial carcinoma cell line) responded more to underground PM than to ambient PM or control particles (titanium dioxide) in terms of release of the neutrophil chemoattractant/activating cytokine IL-8 which is, along with IL-6 and TNFα one of the most commonly measured cytokines in such *in vitro* studies, and also with greater levels of DNA damage (by plasmid scission assay). The increase in IL-8 release could be abrogated by chelation (the exact method is not stated) implying that PM surface or soluble transition metals are responsible for this effect. Furthermore, the use of welding dust as a comparator is not ideal given the fact that much of the underground PM is generated by abrasion rather than high temperature processes generating fume.

The three papers by Karlsson and colleagues [29, 30, 31] detail some experiments similar to those of Seaton *et al*, but performed in greater depth. Data from a paper in 2005 showed that Stockholm underground PM was more potent that urban street PM in causing A549 cell DNA strand breakage and oxidation, that the involvement of iron was greater for underground PM than for urban PM and that, while over half of the effect on DNA oxidation by street PM was due to water-soluble PM components, only a small proportion of the activity of underground PM was water-soluble [29]. They also used x-ray diffraction to study the crystal structure of the PM, showing that the majority of underground PM iron was in the form of magnetite (Fe_3O_4), while haematite (Fe_2O_3) was prevalent in urban PM. This is potentially important, because while iron in haematite exists purely in the ferric Fe^{3+} (Fe(III)) form, iron in magnetite exists as a mix of ferric and ferrous iron (Fe(II,III)). Generation of ROS by PM is one of the key mechanisms by which PM is thought to have its effects, and this requires an electron donor to reduce molecular oxygen to superoxide, superoxide to peroxide, and peroxide to hydroxyl radical. This last step is the Fenton reaction, catalysed especially efficiently by iron, and shown here to be at play by the effect of addition of H_2O_2 resulting in a large increase in DNA oxidation with PM but not in control

cultures. Because the oxidation of Fe(II) to Fe(III) is much more energetically favourable than Fe(III) to Fe(IV), ferrous iron is much more able to generate ROS, and thus magnetite is a potent generator of ROS, whereas haematite is not. Similar findings of a predominance of ferrous iron have been noted in the Shanghai underground [32]. The predominance of magnetite is not universally observed, however. Querol and colleagues found predominance of haematite in the Barcelona underground, with only a small fraction of magnetite, which has also been noted for the Budapest underground [2, 33]. However, in a discussion of their findings of the predominance of magnetite and maghaemite (Fe_2O_3 with a different crystal structure to Fe_2O_3 in haematite), Jung and colleagues assert that while magnetite and haematite can be differentiated by spectroscopic techniques, this is not possible for magnetite *vs.* maghaemite [34]. The implication of this is that ferrous and ferric iron cannot be accurately distinguished by x-ray spectroscopy if the ferric iron is in the form of maghaemite rather than haematite. The discussion is further complicated by the suggestion that dissolved ferric iron can be reduced back to the more toxic ferrous form by antioxidants present in lung lining fluid, at least *in vitro* [35].

The fact that more of the activity was water soluble in urban PM than underground PM is possibly due to a greater proportion of iron/other transition metals in urban PM than in underground PM being in the form of water-soluble salts (eg sulphate), thus increasing the effective metal ion concentration, which in insoluble metal/metal oxides is simply a function of particle surface area. Increased water solubility may modulate the toxicity of these particles, but it is not clear whether it would increase or decrease.

The 2006 paper by the same authors performed similar experiments but extended to compare underground PM_{10} to PM from wood burners (total PM) and tyre wear simulators (PM_{10}, with one additional $PM_{2.5}$ sample) representing different types of burner/fuel and road, respectively, and also “street PM_{10}” collected in a Stockholm city centre street [30]. This showed different effect rankings by endpoint: by comet assay, DNA damage in A549 cells was significantly higher with underground PM than any other PM tested, while underground PM induced only relatively small increases in human monocyte-derived macrophage release of IL-6, IL-8 (both non-significant) and TNFα (significant), compared to the street PM. The release of IL-8 in this study is lower for underground PM than seen by Seaton *et al* [27] (~3-fold *vs.* ~2-fold), but a greater difference is seen for street PM (~11-fold *vs.* ~2-fold). This is discussed in the Summary below. However, it is also notable that the PM in this study was collected on glass fibre filters, which the authors show caused a 5-15-fold increase in cytokine release in the blank filter control cultures. Although the authors attempted to correct for this by comparing cytokine release in the presence of PM to that seen in the filter blank-treated cultures, this makes it difficult to draw robust conclusions from the study, especially given that it seems likely that the ability of macrophages to phagocytose the PM would be impair to an extent by the presence of glass fibres (in this respect, it would be interesting to see the responses in A549 cells). Furthermore, it is also difficult to know whether the inflammatory state clearly induced by the presence of these fibres would have an equal effect on the response to each different PM type, thus making the comparison between different PM types difficult.

Karlsson *et al* (2008) performed similar experiments, but this time looking at different endpoints, and incorporating homogenous/pure PM as well as environmental PM,

here in A549 cells [31]. Underground PM_{10} caused an approximately 4-fold increase over control in both mitochondrial depolarisation (similar to wood and diesel PM, greater than street PM and tyre/roadwear PM) and intracellular reactive oxygen species (ROS) as measured by DCF fluorescence, being the only PM type which increased ROS. To examine the mechanism of these effects, the effect of underground PM was compared to that of magnetite, the principle iron component of the underground PM. Underground PM increased DNA structural damage (measured by single strange breaks and alkaline labile sites) and DNA oxidative damage (by FPG sites) but magnetite PM had only a small effect (trending towards significance) on the former, and none on the latter. These effects were then shown to be ascribable to non-water-/non-citrate-soluble underground PM components, and also more genotoxic than haematite particles or copper-zinc particles, which were also observed in the underground PM samples. Genotoxicity could, however, not be explained by the main component (magnetite), by water-soluble metals, or by intracellular mobilized iron. The genotoxicity is most likely caused by other reactive surfaces giving rise to oxidative stress. The authors conclude that the genotoxicity of underground PM "cannot… be explained by the main component magnetite, by water-soluble metals, or by intracellularly mobilized iron" and that factors responsible for the effects of underground PM may include "Fe coordination at the surface, perfection of the microcrystals, and the arrangements of Fe ions at the surface". However, there is also evidence that the combination of iron and copper, as found in underground PM, can be especially potent in the generation of hydroxyl radicals, (see summary, below) and while this series of papers clearly shows the potential effects of underground PM, it does not provide any conclusive evidence of why the underground PM is toxic, and nor that it is necessarily more toxic than other PM types, at least on an equal-mass basis. Furthermore, such *in vitro* studies do not necessarily indicate the likelihood of toxicity *in vivo*. The authors also suggest that the shard-like shape of a portion of the PM may endow the PM with a pro-inflammatory potential. However, although this is a noted feature of the macrophage response to PM which are particularly larger in one dimension than another (ie have a high aspect ratio), whereby phagocytosis begins but is unable to complete leading to "frustrated phagocytosis" with consequent release of inflammatory cytokines, to the author's knowledge there is no published evidence that this occurs in epithelial cells.

The paper of Lindbom *et al* (2006) clearly illustrates a hazard in comparing PM_{10} types – cell selection [36]. Work in monocyte-derived macrophages showed that underground PM was poor at eliciting release of IL-6, IL-8, and TNFα compared to two types of roadwear PM and water and methanol extracts of diesel PM. However, underground PM was more effective than any other PM type in eliciting TNFα release from bronchial epithelial cells (BEAS-2B), and no PM was able to elicit cytokine release from RPMI2640 nasal epithelial cells. The same group performed some similar experiments in RAW264.7 macrophages, comparing street PM to underground PM, and PM from studded tyre wear on both quartz and granite pavements [37]. In brief, street PM tended to be considerably more inflammogenic in terms of the "traditional" markers IL-6 and TNFα, although underground PM elicited a greater release of arachidonic acid, indicating the potential for a greater effect via the eicosanoid pathway. However, in terms of various measures of ROS, either directly (by cell-free oxidation of DTT) or indirectly (by measurement of thiobarbituric acid-reactive substances as a proxy for lipid peroxidation), underground PM generated a

significantly greater response than did street PM. In general, granite and quartzite pavement wear particles elicited less response over all outcome measures than did the street and underground PM.

The paper by Bachoual *et al* (2007) is interesting and unusual in that it compares two underground PM_{10} types – those from the Paris metro, which uses rubber/pneumatic tyres and "wooden" brakes (PM_{10} Fe=42%, Mn=<1%), and those from the suburban RER system, which uses metallic components (PM_{10} Fe=61%, Mn=7%) [38]. Carbon black, titanium dioxide, and diesel exhaust particulate matter were used as comparators. No source-specific difference in cell death was seen, while the two railway PM sets were the most effective at inducing release of MIP-2 and TNFα from RAW 264.7 murine macrophages, within 3 and 8 hours, respectively, and persisting until at least 24 hours. Conversely, none of the particles tested induced increased mRNA expression of matrix metalloprotease-(MMP) 2 or 9, and all induced MMP-12 to a roughly equivalent extent. The iron chelator desferrioxamine (DFX) reduced release of TNFα by RER PM by ~50%, but not had little effect on Metro PM, while no effect on MIP-2 response was seen with DFX, suggesting differential release mechanisms for these two cytokines. In testing in mice, RER PM, but not carbon black or DEP, caused increased bronchoalveolar lavage (BAL) protein, used as a marker of airway epithelial leakage/damage, with increased BAL total cell and neutrophil percentage, which was also seen to a lesser extent with DEP, but not carbon black. Similarly to the *in vitro* tests, RER PM but not carbon black or DEP induced increased TNFα and MIP-2 release in mice (measured in BALF) within 8 h, an increase in expression of MIP-12 but not MIP-2 or MIP-8. RER PM also induced an increased expression of the anti-oxidant gene HO-1, one of the most commonly used markers of antioxidant response to oxidative stress, which was not noted with carbon black or DEP. Therefore, this study suggests that PM from the Paris underground is generally more inflammatory then the other PM types tested, and a portion of this activity derived from the metallic nature of RER PM which is likely not as present in Metro PM. However, there is also a significant component of the effect of underground PM which does not appear to be related simply to the iron content of the PM. The authors also suggest that the RER environment is more worthy of investigation on account of its higher PM concentration (3.61µg/m^3 *vs.* 68 µg/m^3 in the metro).

The paper by Jung *et al* (2012) is unusual in studying the organic composition of underground PM_{10} [39]. This extract was able to cause significant cell death at concentrations tested in Chinese hamster ovary (CHO-K1) cells, but not BEAS-2B bronchial epithelial cells. Both the organic components, and their metabolic breakdown products, were shown to be able to induce micronucleus formation, indicative of DNA damage, and DNA breakage, in both cell types, which could be ameliorated by scavengers of superoxide, peroxide, and hydroxyl radical, with accompanying intracellular ROS generation noted. GC-MS-MS analysis of the organic extract showed the presence of ten of the US EPA sixteen criteria carcinogenic polyaromatic hydrocarbons (PAHs), although analysis of CYP1A1 expression, which is usually significantly upregulated by the presence of such PAHs, suggested that this underground PM_{10} was not sufficiently PAH-rich as to be able to upregulate CYP1A1 mRNA expression.

Loxham *et al*'s study in 2015 observed similar effects to the above studies, with PM concentration-dependent increases in IL-8 release and ROS generation, along with

upregulation of the antioxidant gene HO-1 [40]. However, the study was unusual in using primary cells differentiated at the air-liquid interface. This technique, using Transwell culture membranes, causes the cell culture to represent the *in vivo* epithelium more accurately than seen in a standard monolayer culture of epithelial cells grown in a plastic well. This can be seen especially in the formation of functional cilia and, of particular relevance to PM toxicology, mucus-secreting goblet cells. Thus, the apical surface of the culture is coated with a layer of mucus. However, underground PM (from Schiphol station outside Amsterdam) was able to cross this barrier and exert the above effects, as well as entering cells. This study is also unusual in examining the ultrafine fraction of underground PM, which is generally neglected by other studies. This fraction was found to be as iron/metal-rich as the other fractions and generated a larger ROS and IL-8 response than the larger size fractions [6, 40]. While the ultrafine fraction was present in a lower mass concentration than the coarse and fine fractions in the air of the underground station, this should be seen in the context of the overall greatly raised PM concentration underground, and thus the exposure to ultrafine PM underground is of potential relevance to the health impacts of underground railway air pollution.

The paper by Spagnolo *et al* looked at $PM_{10\text{-}2.5}$, $PM_{2.5\text{-}1}$, $PM_{1\text{-}0.5}$ and $PM_{0.5\text{-}0.25}$ collected at an underground platform, underground/intermediate commercial area, and outdoor site [41]. By MTT assay, the platform PM was more cytotoxic to H727 bronchial epithelial carcinoma cells than the commercial underground area PM (which showed no significant cytotoxicity), but the three smallest outdoor fractions were the most cytotoxic of all. At all three sites, larger PM fractions were better generators of ROS than the smaller fractions, although the $PM_{0.5\text{-}0.25}$ fraction of platform PM nonetheless generated significantly increased levels of ROS, which was not the case for the same smallest fraction of intermediate or outdoor PM. Levels of ROS generation were strongly correlated with a panel of metals (Mn, Cr, Ti, Fe, Cu, and Zn, and Ni and Mo). These are metals typically found elevated in PM in underground stations, although this paper did not show any mechanistic work to investigate causality of these relationships.

Moreno *et al* (2017) looked at the ability of PM collected from a range of different underground railway locations to deplete ascorbic acid and uric acid and to oxidise glutathione, both of which are measures of the oxidative potential of PM [42]. Other measures of oxidative potential have been evaluated, such as evaluation by electron spin resonance and oxidation of dithiothreitol, but ascorbic acid in particular has been shown to be responsive to the typical components of underground PM [43]). The Moreno study found that while $PM_{2.5}$ mass was not significantly correlated with OP, and iron was also not significantly linked to increased OP (in fact, it was statistically significantly negatively correlated with ascorbic acid depletion), OP was linked to copper, arsenic, manganese, zinc, and barium. While it has previously been suggested that in this type of study GSH is not susceptible to iron, ascorbic acid depletion certainly seems to be [44], and therefore it seems possible that the iron in this study was not redox-active, perhaps existing as haematite. The authors suggest that these correlations, along with the compositions of the most oxidative PM, indicate that a significant source of oxidative potential comes from brake and catenary wear. They also note that the lower oxidative potential is found in the newest station which has platform-edge doors (PEDs) – while PED's are shown to reduce pollution on the platform [34, 45], it is possible that the age of the station also plays a role in this particular paper.

In a similar manner, Gali and colleagues investigated the redox characteristics of PM from above ground and below ground routes of the Hong Kong underground, and compared this with PM collected from journeys on an overground train route, bus, and ambient PM [46]. Unlike other studies detailed here, not only was the underground concentration of coarse or fine PM not significantly elevated compared to above ground locations (it was actually lower than found in above ground train routes, with the lower concentrations underground attributed to the use of platform-edge doors), but coarse underground PM was seen to be more potent in reducing cell viability than coarse PM from above ground or bus journeys; for fine PM, there was little difference across samples, although fine PM was generally more cytotoxic than coarse PM. On a mass basis, underground PM was generally more potent in generating extracellular ROS than overground train and bus PM, and underground coarse PM was slightly more potent than underground fine PM in the generation of intracellular ROS, although there was general equipotency in terms of intracellular ROS generation by the fine PM samples. However, when considered on the basis of airborne PM concentration, by volume, overground train PM was more a more potent generator of ROS than underground PM. In underground PM, intracellular ROS generation was associated with mass concentration of aluminium, barium, copper, manganese, molybdenum, nickel, vanadium, magnesium, and sodium. Interestingly, there was no correlation with iron concentration, in agreement with Moreno and colleagues [42]. Furthermore, ROS generation by underground PM (and, indeed, overground rail and bus PM) was significantly lower than PM from urban sites in a previous study by the same authors – the implication is that metals are less soluble and therefore less bioavailable in underground PM, and this limits the ability of underground PM to generate intracellular ROS. However, the results of this study are difficult to compare to London underground, given the somewhat low concentration of PM and the relatively metal-poor composition of the PM.

Summary

A number of conclusions can be drawn from these studies. Those which have examined the effect on release of inflammatory cytokines from cell cultures exposed to PM (*in vitro*) indicate that underground PM is able to elicit release of the commonly studied inflammatory cytokines, including IL-6, IL-8, and TNFα. Where studies have directly compared the effects of underground PM to other types of PM, it seems that underground PM is more potent in this regard than typically used "control" particles, such as carbon black or titanium dioxide, and also compared to roadwear particles. However, it also appears that the effect of underground PM is less than that of urban/street PM. Reasons for this may include the presence of increased levels of endotoxin/lipopolysaccharide on urban PM, or the presence of organic species such as polyaromatic hydrocarbons. It is also possible that the greater particle number concentration of urban PM *vs.* underground PM plays a role in this effect. Regarding endotoxin, which Karlsson *et al* [30] suggested as a potential reason for the response to street PM, this may relate to the cells used – Karlsson *et al*'s use of macrophages represents a cell expressing the lipopolysaccharide co-receptor CD14, whereas epithelial cells, including A549, do not express CD14, and thus unless there is soluble CD14 in the culture medium, epithelial cells tend to respond much less avidly to lipopolysaccharide. Other studies here also follow this trend – macrophage responses to street PM tend to be higher than to underground PM, whereas epithelial responses do not, suggesting that endotoxin content of street PM may underlie this difference.

It is also important to take into consideration PM concentration/dose metrics. In *in vitro* studies, it is common to test identical mass concentration of PM, but inhaled air contains different PM mass concentrations – underground PM mass concentrations may be 10-25 times greater than those above ground (this varies greatly depending on the city and underground system, however). As such, a study showing that street PM is twice as potent at inducing inflammatory cytokine release as underground PM at an equal PM mass concentration implies that the PM load of the underground station air is more inflammogenic, although one cannot simply apply a multiplication as a correction factor as this assumes linearity of the concentration-response relationship across the concentrations involved.

Another observation is that underground PM appears to have a greater oxidative potential and a greater ability to exert oxidative damage *in vitro* than urban PM. Interestingly, one paper from the RAPTES study [47] found that PM oxidative potential was significantly negatively correlated with cell viability (as measured by MTT reduction assay), and that the ability of PM to induce inflammatory cytokine release from RAW264.7 macrophages *in vitro* was only significantly positively correlated with oxidative potential if the underground PM was excluded from the model (this might be related to the endotoxin content of PM affecting outcomes, as above). This, suggests that there are a number of factors which contribute to inflammatory cytokine release, and that it is not simply oxidative potential which elicits this response, while there are effects outside of inflammatory cytokine release which are related to oxidative potential. In this regard, the observation by some of the above studies that underground PM can elicit damage to DNA (through oxidised bases or strand breaks) and lipid (per)oxidation (by production of thiobarbituric acid-reactive substances (TBARS)) is important.

It is interesting that oxidative potential, as measured by the depletion of one or more of a number of antioxidants *in vitro*, appears to be a good predictor of cellular response with respect to certain endpoints *in vitro*, there is much less evidence to link it directly to effects *in vivo* (for the best example of this over a range of outcome measures, see the series of papers from the RAPTES study) [47, 48, 49].

The question arises as to the component(s) of underground PM important for its effect. In this regard, the fact that a number of papers show that iron chelation/redox inactivation is able to significantly reduce the effects of underground PM indicates that the iron content of underground PM (often in the range of 20-60% by mass) is important. This can also be seen where two underground systems in the same city but with different components are studied [38]. However, the finding that magnetite and haematite are unable to replicate these effects also suggests that there may be other important components, perhaps “working” in concert with the iron-containing species. One possibility is that there are other metals also involved – the Moreno study suggests that the PM responsible for the overall oxidative potential of underground PM may originate from brake and catenary wear, which implicates copper and barium, amongst others. There are suggestions that while iron-driven Fenton chemistry is critical in the generation of the hydroxyl radical from peroxide, copper may be more efficient in generation of other ROS [50, 51]. Thus, it is suggested that iron in a mixture with a lesser amount of copper may be the most efficient for overall driving of ROS-generating processes – it is potentially important to note that this is exactly what is found in underground PM. Furthermore, a requirement for multiple species for more efficient ROS generation would also

explain why (1) iron chelation significantly but often incompletely reduces the effects of underground PM, and (2) why insoluble metal PM in isolation or in a mixture which is dissimilar to underground PM (eg magnetite, haematite, or copper-zinc PM) cannot replicate the effects of underground PM. There is also the possibility that organic species may contribute, although since these species likely derive predominantly from the outside environment where trains run on electricity, and air exchange with the outside environment in the absence of active air conditioning is more limited as station depth increases, this may not apply to all stations, especially those on deep-lying lines as found in the deep-level “tube” lines in central London.

The ability of metal-rich PM to generate ROS is of relevance in asthma, where even in the mild form of the disease there is thought to be dysregulated antioxidant defence in the airways [52], and where *in vitro* evidence suggests that the airway epithelium may be more susceptible to oxidant-induced damage [53].

Although many papers on PM toxicology suggest than zinc is a transition metal, this is not true, since one defining feature of a transition metal is that it has an incompletely filled d-shell, which is not the case for zinc, where the d-shell is completely occupied. Furthermore, zinc exists almost exclusively in a single oxidation state (Zn(II)) – while the Zn(I) oxidation state is possible under certain conditions, these conditions are never attained in conditions where PM zinc would be found, and thus zinc cannot participate in ROS generating reactions as it cannot donate an electron to reduce molecular oxygen or peroxide. The confusion likely arises because zinc is located in the d-block of the periodic table, along with the true transition metals.

Overall, the papers in this section suggest that:

1. Underground PM is able to elicit an inflammatory response *in vitro* as measured by a number of cytokines.
2. This response may be outweighed by the response to street PM, but this is quite possibly due to the endotoxin concentration of street PM (underground PM appears to be endotoxin-poor), and thus this response is generally limited to macrophages rather than epithelial cells.
3. Underground PM can also elicit oxidative DNA damage.
4. The effect of underground PM is, in part, attributable to redox-active iron.
5. However, other components of underground PM, perhaps related to braking and electrical components, may well be of importance as well, and these components may act in concert.

(3) *In vitro-In vivo* extrapolation for Risk Assessment

There are a small number of studies which attempt to quantify health impacts of underground PM from data generated in *in vitro* experiments. Kam and colleagues assessed the health effects of PM on the Los Angeles underground in three studies. In the first, they compared the composition and ROS generating capacity of coarse and fine PM from an underground railway (combined collection from stations and on trains, to represent real-life exposure), overground railway (also stations and on trains), and ambient site at University of Southern California [54]. As with the great majority of similar studies, PM concentrations were significantly lower than in the London Underground. Underground PM_{10} concentrations were highest in the underground station, but this was driven by fine PM mass concentration, whereas coarse PM underground was higher than the overground station but similar to ambient concentrations. In terms of composition, the most notable difference was enrichment of iron in the underground samples compared to overground and ambient samples. Other non-crustal species enriched in the underground included manganese, chromium, cobalt, nickel, copper, barium, molybdenum, cadmium and europium. These elements tended to be more enriched in fine PM than in coarse PM. This enrichment was seen both on a PM mass and a PM volume concentration basis, being attributed to the specific sources of these elements underground and the enclosed environment of the underground, respectively. Ions and organic carbon species underground were suggested to have derived from outdoor sources. Crustal species were similar in coarse PM underground and above ground, but there was suggested to be an additional source of aluminium and calcium in fine PM underground. Correlation analyses suggested that aluminium and calcium, along with the aforementioned non-crustal species, likely derive from a single railway-specific source present above and below ground, as there was a generally high correlation between these elements. However, the authors also suggest additional other sources for barium (brake wear), copper, and zinc (sources for the copper are not suggested, although they possibly come from electrical contact components, while it is implied that zinc may also originate from above-ground vehicular emissions). Interestingly, when the water-solubility of these elements was examined, in both coarse and fine PM there was generally a lower solubility in underground PM than overground or ambient PM. When taking airborne PM mass concentration into account, this meant that only water-soluble iron and barium were enriched in underground PM compared to the other two sites. Across all sites, ROS generation was strongly correlated with water-soluble iron, nickel, chromium, cadmium (which is not redox active) and organic carbon. Further analysis indicated that, across all sites and PM types, 94% of ROS generating variability could be explained by water soluble iron and organic carbon concentrations. Fine PM across all sites possessed greater ROS-generating capacity than coarse PM. On a PM mass basis, underground coarse PM generated slightly more ROS than the other PM samples, while for fine PM overground railway generated more ROS than underground and ambient PM, which were approximately equipotent. When taking airborne concentration into account, on an air-volume basis, both coarse and fine PM at the underground site generated more ROS than the overground rail and ambient sites, but the magnitude of this difference was not as great as might be expected if only the elemental concentration of PM samples were considered.

A second study by the same group compared the underground and overground lines with two roads, one with low HGV usage, and one with high HGV usage [55].

Analysis of the potential lung cancer risk due to polyaromatic hydrocarbon exposure was performed, suggested that the lung cancer risk was highest from the HGV-high roadway, on account of the relatively higher concentrations of PAH. While the underground line had the lowest PAH concentration, it was suggested that the overground rail line, which had a PAH concentration almost as low as the underground line, along with a lower PM load and metal concentration than the underground line, may represent the safest route with its combination of low PAH and low PM.

However, it is also recognised that PAH are not the only carcinogen/potential carcinogen in airborne PM. Therefore, the same group performed another study, which attempted to evaluate the effects of metals within underground $PM_{2.5}$, both in terms of carcinogenicity and non-carcinogenic toxicity, and set these effects within the context of the risks from PAH exposure [56]. This was done by measuring concentrations of organic carbon and metal species in underground and overground railway systems, as well as the two roadway classes as in the previous study, and deriving the cancer-causing and toxicity potentials from values established by the US EPA and the California Office of Environmental Health Hazard Assessment. Notably, the metals found to be especially enriched in underground $PM_{2.5}$ were found to contribute to a greater increase in cancer and non-cancer risk than the PAHs enriched in traffic-derived PM. This was especially true for chromium, which was assumed to exist wholly in the highly carcinogenic hexavalent Cr(VI) form [57], as well as nickel, cobalt, and cadmium. For non-cancer risk (termed the “hazard quotient”, although it is not stated what these non-cancer risks are) the increased risk of underground PM exposure was driven by cadmium, chromium, nickel, and manganese. The cancer and non-cancer health risks were approximately one order of magnitude higher for underground exposure compared to roadway exposure. The authors note that while the permissible exposure levels set by the US Occupational Health and Safety Administration are not exceeded, the excess cancer risk, at 10^{-5} over a lifetime of exposure, is greater than that permitted 10^{-6}. This is driven primarily by chromium levels in the underground, which were found to be 100-1000 times greater than ambient concentrations. However, the conclusions of this paper rely partly on the key assumption that underground chromium in $PM_{2.5}$ exists in the Cr(VI) form on account of its high temperature formation – this may be incorrect, although a similar assumption has been made elsewhere [58]. Nonetheless, this study, and those above, provide evidence to support the assertion that underground PM should not be regarded as simply an iron-rich particle, but that consideration should be given to other metal constituents which may also play a role in PM toxicity, as well as the solubility (and hence bioavailability) of the metals, which may be lower in underground PM than in ambient PM.

Cao and colleagues monitored $PM_{2.5}$ and NO_2 concentrations in five railways stations in Suzhou, China, with different characteristics such as overground, underground, urban centre, and industrial area [59]. They found increased concentrations of $PM_{2.5}$ and decreased NO_2 in underground stations compared to overground stations, and in underground stations in urban areas compared to those in green areas. The observed underground platform $PM_{2.5}$ concentration of 265 $\mu g/m^3$ is much closer to that found on the London underground than is seen in most other studies of underground PM concentrations. Furthermore, underground $PM_{2.5}$ and NO_2 concentrations increased in rush hour. $PM_{2.5}$ concentrations were lower in carriages than on platforms, an observation attributed to the use of in-carriage air

filters. Underground PM2.5 in the summer was significantly lower than in the spring, which was suggested to be due to increased humidity in the summer, and demonstrated by significant negative correlation between the two. The authors then attempted to derive inhaled dose and use this value, along with underground PM= and NO_2 concentrations and journey numbers to calculate disability adjusted life years (DALYs), arriving at a value of 6390 DALYs in 2015, equating to 375 premature deaths, or 1% of the total deaths in the city. However, the authors did not consider a similar calculation for above-ground exposure, nor did they account for the unusual chemistry of $PM_{2.5}$ in the underground railway, or the likelihood that underground passengers may represent a relatively healthy, and therefore less susceptible, subgroup of the population. Thus, such figures cannot be taken as indicative.

Summary

The studies performed on the Los Angeles transport network illustrate the importance of considering the totality of PM composition, rather than focusing on a small number of PM constituents. The initial finding of enrichment of several metallic elements in underground railway PM is in line with the great majority of other studies, and the finding that this metal was relatively water insoluble in comparison to PM from urban sources is also consistent with other studies. It is interesting, however, that underground PM was not as potent in the generation of ROS as might have been expected given its transition metal content – this is likely due to its relative insolubility. Furthermore, given this lower-than-expected ROS generating capacity, initial calculations suggested that roadway PM may possess greater carcinogenic risk on the basis of its PAH content. The US EPA recognises 16 PAHs as carcinogenic and requiring of monitoring, and it was on this basis that the authors initially restricted their calculations to this group. However, they also considered metals, and in particular metals exerting toxicity by non-ROS mechanisms. Of particular relevance here is the formation of DNA adducts by Cr(III) formed from the intracellular reduction of Cr(VI). The paper illustrates that the carcinogenic and non-carcinogenic effects of non-ferrous metals in the underground PM outweighed significantly the effects of the PAH in the HGV-heavy road PM, albeit with certain assumptions being made. This assertion is unusual, in that it is a departure from the usually-considered chemical features of underground PM and mechanisms of its toxicity, specifically iron/steel-associated elements and ROS generation, respectively. While the specific risk factors, attributed DALYs, and similar derived values may not be reliable given their reliance on a number of assumptions, and the potential predominance of the underground passenger population towards a healthy demographic, such studies serve to highlight the potential effects of underground PM exposure on large populations, and the discussed studies in particular illustrate the diversity of potential toxicants within underground PM.

(4) Studies of the effects of exposure to iron-rich particles generated by processes such as grinding, polishing, and milling

In addition to a small number of studies on the *in vivo* effects of exposure to underground PM (section (1)), there are studies examining the effects of exposure to PM which, while not originating from an underground station, may be expected to be similar to underground PM in terms of chemical composition, or at least more so than ambient PM. While the original IOM report suggested that welding fume might represent a surrogate for underground PM, the majority of the mass of PM on the underground is likely to derive from shearing and abrasion, rather than formation at temperatures which may result in vaporisation of the metal. This may result in different particle characteristics, including size, number concentration, and surface chemistry. Similar processes take place in steel mills, and therefore studies of human exposure to steel mill emissions were examined as part of this working paper.

In the paper of Cakmak *et al* (2014), $PM_{2.5}$ iron concentrations near a steel plant were a mean 394 ng/m3 [60]. This is at least 2 orders of magnitude lower than might be seen in a London underground station. Furthermore, levels of other transition metals are probably even further away from what one might expect in an underground station. There were associations, dependent on the adjustments applied to the model, between metals and increased heart rate (calcium, tin, strontium), diastolic (cadmium, aluminium, calcium, manganese) and systolic blood pressure (cadmium, lead). Cadmium was also associated with decreases to a number of parameters of lung function, but only zinc and vanadium were also associated with decreased lung function. All changes were described by the authors as “small”, and no effect specific to $PM_{2.5}$ iron content was observed. This paper does not provide any evidence for or against the effect of iron in underground PM on the basis of the relatively iron poor nature of the $PM_{2.5}$ studied, and also in terms of the predominant effects of cadmium, manganese, vanadium, and tin, although it should also be noted that manganese is generally found in elevated levels in underground PM on account of its use in steel. This paper was a follow-up to an earlier paper where the effects of spending 5 consecutive 8-hour days near to a steel mill *vs.* distant from a steel mill was associated with small but significant decreases in a range of parameters of lung function. With respect to specific components of air pollution, which were generally slightly higher at the near-steel plant site, across both sites fine PM concentration was significantly associated with decreased FEV_1, FEF_{25-75}, TLC, and RV, while ultrafine PM concentration was significantly associated with decreased FVC, FEF_{25-75}, and TLC. However, there were also negative associations between these lung function parameters and concentrations of sulphur dioxide and nitrogen dioxide. While FEV_1/FVC, FEF_{25-75}, TLC, FRC, and RV were lower at the near-steel plant site, there was only a non-significant trend towards these effects being more pronounced when the subjects were downwind, rather than upwind, of the study site. Therefore, it is impossible to conclude that the subtle effects on lung function at the near-steel plant site were due to PM (since PM concentrations were not shown to be independent of NO_2/SO_2 concentrations), or that they related to emissions from the steel mill, rather than from another source near the steel mill. [61]. A recently published cross-over study by the same group (seemingly using the same subjects as the previous two studies), at the same two sites, similarly found associations between raised pollutant concentrations at the near-plant site and

decreased heart rate variability [62]. However, the authors also found that several of the pollutants were correlated with each other. As such, these findings cannot be taken to be suggestive of an effect of steel mill PM emissions specifically, and are therefore not suitable for understanding the effect of underground railway PM.

The paper by Pavanello [63] shows steel workers whose exposure to PM_{10} and PM_1 is roughly similar to what might be expected in an average underground station, although it is notable that the proportion of iron in this PM (mean 32 $\mu g/m^3$ in a mean PM_{10} load of 233 $\mu g/m^3$) is somewhat lower than that in underground PM. 17 extracellular vesicular microRNAs were significantly correlated with metal exposure (it appears that a total of 88 miRs were evaluated), all of which were positive correlations except for the association between mir30a and barium. Mir196b was the most strongly positively associated with metal exposure. A number of papers in the literature suggest that this microRNA is linked to a poor prognosis in a number of cancers, with a role in epithelial-to-mesenchymal transition and thus metastatis [64], and also a potential role in insulin biosynthesis [65]. When inflammatory, coagulation, and oxidative outcomes were examined, 3 microRNAs were significantly linked to outcomes, but levels of these microRNAs were only infrequently linked to metal exposure (2/3 linked to cadmium, 1/3 to each of aluminium, lead, and zinc). Analysis of the network of microRNAs with altered regulation showed alteration of networks related to (amongst others) connective tissue disorders, cell morphology, and cell development, and further analysis of these networks suggested a potential effect on platelet activation. Another analysis indicated that toxicological pathways modified may include cardiac hypertrophy, cardiac necrosis/cell death, p53 signalling, renal necrosis/cell death and hypoxia-inducible factor signalling. While these findings are interesting, they are arguably simply grounds for further research. Omics studies are excellent sources of hypotheses for future work, but the extent of their reliance on computer algorithms means that they cannot be regarded as being of use in coming to reliable conclusions about effects on health, especially because this study did not further the findings of the omics analysis with no validation of the results in terms of physiological endpoints related to the identified perturbations in microRNA expression, for example measurement of blood coagulation in exposed workers. The Pavanello group had previously examined changes in blood microvesicle miRNAs [66] and found upregulation of miR-302c and miR-128 after 4 days of work following a 2 day rest period, with miR-28-3p, let-7 g, miR-125a-5p and miR-181a up regulated but not crossing the significance threshold after false discovery rate correction for multiple testing. Algorithms mapping microRNAs onto their targets suggested that miR-302c and miR-128 might overlap to affect NF-kB signalling, and that they may be involved in toxicity manifesting as cardiac hypertrophy, cardiac dysfunction, and dysregulated cholesterol metabolism, amongst others. Treatment of A549 cells with urban PM produced increased miR-128 expression, but no detectable miR-302c. However, the relevance of this part of the work to underground PM is unclear, since it used SRM1648a, a NIST standard urban PM collected from St Louis in the 1970s. This PM is less metal rich than steel mill PM and underground PM, and therefore this finding is of uncertain significance in understanding the effects of steel mill and underground PM.

Tarantini *et al* (2014) looked at the methylation of a number of blood coagulation-related genes in steel plant workers [67]. Increased gene methylation (hypermethylation) usually acts to repress gene transcription, whereas decreased

gene methylation (hypomethylation) increases gene transcription. In their study of what appears to be the same group of workers as the paper of Pavanello *et al* (above), PM_{10} and PM_1 levels were associated with decreased methylation of NOS3, coding for endothelial nitric oxide synthase (a key regulating pathway for cardiovascular homeostasis, which is impaired in cardiovascular disease), as was total zinc concentration, which was also associated with decreased EDN1 (coding for endothelin-1) methylation. Iron was borderline-significantly associated with decreased NOS3 methylation. Blood endogenous thrombin potential (ETP), which represents the pro-coagulant/anticoagulant balance in blood, increased with overall PM_{10} and PM_1 exposure and also zinc exposure. Decreases in EDN1 and NOS3 methylation were both linked to increased ETP, but there was no statistical link to C-reactive protein levels in the same population, suggesting that these methylation/ETP effects occur separately from systemic inflammation. Using the same group of workers, Cantone *et al* (2011) showed that, in blood leukocytes, there was increased demethylation of histone 3 lysine 4 (H3K4), and acetylation of histone 3 lysine 9 (H3K9) with increasing length of employment in the steel plant (irrespective of subject age) [68]. Furthermore, H3K4 demethylation was positively associated with nickel, arsenic, and iron exposure, while H3K9 acetylation was positively associated with nickel and iron, although neither reached significance. After determination of approximate cumulative exposure to metals by accounting for employment duration and individual exposure during the study period, H3K4 dimethylation and H3K9 acetylation were positively and significantly associated with nickel and arsenic exposure, while a non-significant association of both to cumulative iron exposure was also noted. However, no attempt was made to examine these changes in lung tissue. Although the authors state that there is evidence that these histone modifications can lead to relaxation of chromatin structure, potentially altering gene transcription to favour carcinogenesis, there is no obvious link which suggests *per se* that these changes may induce cancer. Furthermore, the exposures in these studies are sufficiently different to underground PM to make them relatively unrepresentative, although they are perhaps the closest which could be achieved, in terms of the generation of iron/transition metal-rich PM by friction and shearing, without actually performing these experiments in underground systems.

The work of Lall *et al* (2011) examined associations between $PM_{2.5}$ and respiratory and cardiovascular hospital admissions in New York in 2001 and 2002 [69]. Source apportionment of the $PM_{2.5}$ by positive matrix factorisation allowed the study to examine the effects on hospital admissions of different sources of $PM_{2.5}$. They show that steel emissions, correlated with iron and manganese, and likely increased by World Trade Center site welding and construction, were significantly associated with increased respiratory admissions with a 0 and 3 day lag (but not 1 and 2 day lag), and further investigation found links to asthma admissions on the same day as pollution exposure, and pneumonia 3 days after exposure. Conversely, traffic PM, which was not associated with respiratory admissions, was linked to total cardiovascular admissions with 0 days lag, while cardiovascular admissions were not linked to steel emissions. The link between steel emissions and respiratory admissions was strongest in Manhattan, followed by the Bronx, but not linked to those in Queens or Staten Island, suggesting that the WTC may have been the source, although the link was robust even when cleanup days following the WTC attack were excluded. The main conclusion from this study is that although steel and traffic $PM_{2.5}$ both have effects on hospital admissions, their different effects suggest

different mechanisms, thus implying that simply mass based metrics for $PM_{2.5}$ limits was be insufficient to protect health.

Summary

These studies, which generally focus on steel production-associated PM, and PM sources with a steel signature, were originally suggested for inclusion in the Sub-Committee's discussions as offering a potential alternative to exposure to welding fume, which does not offer an ideal comparator to underground PM on account of the shear/friction generation of the iron-rich PM underground, compared to the high temperature spark generation of iron to vapour, which then condenses to form iron-rich PM in welding. There are some reasons to think that this hypothesis may be true:

1. The level of PM to which the iron workers are exposed in the Italian steel production-plant studies here are not dissimilar to those which might be found in a typical underground station, and the PM is certainly enriched in iron compared to that which would be seen above ground.

2. This enrichment is clearly not simply a fume-derived process as is the case for welding fume, and thus the toxicity might not be expected to be as high as that from welding fume which, on account of its generation method, results in large numbers of PM of a small diameter, with consequent high particle number concentration and total particle surface area.

3. When studying effects *in vivo* on workers in steel plants and underground trains, it might be reasonable to assume that both groups would likely be composed of mainly young and middle aged workers and, if sex is taken into account, both underground drivers and steel production workers are predominantly male. However, the latter may not apply to staff elsewhere on the underground eg ticket office and platform staff.

Therefore, it is true that these studies provide an alternative, and perhaps more physicochemically similar comparator to underground PM rather than does welding fume. As such, it is reasonable to suggest that steel plant/steel mill PM studies should be considered at when trying to better understand the effects of underground PM. However, there are also a number of reasons to think that they do not provide a close enough approximation to allow general extrapolation of results without clear caveats. There are a few reasons for this:

1. The proportion of iron in the PM to which the workers were exposed is around 14% (according to the Italian studies here), which is rather less that what would be expected in an underground environment (underground PM seems to be ~40-65% iron).

2. In the underground, as well as PM deriving from wheel and rail interactions, there is also PM deriving from catenary/third rail shoe interactions, and also braking systems, which is likely to enrich the environment in metallic elements other than those found in steel. In some of the papers detailed in other sections of this working paper, these other elements have been suggested to be important drivers of some of the effects of underground PM. This is a potentially crucial difference.

3. Four of these six papers (ie Pavanello, Tarantini, Bollati, Cantone [63, 66, 67, 68]) derive from studies of the same group of 63 workers in the same steel production plant in Italy. Thus any conclusions will unavoidable be biased towards the characteristics of this study population and/or the working environment.

In terms of the findings of the studies, their evidence for an effect of underground PM is generally minimal: Cakmak *et al* show links between small changes in respiratory and cardiovascular function with certain elements, but none of these elements predominate in underground PM. The microRNAs in extracellular vesicles in the papers by Pavanello and Bollati suggest effects on cancer and cardiovascular outcomes, but such studies should serve as the basis for focusing of future work, and not as and end point in themselves (they are effectively hypothesis-generating studies), and the same is true of the epigenetic studies by Tarantini and Pavanello. They are certainly interesting studies and should lead to further work, but no conclusions about the end effects of steel production PM, let alone underground PM, can be drawn. The paper of Lall *et al* is perhaps the most interesting, in that it shows Fe/Mn (which was used as a signature for steel) linked to adverse respiratory admissions, and that the effect seems to be different to street PM. However, it must be taken into account that these extra admissions may well have been restricted to vulnerable subgroups who would be unlikely to use the underground. Nonetheless, this paper is of interest in showing a link between Fe/Mn rich PM and adverse respiratory outcomes.

(5) RAPTES - Risk of Airborne Particles, a Toxicological-Epidemiological hybrid Study

The RAPTES study was a multi-part study of the effects of exposure to PM from a range of locations in the Netherlands, attempting to link source and composition to effect. Although these studies are mainly performed through human exposures *in vivo*, they merit separate consideration given that the same sites are used for analysis from PM chemistry, through *in vitro* effects, to *in vivo* effects, allowing comparisons to be drawn between conclusions across different experimental setups. Sites used in the study were an underground railway station, urban background site, farm, three traffic sites (continuous traffic, stop-go traffic, and truck traffic), a harbour, and an area near a steelworks. Mass concentration of PM_{10} and $PM_{2.5}$ was found to be much higher at the underground station than other sites (394 and 137 $\mu g/m^3$, respectively), although particle number concentration was lower than at the road traffic sites. On a volume basis, the concentration of PM iron and copper was approximately two orders of magnitude higher than any other site, nickel was one order of magnitude higher, while vanadium concentration was comparable to the road and harbour sites. Underground air was 10 times more EC-rich in the coarse PM fraction than the other sites, and had an EC/OC ratio of 2.5, much higher than any other site., while the fine PM (per volume of air) was also more EC rich, although to a lesser extent) and had an EC/OC ratio comparable to the traffic sites [70]. Across coarse, fine, and quasi-ultrafine (<0.18 µm) fractions, underground PM had a greater effect on RAW 264.7 macrophage viability assessed by MTT reduction. However, although coarse underground PM was most able of all coarse PM sources to elicit TNFα and MIP-2 release, underground PM was less active in this regard for fine and quasi-ultrafine fractions (traffic fractions were notably more potent). Moreover, underground PM appeared unable to elicit IL-6 release across fractions, although this appeared true for other PM fractions except fine and ultrafine traffic and steelworks PM. While across all samples there was no association between PM oxidative potential and cytokine release, a positive association was seen when underground PM was excluded, suggesting that underground PM possesses fundamentally different chemistry from the other PM types [47].

Following *in vitro* studies, five of the sites were used for human exposure studies (truck traffic, steelworks, and harbour sites were excluded). Unfortunately, the raw data of exposure endpoints at these sites was not published; rather, the studies focuses on understanding the specific component(s) of air pollution driving the observed responses. In human exposure, fraction of exhaled nitric oxide (FE_{NO}), representing eosinophilic airway inflammation [71], was associated with PM iron, vanadium, copper, and water-soluble nickel, while iron, copper, and water-soluble nickel were also associated with loss of lung function as measured by FVC and FEV_1. However, changes in lung function were not associated with PM_{10} mass concentration or oxidative potential [49]. A study of markers of inflammation in nasal lavage after exposure at the different study sites found that concentrations of the inflammatory cytokines IL-6 and IL-8 were associated with organic carbon, NO_2 and endotoxin levels. However, levels of lactoferrin (a metal-binding protein with both pro- and anti-inflammatory properties) were seen to be related to the high metal concentration found at the underground site [72]. Similarly, levels of markers of cardiovascular risk, including C-reactive protein, fibrinogen, von Willebrand factor, and tissue plasminogen activator/plasminogen activator inhibitor-1 complex, as well as platelet count, were associated with exposure to organic carbon, nitrate, and

sulphate (although the latter two may reflect increased bioavailability of metals) [73]. In extension of this, thrombin generation (a marker of propensity for blood coagulation, and thus a cardiovascular risk marker) in blood taken from exposed volunteers, was associated with NO_2 concentration, and also PM nitrate and sulphate [74]. Examination of circulating white blood cells showed that after 2 h exposure, there was an increase in neutrophil count, while after 18 h exposure there was an increase in circulating monocytes [75]. These effects were linked to mass concentration of PM_{10} and $PM_{2.5}$, elemental carbon, and PM oxidative potential. These associations were, however, driven by underground exposure, and were not present in when underground exposures were excluded from analyses. Because of the consistently much higher concentration of certain PM characteristics at the underground site compared to the other study sites, it was not possible to determine the characteristics driving this response, but it is notable that most factors other than PM oxidative potential were excluded.

Summary

The RAPTES series of studies identified various PM characteristics which vary according to sampling site. A range of *in vitro* and *in vivo* endpoints were studied to determine PM (as well as gaseous pollutant) characteristics which may be associated with these endpoints. Interestingly, different endpoints were responsive to different PM characteristics. Furthermore, it can be seen that short term (2 h) exposures were sufficient to induce measurable changes *in vivo*, which may be important in understanding the potential effects of underground air exposure on passengers whose exposures are likely shorter than workers. While it was noted that some (although not all) of these endpoints were especially responsive to underground PM, it is also notable that in several cases, correlations between PM characteristics and endpoint were driven by the presence/absence of underground PM in the analyses, implying that underground PM represented a distinct type of PM compared to the other PM types analysed. This is not to say, however, that the endpoints measured were always positively correlated with factors enriched in underground PM – for example, in several cases, outcomes were associated with organic carbon concentrations. Furthermore, the fact that the underground PM was apparently so different to the other PM samples tested, means that it was difficult to delineate the specific components of underground PM responsible for its effects.

Conclusions

The studies described in this working paper use a variety of techniques to investigate the effects of underground PM. From *in vitro* studies using cell lines, through *in vitro-in vivo* extrapolation from chemical composition, to controlled exposure studies *in vivo*, and epidemiological studies of underground railway workers, there are some conclusions in common across most of the studies in which they are addressed:

1. Underground railways generally have an increased airborne PM mass concentration (ie micrograms of PM per cubic metre of air).

2. Factors which increase the extent to which underground PM is different from ambient PM have been shown/suggested to include station depth, station age, wheel/rail type, air conditioning system, and distance from portal.

3. Underground PM is generally transition metal-rich, whether measured per mass of PM, or per volume of air. Iron is the predominant element, but there is a general enrichment of steel-associated elements, and also elements associated with train brake wear, electrical components, and lubricants.

4. The proportion of the mass of transition metals in underground PM which is water-soluble is notably lower than in urban PM. As such, the airborne mass concentration of water-soluble transition metal within underground PM is not necessarily as elevated above ambient PM as might be expected, were only the airborne PM mass concentration and total metal concentration to be considered.

5. *In vitro*, underground PM appears to be better able to elicit ROS generation/antioxidant depletion than does ambient PM. This is frequently shown to be dependent on underground PM transition metal composition.

6. This metal-related ROS generation appears at least partly to underlie the oxidative damage of DNA, and induction of antioxidant expression, which is generally observed in cells exposed to underground PM.

7. Per mass of PM, underground PM is generally poor in organic carbon and anions such as sulphate and nitrate. The latter may be underlie the poor solubility of metals in underground PM.

8. The apparent potency of underground PM in eliciting inflammatory cytokine release may depend on whether the cell type used is sensitive to endotoxin, which is found to be enriched in ambient PM compared to underground PM. Thus, endotoxin-sensitive cells (eg macrophages), may appear to be especially sensitive to ambient PM, while endotoxin-insensitive cells (eg epithelial cells) may appear to be relatively less sensitive to ambient PM, and relatively more sensitive to underground PM.

9. The effects of underground PM which are dependent on PM composition may not solely relate to simple elemental composition, but may also be a function of elemental oxidation state. For example, elements existing in their lower oxidation states (where multiple states are possible) can exert oxidative stress through electron donation, and other elements may be able to enter cells and exert effects through oxidation state-selective entry mechanisms (as is the case for the carcinogenicity of Cr(VI) *vs.* Cr(III)). As such, while understanding

the elemental composition of PM may aid understanding of potential toxicity/toxic mechanisms of PM, knowledge of PM oxidation state aids this further. However, oxidation state may also be altered through the highly reducing environment of the airways (principally antioxidants in the respiratory tract/lung lining fluid), and so oxidation states of elements in collected PM may not necessarily mirror that of the same elements when they reach the cell surface.

10. The association of transition metals and ROS generation with various endpoints *in vitro* is not obviously consistently apparent in studies *in vivo*. This may be due to the way that complex tissues and organs, comprised of different, interacting cell types, respond, to the insult of PM compared to the response *in vitro*.

11. It is also important to note that cell exposure studies *in vitro* generally use concentrations of PM which are significantly greater than those which are likely to result from *in vivo* exposure, often to the extent that such concentrations would be unattainable in normal exposure scenarios. Such excessively high concentrations may trigger mechanisms which are not physiologically relevant to real-life exposures.

12. As well as having a significantly increased PM mass concentration and PM metal concentration, underground railways also tend to have lower concentrations of traffic-related gaseous pollutants (eg NO_2) compared to above-ground locations.

13. Calculations of the potential health risks of underground PM on the basis of PM composition have suggested that exposure to underground PM may be associated with an increased risk of carcinogenicity and non-cancer health effects. This is on account of the metal-rich composition of underground PM, which outweighs the toxicity of PAH in ambient PM. This generally, but not exclusively, relates to transition metals. Such studies have served to highlight the potential risks associated with non-ferrous metals in underground PM, such as chromium, nickel, cobalt, manganese and cadmium, although there is little evidence that airborne concentrations of these metals exceed exposure limits.

14. Experimental exposure studies in underground stations have shown some associations of metals with respiratory endpoints, although these were not always associated with PM oxidative potential. However, the same studies have found other endpoints unrelated to PM metals, and instead related to endpoints such as PM organic carbon and anions, both of which comprise relatively ow proportions of underground PM mass compared to ambient PM.

15. Studies of underground railway workers have generally found little to no association of disease endpoints with working in the underground, which may relate in part to the relatively small sample sizes being used, the paucity of such studies, or the fact that the working populations studied represent a relatively non-susceptible subset of the population. However, it also cannot be discounted that there would be no association found in the general population either. Furthermore, no consistent modifying effect of asthma on these findings has been noted.

16. Studies of workers in steel mills/steel plants, whose exposures have been suggested to represent an approximation to exposures in underground networks, have also found no consistent effects. However, exposures in these studies are generally lower in PM mass concentration and PM metal content than underground PM exposures, and also generally higher in pollutant gases (eg NO_2, SO_2) than underground.

17. Steel mill studies have noted effects of mill-related exposures on lung function following relatively short term exposure, and differential expression of microRNAs and altered DNA methylation inn steel mill workers. However, none of these studies could attribute such changes to the transition metal components of steel mill PM emissions, nor is the physiological manifestation (if any) of the molecular changes known. One study did show association of a steel-type iron-manganese signature in PM in New York City with hospital respiratory admissions, but not cardiovascular admissions. This is in broad agreement with the RAPTES studies, which found transition metals were associated with effects on lung function, but that cardiovascular endpoints were more associated with components in which underground PM is poor. However, these studies in isolation do not provide evidence for a likely effect of underground PM exposure on health, although they do suggest that further research should be performed.

Overall, while both the increased PM mass concentration and metal content are suggestive of, and seen to be responsible for, effects on various toxicological endpoints *in vitro*, there is much less evidence to indicate overt toxicity of underground PM exposures *in vivo*. While it appears that the unusual composition of underground PM may underlie some of its effects, and that some of the effects of underground PM may be different to the effects of ambient PM, it is certainly not the case that these effects of underground PM are necessarily of a greater magnitude than those of ambient PM. Furthermore, there are pollutants such as PAHs and NO_2 which are associated with toxicity through various mechanism which may be found at higher concentrations in ambient air compared to underground air.

There is little evidence, from the small number of studies, that the physicochemical characteristics of underground PM translate to a significantly increased risk of disease in workers, although it is clear that further work in this area is required, especially well-designed studies with study populations significantly larger than those hitherto used. From a mechanistic viewpoint, more attention needs to be paid to the non-ferrous components of underground PM, ROS- and non-ROS-related mechanisms of toxicity of underground PM, alternative endpoints of PM toxicity as they become identified by epidemiological research, and the distinct effects (if any) of acute exposure *vs.* chronic, repeated exposure over a lifetime, however short the individual exposures may be.

References

1. Sahin UA, Onat B, Stakeeva B, Ceran T, Karim P. PM10 concentrations and the size distribution of Cu and Fe-containing particles in Istanbul's subway system. Transport Res D-Tr E. 2012;17 1:48-53;

2. Querol X, Moreno T, Karanasiou A, Reche C, Alastuey A, Viana M, et al. Variability of levels and composition of PM10 and PM2.5 in the Barcelona metro system. Atmospheric Chemistry and Physics. 2012;12 11:5055-76;

3. Jung HJ, Kim B, Ryu J, Maskey S, Kim JC, Sohn J, et al. Source identification of particulate matter collected at underground subway stations in Seoul, Korea using quantitative single-particle analysis. Atmos Environ. 2010;44 19:2287-93;

4. Colombi C, Angius S, Gianelle V, Lazzarini M. Particulate matter concentrations, physical characteristics and elemental composition in the Milan underground transport system. Atmos Environ. 2013;70:166-78;

5. Moreno T, Perez N, Reche C, Martins V, de Miguel E, Capdevila M, et al. Subway platform air quality: Assessing the influences of tunnel ventilation, train piston effect and station design. Atmos Environ. 2014;92:461-8;

6. Loxham M, Cooper MJ, Gerlofs-Nijland ME, Cassee FR, Davies DE, Palmer MR, et al. Physicochemical characterization of airborne particulate matter at a mainline underground railway station. Environ Sci Technol. 2013;47 8:3614-22;

7. Eom H-J, Jung H-J, Sobanska S, Chung S-G, Son Y-S, Kim J-C, et al. Iron Speciation of Airborne Subway Particles by the Combined Use of Energy Dispersive Electron Probe X-ray Microanalysis and Raman Microspectrometry. Anal Chem. 2013;85 21:10424-31;

8. Xu B, Hao JL. Air quality inside subway metro indoor environment worldwide: A review. Environ Int. 2017;107:33-46;

9. Klepczynska Nystrom A, Svartengren M, Grunewald J, Pousette C, Rodin I, Lundin A, et al. Health effects of a subway environment in healthy volunteers. Eur Respir J. 2010;36 2:240-8;

10. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. Nature Reviews Immunology. 2008;8 7:523-32;

11. Akdis M, Aab A, Altunbulakli C, Azkur K, Costa RA, Crameri R, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: Receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2016;138 4:984-1010;

12. Deiuliis JA, Kampfrath T, Zhong JX, Oghumu S, Maiseyeu A, Chen LC, et al. Pulmonary T cell activation in response to chronic particulate air pollution.

American Journal of Physiology-Lung Cellular and Molecular Physiology. 2012;302 4:L399-L409;

13. Nadeau K, McDonald-Hyman C, Noth EM, Pratt B, Hammond SK, Balmes J, et al. Ambient air pollution impairs regulatory T-cell function in asthma. J Allergy Clin Immunol. 2010;126 4:845-U280;

14. Danesh J, Lewington S, Thompson SG, Lowe GDO, Collins R, Collaboration FS. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality - An individual participant meta-analysis. Jama-J Am Med Assoc. 2005;294 14:1799-809;

15. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate Matter Air Pollution and Cardiovascular Disease An Update to the Scientific Statement From the American Heart Association. Circulation. 2010;121 21:2331-78;

16. Ljungman P, Bellander T, Schneider A, Breitner S, Forastiere F, Hampel R, et al. Modification of the Interleukin-6 Response to Air Pollution by Interleukin-6 and Fibrinogen Polymorphisms. Environ Health Perspect. 2009;117 9:1373-9;

17. Bigert C, Alderling M, Svartengren M, Plato N, de Faire U, Gustavsson P. Blood markers of inflammation and coagulation and exposure to airborne particles in employees in the Stockholm underground. Occup Environ Med. 2008;65 10:655-8;

18. Bigert C, Klerdal K, Hammar N, Gustavsson P. Myocardial infarction in Swedish subway drivers. Scand J Work Environ Health. 2007;33 4:267-71;

19. Lundstrom SL, Levanen B, Nording M, Klepczynska-Nystrom A, Skold M, Haeggstrom JZ, et al. Asthmatics Exhibit Altered Oxylipin Profiles Compared to Healthy Individuals after Subway Air Exposure. PLoS One. 2011;6 8;

20. Gouveia-Figueira S, Karimpour M, Bosson JA, Blomberg A, Unosson J, Pourazar J, et al. Mass spectrometry profiling of oxylipins, endocannabinoids, and N-acylethanolamines in human lung lavage fluids reveals responsiveness of prostaglandin E2 and associated lipid metabolites to biodiesel exhaust exposure. Anal Bioanal Chem. 2017;409 11:2967-80;

21. Grass DS, Ross JM, Family F, Barbour J, Simpson HJ, Coulibaly D, et al. Airborne particulate metals in the New York City subway: A pilot study to assess the potential for health impacts. Environ Res. 2010;110 1:1-11;

22. Mehrdad R, Aghdaei S, Pouryaghoub G. Urinary 8-hydroxy-deoxyguanosine as a biomarker of oxidative DNA damage in employees of subway system. Acta Med Iran. 2015;53 5:287-92;

23. Liu WT, Ma CM, Liu IJ, Han BC, Chuang HC, Chuang KJ. Effects of commuting mode on air pollution exposure and cardiovascular health among young adults in Taipei, Taiwan. International Journal of Hygiene and Environmental Health. 2015;218 3:319-23;

24. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. Int J Cardiol. 2010;141 2:122-31;

25. Kelly FJ, Fussell JC. Role of oxidative stress in cardiovascular disease outcomes following exposure to ambient air pollution. Free Radic Biol Med. 2017;110:345-67;

26. Klepczynska-Nystrom A, Larsson BM, Grunewald J, Pousette C, Lundin A, Eklund A, et al. Health effects of a subway environment in mild asthmatic volunteers. Respir Med. 2012;106 1:25-33;

27. Seaton A, Cherrie J, Dennekamp M, Donaldson K, Hurley JF, Tran CL. The London Underground: dust and hazards to health. Occup Environ Med. 2005;62 6:355-62;

28. Peters A, Wichmann HE, Tuch T, Heinrich J, Heyder J. Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med. 1997;155 4:1376-83;

29. Karlsson HL, Nilsson L, Moller L. Subway particles are more genotoxic than street particles and induce oxidative stress in cultured human lung cells. Chem Res Toxicol. 2005;18 1:19-23;

30. Karlsson HL, Ljungman AG, Lindbom J, Moller L. Comparison of genotoxic and inflammatory effects of particles generated by wood combustion, a road simulator and collected from street and subway. Toxicol Lett. 2006;165 3:203-11;

31. Karlsson HL, Holgersson A, Moller L. Mechanisms related to the genotoxicity of particles in the subway and from other sources. Chem Res Toxicol. 2008;21 3:726-31;

32. Lu SL, Liu DY, Zhang WC, Liu PW, Fei Y, Gu Y, et al. Physico-chemical characterization of PM2.5 in the microenvironment of Shanghai subway. Atmos Res. 2015;153:543-52;

33. Salma I, Posfai M, Kovacs K, Kuzmann E, Homonnay Z, Posta J. Properties and sources of individual particles and some chemical species in the aerosol of a metropolitan underground railway station. Atmos Environ. 2009;43 22-23:3460-6;

34. Jung HJ, Kim B, Malek MA, Koo YS, Jung JN, Son YS, et al. Chemical speciation of size-segregated floor dusts and airborne magnetic particles collected at underground subway stations in Seoul, Korea. J Hazard Mater. 2012;213:331-40;

35. Vidrio E, Jung H, Anastasio C. Generation of hydroxyl radicals from dissolved transition metals in surrogate lung fluid solutions. Atmos Environ. 2008;42 18:4369-79;

36. Lindbom J, Gustafsson M, Blomqvist G, Dahl A, Gudmundsson A, Swietlicki E, et al. Exposure to wear particles generated from studded tires and pavement induces inflammatory cytokine release from human macrophages. Chem Res Toxicol. 2006;19 4:521-30;

37. Lindbom J, Gustafsson M, Blomqvist G, Dahl A, Gudmundsson A, Swietlicki E, et al. Wear particles generated from studded tires and pavement induces inflammatory reactions in mouse macrophage cells. Chem Res Toxicol. 2007;20 6:937-46;

38. Bachoual R, Boczkowski J, Goven D, Amara N, Tabet L, On D, et al. Biological effects of particles from the Paris subway system. Chem Res Toxicol. 2007;20 10:1426-33;

39. Jung MH, Kim HR, Park YJ, Park DS, Chung KH, Oh SM. Genotoxic effects and oxidative stress induced by organic extracts of particulate matter (PM10) collected from a subway tunnel in Seoul, Korea. Mutat Res-Gen Tox En. 2012;749 1-2:39-47;

40. Loxham M, Morgan-Walsh RJ, Cooper MJ, Blume C, Swindle EJ, Dennison PW, et al. The effects on bronchial epithelial mucociliary cultures of coarse, fine, and ultrafine particulate matter from an underground railway station. Toxicol Sci. 2015;145 1:98-107;

41. Spagnolo AM, Ottria G, Perdelli F, Cristina ML. Chemical Characterisation of the Coarse and Fine Particulate Matter in the Environment of an Underground Railway System: Cytotoxic Effects and Oxidative Stress-A Preliminary Study. Int J Env Res Pub He. 2015;12 4:4031-46;

42. Moreno T, Kelly FJ, Dunster C, Oliete A, Martins V, Reche C, et al. Oxidative potential of subway PM2.5. Atmos Environ. 2017;148:230-8;

43. Janssen NA, Yang A, Strak M, Steenhof M, Hellack B, Gerlofs-Nijland ME, et al. Oxidative potential of particulate matter collected at sites with different source characteristics. Sci Total Environ. 2014;472:572-81;

44. Zielinski H, Mudway IS, Berube KA, Murphy S, Richards R, Kelly FJ. Modeling the interactions of particulates with epithelial lining fluid antioxidants. American Journal of Physiology-Lung Cellular and Molecular Physiology. 1999;277 4:L719-L26;

45. Kim KH, Ho DX, Jeon JS, Kim JC. A noticeable shift in particulate matter levels after platform screen door installation in a Korean subway station. Atmos Environ. 2012;49:219-23;

46. Gali NK, Jiang SY, Yang FH, Sun L, Ning Z. Redox characteristics of size-segregated PM from different public transport microenvironments in Hong Kong. Air Qual Atmos Hlth. 2017;10 7:833-44;

47. Steenhof M, Gosens I, Strak M, Godri KJ, Hoek G, Cassee FR, et al. In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands

is associated with PM composition, size fraction and oxidative potential - the RAPTES project. Part Fibre Toxicol. 2011;8:26;

48. Ayres JG, Borm P, Cassee FR, Castranova V, Donaldson K, Ghio A, et al. Evaluating the toxicity of airborne particulate matter and nanoparticles by measuring oxidative stress potential - A workshop report and consensus statement. Inhal Toxicol. 2008;20 1:75-99;

49. Strak M, Janssen NAH, Godri KJ, Gosens I, Mudway IS, Cassee FR, et al. Respiratory Health Effects of Airborne Particulate Matter: The Role of Particle Size, Composition, and Oxidative Potential-The RAPTES Project. Environ Health Perspect. 2012;120 8:1183-9;

50. Shen HY, Anastasio C. A comparison of hydroxyl radical and hydrogen peroxide generation in ambient particle extracts and laboratory metal solutions. Atmos Environ. 2012;46:665-8;

51. Charrier JG, Anastasio C. Impacts of antioxidants on hydroxyl radical production from individual and mixed transition metals in a surrogate lung fluid. Atmos Environ. 2011;45 40:7555-62;

52. Kelly FK, Mudway J, Blomberg A, Frew A, Sandstrom T. Altered lung antioxidant status in patients with mild asthma. Lancet. 1999;354 9177:482-3;

53. Bucchieri F, Puddicombe SM, Lordan JL, Richter A, Buchanan D, Wilson SJ, et al. Asthmatic bronchial epithelium is more susceptible to oxidant-induced apoptosis. Am J Respir Cell Mol Biol. 2002;27 2:179-85;

54. Kam W, Ning Z, Shafer MM, Schauer JJ, Sioutas C. Chemical Characterization and Redox Potential of Coarse and Fine Particulate Matter (PM) in Underground and Ground-Level Rail Systems of the Los Angeles Metro. Environ Sci Technol. 2011;45 16:6769-76;

55. Kam W, Delfino RJ, Schauer JJ, Sioutas C. A comparative assessment of PM2.5 exposures in light-rail, subway, freeway, and surface street environments in Los Angeles and estimated lung cancer risk. Environ Sci-Proc Imp. 2013;15 1:234-43;

56. Lovett C, Shirmohammadi F, Sowlat MH, Sioutas C. Commuting in Los Angeles: Cancer and Non-Cancer Health Risks of Roadway, Light-Rail and Subway Transit Routes. Aerosol Air Qual Res.

57. Costa M. Toxicity and carcinogenicity of Cr(VI) in animal models and humans. Crit Rev Toxicol. 1997;27 5:431-42;

58. Chillrud SN, Epstein D, Ross JM, Sax SN, Pederson D, Spengler JD, et al. Elevated airborne exposures of teenagers to manganese, chromium, and iron from steel dust and New York City's subway system. Environ Sci Technol. 2004;38 3:732-7;

59. Cao SJ, Kong XR, Li LY, Zhang WR, Ye ZP, Deng YL. An investigation of the PM2.5 and NO2 concentrations and their human health impacts in the metro subway system of Suzhou, China. Environ Sci-Proc Imp. 2017;19 5:666-75;

60. Cakmak S, Dales R, Kauri LM, Mahmud M, Van Ryswyk K, Vanos J, et al. Metal composition of fine particulate air pollution and acute changes in cardiorespiratory physiology. Environmental Pollution. 2014;189:208-14;

61. Dales R, Kauri LM, Cakmak S, Mahmud M, Weichenthal SA, Van Ryswyk K, et al. Acute changes in lung function associated with proximity to a steel plant: A randomized study. Environ Int. 2013;55:15-9;

62. Shutt RH, Kauri LM, Weichenthal S, Kumarathasan P, Vincent R, Thomson EM, et al. Exposure to air pollution near a steel plant is associated with reduced heart rate variability: a randomised crossover study. Environ Health-Glob. 2017;16;

63. Pavanello S, Bonzini M, Angelici L, Motta V, Pergoli L, Hoxha M, et al. Extracellular vesicle-driven information mediates the long-term effects of particulate matter exposure on coagulation and inflammation pathways. Toxicol Lett. 2016;259:143-50;

64. Yu SL, Lee DC, Sohn HA, Lee SY, Jeon HS, Lee JH, et al. Homeobox A9 directly targeted by miR-196b regulates aggressiveness through nuclear Factor-kappa B activity in non-small cell lung cancer cells. Mol Carcinog. 2016;55 12:1915-26;

65. Panda AC, Sahu I, Kulkarni SD, Martindale JL, Abdelmohsen K, Vindu A, et al. miR-196b-Mediated Translation Regulation of Mouse Insulin2 via the 5 ' UTR. PLoS One. 2014;9 7;

66. Bollati V, Angelici L, Rizzo G, Pergoli L, Rota F, Hoxha M, et al. Microvesicle-associated microRNA expression is altered upon particulate matter exposure in healthy workers and in A549 cells. J Appl Toxicol. 2015;35 1:59-67;

67. Tarantini L, Bonzini M, Tripodi A, Angelici L, Nordio F, Cantone L, et al. Blood hypomethylation of inflammatory genes mediates the effects of metal-rich airborne pollutants on blood coagulation. Occup Environ Med. 2013;70 6:418-25;

68. Cantone L, Nordio F, Hou LF, Apostoli P, Bonzini M, Tarantini L, et al. Inhalable Metal-Rich Air Particles and Histone H3K4 Dimethylation and H3K9 Acetylation in a Cross-sectional Study of Steel Workers. Environ Health Perspect. 2011;119 7:964-9;

69. Lall R, Ito K, Thurston GD. Distributed Lag Analyses of Daily Hospital Admissions and Source-Apportioned Fine Particle Air Pollution. Environ Health Perspect. 2011;119 4:455-60;

70. Strak M, Steenhof M, Godri K, Gosens I, Cassee F, Hoek G, et al. Physical, Chemical, and Oxidative Characterization of Particles From Locations With

Contrast in Local Source Emissions: Exposure and Health Assessment in the Raptes Study. Epidemiology. 2011;22 1:S219-S;

71. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. Am J Respir Crit Care Med. 2011;184 5:602-15;

72. Steenhof M, Mudway IS, Gosens I, Hoek G, Godri KJ, Kelly FJ, et al. Acute nasal pro-inflammatory response to air pollution depends on characteristics other than particle mass concentration or oxidative potential: the RAPTES project. Occup Environ Med. 2013;70 5:341-8;

73. Strak M, Hoek G, Godri KJ, Gosens I, Mudway IS, van Oerle R, et al. Composition of PM Affects Acute Vascular Inflammatory and Coagulative Markers - The RAPTES Project. PLoS One. 2013;8 3;

74. Strak M, Hoek G, Steenhof M, Kilinc E, Godri KJ, Gosens I, et al. Components of ambient air pollution affect thrombin generation in healthy humans: the RAPTES project. Occup Environ Med. 2013;70 5:332-40;

75. Steenhof M, Janssen NA, Strak M, Hoek G, Gosens I, Mudway IS, et al. Air pollution exposure affects circulating white blood cell counts in healthy subjects: the role of particle composition, oxidative potential and gaseous pollutants - the RAPTES project. Inhal Toxicol. 2014;26 3:141-65;