Health Effects of Particulate Matter Air Pollution in Underground Railway Systems – A Critical Review of the Evidence

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Abstract

Background

Exposure to ambient airborne particulate matter is a major risk factor for mortality and morbidity, associated with asthma, lung cancer, heart disease, myocardial infarction, and stroke, and more recently type 2 diabetes, dementia and loss of cognitive function. Less is understood about differential effects of particulate matter from different sources. Underground railways are used by millions of people on a daily basis in many cities. Poor air exchange with the outside environment means that underground railways often have an unusually high concentration of airborne particulate matter, while a high degree of railway-associated mechanical activity produces particulate matter which is physicochemically highly distinct from ambient particulate matter. The implications of this for the health of exposed commuters and employees is unclear.

Main body

A literature search found 27 publications directly assessing the potential health effects of underground particulate matter, including *in vivo* exposure studies, *in vitro* toxicology studies, and studies of particulate matter which might be similar to that found in underground railways. The methodology, findings, and conclusions of these studies were reviewed in depth, along with further publications directly relevant to the initial search results.

In vitro studies suggest that underground particulate matter may be more toxic than exposure to ambient/urban particulate matter, especially in terms of endpoints related to reactive oxygen species generation and oxidative stress. This appears to be predominantly a result of the metal-rich nature of underground particulate matter, which is suggestive of increased health risks. However, while there are measureable effects on a variety of endpoints following exposure *in vivo*,

there is a lack of evidence for these effects being clinically significant as may be implied by the *in vitro* evidence

Conclusion

There is little direct evidence that underground railway PM exposure is more harmful than ambient PM exposure. This may be due to disparities between *in vivo* exposures and *in vitro* models, and differences in exposure doses, as well as statistical under-powering of *in vivo* studies of chronic exposure. Future research should focus on outcomes of chronic *in vivo* exposure, as well as further work to understand mechanisms and potential biomarkers of exposure.

Keywords

Underground railway, subway, particulate matter, transition metal, steel, iron, reactive oxygen species, oxidative stress, inflammation.

35 **Background**

36 Underground railways are mass transit systems used for several million passenger journeys per day
37 in many of the world's most populous cities [1]. Depending on station depth, proximity to the
38 nearest transition from tunnel section to the outside environment, and air conditioning system,
39 there may be relatively little exchange of air with the outside environment, and this may be further
40 modulated by station design [2, 3, 4, 5, 6, 7]. As such, the airborne particulate matter (PM) load in
41 underground railway stations has the potential to be (1) significantly greater, on a mass
42 concentration basis, than the outside environment, and (2) influenced principally by source
43 materials and generation methods specific to the underground railway. These processes include
44 wear of wheels and brakes, and arcing of electrical current between the third rail or catenary and
45 the current collecting apparatus [8, 9]. As a result, PM generated in underground railway systems
46 tends to be rich in metals, especially Fe, but also Cr, Ni, Co, Mn, and Cd, across coarse ($PM_{10\text{-}2.5}$;
47 median aerodynamic diameter 10-2.5 μm), fine ($PM_{2.5}$; <2.5 μm), and ultrafine (UFPM/$PM_{0.1}$;
48 <0.1 μm) fractions [8, 10, 11, 12]. Given the potential for underground PM to differ significantly
49 in composition, as well as in physical characteristics such as particle number concentration (PNC),
50 and the number of people potentially exposed on a regular basis through either employment in
51 underground railway systems or regular commuting, the question of whether underground PM
52 exerts effects on health and, if so, the mechanisms involved, warrants urgent attention.

53 Air quality in underground systems has been the focus of previous reviews, but these have centred
54 around pollution characterisation and chemistry, rather than potential health effects [13, 14].
55 Therefore, the focus of this review is the critical examination of the evidence for potential health
56 effects of PM in underground stations, and PM which may be similar to that found in underground
57 stations. The review is divided into four principal sections, respectively focusing on evidence for
58 effects of exposure to underground PM *in vivo*, evidence from *in vitro* studies, studies which use
59 various derived risk factors to evaluate *in vivo* risk, and evidence from *in vivo* studies of exposure to

PM not from underground railways, but which may approximate the physicochemical characteristics of underground PM.

Main Text

Search Strategy and Review Structure

The search strategy for this review is shown in Figure 1. An initial literature search was performed by Dr Sarah Robertson (Public Health England, UK) forming the basis for a report by a subcommittee of the UK Committee on the Medical Effects of Air Pollution (COMEAP). The literature search was performed across Ovid MEDLINE, Embase, CINAHL, and Google databases, to include all published, Epub ahead of print, in-process, and non-indexed citations. The search was designed to find papers on all aspects of underground railway air quality, not limited to those studying the health effects, but also those relevant to pollution chemistry and engineering. The search was carried out for all entries containing (1) EITHER "underground" OR "enclosed railway" OR "subway" OR "metro" OR "metropolitan", AND (2) the subject heading "transportation" OR the subject heading "railroads", AND (3) the subject heading "air pollution" OR the subject heading "air pollutants" OR the subject heading "particulate matter". Results containing the term "coal" were excluded. This yielded 203 results. Initial refining of results to remove duplicates and those not relevant to the underground left 51 exposure studies, 8 *in vivo*/*in vitro* toxicity studies, and 14 health studies. These results were supplemented using references from a previous review by Xu and Hao [13] and the EU-Life funded project IMPROVE. Further refining to remove reviews and those which did not have a direct health component, and rearranging into the categories used in this review, yielded 6 short term exposure studies on volunteers, 4 long term/occupational exposure studies, 11 *in vivo*/*in vitro* toxicity studies, and 6 studies focusing on steel mill or other steel-related PM. These studies form the basis of this

84 review, with additional references selected only where specifically addressing *in vitro* or *in vivo* effects
85 of exposure, or directly linked to/relevant to such studies.

86 Throughout this review, the terms "underground" and "underground railway" are used for
87 consistency. This is equivalent to terms such as "subway" and "metro" used elsewhere, with the
88 key distinction required for the use of the term "underground" being that the station/section in
89 question is subterranean, unless explicitly stated.

90

91 **(1) *In vivo* evidence for health effects of exposure to underground air pollution/PM**

92 *Acute exposure*

93 Klepczynska Nystrom *et al* exposed 20 healthy volunteers to a Stockholm underground
94 environment or a control environment for 2h and investigated effects of this exposure on lung
95 function and inflammation in the lower airways and blood. Lung function tests found no effect
96 of underground exposure on vital capacity (VC), forced vital capacity (FVC), forced expiratory
97 volume in 1 second (FEV_1), exhaled NO, or peak expiratory flow (PEF). They found statistically
98 significantly increased levels of fibrinogen in plasma of those exposed in the underground, with
99 increased counts of CD4/CD25/FOXP3 T cells [15]. The increase in CD4 T cells positive for
100 FOXP3, or FOXP3 and CD25 (in blood but not bronchoalveolar lavage fluid (BALF)) indicates
101 an increase in the pool of regulatory T cells (Treg) at 14 h following underground exposure. A key
102 function of Treg cells is to reduce the inflammatory response, which can be achieved through
103 secretion of anti-inflammatory cytokines and effects on other immune cells [16, 17]. As such, the
104 increase in Treg numbers may be a response to the systemic inflammation indicated by increased
105 fibrinogen release. Concentration of the anti-inflammatory cytokine IL-10, released by Tregs, were
106 assayed only in the BALF (where there was no increase in Tregs) and not in the blood (where

Tregs were increased). Similar increases in systemic Tregs but not lung Tregs have been found following $PM_{2.5}$ exposure in mice [18]. Post-exposure blood tests also showed slight but significant increases in other markers of T cell activation. This study did not consider the ability of Tregs to suppress inflammation, which has been seen to be impaired by ambient air pollution exposure in children, especially those with asthma [19]. Therefore, while the increase in peripheral blood Treg cells in this study may represent a natural consequence of the systemic inflammation of which increased fibrinogen concentrations are a marker, it is impossible to determine whether there is any effect of underground railway pollution on Treg functioning.

The lack of effect on lung function parameters was also found in a study by Bigert *et al*, who investigated effects of short-term exposure of employees in the Stockholm underground by measuring before-work and after-work fraction of exhaled NO (F_ENO), and taking regular measurements of PEF and FEV_1 [20]. These employees were mainly non-asthmatic (74/81), and were either platform workers, train drivers, or ticket office staff, in order of decreasing $PM_{2.5}$ exposure. The authors found no change in F_ENO over the working day in any of the occupation/exposure groups, nor was there a significant decrease in PEV/FEV_1 ratio over the working day, which suggests no obvious decline in airway function. The authors do, however, suggest that their study design might not have allowed a sufficient washout for the inflammatory effects of previous exposures to disappear before the before-work F_ENO testing, or that the after-work test may have been too soon to observe the response to the day's exposure. Nonetheless, the results are in broad agreement with other tests presented here where lung function testing is used to assess responses to short-term underground exposure.

In a follow-up to their study on the effects of underground exposure in healthy individuals, Klepczynska Nystrom *et al* observed that mild asthmatics similarly exposed had 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) [21]. 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. This might reflect increased deposition of PM in the upper airways in asthma as a result of increased mucus production and/or reduced airway patency, with reduced penetration of PM to the lower airways and alveoli, perhaps due to increased mucus or airflow turbulence as a result of the disease. However, the overall conclusions of the authors suggest that, although there were some changes in markers indicative of systemic inflammation after 2 h exposure to underground PM (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 caused only slight acute effects, with changes in biomarkers being of uncertain consequence in terms of immune system functioning. Moreover, these two studies found no significant increase in the concentrations of any inflammatory cytokines (IL-1β, IL-6, IL-8, and, TNFα) or anti-inflammatory cytokine (IL-10) in BALF, suggestive of a lack of acute inflammatory response [15, 21]. Furthermore, these studies were not designed to study the effect of persistent exposure over longer periods.

Changes in systemic markers in healthy, but not asthmatic, subjects were also seen in these studies in terms of plasma fibrinogen concentrations, which increased slightly, but nonetheless significantly, in healthy volunteers from 2.2 g/l to 2.3 g/l, but did not change in mild asthmatics, although the physiological relevance of this small increase in terms of adverse cardiovascular outcomes is unclear. Fibrinogen undergoes enzymatic cleavage by thrombin to form fibrin, which then forms the "meshwork" for clot formation [22]. 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 [23]. 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

157 the activation of an enzyme cascade involved in clot breakdown [24]. This study sampled blood
158 14 h after exposure, which is within the normal time frame for acute response proteins to be
159 increased in the blood, although it is possible that slightly later timepoints may reveal increased
160 levels of such markers not seen at 14 h. In addition to C-reactive protein and various inflammatory
161 cytokines (e.g. IL-6), fibrinogen is a commonly assayed blood-borne marker of the effects of air
162 pollution in humans, specifically as a marker of systemic inflammation, where it is sometimes noted
163 to be correlated with circulating levels of C-reactive protein (reviewed by [25]). The increase in
164 fibrinogen has been suggested to be dependent on the genotype of the individual concerned, for
165 exposure to gaseous pollutants, although the same may not necessarily be true for PM pollution
166 [26]. Bigert and colleagues found increased fibrinogen in underground ticket sellers and PAI-1 in
167 underground train drivers after 2-days of work following a work-free wash-out period of at least
168 2 days [27]. However, baseline levels were highest in platform workers, who showed no acute
169 increases in these markers, suggesting that acute changes were not due to PM exposure. This is in
170 line with their findings in the same cohort used for their previous study, detailed above, which
171 showed no change in lung function of inflammation by F_ENO or PEV/FEV_1 measurement [20].
172 Therefore, while fibrinogen is commonly used as a biomarker for systemic inflammation as a result
173 of short term exposure to PM, the evidence for a biologically significant effect of underground
174 PM on fibrinogen is lacking.

175 In contrast Lundstrom *et al* [28] used lipid mediators in BALF to study the effects of exposure to
176 underground air. These are not commonly used markers in such studies, perhaps on account of
177 the numerous analytes studied, the need for lavage, the requirement for specialised mass
178 spectrometry techniques for their measurement, of their lack of comparability to other studies.
179 The study showed an increased level of nine oxylipins out of sixty-four assayed in healthy
180 volunteers compared to mild asthmatic volunteers, following 2 h exposure in the Stockholm
181 underground. Levels of these oxylipins increased in the healthy volunteers, while tending to

182 remain the same or slightly decrease in the mild asthmatic volunteers. These nine oxylipins were
183 prostaglandin E2 (a product of arachidonic acid metabolism by cyclooxygenase), and eight
184 products of biosynthesis from linoleic or α-linolenic acids *via* 15-lipoxygenase, suggesting a
185 common mechanism of regulation. The authors suggested that, given the bronchoprotective
186 effects of the oxylipins increased in healthy individuals but not asthmatics following exposure to
187 underground air, there is evidence of a differential effect depending on asthma status. This is
188 supported by the increased BAL cell expression of cyclooxygenase-1 in healthy volunteers only.
189 However, while these results imply asthma status-specific effects, the pathways of these
190 metabolites are significantly complex that it is difficult to draw any robust conclusions regarding
191 their implications for acute responses to underground air exposure. Furthermore, the effects of
192 the observed changes in their concentration is unclear, and it is also noteworthy that changes were
193 observed in only a small proportion of the mediators assayed. Lipid mediators and lipid oxidation
194 in general represent an interesting and currently under-investigated avenue for PM research, but
195 this study is not sufficient to draw any relevant conclusion other than as an illustration that some
196 lipid mediators may change in response to underground air exposure, and that there are asthmatic
197 *vs.* healthy differences in response to the underground environment. The lack of research into
198 lipid mediators following pollution exposure is underlined in a recent paper on the effects of
199 biodiesel exhaust [29], where healthy volunteers were exposed to biodiesel exhaust at a PM level
200 not dissimilar to the PM level of the Stockholm underground. They found increased
201 concentrations in BALF of PGE2, 13-hydroxyoctadecadienoic acid (13-HODE), and 12,13-
202 dihydroxyoctadecadienoic acid (12,13-diHOME) following biodiesel exhaust exposure.
203 Interestingly, while PGE2 and 13-HODE were significantly more responsive to underground air
204 exposure in healthy *vs.* asthmatic volunteers in the Stockholm underground study and 12,13-
205 diHOME was close to significance ($p=0.1$), none of these were significantly increased in the
206 Stockholm underground following exposure *vs.* no exposure, and further examination of the
207 Stockholm underground study shows that the significance threshold for analysis between asthma

208 status was likely attained not simply because of an increase in levels of these oxylipins in healthy
209 lavage fluid, but also because of a decrease in their levels in asthmatics. Indeed, considering the
210 asthmatic and healthy groups separately, only two oxylipins were significantly altered by exposure
211 to underground air (one of these overlapped both groups). This further reinforces the conclusion
212 that the Stockholm study, while suggesting some disease-related differences, does not provide
213 strong evidence *per se* for any significant effect of underground air, but rather evidence of some
214 more subtle asthmatic *vs.* non-asthmatic differences.

215 A study by Liu *et al* used heart rate variability (HRV) as an endpoint [30]. HRV is often used as an
216 endpoint to assess the effects of pollution, representing a risk factor, both in itself and as a marker
217 of autonomic imbalance, for a number of adverse cardiovascular outcomes [31], and is thought to
218 be influenced by effects of pollution on the autonomic nervous system, resulting in disturbed
219 sympathetic tone, and thus altered sympathetic/parasympathetic balance. This study found a
220 decrease in HRV with increasing $PM_{2.5}$ exposure, as has been noted elsewhere (for a
221 comprehensive overview, see recent review by [32]). Interestingly, the study noted that the
222 decrease in HRV seen by participants using the underground for a 1 h commute was less than seen
223 for those walking or using the car or bus. However, the concentrations of $PM_{2.5}$ to which
224 participants were exposed shows that the underground air was significantly less polluted than
225 environments used for walking or bus exposures, and trended to a lower concentration compared
226 to car-based exposures. This was also true for exposure to PM_{10} and total volatile organic
227 compounds. The finding that the underground was the least PM-loaded environment is somewhat
228 unusual - one reason for the relatively low PM concentration noted in the Taipei underground
229 here may be the use of an air conditioning system. Therefore, it is difficult to extrapolate these
230 results to understanding the effects of exposures in other underground networks, where the air
231 tends to be more PM-rich, although the study does imply that when similar PM_{10} and $PM_{2.5}$ mass

concentrations are involved, air in the underground is not necessarily more detrimental than other modes of transport, at least in terms of HRV.

Chronic exposure

There is a paucity of studies of the effects of chronic exposure to underground railway pollution. Bigert and colleagues found no significant increased risk of myocardial infarction in 304 underground train drivers amongst a study population of 153,807 men aged 40-69 in Stockholm County [33]. Similarly, Gustavsson and colleagues found that the incidence of lung cancer was not increased in 348 Stockholm underground drivers compared to 319,979 employed males in the Stockholm area [34]. However, these studies may have been insufficiently powered to uncover an effect, on account of the relatively small number of underground train drivers and low incidence of disease (54 myocardial infarction cases and 9 lung cancer cases in the two studies, respectively). Indeed, both studies have somewhat large confidence intervals of relative risk. 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 $PM_{2.5}$ 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 [35]. 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 [36]. 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.

Summary

These studies are summarised in Table 1. Studies aimed at understanding the effects of exposure to underground railway air pollution have generally found no consistent, convincing evidence for significant effects on the health of either acute (2-8 h) or chronic exposure. Studies of acute exposure in the Stockholm underground found no effect on lung function, although there were

some reported lower (healthy volunteers) and upper (asthmatic volunteers) airway symptoms. These studies did show effects on Treg cell populations following exposure, but the clinical significance of this, along with the observed slight increase in circulating fibrinogen, is unclear, especially in light of the lack of other observable effects. Increased concentrations of a relatively small number of oxylipins in BALF may suggest differential effects depending on asthma status, but again the clinical significance is unclear. Studies of the effects of chronic exposure have been similarly lacking in evidence, suggesting no increased risk of myocardial infarction or lung cancer, and noting no obvious effect of underground exposure on a range of circulating biomarkers which one might hypothesise to be affected, were underground exposure to be a significant cause for concern. However, it is also possible that these studies are underpowered, with only a small number of cases, and commensurately large confidence intervals in their results. Therefore, although these studies provide no clear evidence for significant health effects of acute or chronic exposure in the underground, there is clearly a need for larger studies and studies more powered to analyse differential effects on those with underlying airway and cardiovascular disease. Furthermore, there is clearly a disconnect between the results of these *in vivo* studies, and those which use a range of other techniques, such as *in vitro* models and *in vitro-in vivo* extrapolation, as detailed the in the sections which follow.

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

Several non-human studies (mainly *in vitro*) have been conducted to examine the toxicity of underground PM and mechanisms involved. While they cannot give the same insight into potential effects on exposed humans as those studies in the previous section, they may be better able to shed light on the potential cellular mechanisms at work, and how they relate to the composition

303 of the PM, which is important in understanding whether underground PM may exert effects
304 different to those caused by urban PM, and why [37].

305 Seaton *et al* examined the characteristics of PM on the London Underground from a size and
306 compositional point of view, with PM collection and monitoring at three deep-level underground
307 stations (Oxford Circus, Holland Park, Hampstead) [38]. The mass concentration of $PM_{2.5}$
308 underground was noted to be much higher than would be seen above ground, but there was not a
309 correspondingly higher level of PM by PNC. In fact, the number concentration was lower than
310 might be expected in a city street. Overall, the size distribution of underground PM *vs.* urban PM
311 was shifted towards the larger size of the PM spectrum. If composition is disregarded and only
312 size/PNC is considered, this observation suggests that underground PM may well pose less of a
313 health risk that that above ground, where higher numbers of particulates persist, given that PNC
314 has been suggested to be more important a dose metric than particle mass [39]. Furthermore, the
315 transition metal-rich nature of underground PM means that it is likely denser than urban PM, and
316 thus would have a lower PNC, even if the size distribution and mass concentrations were identical.
317 However, Seaton and colleague's work also showed that, *in vitro*, A549 cells (a type II alveolar
318 epithelial carcinoma cell line) were more affected by underground PM than ambient PM or control
319 particles (TiO_2) in terms of release of the neutrophil chemoattractant/activating cytokine IL-8 and
320 extent of DNA damage, as determined by plasmid scission assay. The increase in IL-8 release
321 could be abrogated by chelation (the exact method is not stated) implying that PM surface or
322 soluble transition metals are responsible for this effect. Furthermore, the use of welding dust as a
323 comparator in this study was not ideal given the fact that much of the underground PM is
324 generated by abrasion rather than high temperature processes generating fume.

325 Three papers by Karlsson and colleagues detail experiments similar to those of Seaton *et al*, using
326 PM from the Stockholm underground, but with additional analyses [40, 41, 42]. Data from their
327 first paper in 2005 showed that Stockholm underground PM was more potent than urban street

PM in causing A549 cell DNA strand breakage and oxidation, that the involvement of Fe 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 [40]. They also used x-ray diffraction to study the crystal structure of the PM, showing that the majority of underground PM Fe 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 Fe in haematite exists purely in the ferric Fe^{3+} (Fe(III)) form, Fe in magnetite exists as a mix of ferrous (Fe^{2+}) and ferric Fe (Fe(II,III)). Generation of reactive oxygen species (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 [43]. This last step is the Fenton reaction, catalysed especially efficiently by Fe, and shown in Karlsson's study to be involved 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) liberates the electron required for generation of ROS, ferrous Fe is much more able to generate ROS, and thus magnetite is a potent generator of ROS, whereas haematite is not. This is, however, complicated by the observation that dissolved ferric Fe can be reduced back ferrous Fe by antioxidants present in lung lining fluid, at least *in vitro* [44], given that the lung lining fluid presents a potent reducing environment [45, 46]. Similar to the Stockholm underground, a predominance of ferrous Fe has been noted in the Shanghai and Seoul underground systems [9, 47]. 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 [3, 48]. However, in a discussion of their findings of the predominance of magnetite and maghaemite (γ-Fe_2O_3 with a different crystal structure to α-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 [49]. The implication of this is that

354 ferrous and ferric Fe cannot be accurately distinguished by x-ray spectroscopy if the ferric Fe is in
355 the form of maghaemite rather than haematite.

356 The fact that more of the activity was water soluble in urban PM than underground PM is possibly
357 due to a greater proportion of Fe/other transition metals in urban PM than in underground PM
358 being in the form of water-soluble salts (e.g. sulphate (SO_4^{2-})), thus increasing the effective metal
359 ion concentration, which in insoluble metal/metal oxides is simply a function of particle surface
360 area. Increased water solubility may modulate the toxicity of these particles, although it is not clear
361 whether it would increase or decrease.

362 The 2006 paper by the same authors compared Stockholm underground PM_{10} to PM from wood
363 burners (total PM) and tyre wear simulators (PM_{10}, with one additional $PM_{2.5}$ sample) representing
364 different types of burner/fuel and road, respectively, and also street PM_{10} collected in a Stockholm
365 city centre street [41]. This showed different effect rankings by endpoint: by comet assay, DNA
366 damage in A549 cells was significantly higher with underground PM_{10} than any other PM tested,
367 while underground PM induced only relatively small increases in release of TNFα (significant) and
368 IL-6, IL-8 (both non-significant) from human monocyte-derived macrophages, compared to street
369 PM. The release of IL-8 in this study was lower for underground PM than seen by Seaton *et al*
370 (~3-fold *vs.* ~2-fold) [38], but a greater difference for street PM (~11-fold *vs.* ~2-fold). The
371 authors suggest that this may be due to the greater concentration of endotoxin in the street PM,
372 to which cultured macrophages are sensitive, but cultured epithelial cells are relatively insensitive.
373 This is because macrophages express the lipopolysaccharide co-receptor CD14, whereas epithelial
374 cells, including A549, do not express CD14, and thus unless there is soluble CD14 in the culture
375 medium, epithelial cells tend to respond much less avidly to lipopolysaccharide [50]. Furthermore,
376 the PM in this study was collected on glass fibre filters, which caused a 5-15-fold increase in
377 cytokine release in the blank filter control cultures. Although the authors attempted to correct for
378 this by comparing cytokine release in the presence of PM to that seen in the filter blank-treated

379 cultures, it is possible that the ability of macrophages to phagocytose the PM would be impaired
380 to an extent by the presence of glass fibres. Furthermore, it is unclear whether the inflammatory
381 state clearly induced by the presence of these fibres would have an equal effect on the response to
382 each different PM type.

383 The same authors performed similar experiments in a 2008 study, but examining different
384 endpoints, and incorporating chemically homogenous PM into the work alongside environmental
385 PM, here in A549 cells [42]. Stockholm underground PM_{10} caused an approximately 4-fold
386 increase over control in mitochondrial depolarisation (similar to wood and diesel PM, greater than
387 street PM and tyre/roadwear PM) and intracellular ROS as measured by dichlorofluorescein
388 (DCF) fluorescence, being the only PM type which increased ROS. To examine the mechanism
389 of these effects, the activity of underground PM was compared to that of magnetite, the principal
390 Fe component of the underground PM. Underground PM increased DNA structural damage,
391 measured by single strange breaks and alkaline labile sites, and DNA oxidative damage, assessed
392 by the number of recognition sites for the enzyme formamidopyrimidine DNA glycosylase (FPG),
393 which repairs oxidatively damaged DNA. Conversely, magnetite particles had only a small effect
394 (trending towards significance) on the former, and none on the latter. These effects were then
395 shown to be ascribable to non-water-/non-citrate-soluble underground PM components, and also
396 more genotoxic than haematite particles or Cu-Zn particles, which were also observed in the
397 underground PM samples. Genotoxicity could not, however, be explained by the main component
398 (magnetite), by water-soluble metals, or by intracellular mobilized Fe. The authors suggest that
399 factors responsible for the effects of underground PM may include surface Fe coordination,
400 microcrystal morphology, and surface Fe ion arrangements. However, there is also evidence that
401 the combination of Fe and Cu, as found in underground PM, can be especially potent in the
402 generation of hydroxyl radicals. While Fe-driven Fenton chemistry is critical in the generation of
403 the hydroxyl radical from peroxide, Cu may be more efficient in generation of other ROS [51, 52].

404 Thus, Fe in a mixture with a lesser amount of Cu may be the most efficient for overall driving of
405 ROS generating processes – it is perhaps important to note that this is generally what is found in
406 underground PM. This would explain why insoluble metal PM in isolation or in a mixture which
407 is dissimilar to underground PM (e.g. magnetite, haematite, or Cu-Zn PM) cannot replicate the
408 effects of underground PM.

409 While this series of papers clearly shows the potential effects of underground PM, it does not
410 provide any conclusive evidence of why the underground PM is toxic, nor that it is necessarily
411 more toxic than other PM types, at least on an equal-mass basis. The authors also suggest that the
412 shard-like shape of a portion of the PM may endow the PM with a pro-inflammatory potential.
413 However, although this is a noted feature of the macrophage response to PM which are particularly
414 larger in one dimension than another (i.e. have a high aspect ratio), whereby phagocytosis begins
415 but is unable to complete leading to "frustrated phagocytosis" with consequent release of
416 inflammatory cytokines [53, 54], to our knowledge there is no published evidence that such an
417 effect occurs in epithelial cells.

418 The study of Lindbom *et al* (2006) illustrates the difference in responses to PM_{10} of different cell
419 types [55]. Data from monocyte-derived macrophages showed that underground PM was poor at
420 eliciting release of IL-6, IL-8, and TNFα compared to two types of roadwear PM and water and
421 methanol extracts of diesel PM. However, underground PM was more effective than any other
422 PM type in eliciting TNFα release from bronchial epithelial cells (BEAS-2B), while no PM was
423 able to elicit cytokine release from RPMI2640 nasal epithelial cells. Similar experiments in RAW
424 264.7 macrophages found that street PM_{10} tended to be considerably more inflammogenic in terms
425 of IL-6 and TNFα, although underground PM_{10} elicited a greater release of arachidonic acid,
426 indicating the potential for a greater effect *via* the eicosanoid pathway [56]. However, underground
427 PM_{10} generated a significantly greater response than did street PM_{10} in terms of various measures
428 of ROS, either directly (by cell-free oxidation of dithiothreitol; DTT) or indirectly (by measurement

429 of thiobarbituric acid-reactive substances as a proxy for lipid peroxidation). In general, granite and
430 quartzite pavement wear particles elicited less response over all outcome measures than did the
431 street and underground PM_{10}.

432 The paper by Bachoual *et al* (2007) is interesting and unusual in that it compares two underground
433 PM_{10} types – those from the Paris Metro, which uses rubber/pneumatic tyres and "wooden"
434 brakes (PM_{10} Fe=42%, Mn=<1%), and those from the suburban Réseau Express Régional (RER)
435 system, which uses metallic components (PM_{10} Fe=61%, Mn=7%) [57]. Carbon black, titanium
436 dioxide, and diesel exhaust PM were used as comparators. No source-specific difference in cell
437 death was seen, while the two railway PM sets were the most effective at inducing release of MIP-
438 2 and TNFα from RAW 264.7 murine macrophages, within 3 and 8 h, respectively, and persisting
439 until at least 24 h. Conversely, none of the particles tested induced increased mRNA expression
440 of matrix metalloprotease-(MMP) 2 or 9, and all induced MMP-12 to a roughly equivalent extent.
441 The Fe chelator desferrioxamine (DFX) reduced release of TNFα by RER PM by ~50%, but had
442 little effect on the response to Metro PM, while no effect on MIP-2 response was seen with DFX,
443 suggesting differential release mechanisms for these two cytokines. In mice intratracheally instilled
444 with PM at 0.22-4.48 mg/kg body weight, RER PM but not carbon black or diesel exhaust PM
445 (DEP) caused increased BAL protein, used as a marker of airway epithelial leakage/damage, with
446 increased BAL total cell and neutrophil percentage. These increased cell counts were seen to a
447 lesser extent with DEP, but not seen following carbon black. Similarly to the *in vitro* tests, RER
448 PM but not carbon black or DEP induced increased TNFα and MIP-2 release in mice (measured
449 in BALF) within 8 h, an increase in expression of MIP-12 but not MIP-2 or MIP-8. RER PM also
450 induced increased expression of the antioxidant gene haemoygenase-1 (HO-1), one of the most
451 commonly used markers of antioxidant response to oxidative stress, which was not noted with
452 carbon black or DEP. Therefore, this study suggests that PM from the Paris underground is
453 generally more inflammatory then the other PM types tested, and a portion of this activity derived

454 from the metallic nature of RER PM which was not seen in Metro PM. However, there is also a
455 significant component of the effect of underground PM which does not appear to be related simply
456 to the Fe content of the PM. The authors also suggest that the RER environment is more worthy
457 of investigation on account of its higher PM concentration (361 $\mu g/m^3$ *vs.* 68 $\mu g/m^3$ in the Metro).

458 The organic composition of underground PM_{10} is rarely studied, probably on account of the
459 general consensus that the principal sources of organic PM, such as road vehicle exhaust emissions
460 and combustion, contribute little to the underground PM load. However, this was the focus of a
461 study by Jung *et al* in which the organic extract of underground PM_{10} was able to elicit significant
462 cell death in Chinese hamster ovary (CHO-K1) cells, but not BEAS-2B bronchial epithelial cells
463 [58]. The organic components, and their metabolic breakdown products, were shown to be able
464 to induce micronucleus formation, indicative of DNA damage, and DNA strand breakage in both
465 cell types, and this could be ameliorated by scavengers of superoxide, peroxide, and hydroxyl
466 radical. GC-MS-MS analysis of the organic extract showed the presence of ten of the sixteen US
467 Environmental Protection Agency (EPA) criteria carcinogenic PAHs, although analysis of
468 cytochrome p450 1A1 (CYP1A1) expression, which is usually significantly upregulated by the
469 presence of such PAHs through the aryl hydrocarbon receptor [59], suggested that this
470 underground PM_{10} was not sufficiently PAH-rich as to be able to upregulate CYP1A1 mRNA
471 expression.

472 The study of Loxham *et al* in 2015 observed similar effects to the above studies, with PM
473 concentration-dependent increases in IL-8 release and ROS generation, along with upregulation
474 of the antioxidant gene HO-1 [60]. However, the study was unusual in using primary cells
475 differentiated at the air-liquid interface on Transwell culture membranes. This facilitates formation
476 of functional cilia and mucus-secreting goblet cells, with consequent apical mucous layer, and thus
477 is more representative of the *in vivo* airway epithelium than is a standard monolayer culture [61].
478 Underground PM (from a European mainline underground station) was nonetheless able to cross

479 the mucous barrier and exert the above effects, as well as entering cells. This study is also unusual
480 in examining the ultrafine fraction of underground PM, which is generally neglected by other
481 studies. This fraction was found to be as Fe/metal-rich as the other fractions and generated a
482 larger ROS and IL-8 response than the larger size fractions on an equal PM concentration basis
483 [8, 60]. While the ultrafine fraction (44 $\mu g/m^3$) was present in a lower mass concentration than
484 the coarse and fine fractions (180 and 71 $\mu g/m^3$, respectively) in the air of the underground station,
485 this should be seen in the context of the overall greatly raised PM concentration underground, and
486 thus the exposure to UFPM underground is of potential importance to the health impacts of
487 underground railway air pollution.

488 Spagnolo *et al* considered $PM_{10\text{-}2.5}$, $PM_{2.5\text{-}1}$, $PM_{1\text{-}0.5}$ and $PM_{0.5\text{-}0.25}$ collected at an unnamed
489 underground platform, underground/intermediate commercial area, and outdoor site [62]. By
490 MTT assay, the platform PM was more cytotoxic to H727 bronchial epithelial carcinoma cells than
491 the commercial underground area PM (which showed no significant cytotoxicity), but the three
492 smallest outdoor fractions were the most cytotoxic of all. In contrast to the study of Loxham *et al*
493 [60], larger PM fractions were better generators of ROS than the smaller fractions at all three sites,
494 although the $PM_{0.5\text{-}0.25}$ fraction of platform PM nonetheless generated significantly increased levels
495 of ROS, which was not the case for the same smallest fraction of intermediate or outdoor PM.
496 Levels of ROS generation were strongly correlated with a panel of metals (Mn, Cr, Ti, Fe, Cu, Zn,
497 Ni, Mo) typically found elevated in underground stations PM, although this study did not
498 undertake any mechanistic work to investigate causality of these relationships.

499 It has been suggested that a key mechanism by which PM exerts toxic effects is through the
500 generation of ROS, and therefore assays which monitor the depletion of antioxidants, as a proxy
501 for the ability of PM to oxidise biomolecules, termed "oxidative potential" (OP), may be useful in
502 measuring potential toxicity of PM [63, 64, 65]. Moreno *et al* (2017) looked at the ability of PM
503 collected from a range of different underground railway locations to deplete ascorbic acid and uric

504 acid and to oxidise glutathione, all of which are measures of the OP of PM [66]. Other measures
505 of OP have been evaluated, such as electron spin resonance and oxidation of dithiothreitol, but
506 ascorbic acid in particular has been shown to be responsive to the typical components of
507 underground PM [67]. The Moreno study found that $PM_{2.5}$ mass was not significantly correlated
508 with OP, and Fe was significantly negatively correlated with ascorbic acid depletion. Conversely,
509 OP was linked to PM Cu, As, Mn, Zn, and Ba concentrations. While it has previously been
510 suggested that in this type of study glutathione (GSH) is not susceptible to the presence of Fe,
511 ascorbic acid depletion does seem to be [63]. Thus, one explanation for the lack of effect of Fe in
512 this study is that is existed in a non-redox-active form such as haematite. The authors suggest that
513 these correlations, along with the compositions of the most oxidative PM samples, indicate that a
514 significant source of PM OP derived from brake and catenary wear. They also note that the lowest
515 OP is found in the newest station which has platform-edge doors (PEDs), which have been seen
516 to reduce platform PM concentrations [49, 68].

517 Similarly, Gali and colleagues investigated the redox characteristics of PM from personal samplers
518 of passengers making journeys on above-ground and below-ground routes of the Hong Kong
519 underground (including time on the train and waiting on the platform), and compared this with
520 PM collected from journeys on an overground train route, bus, and ambient PM [69]. This study
521 was unusual in that the underground concentration of coarse and fine PM was lower than found
522 in above ground train routes, with the lower concentrations underground attributed to the use of
523 platform-edge doors, while the underground PM samples were also less metal-rich than seen in
524 other studies, for example the Fe concentration of $PM_{2.5}$ being ~0.2% by mass. Coarse
525 underground PM was seen to be more potent in reducing cell viability than coarse PM from above
526 ground or bus journeys, but for fine PM, there was little difference across samples, although fine
527 PM was generally more cytotoxic than coarse PM. On a mass basis, underground PM was generally
528 more potent in generating extracellular ROS than overground train and bus PM, and underground

529 coarse PM was slightly more potent than underground fine PM in the generation of intracellular
530 ROS, although there was general equipotency in terms of intracellular ROS generation by the fine
531 PM samples. However, when considered on the basis of airborne PM concentration, by volume,
532 overground train PM was a more potent generator of ROS than underground PM. In underground
533 PM, intracellular ROS generation was associated with mass concentration of Al, Ba, Cu, Mn, Mo,
534 Ni, V, Mg, and Na. Interestingly, there was no correlation with Fe concentration, in agreement
535 with Moreno and colleagues [66]. Furthermore, ROS generation by underground PM was
536 significantly lower than seen in PM from urban sites in a previous study by the same authors [70].
537 This suggests that underground PM metals may be less soluble and therefore less bioavailable in
538 underground PM, and this limiting the ability of underground PM to generate intracellular ROS.

539

540 *Summary*

541 These studies are summarised in Table 2. Studies which have examined release of inflammatory
542 cytokines from cell cultures exposed to PM (*in vitro*) indicate that underground PM is able to elicit
543 release of the commonly studied inflammatory cytokines, including IL-6, IL-8, and TNFα. Where
544 studies have directly compared the effects of underground PM to other types of PM, it seems that
545 underground PM is more potent in this regard than carbon black or titanium dioxide, and also
546 roadwear particles, but less so than urban PM. The increased pro-inflammatory potency of urban
547 PM may relate to the increased concentration of PAHs or other organic species, or of
548 endotoxin/lipopolysaccharide, the latter evidenced by a trend for heightened macrophage, but not
549 epithelial, pro-inflammatory responses to street/urban PM compared to underground PM. It is
550 also possible that the greater PNC of urban PM *vs.* underground PM plays a role in this effect,
551 given the suggested importance of PNC in driving particle toxicity and the known effects of UFPM
552 and nano-sized PM [39, 71].

553 It should also be considered that while *in vitro* studies tend to test identical mass concentration of
554 PM types, inhaled air contains different PM mass concentrations – underground PM mass
555 concentrations may be several times greater than those above ground, although one cannot
556 necessarily apply a multiplicative correction factor as this would assume linearity of the
557 concentration-response relationship. There is some evidence that such linearity may exist at
558 ambient PM concentrations, but this may break down at PM concentrations found underground,
559 and may also depend on the endpoint and the nature of the PM [72].

560 Another observation is that underground PM appears to have a greater OP and a greater ability to
561 exert oxidative damage *in vitro* than urban PM. The observation that underground PM can elicit
562 damage to DNA (through oxidised bases or strand breaks) and lipid (per)oxidation, in terms of
563 production of thiobarbituric acid-reactive substances (TBARS) is important. It is interesting that
564 OP, as measured by the depletion of one or more antioxidants *in vitro*, appears to be a good
565 predictor of cellular response with respect to certain endpoints *in vitro*, but that there is much less
566 evidence to link it directly to effects *in vivo* (for the best example of this over a range of outcome
567 measures, see the series of papers from the RAPTES study (section 3)).

568 The question arises as to the component(s) of underground PM important for its effect. In this
569 regard, the fact that a number of studies show that Fe chelation/redox inactivation is able to
570 significantly reduce the effects of underground PM indicates that the Fe content of underground
571 PM (generally in the range of 20-60% by mass) is important. This can also be seen where two
572 underground systems in the same city but with different components are studied [57]. However,
573 the finding that magnetite and haematite are unable to replicate these effects also suggests that
574 there may be other important components, perhaps "working" in concert with the Fe-containing
575 species. One possibility is that there are other metals also involved, and maybe even predominating
576 in determining OP, such as brake and catenary wear Cu and Sb [66]. This would explain why Fe
577 chelation can significantly but nonetheless only partially reduce the effects of underground PM,

578 and also why correlations between ROS generation and PM elemental concentrations often
579 highlight the importance of non-ferrous metals. There is also the possibility that organic species
580 may contribute [58], although since these species likely derive predominantly from the outside
581 environment, this may not apply to deep-lying stations with poor air exchange [73, 74]. Notably,
582 the greater effects of underground PM on various markers of oxidative stress and oxidative damage
583 occur despite the PNC of underground PM being lower than that of urban PM, thus strongly
584 implicating a composition component of underground PM, of which transition metals are the most
585 obvious candidate. The ability of metal-rich PM to generate ROS may be of relevance in asthma,
586 where even in the mild form of the disease there is thought to be dysregulated antioxidant defence
587 in the airways [75], and where *in vitro* evidence suggests that the airway epithelium may be more
588 susceptible to oxidant-induced damage [76].

589

590 **(3) RAPTES - Risk of Airborne Particles, a Toxicological-Epidemiological hybrid Study**

591 The RAPTES study was a multi-part study of the effects of exposure to PM from a range of
592 locations in the Netherlands, attempting to link PM source and composition to effect. Sites used
593 in the study were an underground railway station, urban background site, farm, three traffic sites
594 (continuous traffic, stop-go traffic, and truck traffic), a harbour, and an area near a steelworks.
595 The underground site had a much higher mass concentration of PM_{10} and $PM_{2.5}$ than other sites
596 (394 and 137 $\mu g/m^3$, respectively), but lower PNC than the road traffic sites. In the coarse PM
597 fraction, mass/volume concentrations of Fe and Cu were approximately two orders of magnitude
598 higher underground than any other site, and Ni one order of magnitude higher, along with
599 elemental carbon (EC) one order of magnitude higher, and EC/organic carbon (OC) ratio higher
600 than any other site. In the fine fraction, underground PM was also more EC rich but had an
601 EC/OC ratio comparable to the traffic sites [77]. Across coarse, fine, and quasi-ultrafine (<0.18

μm) fractions, underground PM had a greater effect on RAW 264.7 macrophage viability, and coarse underground PM was the most potent coarse PM in eliciting release of TNFα and MIP-2, although underground PM was less active in this regard for fine and quasi-ultrafine fractions compared to traffic PM. Only traffic and steelworks fine and UFPM elicited IL-6 release. While across all samples there was no association between PM OP and cytokine release, a positive association was seen when underground PM was excluded from correlation analysis, suggesting that underground PM possesses fundamentally different chemistry from the other PM types [78].

Following *in vitro* studies, five of the sites were used for human exposure studies (underground, urban background, continuous traffic, stop-go traffic, and farm). These studies found associations between F_ENO, which represents eosinophilic airway inflammation [79], and PM Fe, V, Cu, and water-soluble Ni, and between loss of lung function (by FVC and FEV_1) and Fe, Cu, and water-soluble Ni, but not PM_{10} mass concentration or OP [80]. In nasal lavage, IL-6 and IL-8 were associated with organic carbon, NO_2 and endotoxin concentrations. Concentrations of lactoferrin, a metal-binding protein with both pro- and anti-inflammatory properties, were associated with the high PM metal concentration found at the underground site [81]. Plasma concentrations of cardiovascular risk markers C-reactive protein (CRP), fibrinogen, von Willebrand factor (vWF), and tissue plasminogen activator/plasminogen activator inhibitor-1 complex, as well as platelet count, were associated with PM OC, nitrate (NO_3^-), and SO_4^{2-}, although the latter two may reflect increased bioavailability of metals rather than being the causative species *per se* [82], while thrombin generation in blood taken from exposed volunteers was associated with PM NO_3^- and SO_4^{2-}, as well as NO_2 concentration [83]. After 2 h exposure, there was an increase in neutrophil count, while 18 h after 5 h exposure there was an increase in circulating monocytes, associated with PM_{10} and $PM_{2.5}$ mass concentrations, EC, and PM OP [84]. These associations were, however, driven by underground exposure, and were not present in when underground exposures were excluded from analyses, further emphasising the atypical nature of

627 underground PM. Because of the consistently higher concentration of certain PM characteristics
628 at the underground site compared to the other study sites, it was not possible to determine the
629 characteristics driving this response, but it is notable that most factors other than PM OP were
630 excluded.

631 Over the RAPTES studies (summarised in Table 3), it is noteworthy that different endpoints were
632 responsive to different PM characteristics. Furthermore, it can be seen that short term (2 h)
633 exposures were sufficient to induce measurable changes *in vivo*, which may be important in
634 understanding the potential effects of underground air exposure on passengers, as well as workers
635 who may be exposed for longer periods. While it was noted that some (although not all) of these
636 endpoints were especially responsive to underground PM, it is also notable that in several cases,
637 correlations between PM characteristics and endpoint were driven by the presence/absence of
638 underground PM in the analyses, implying that underground PM represents a distinct type of PM
639 compared to the other PM types analysed. However, the endpoints measured were not always
640 associated with factors enriched in underground PM; in several cases, outcomes were associated
641 with organic carbon concentrations. The degree of difference between underground PM and the
642 other PM samples renders it difficult to delineate the specific components of underground PM
643 responsible for its effects.

644

645 **(4) *In vitro-In vivo* extrapolation for risk assessment**

646 A small number of studies have attempted to quantify health impacts of underground PM from
647 data generated in *in vitro* experiments. The group of Constantinos Sioutas and colleagues assessed
648 the health effects of PM on the Los Angeles underground in three studies [85, 86, 87]. In the first,
649 they compared the composition and ROS generating capacity of coarse and fine PM from an
650 underground railway and an overground railway, collected from stations and on trains to represent

real-life exposure, and an ambient site at University of Southern California [85]. 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 Fe in the underground samples compared to overground and ambient samples along with enrichment of non-crustal species such as Mn, Cr, Co, Ni, Cu, Ba, Mo, Cd, and Eu, with the enrichment more pronounced in the fine fraction than the coarse fraction on both PM mass/mass and mass/volume concentration measures. This concentration enrichment was attributed to the specific sources of these elements underground and the enclosed environment of the underground, respectively. Secondary ions and organic carbon species underground were suggested to have derived from outdoor sources. Crustal species concentrations were similar in coarse PM underground and above ground, but there was suggested to be an additional source of Al and Ca in fine PM underground. Correlation analyses suggested that Al and Ca, along with the aforementioned non-crustal species, likely derived 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 Ba (brake wear), Cu (sources not suggested, although may be electrical contact components), and Zn (may originate from above-ground vehicular emissions). As noted in other studies earlier, these elements generally exhibited lower water solubility in underground PM than in overground or ambient PM, across both coarse and fine PM. When taking airborne PM mass concentration into account, Fe and Ba were the only water-soluble components enriched in underground PM compared to the other two sites. Across all sites, ROS generation in DCF-loaded PM-exposed macrophages was strongly correlated with water-soluble Fe, Ni, Cr, Cd (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 Fe 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

677 slightly more ROS than the other PM samples, while in the fine fraction overground railway PM
678 generated more ROS than underground and ambient PM, which were approximately equipotent.
679 When taking airborne concentration into account, on a PM mass/volume basis, coarse and fine
680 PM at the underground site generated more ROS than the overground rail and ambient sites, but
681 the magnitude of this difference was not as great as might have been expected if only the elemental
682 concentration of PM samples were considered.

683 A second study by the same group compared PM on the underground and overground lines with
684 two roads, one with low heavy goods vehicle (HGV) usage and one with high HGV usage [86].
685 Analysis of the potential lung cancer risk due to PAH exposure was performed, suggested that the
686 lung cancer risk was highest from the HGV-high roadway, on account of the relatively higher
687 concentrations of PAH. While the underground line had the lowest PAH concentration, it was
688 suggested that the overground rail line, which had a PAH concentration almost as low as the
689 underground line, along with a lower PM load and metal concentration than the underground line,
690 may represent the safest route with its combination of low PAH and low PM. The US EPA
691 recognises 16 PAHs as carcinogenic and requiring of monitoring [88], and it was on this basis that
692 the authors initially restricted their calculations to this group.

693 However, it is also recognised that PAH are not the only carcinogen/potential carcinogen in
694 airborne PM. Therefore, the same group performed a further study to evaluate the effects of
695 metals within underground $PM_{2.5}$, both in terms of carcinogenicity and non-carcinogenic toxicity,
696 and set these effects within the context of the carcinogenicity from arising from PAH exposure
697 [87]. This was done by measuring concentrations of organic carbon and metal species in
698 underground and overground railway systems, as well as the two roadway classes in the previous
699 study, and deriving the carcinogenicity and toxicity potentials from values established by the US
700 EPA and the California Office of Environmental Health Hazard Assessment. Notably, the
701 contribution to increased cancer and non-cancer risk was found to be greater for metals especially

enriched in underground $PM_{2.5}$ compared to the contribution for PAHs enriched in traffic-derived PM. This was especially true for Cr, which was assumed to exist wholly as hexavalent Cr(VI), which is highly carcinogenic [89], as well as Ni, and Cd. This carcinogenicity is due to the ability of Cr(VI) to enter cells as a result of the similarity of the hexavalent Cr oxyanion (CrO_4^{2-}) to phosphate (PO_4^{2-}) and SO_4^{2-} anions and the relatively non-selective anion uptake channels used [89, 90]. Cr(VI) is then reduced intracellularly to Cr(III), which forms DNA adducts. For non-cancer risk (termed the "hazard quotient") the increased risk of underground PM exposure was driven by Cd, Cr, Ni, and Mn. 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 were not exceeded, the excess lifetime cancer risk (ELCR), at 10^{-5} over a lifetime of exposure, is an order of magnitude greater than the permitted value of 10^{-6}. This is driven primarily by Cr concentrations 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 Cr in $PM_{2.5}$ exists in the Cr(VI) form on account of its high temperature formation – this was not verified, although a similar assumption has also been made elsewhere [91].

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 [92]. They found increased concentrations of $PM_{2.5}$ and decreased NO_2 in underground stations compared to overground stations, and increased $PM_{2.5}$ in underground stations in urban areas compared to those in green areas. The observed average underground platform $PM_{2.5}$ concentration during rush hour periods of 265 $\mu g/m^3$ is higher than in most other studies. Furthermore, underground $PM_{2.5}$ and NO_2 concentrations increased in rush hour. $PM_{2.5}$ concentrations were lower in carriages than on platforms, a finding attributed to the use of in-

carriage air filters. Underground $PM_{2.5}$ in the summer was significantly lower than in the spring, suggested to be due to increased humidity in the summer. The authors then attempted to derive inhaled dose and use this value, along with underground $PM_{2.5}$ and NO_2 concentrations and journey numbers to calculate DALYs, arriving at a value of 6390 DALYs in 2015, equating to 375 premature deaths, or 1% of the total deaths in the city as a result of underground air pollution exposure. 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 across a whole population.

Summary

These studies (summarised in Table 4) provide evidence to support the assertion that underground PM should not be regarded as simply an Fe-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 of the metals, which may be lower in underground PM than in ambient PM [85]. This work illustrates that the carcinogenic and non-carcinogenic effects of non-ferrous metals in underground PM may outweigh the effects of the PAH in the heavily trafficked roads. This also lends further support to the studies featured in Section 2, which are suggestive of important roles for non-ferrous metals in the toxicity of underground PM [66, 69]. While the specific risk factors, attributed disability adjusted life years (DALYs), and similar derived values rely on a number of assumptions, such studies serve to highlight the potential effects of underground PM exposure on large populations, and illustrate the diversity of potential toxicants within underground PM, thus emphasising the

importance of considering the totality of PM composition, rather than solely the predominant components.

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

In addition to studies on the *in vivo* effects of exposure to underground PM (section 1), there are studies examining the *in vivo* effects of exposure to PM which, while not originating from an underground station, might be expected to be similar to underground PM in terms of chemical composition, or at least more so than ambient PM. A report by the Institute of Occupational Medicine suggested that welding fume may represent a surrogate for underground PM [38, 93]. However, the majority of the mass of PM on the underground is likely to derive from shearing and abrasion as takes place in steel mills, rather than through vaporisation, which is the case with welding fume generation [94].

These studies may offer insight into the potential effects of underground railway PM, because they feature exposure to PM which is (1) enriched in Fe compared to that above ground, and (2) at concentrations closer to those which might be found in a typical underground station compared to urban PM. Furthermore, this enrichment is not simply a fume-derived process as is the case for welding fume which, on account of its generation method, results in large numbers of particulates of a small diameter, with consequent high PNC and total particle surface area. In terms of effects on underground staff, it is reasonable to assume that underground drivers and steel mill workers would likely be composed of mainly young and middle aged workers, predominantly male, although the latter may not apply to staff elsewhere on the underground such as ticket office and platform staff.

773 Studies performed near a Canadian steel mill observed associations between various airborne
774 metals and markers of adverse cardiac effects [95], having previously observed that spending 8-
775 hour periods near to a steel mill was associated with small decreases in lung function [96].
776 However, airborne concentrations of Fe and other transition metals near the steel mill were at least
777 2 orders of magnitude lower than might be seen in an underground station, and no association of
778 health effects with Fe was noted. Furthermore, these findings were not consistent with wind
779 direction from the steel mill, and confounded by associations with SO_2 and NO_2 concentrations,
780 which was also the case for the observed association with decreased HRV in a follow-up cross-
781 over study [97]. A follow-up study did find an association between the increased UFPM PNC at
782 the near-steel mill site and urinary 8-isoprostane with a 2 day lag, and also between 8-OHdG and
783 NO_2 and NO with a 1 day lag, suggesting a delayed effect of steel mill emissions on urinary
784 biomarkers of oxidative stress [98], but this does not necessarily implicate Fe in the association.

785 Studies on workers at an Italian steel mill showed exposures to PM_{10} and PM_1 similar to those
786 which might be expected in an average underground station, although with a notably lower
787 proportion of Fe (mean 32 $\mu g/m^3$ in a mean airborne PM_{10} load of 233 $\mu g/m^3$) [99]. 17/88
788 extracellular vesicular microRNAs evaluated in blood were significantly correlated (16 positive, 1
789 negative) with PM and metal exposure [99], principally miR196b which is linked to poor prognosis
790 in a number of cancers, with a role in epithelial-to-mesenchymal transition and thus metastasis
791 [100], and also a potential role in insulin biosynthesis [101]. Previous studies at the same site found
792 changes in blood microvesicle miRNAs and upregulation of miR-302c and miR-128 after 4 days
793 of work following a 2 day rest period [102], and also associations between PM_{10}, PM_1, and Zn
794 concentrations and increased blood endogenous thrombin potential (ETP), methylation of NOS3
795 (nitric oxide synthase-3), and decreased EDN1 (endothelin-1) methylation (Zn only) [103]. As
796 length of employment increased (irrespective of donor age), there was increased demethylation of
797 histone 3 lysine 4 (H3K4) and acetylation of histone 3 lysine 9 (H3K9), both associated with Ni

798 and As exposure [104]. All of these results are interesting and suggest the need for future work,
799 but do not suggest any specific health effects of exposure to steel mill PM *per se*.

800

801 Studies using the closure of a steel mill in the Utah valley to investigate PM_{10} concentrations and
802 health outcomes were performed in the 1980s. These showed an 89% increase in hospital
803 respiratory admissions amongst children, and a 47% increase amongst adults, when PM_{10} exceeded
804 50 $\mu g/m^3$, while PM_{10} concentrations decreased when the steel mill closed [105]. However, it is
805 unclear from this study whether these effects were due to decreased Fe-rich or combustion-related
806 emissions. Water-soluble extracts of ambient total suspended PM (TSP) samples from the local
807 area taken when the steel mill was open were also more able to cause apoptosis, increased BAL
808 fluid cell count, and airways hyperresponsiveness when instilled in rat airways compared to PM
809 samples collected when the mill was closed [106]. Lung injury and inflammatory mediator release
810 *in vitro* induced by TSP collected nearby in 1982 were reduced in when the PM was pre-treated
811 with a chelator [107]. Importantly, effects on cell injury and intracellular signalling *in vitro* caused
812 by Utah PM could be recapitulated by treating cells with simple mixtures of the most common
813 metals in the PM samples [108]. However, no clear picture has emerged as to which metal is most
814 likely to be particularly relevant to health or if elemental interactions play a role. In this regard,
815 the latter paper above suggests that it is the combination of metals, rather than their individual
816 presence, which is of critical importance in determining outcome.

817 It is noteworthy that in most cases, these studies find associations of health effects with non-
818 ferrous metals rather than ferrous metal, suggesting that other metals associated directly or
819 indirectly with steel manufacture may drive health effects. Similarly, in several of the papers
820 detailed in this review, these other elements have been suggested to be important drivers of some
821 of the effects of underground PM, and would likely be less enriched in steel mill PM.

822 Nonetheless, if it is assumed, with caveats, that such steel mill investigations might provide insight
823 into the potential effects of underground PM, their evidence for an effect of underground PM is
824 generally minimal – small changes in respiratory and cardiac function with elements not associated
825 with underground PM, and effects on microRNA and DNA methylation without obvious
826 manifestations *in vivo*. Therefore, no clear conclusions about the end effects of underground PM
827 can be drawn from these studies, but the potential effects of steel-associated PM do merit further
828 study. In this regard, it is notable that a study of the associations between $PM_{2.5}$ and respiratory
829 and cardiovascular hospital admissions in New York in 2001 and 2002 showed that steel emissions,
830 characterised by Fe and Mn, likely from the World Trade Center construction site, were
831 significantly associated with increased respiratory admissions with 0 and 3 days lag, for asthma and
832 pneumonia, respectively [109]. Conversely, traffic PM was associated with total cardiovascular
833 admissions with 0 days lag, although these extra admissions may have been restricted to vulnerable
834 subgroups who would be less likely to use the underground.

835

836 **(6) Other studies of PM metal composition**

837 In addition to work on steel mill exposures, there are many other studies examining the
838 contribution of specific $PM_{2.5}$ components on health outcomes. These are the subject of an
839 excellent review by Morton Lippman [110], which also covers the National Particle Component
840 Toxicity (NPACT) series of studies, and will not be discussed at length here. In short, while it is
841 clear that transition metals are one of the components of $PM_{2.5}$ consistently linked to adverse
842 effects on health following acute exposure, the specific metals implicated are much less consistent
843 across studies. In terms of sources, short-term effects are generally associated with road vehicle
844 and ship emissions (e.g. OC, EC, Cu for traffic, and Ni and V for ship marine oil combustion),
845 whereas longer-term effects are more associated with coal combustion. In ambient exposures,

correlation between many of the constituents means that identification of the most toxic specific chemical species, rather than sources, is difficult. This makes extrapolation of the results to underground railways difficult because underground PM is very rarely included in such studies. Thus, it is difficult to determine whether any element present in elevated concentrations underground may pose a specific risk to health based on findings from ambient PM because such an extrapolation requires knowledge of causation rather than simply association, unless the key sources are the same. Even if certain elements in ambient air could be conclusively shown to cause certain health outcomes, these elements may not exist in the same form (e.g. metallic/oxide/sulphate/nitrate), oxidation state, or combination as underground – these factors could potentially influence ROS generating capacity, which is likely an important modulator of toxicity. Furthermore, the mass concentration and exposure rate to PM underground is much different to above ground, which may have consequences for biological response.

Conclusion

From *in vitro* studies using cell lines, through *in vitro-in vivo* extrapolation from chemical composition, to controlled short-term exposure studies *in vivo*, and epidemiological studies of underground railway workers exposed chronically, there are several conclusions which can be drawn about the effects of underground railway PM:

1. Studies which compare airborne PM mass concentrations in underground railways with those above ground generally find increased PM concentrations underground. However, such studies tend to focus on a relatively small number of networks, in cities with relatively low ambient PM concentrations, and older underground railway networks which are less likely to have air conditioning systems installed. Further research is needed to understand whether these findings also apply to newer air conditioned networks in more heavily-

polluted cities. Other factors which may increase the extent to which underground PM characteristics and concentration are different from those above ground include station depth, station age, wheel/rail type, air conditioning system, and distance from portal where the trains enter/leave the outside environment.

2. Underground PM is generally transition metal-rich, whether measured per mass of PM or per volume of air. Fe predominates, but there is a general enrichment of steel-associated elements, and also elements associated with train brake wear, electrical components, and lubricants, such as Cu, Sb, and Ba.
3. The proportion of the mass of transition metals in underground PM which is water-soluble is markedly lower than in urban PM. As such, the airborne mass concentration of water-soluble transition metals within underground PM is not necessarily greatly elevated over ambient PM. Consequently, the concentration of bioavailable metal in underground PM compared to ambient PM may be overestimated if only total PM metal concentration is considered.
4. Per mass of PM, underground PM is generally poor in organic carbon and secondary anions such as NO_3^- and SO_4^{2-}, the latter potentially underlying the poor solubility of metals in underground PM.
5. 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 (e.g. NO_2) compared to above-ground locations.
6. *In vitro*, underground PM appears to be better able to elicit ROS generation/antioxidant depletion than is ambient PM, likely related to underground PM transition metal composition.
7. Metal-related ROS generation appears at least partly to underlie the oxidative damage of DNA, and induction of antioxidant expression.

8. *In vitro*, inflammatory cytokine release in response to underground PM relative to ambient PM may depend on whether the cell type used is sensitive to endotoxin, which is generally less concentrated in underground PM compared to ambient PM. Thus, endotoxin-sensitive cells (e.g. macrophages), may appear to be especially sensitive to ambient PM, while endotoxin-insensitive cells (e.g. epithelial cells) may appear to be relatively less sensitive to ambient PM.
9. The heightened effects of underground PM compared to ambient PM, in terms of oxidative damage and effects where endotoxin is unlikely to be involved, are clearly able to outweigh the burden of toxicity which may be expected in urban PM as a result of increased PNC, compared to the lower PNC of an equal mass of underground PM.
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 intrinsic differences between complex tissues and organs, comprising multiple interacting cell types, and simple cell cultures, or the use of PM concentrations in studies *in vitro* which are unlikely to be attained in normal *in vivo* environmental exposures.
11. On the basis of PM composition, underground PM may be associated with an increased risk of carcinogenicity and non-cancer health effects due to its metal-rich composition, which may outweigh the toxicity of PAH in ambient PM. This mainly relates to transition metals; studies have highlighted the potential risks associated with not only Fe, but also non-ferrous metals in underground PM, such as Cr, Ni, Co, Mn, and Cd, although these do not generally exceed exposure limits.
12. Controlled studies of short term exposure in underground stations have shown some associations of metals with respiratory endpoints, although these were not always associated with PM OP. However, the same studies have found other endpoints unrelated to PM metals, and instead related to components such as PM organic carbon and anions,

both of which comprise relatively low proportions of underground PM mass compared to ambient PM.

13. Studies of underground railway workers, who are chronically exposed to air pollution in underground railway systems, have generally found little or no association of disease endpoints or exacerbations with working in the underground. However, these studies, of which there are only a small number, generally suffer from the use of relatively small sample sizes, while the working populations studied may represent a relatively non-susceptible subset of the population. Therefore, it is difficult to draw robust conclusions from them, especially in terms of how the commuting population may be affected.
14. The closest approximation to underground PM exposures elsewhere may be in steel mills, although these are generally lower in PM mass concentration and PM metal content than underground PM exposures, and also generally higher in pollutant gases (e.g. NO_2, SO_2) than underground. Notably, as for the underground, studies of workers in steel mills/steel plants have also found no consistent clinically significant effects.
15. The RAPTES studies and a study of the effects of a steel-type Fe-Mn signature in ambient PM in New York City have suggested that transition metals may be more associated than ambient PM with effects on lung function, but that cardiovascular endpoints seem to be driven by PM components less in which underground PM is less rich. However, these studies *per se* do not provide sufficient evidence for a likely effect of underground PM exposure.

Overall, while both the increased PM mass concentration and metal content of underground railway PM are suggestive of, and seem to be responsible for, effects on various toxicological endpoints *in vitro*, there is much less evidence to indicate overt toxicity of underground PM exposure *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

945 of ambient PM, it is certainly not the case that these effects of underground PM are necessarily of
946 a greater magnitude than those of ambient PM. Furthermore, there are PM components such as
947 PAHs, and gaseous pollutants such as NO_2, which are generally found at higher concentrations in
948 ambient air compared to underground air, and which are also associated with health effects.

949 From the small number of studies, there is little evidence that the physicochemical characteristics
950 of underground PM translate to a significantly increased risk of adverse health effects in
951 underground railway workers or commuters, although it is clear that further work in this area is
952 required. From a mechanistic viewpoint, more attention needs to be paid to the non-ferrous
953 components of underground PM, ROS- and non-ROS-related mechanisms of toxicity of
954 underground PM, alternative endpoints of PM toxicity as they become identified by
955 epidemiological and omics research, and the distinct effects (if any) of acute exposure *vs.* chronic,
956 repeated exposure over a lifetime, however short the individual exposures may be. In terms of
957 understanding the effects of exposure *in vivo*, there is a particular need for well-designed studies
958 with study populations significantly larger than those hitherto used, and where possible using
959 different population groups stratified factors such as age, sex, and underlying disease.
960 Furthermore, beyond the established PM-associated diseases of cardiovascular disease, asthma,
961 and lung cancer, and given the potential for relatively-insoluble PM to cross the air-blood barrier
962 [111, 112], studies of the effects of chronic exposure to underground PM should also examine
963 other more recent associations with PM exposure, including idiopathic pulmonary fibrosis [113,
964 114], type 2 diabetes [115, 116], Alzheimer's disease [117], and decreased cognitive function [118].

965 **Abbreviations**

966 8-OHdG – 8-hydroxy-2'-deoxyguanosine; BALF – bronchoalveolar lavage fluid; CYP –
967 cytochrome p450; DCF – dichlorofluorescein; DEP – diesel exhaust particulate matter; DTT –
968 dithiothreitol; EC – elemental carbon; F_ENO – fraction of exhaled nitric oxide; FEV1 – forced
969 expiratory volume in 1 second; FVC – forced vital capacity; HRV – heart rate variability; OC –
970 organic carbon; OP – oxidative potential PAH – polyaromatic hydrocarbon; PAI-1 plasminogen
971 activator inhibitor-1; PEF – peak expiratory flow; PM – particulate matter; PNC – particle number
972 concentration; ROS – reactive oxygen species; UFPM – ultrafine particulate matter.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

ML is a co-opted member of a subcommittee of the Committee on the Medical Effects of Air Pollution (COMEAP), who have prepared a report for Transport for London on the potential health effects of air pollution in the London Underground. MJN received payment from Transport for London for preparing a summary of literature relevant to underground railway air quality.

Funding

ML is funded by a Future Leader Fellowship from the Biotechnology and Biological Sciences Research Council (BBSRC [UK] Ref: BB/P011365/1) and a Senior Fellowship from the National Institute of Health Research (NIHR) Southampton Biomedical Research Centre. ML's attendance at relevant meetings was funded by Public Health England.

Authors' contributions

ML read the manuscripts found in the literature search, searched the literature for further relevant references, and wrote the manuscript. MJN searched the literature for further references and made changes to the manuscript. Both authors read and approved the final manuscript.

Acknowledgements

The authors are grateful to Dr Sarah Robertson (Public Health England, UK), who performed the initial literature search and contributed to discussions on the topics arising. In addition, the authors are grateful to other members of the London Underground subcommittee of the Committee on the Medical Effects of Air Pollution, for discussions on various aspects of air pollution in underground – Dr Nicola Carslaw (University of York, UK), Dr David C. Green (Kings College London, UK), Prof Alan R. Boobis, Prof Deborah Jarvis, and Prof Paul Cullinan (all Imperial College, UK), John R. Stedman (Ricardo Plc, UK), Dr Alison M. Gowers and Dr Christina Mitsakou (Public Health England, UK), and to members of the full committee of COMEAP, in particular Dr Mark R. Miller (University of Edinburgh, UK), Prof Frank Kelly (Kings College London, UK), Fintan Hurley (Institute of Occupational Medicine, Edinburgh, UK), and Prof Robert L. Maynard (University of Birmingham, UK). They are also grateful to Prof Stephen T. Holgate, Dr Elizabeth R. Davies, Marieke Wandel, and Dawn M. Cooper (all University of Southampton, UK) for their helpful advice and comments regarding the manuscript.

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Figure Legends

Figure 1. Literature search methodology. Papers were selected for inclusion in the review by combining each of the search boxes in the figure with the Boolean operator "AND", across four databases. The 203 papers yielded by this initial search were refined to include only those of relevance to underground railways and health, and supplemented by relevant papers used in the review of Xu and Hao [13] and also the IMPROVE study. This yielded the 27 papers which form the core body of literature for this review. Further studies have been included and cited where appropriate.

Table 1. Studies investigating the health effects of exposure to underground railway air pollution *in vivo*.

First Author	Publication Year (Study Dates)	Underground	[Airborne PM] (μg/m³ unless stated)	PM Composition	Exposure Period	Sample Size	Effects of Underground Exposure
Klepczynska Nystrom [15]	2010 (October 2006-March 2007)	Stockholm, Sweden	PM_{10}=242±40; $PM_{2.5}$=77±10; $PM_{0.1}$ PNC=8283±1716/cm³	PM_{10}: Fe=58.6±21/0%; Ba=1.0±0.4%; Cu=0.8±0.4%; Mn=0.5±0.2%	2 h, afternoon rush hour	20 healthy non-smoking volunteers (13M 7F), mean age 27 y (range 18-46)	No change in lung function or airway cellular parameters, increased plasma fibrinogen, increased blood Treg count.
Bigert [20]	2011 (November 2004-March 2005)	Stockholm, Sweden	PM_{10-1} DataRAM and $PM_{2.5}$ ticket office=13±3, 10±3; train drivers=33±12, 19±3; platform workers=182±57, 63±12	Not stated	~8 h working day	81 non-smoking workers (55M 26F), mean age 38 y (range 25-50)	No changes in F_ENO or lung function over working day.
Klepczynska Nystrom [21]	2012 (mid November-early April, year not stated)	Stockholm, Sweden	PM_{10}=232±51; $PM_{2.5}$=71±13; $PM_{0.1}$ PNC=8960±660/cm³	PM_{10}: Fe=49.3±7.3%; Ba0.7±0.1%; Cu=ND; Mn=0.4±0.1	2 h, afternoon rush hour	16 mild asthmatic non-smoking volunteers (5M 11F), mean age 26 y (range 18-52)	Increased activated T cells in BALF, no effect on blood T cell counts or coagulation markers.
Bigert [27]	2008 (November 2004-March 2005)	Stockholm, Sweden	PM_{10-1} DataRAM and PM2.5 ticket sellers=13±3, 10±3; train drivers=33±12, 19±3; platform cleaners=256±97, 79±17; platform ticket collectors=108±26, 50±8	Not stated	48 h (over 2 working days)	79 non-smoking workers (54M, 25F), mean age 38 y (range 25-50)	Increased PAI-1 in ticket sellers, increased fibrinogen in train drivers. More exposed platform workers had higher baseline PAI-1 and hsCRP, but no effect over exposure period. No obvious PM effect.
Lundstrom [28]	2011 (mid November-early April, year not stated)	Stockholm, Sweden	Not stated – see [15] and [21]	Not stated – see [15] and [21]	2 h, afternoon rush hour	18 healthy, 15 mild intermittent asthmatic non-smoking volunteers (17M, 16F), mean age 26 y (range 18-52)	9/64 oxylipins assayed in BALF increased in healthy *vs.* asthmatic, volunteers, principally 15-lipoxygenase-generated derivatives of linoleic and α-linolenic acids.
Liu [30]	2015	Taipei, Taiwan	PM_{10}, $PM_{2.5}$ underground=32±12, 22±7; bus=40±16, 32±12; car=34±13, 29±11; walking=50±21, 42±18	Not stated	1 h morning commute	120 healthy volunteers (58M, 62F), mean age 21 y (range 19-24)	Underground commute showed lowest $PM_{2.5}$ exposure and lowest effect on heart rate variability *vs.* bus, car, or walk.
Bigert [33]	2007 (data from 1976-1996)	Stockholm, Sweden	Not stated	Not stated	Chronic workplace exposure	131,496M (250 underground drivers), 22,311 myocardial	No increased risk of myocardial infarction in underground drivers (RR=0.92 [95% CI 0.68-1.25] *vs.* manual

						infarction cases (54 underground drivers)	workers, 1.06 [95% CI 0.78-1.43] vs. other employed males).
Gustavsson [34]	2008 (subjects followed from 1970-1989)	Stockholm, Sweden	Not stated	Not stated	Chronic workplace exposure	319,979M (348 underground drivers), 4,731 lung cancer cases (9 underground drivers)	No increased risk of lung cancer in underground drivers (standardised incidence ratio 0.82 [95% CI 0.38-1.56]).
Grass [35]	2010 (November 2004-February 2005)	New York City, USA	$PM_{2.5}$ exposure median across all subway roles=27 (5th-95th %ile=8-112)	$PM_{2.5}$: median Fe=27%	Chronic workplace exposure	39M underground drivers (median age 48 y, IQR 38-53), 11M bus drivers (45 y, 41-48), 25M office workers (44 (37-51))	Across a wide range of chemical and biomarker assays in blood and urine, only urinary 8-isoprostate was associated with (cumulative) underground exposure.
Mehrdad [36]	2015 (September-October 2012)	Tehran, Iran	Not stated	Not stated	Chronic workplace exposure	81M healthy underground workers, mean age 32±7 y	Increased urinary 8-OHdG in underground tunnel workers *vs.* underground non-tunnel workers.

Table 2. ***In vitro*** **studies of the toxicology of underground railway particulate matter.**

Author	Publication Year	Underground	[Airborne PM] (μg/m³ unless stated)	Underground PM Composition	Comparator PM	Model	Exposure Conc/Time	Findings
Seaton [38]	2005	London, UK	$PM_{2.5}$=270-480; PNC=14,000-29,000/cm³	$PM_{2.5}$: Fe=64-71%; Cr=0.1-0.2%; Mn=0.5-1%; Cu=0.1-0.9%; quartz=1-2%	Urban PM_{10}; TiO_2; welding fume	A549	$PM_{2.5}$ 1-100 μg/ml, 8-24 h	Underground $PM_{2.5}$ caused concentration-dependent increase in IL-8 release, LDH release, plasmid damage.
Karlsson [40]	2005	Stockholm, Sweden	Not stated	PM_{10}: Fe=39% (mainly Fe_3O_4); Si=6%; Al=3%; Ca=1%; Cu<1%; Ba<1%; Mn<1%	Urban street PM_{10}	A549	PM_{10} 9-70 μg/ml (5-40 μg/cm²), 4 h	Underground PM_{10} more genotoxic and oxidative-stress inducing than urban PM_{10}.
Karlsson [41]	2006	Stockholm, Sweden	Not stated	Not stated (may be same as [40])	Wood boiler PM; tyre wear PM_{10}/$PM_{2.5}$; urban PM_{10}	A549; monocyte-derived macrophages	PM_{10} 70 μg/ml (40 μg/cm²), 4 h	Underground PM_{10} induced more DNA damage in A549 cells than other PM tested. In macrophages, urban PM_{10} was most potent inducer of inflammatory mediator release.
Karlsson [42]	2008	Stockholm, Sweden	Not stated	"	Wood boiler PM; tyre wear PM_{10}, urban PM_{10}; diesel PM; Fe_3O_4; Fe_2O_3; CuO; Cu-Zn	A549	PM_{10} 35-70 μg/ml (20-40 μg/cm²), 2-8 h	For mitochondrial depolarisation by PM_{10}, DEP>underground=wood>street>tyre. Underground PM_{10} most potent ROS generator, and increased FPG sites and DNA damage more than Fe_3O_4, Fe_2O_3, CuO, Cu-Zn.
Lindbom [55]	2006	Stockholm, Sweden	PM_{10}=469; $PM_{2.5}$=258	Predominantly Fe, with some Si, Ca, Ba, Cu	Roadwear PM_{10}; street PM_{10}; DEP	Monocyte-derived macrophages; RPMI 2650 nasal epithelial cells; BEAS-2B	PM_{10} 10-500 μg/ml, 18 h	Underground PM_{10} was less potent in eliciting IL-6, IL-8, TNFα release from macrophages, but most potent in eliciting their release from BEAS-2B.
Lindbom [56]	2007	Stockholm, Sweden	"	"	Roadwear PM_{10}, street PM_{10}	RAW 264.7 macrophages	PM_{10} 1-100 μg/ml, 18 h	For inflammatory mediator release by PM_{10}, street>underground>roadwear. For arachidonic acid release and measures of oxidative stress (DTT, TBARS), underground>street>roadwear.

Bachoual [57]	2007	Metro and RER, Paris, France	PM_{10} Metro=67; RER=3609	PM_{10} Metro: Fe=41.8%; Mn<1%; Ca=1.25%; Cu=1.2%; S=2.2%; Si=1.45%; PM_{10} RER: Fe=61%; Mn=7%; Ca=0.2%; Cu=0.45%; S=1.95%; Si=1.8%	Carbon black; TiO_2; DEP	RAW 264.7 macrophages; C57BL/6 mice	PM_{10} RAW 264.7: 0.05-50 μg/ml (0.01-10 μg/cm²), 3-24 h; Mice: 0.22-4.48 mg/kg (5-100 μg/mouse), 8/24 h	RAW 264.7: underground PM_{10} sets elicited most MIP2 and TNFα release. DFX reduced TNFα release by RER but not Metro PM_{10}. Mice: RER PM_{10} but not CB or DEP induced release of TNFα and MIP2, and HO-1 expression.
Jung [58]	2012	Seoul, South Korea	PM_{10}=34; $PM_{2.5}$=4.5	Not stated	None	CHO-K1; BEAS-2B	1.6-100 μg/ml organic extract of PM_{10}	Underground PM_{10} induced significant cell death in CHO-K1, but not BEAS-2B cells. DNA micronucleus formation and strand breakage by underground PM_{10} inhibited by ROS scavengers.
Loxham [60]	2015	Mainline underground station, Europe	$PM_{10\text{-}2.5}$=180; $PM_{2.5}$=71; $PM_{0.18}$=44	$PM_{10\text{-}2.5}$: Fe=32.1%, Cu=1.68%; Mg=1.63%; Ca=1.52%; $PM_{2.5}$: Fe=28.4%; Cu=1.41%; Mg=2.12%; Ca=1.52%; $PM_{0.18}$: Fe=32.9%; Cu=1.71%; Mg=2.56%; Ca=2.20% (see also [8])	None	16HBE14o-; PBEC	$PM_{10\text{-}2.5}$, $PM_{2.5}$, $PM_{0.18}$ 6.25-50 μg/ml (0.6-12.5 μg/cm²), 24 h	PM crosses PBEC mucous barrier to cause concentration-dependent release of IL-8 increasing with smaller PM size. ROS generation and HO-1 induction observed, both inhibited by DFX and NAC.

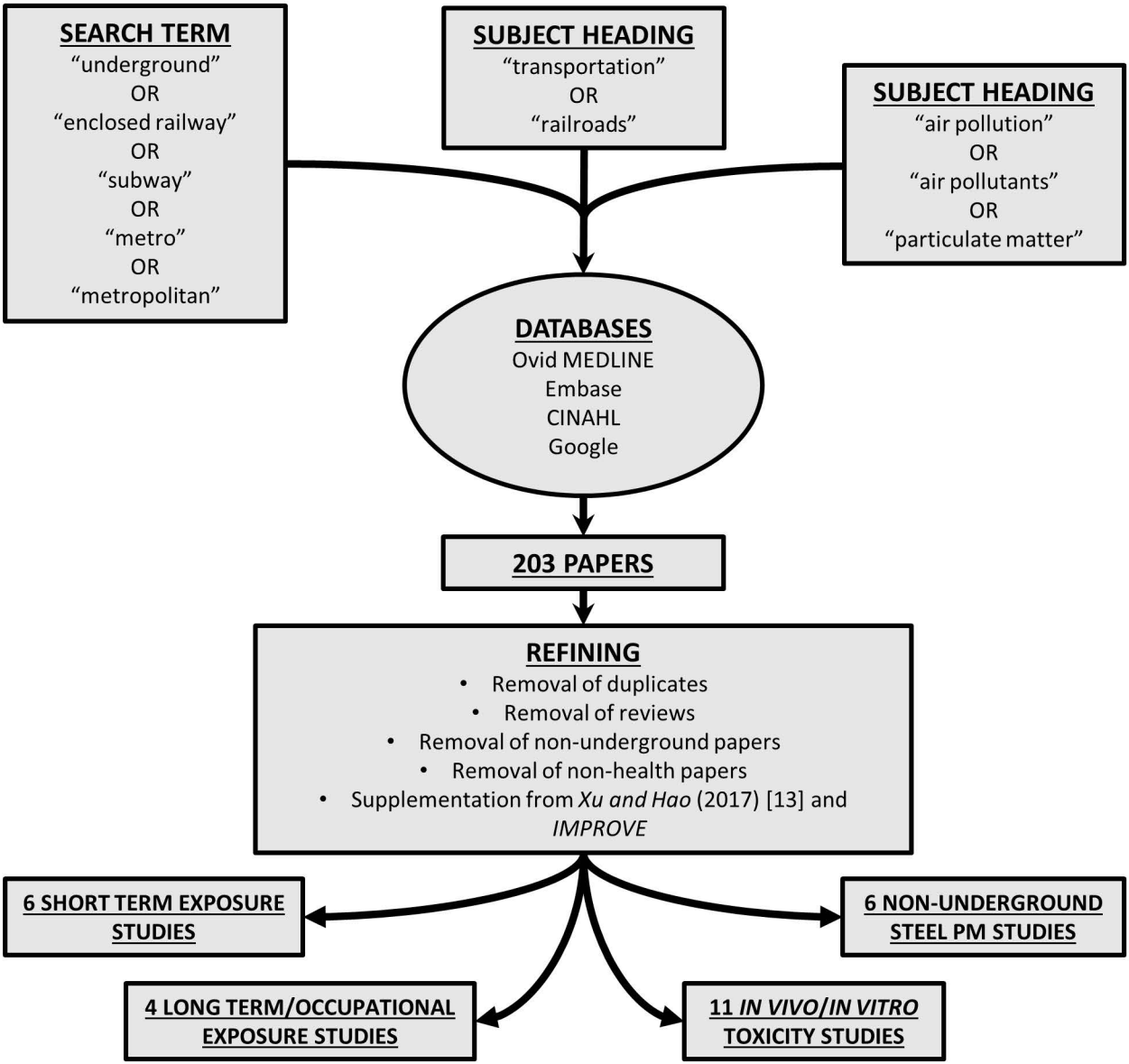
Spagnolo [62]	2015	Not stated	$PM_{10-2.5}$=26; $PM_{2.5-1}$=13; $PM_{1-0.5}$=3.7 μg/m^3; $PM_{0.5-0.25}$=14 μg/m^3	(All ng/m3) $PM_{10-2.5}$: Fe=545, Ca=1568, Ba=122, Cr=15, Cu=14; $PM_{2.5-1}$: Fe=212, Ca=256, Ba=96, Cr=3, Cu=12; PM1-0.5: Fe=71, Ca=58 Ba=99, Cr=2 Cu=4; $PM_{0.5-0.25}$: Fe=31; Ca=30; Ba=99; Cr=ND; Cu=3	Commercial/intermediate station area PM; outdoor PM	NCI-H727	70 μg/ml, 3/6/24 h	Cytotoxicity: platform PM>intermediate area PM, but smallest fractions of outdoor PM most cytotoxic. ROS generation: larger PM sizes>smaller PM sizes. Correlations between transition metals and ROS generation.
Moreno [66]	2017	Barcelona (six stations), Spain	$PM_{2.5}$=33-87 (102 during maintenance activity)	(All ng/m^3) $PM_{2.5}$: Fe=8,000-34,000, Ca=500-1,300 Cu=33-331, Mn=107-301	M120(CB), NIST1648a	Cell-free depletion of ascorbate and GSH	$PM_{2.5}$, cell-free	Antioxidant depletion not associated with PM mass. Antioxidant depletion positively associated with Cu, As, Mn, Zn, Ba, ascorbate depletion negatively associated with Fe
Janssen [67]	2014	Mainline underground station, Europe	PM_{10}=409; $PM_{2.5}$=143	Not stated (see [8] for characterisation of separate samples from same location)	PM_{10} and $PM_{2.5}$: urban background; continuous traffic; stop-go traffic; farm	Cell-free depletion of ascorbate, DTT, ESR	PM_{10} and $PM_{2.5}$, cell-free	Underground PM had greatest oxidative potential of all PM types studied.
Gali [69]	2017	Hong Kong	$PM_{10-2.5}$=10±5; $PM_{2.5}$=48±13	Data as graph only, Fe≈0.2% (similar to other PM sets in study)	$PM_{10-2.5}$ and $PM_{2.5}$: above ground railway journey; bus journey; ambient site	RAW 264.7 macrophages	10-100 μg PM suspension, 4/24 h	Underground $PM_{10-2.5}$ had greatest negative effect on cell viability. Little difference across $PM_{2.5}$ sets. Mass/mass: underground $PM_{10-2.5}$ was best generator of ROS. Mass/volume: above ground PM was more potent. No association with Fe.

Table 3. Papers arising from the RAPTES series of studies.

First Author	Year (sampling/exposure)	Underground	[Airborne PM] (μg/m³ unless stated)	Underground PM Composition	Comparator PM	Model	Exposure Conditions	Sample Size	Findings
Steenhof [78]	2011 (June 2007 - February 2008)	Mainline underground station, Europe (same as [8, 60])	$PM_{10-2.5}$=58; $PM_{2.5-0.18}$=38; $PM_{0.18}$=83; PNC=39,000/cm²	Fe=30.5%; Cu=2.7%; Zn=1.2%	Urban background; continuous traffic; stop-go traffic; truck traffic; farm; steelworks; harbour	RAW 264.7 macrophages	6.25-100 μg/ml (3.68-58.8 μg/cm²), 16 h	N/A	All sizes of underground PM were most potent in reducing cell viability. Coarse underground PM most potent inducer of TNFα and MIP-2 release, otherwise traffic PM generally more pro-inflammatory.
Strak [80]	2012 (March-October 2009)	"	PM_{10}=394; $PM_{2.5}$=140; $PM_{10-2.5}$=252	Fe=154μg/m³; Cu=7μg/m³; Ni=68ng/m³; V=25ng/m³	Urban background, continuous traffic, stop-go traffic, farm	*In vivo* human	5 h	31	F_ENO was associated with PM Fe, V, Cu, and water soluble Ni, and loss of FVC and FEV_1 with Fe, Cu, and water soluble Ni. No association with PM_{10} mass or OP.
Steenhof [81]	2013 (March-October 2009)	"	"	"	"	"	"	"	OC, NO_2, and endotoxin associated with nasal lavage IL-6 and IL-8. Lactoferrin associated with underground PM metal.
Strak [82]	2013 (March-October 2009)	"	"	"	"	"	"	"	Plasma CRP, fibrinogen, VWF, tPA-PAI-1, platelet count associated with PM OC, NO_3^-, SO_4^{2-}.
Strak [83]	2013 (March-October 2009)	"	"	"	"	"	"	"	*Ex vivo* blood thrombin generation associated with PM NO_3^- and SO_4^{2-}.
Steenhof [84]	2014 (March-October 2009)	"	"	"	"	"	"	"	Increase in circulating monocytes associated with PM_{10} and $PM_{2.5}$ mass, EC, and PM OP, mainly driven by atypical characteristics of underground PM.

Table 4. Studies using *in vitro-in vivo* extrapolation for risk assessment of exposure to underground railway air pollution.

First Author	Year	Location	[Airborne PM] (μg/m³)	PM Composition	Comparator PM	Model	Findings
Kam [85]	2011 (sampling May-August 2010)	Los Angeles, USA	$PM_{10-2.5}$=11±2; $PM_{2.5}$=33±1	$PM_{10-2.5}$: Fe=27%; $PM_{2.5}$: Fe=32%	$PM_{10-2.5}$ and $PM_{2.5}$: overground train journey; ambient	Alveolar macrophage	Underground PM enriched in Fe, Mn, Cr, Co, Ni, Cu, Ba, Mo, Cd, Eu, especially in $PM_{2.5-0.1}$. In terms of water-soluble elements, only Fe and Ba were higher in underground PM. For ROS generation, underground>overground>ambient, but difference small.
Kam [86]	2013 (sampling May-August 2010)	"	"	"	$PM_{2.5}$: overground railway; HGV-heavy and HGV-light freeways; stop-go road	N/A	On the basis of airborne PAH concentration, lung cancer risk was: HGV-heavy road>HGV-freeway>stop-go road>overground railway>underground railway
Lovett [87]	2018 (sampling May-August 2010)	"	"	"	"	N/A	Extending [86] to also take metals into account, total hazard quotient from PM exposure greatest on the underground, mainly due to Cr(VI). Overground railway has lowest hazard.
Cao [92]	2017 (measurement March - August 2015)	Suzhou, China	$PM_{2.5}$ regular hours: underground platform=198 (range 86-351); carriages=60 (45-121); $PM_{2.5}$ rush hours: platform=265 (112-365); carriages=79 (75-145)	Not stated	4 underground stations, 1 above ground station	N/A	$PM_{2.5}$ underground stations>overground, especially in urban *vs.* green areas. Underground $PM_{2.5}$ summer>spring. Underground exposure associated with 6,390 DALYs = 375 premature deaths = 1% total deaths in the city.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• Removal of non-underground papers
• Removal of non-health papers
• Supplementation from *Xu and Hao* (2017) [13] and *IMPROVE*
6 SHORT TERM EXPOSURE STUDIES
6 NON-UNDERGROUND STEEL PM STUDIES
4 LONG TERM/OCCUPATIONAL EXPOSURE STUDIES
11 *IN VIVO/IN VITRO* TOXICITY STUDIES