COMEAP Non-Exhaust Emissions

Summary of Toxicology Studies of non-exhaust PM

There is a general paucity of studies considering the potential health effects of the non-exhaust particulate matter from traffic ("NE-PM", eg particulate matter (PM) from brake wear, tyre wear, road wear and re-suspended road dust).

39 papers were reviewed (see Annex D for references).

- 12 were *in vivo* studies:
  - 11 exposures in rodents
    - 5 by instillation
    - 3 by inhalation
    - 3 by inhalation, but of asbestos-containing brake wear
  - 1 using frog embryos.
- 27 were *in vitro* studies:
  - 1 using human specimens
  - 19 using mammalian cells/cell cultures
  - 3 with non-mammalian cells
  - 4 were acellular only (5 in total included acellular measurements)

In vivo studies largely focused on the effects of NE-PM on the lungs, and occasionally blood biochemistry or blood biomarkers. Typically the biological pathways explored/identified are those that are of known importance for urban PM and exhaust-PM, eg oxidative stress and inflammation. There is considerable inconsistency between studies, but in most cases it appears that tyre or brake-wear particles do have the potential to induce both inflammation and oxidative stress.

In vitro studies have employed a number of methodological designs, dose ranges and cell types, although monolayers of A549 cells (a model for bronchial epithelial cells) are the most frequently studied. As with *in vivo* studies, there is inconsistency between findings, although the majority do show that (higher concentrations of) NE-PM can induce cytotoxicity, release of inflammatory cytokines and generate oxidative stress, eg oxidative modification of DNA.

While both *in vivo* and *in vitro* studies show that NE-PM has the ***capacity*** to induce biological action that are indicative of potential health effects, there are a number of caveats that should be emphasised:

1- There is considerable inconsistency between findings. While the published abstracts from these studies tend to emphasise the positive results, more detailed reading of the full papers show that there are often a similar number of endpoints that are not affected by NE-PM.

2- In general, the significant effects tend be found only at higher concentrations of NE-PM.

-Three inhalation studies have been published for NE-PM*, of which detailed text was available for two. One study (Gerlofs-Nijland *et al.* 2019) used a single high exposure (9 mg/m^3) for several hours and saw some modest toxicological effects. The other study (Kreider *et al.* 2012) used lower doses (0.01-0.1 mg/m^3) for 28 days and found no toxicological effects (these studies are discussed further below).

-In *in vivo* studies administering NE-PM by instillation to the lung, the doses employed are in the upper range, or higher (eg 1-2 mg per mouse or rat**), of that typically used for other PM,

-In *in vitro* studies, wider dose ranges are used, but frequently effects are only seen at the higher doses. The relevance of these doses to other toxicological studies or human exposure scenarios is not clear.

- For several study types employing more than one dose, a clear dose-dependent response is not always evident.

* This does not include studies that investigated brake wear PM that contained asbestos. It is assumed that almost all current vehicles on UK roads will not contain asbestos, following a ban in 1999. A review of the toxicology of asbestos-containing brake wear can be found in Poland & Duffin (2019).

** Frequently upper doses for rodent studies are lower than than many of the NE-PM studies discussed here, eg the University of Edinburgh research lab would typically use upper doses of 0.5 mg/rat or 0.05 mg/mouse, and many of the NE-PM used concentrations >1 mg/rat or 0.1 mg/mouse.

3- Particle size. As would be expected from the number of sources (ie tyre, brake or road PM) and constituents, NE-PM can have a varied size distribution. While there is a general assumption that NE-PM is of a larger size distribution (coarse and the upper ranges of fine) than that of exhaust PM, in many cases there is also a small proportion of ultrafine PM present. The greater relative surface area of the ultrafine particles may make a significant contribution to the biological effects, although this has not been adequately addressed within the evidence reviewed.
The degree of penetration to, and deposition in, the lungs is not adequately considered by *in vitro* models. Given the larger particle size overall, the potential translocation into the blood and to other organs is likely to be less than that of the smaller particles in exhaust PM. Thus, the toxicity of the NE-PM observed in studies investigating lung parameters may not be matched by similar effects in other systems.

4- Many studies use PM collected at roadside, that are then separated into different samples based on season of collection, sampling site or broad size fraction. Particle characterisation (usually content of specific metals) is performed to determine which samples are likely to be rich in NE-PM compared to other sources. However, it should still be noted

that even samples with a higher proportion of NE-PM overall would still contain PM from a mixture of other sources. The proportion of non-NE-PM is often not clear, and may vary depending on whether this is determined by mass or particle number.

5- Comparative toxicology. There are very few papers that directly compare the biological effects of NE-PM with other PM types.

- A selection of *in vitro* assays compare PM from different collection sites that are likely to have contrasting levels of NE-PM (see Table). While the fractions containing NE-PM (including metal-rich coarse PM) have oxidative capacity (a possible predictor of how PM could induce harm to cells) it is not necessarily clear if this can be directly attributed to NE-PM, or if the NE-PMs have a greater reactivity than other PM constituents.

-An *in vitro* study by Gustafsson *et al.* 2008 compared NE-PM with that of other PM sources. This study used a machine to directly generate NE-PM from tyres (studded and non-studded) on different road surfaces. A small part of the work investigated the actions of PM on isolated monocytes from human blood. The clearest results were on cytokine release (See Figure below. The ABT/ABS columns are the tyre+road dust using different road surfaces. “Street PM” was PM collected from a roadside where the physicochemical properties were suggestive of a high proportion of NE-PM). The inflammatory response of monocytes varied between NE-PM samples, although some responses to NE-PM were greater than PM from a subway or diesel exhaust particles (DEP). It should be noted, though, that the very weak response to DEP is surprising (extraction of DEP from filter collection may have been a factor) and that cytotoxicity of PM did not follow the pattern of inflammatory response (see Table below).

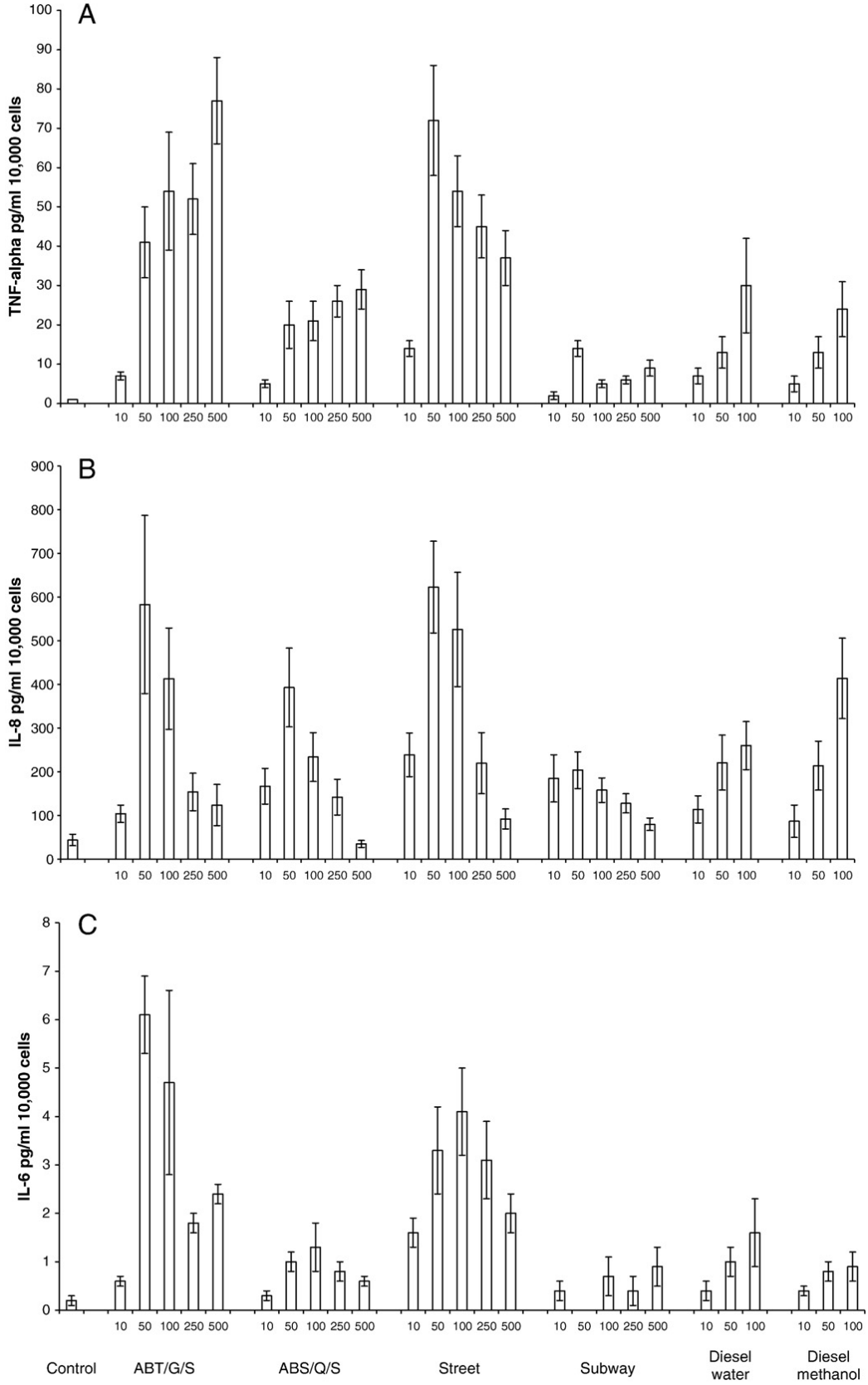
A
TNF-alpha pg/ml 10,000 cells
B
IL-8 pg/ml 10,000 cells
C
IL-6 pg/ml 10,000 cells
10 50 100 250 500
Control
ABT/G/S
ABS/Q/S
Street
Subway
Diesel water
Diesel methanol

Table 5 – Viability of monocyte-derived macrophages as judged by trypan blue exclusion[a]

Particle type	Control	10 μg ml^{-1}	100 μg ml^{-1}	250 μg ml^{-1}	500 μg ml^{-1}
ABT/G/S	96 ± 1.0	93 ± 8.0	91 ± 1.0	78 ± 12	67 ± 15[b]
ABS/Q/S	96 ± 1.0	92 ± 3.0	89 ± 3.1	77 ± 12	77 ± 6[b]
Street	96 ± 1.0	77 ± 23	63 ± 7.5[b]	43 ± 6.0[b]	30 ± 10[b]
Subway	96 ± 1.0	73 ± 21	38 ± 6.3[b]	40 ± 10[b]	33 ± 6[b]
Diesel (w)[c]	96 ± 1.0	–[e]	–[e]	–[e]	–[e]
Diesel (m)[d]	96 ± 1.0	100	100	–[e]	–[e]

[a]The values are expressed as % viable macrophages after 18 h of exposure to 10, 100, 250, and 500 μg ml^{-1} of respective particle type. Values are based on the results from 3 to 4 different experiments except for the diesel (m) value which is based on one experiment due to lack of particles. Comparisons between different types of particles are presented in Results. [b]$p < 0.05$ vs control (unexposed cells). [c]Diesel (w) extracted with water. [d]Diesel (m) extracted with methanol. [e]Not tested due to lack of particles.

-A single study (Gerlofs-Nijland *et al.* 2019) performed 28-day inhalations in mice to study a range of different exposure scenarios including aerosolised brake wear particles. The highest dose used (a dose the authors state to be above real-world scenarios) could induce lung inflammation and increased blood fibrinogen. Comparisons between other exposures showed that in some cases certain brake-wear PMs were more potent than DEP. However, the results were complex and varied greatly between biological parameters. Furthermore, other sources of PM (eg from near a poultry farm) were more consistent in their ability to induce a greater inflammatory response (an example is shown in the Figure below – the further to the left the line is, the more potent the PM for that particular parameter). That there should be such marked differences in potency between these different biological markers of lung inflammation is a little surprising.

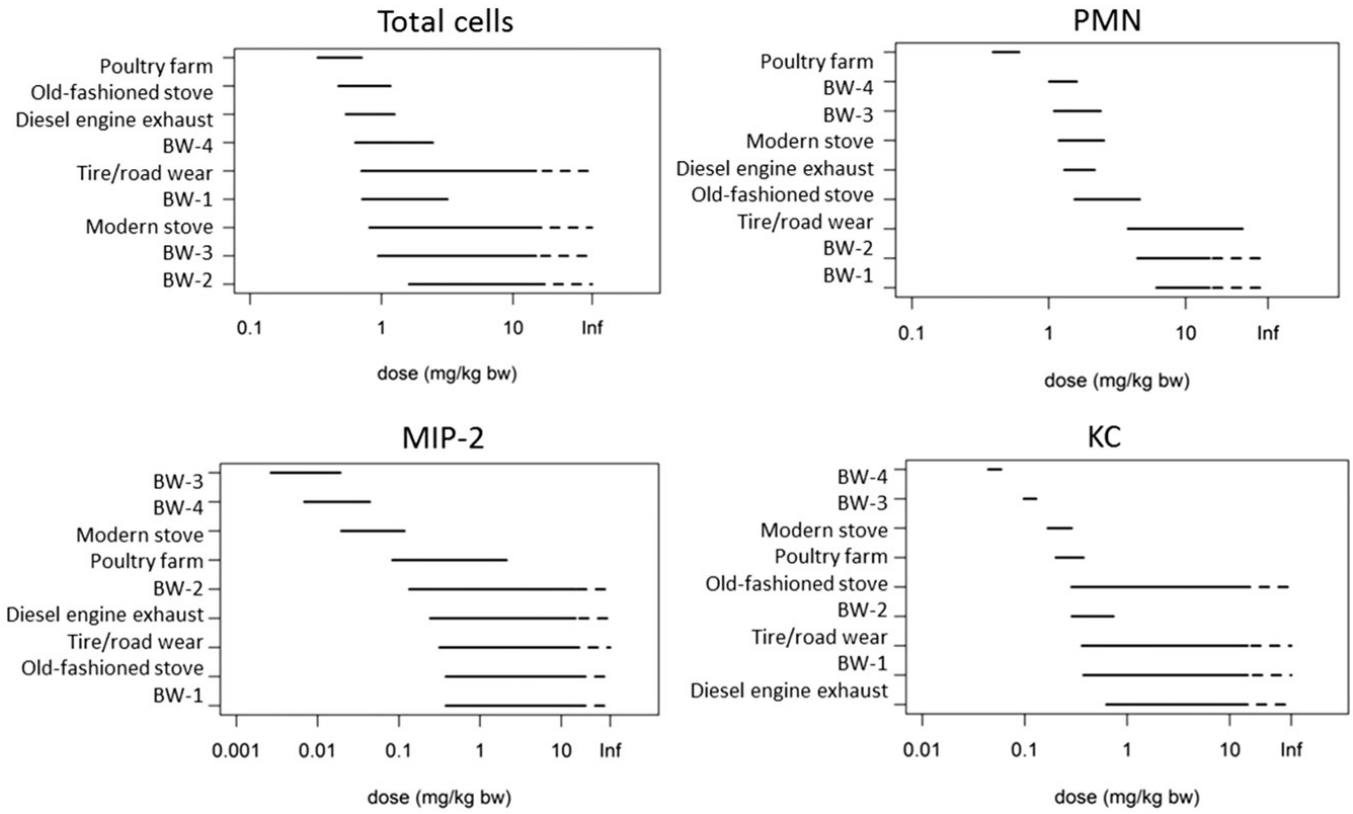

Figure 2. Ranking of PM dose range (BMDL and BMDU) for inflammatory responses in the lung resulting in 20% extra fraction (PMN) or 20% change in response (total cells, MIP-2, KC) compared to controls. Dashed line BMDU infinitive; a dose–response is present, however, at the current doses the in- or decrease in dose–response is too small (<BMR) to derive a BMDU.

-A notable study is that by Kreider *et al.* (2012). Here, a 28 day inhalation exposure to brake wear PM was carried out in rats using 3 concentrations (10, 40 & 100 μg/m^3) the lower doses of which may be relevant to the real world. A large battery of assays was performed and, apart from the occasion foci of lung inflammation in a few mice, no effects were seen for any parameter.

Sub-acute inhalation exposures are considered to be the gold standard with PM testing, thus this study presents strong evidence that brake-wear PM has limited toxicity at relevant concentrations. The authors conclude that the brake wear has a no-adverse exposure level (NOAEL) of 0.112 mg/m^3 (the highest dose they tested), which presumably would be well above the NE-PM expected at roads in the real-world. A later study by the same group (Krieder *et al.* 2019) made extrapolations of this NOAEL for the purposes of human risk assessment, providing a calculated NOAEL of 55 μg/m^3 once species lung deposition parameters and exposure time were taken into consideration. While the study had limitations (eg toxicology and exposure monitoring data were based on only 2 papers by the same group; differences in susceptibility between rodents versus humans and variation in human susceptibility were not considered; the values were based on a limited number of non-respiratory parameters and exposure was intermittent and sub-acute; lack of comparison with other

particle exposures, etc), the progression towards risk assessment is useful.

In relation to comparative toxicology, the Discussion of the Kreider study also shows unpublished preliminary data from rodents instilled with the brake-wear and other PM. While less physiological, this route of administration has the advantage of bypassing the complex nasal cavities of rodents that could limit the amount of PM reaching the lung. Although only a single marker of lung inflammation is reported (LDH in BALF) both SiO_2 nanoparticles and diesel exhaust particles induce lung inflammation, whereas the brake-wear PM (TRWP1 & 2 on graph) results are identical to the control (the solution the PM was suspended in for administration; labelled as “vehicle” on the graph).

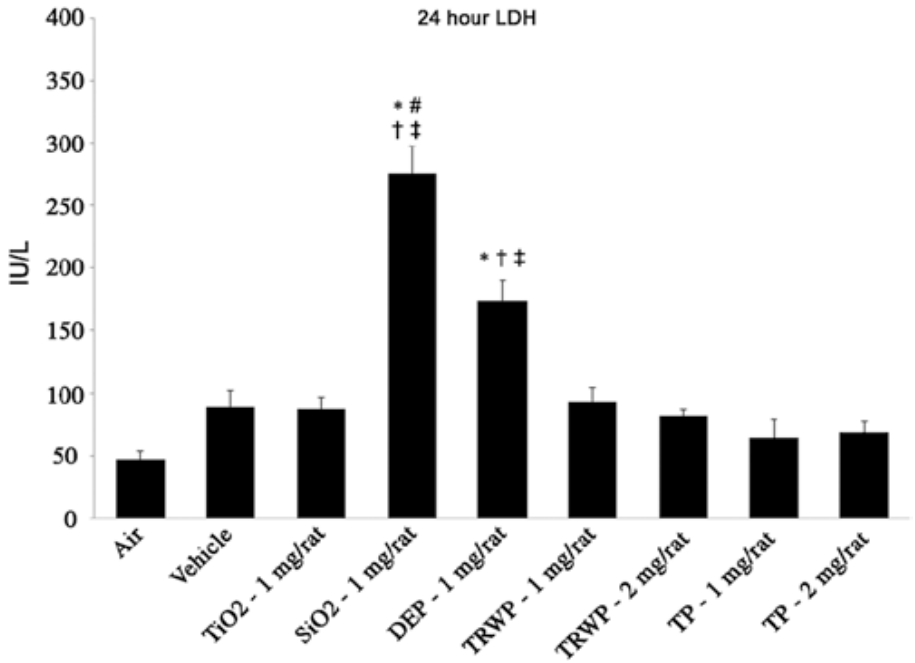

Figure 8. Sample results from comparative toxicity study: Intratracheal instillation. Data are presented as mean ± SE. n = 8 per treatment group (males and females combined). *Statistically different from air control; #Statistically different from vehicle control; †Statistically different from tire and road wear particles (TRWP) (1 mg). ‡Statistically different from tread particles (TPs) (1 mg).

Overall, from the limited studies available, the evidence suggests that while NE-PM can induce inflammation and oxidative stress in biological systems, the evidence as to whether this leads to ‘toxicological actions’ is varied. Where toxicological effects are observed, this may only be seen for some biological parameters and not others. Additionally, often effects are observed only at high doses that are beyond real-world scenarios. There is a need for further studies that directly compare toxicological actions of NE-PM to exhaust PM and investigate the effects of NE-PM in organ systems other than the lung.

Cited References (see Annex D for list of other references reviewed)

Gerlofs-Nijland ME, Bokkers BGH, Sachse H, Reijnders JJE, Gustafsson M, Boere AJF*, et al.* (2019). Inhalation toxicity profiles of particulate matter: a comparison between brake wear with other sources of emission. Inhal Toxicol 31**:** 89-98.

Gustafsson M, Blomqvist G, Gudmundsson A, Dahl A, Swietlicki E, Bohgard M, Lindbom J, & Ljungman A (2008). Properties and toxicological effects of particles from the interaction between tyres, road pavement and winter traction material. Sci Total Environ 393: 226-40.

Kreider ML, Doyle-Eisele M, Russell RG, McDonald JD, & Panko JM (2012). Evaluation of potential for toxicity from subacute inhalation of tire and road wear particles in rats. Inhal Toxicol 24**:** 907-917.

Kreider ML, Unice KM, Panko JM (2019) Human health risk assessment of tire and road wear particles (TRWP) in air. Human Ecologic Risk Assess, https://doi.org/10.1080/10807039.2019.1674633.

Poland CA & Duffin R (2019). The toxicology of chrysotile-containing brake debris: implications for mesothelioma. Crit Rev Toxicol 49: 11-35.