Reviewer's report

Title: Activation of inflammatory responses in human U937 macrophages by particulate matter collected from dairy farms

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Reviewer: Inge Wouters

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The paper by Vogel et al. presents interesting results of a study investigating the inflammatory potential of particulate matter from dairy farms. The authors have conducted a series of experiments to show that PM from dairy farms results in inflammation and that both the TLR4 and NFkBeta pathway are involved. However the authors conclusion that endotoxin components of the particles collected play a major role in mediating an inflammatory response might be debated based on the results shown.

Major compulsory revisions

1. Page 3, sentences with concentrated animal feeding operations (CAFO)... till end of paragraph should be removed. These sentences suggest that the effects of emitted PM of dairy farms will be investigated, which is not the case.

2. Last paragraph of the introduction authors state that the study aims to explore the inflammatory effects of sustained exposure to dairy PM. This suggests that authors would investigate longterm exposure in vitro, however experiments conducted included only short-term exposures to PM, namely ranging from 1hr to 6 hr. This sentence should thus be rephrased.

3. Authors should provide some information about the different dairy farms from which results were presented, e.g. number of cows, type of housing, etc

4. Information on endotoxin analysis should be provided in the methods section. As far as I understood the endotoxin content of PM samples was tested by GC/MS analysis and were expressed as EU/mg of dust. Results of GC/MS analysis are normally not expressed in endotoxin units but in micromols. Can the authors explain how they transferred the GC/MS outcomes to endotoxin units?

5. Figure 1 and Figure 2 might be combined, and outcomes described more shortly.

6. In the different experiments (IL8 expression, effect of TLR4 and NFkB inhibitors on IL8 induction, NFkB receptor activity) different exposure duration to PM ranging between 1hr and 6hr were applied. It is unclear why? and how this would affect the outcomes.

7. In addition to TLR4 and NFkB receptor activity also XRE receptor activity activating the AhR signaling pathway was investigated and reported, which is
mainly associated with polycyclic aromatic hydrocarbons. It is highly unlikely that these are present in high concentration in the PM samples from farms. Most likely the PAH content of non-farm PM is higher. However, this comparison was not made. Results do not have much added value and might be skipped.

8. Authors conclude that the endotoxin content of the PM from farms is of major importance and explaining the inflammatory effects found, this is however not completely proven. Indeed the endotoxin content in urban dust is lower than in farm dust, but other components in farm dust likely differ as well. To prove the endotoxin theory, the authors could have removed the endotoxin from the PM solution by treatment with polymixinB which binds endotoxin, which should result in altered inflammatory induction. Alternatively, authors could plot the inflammatory potential relative to the endotoxin content of the dust, and put this in perspective to the outcomes of the LPS control. Given the endotoxin content. 500 EU/mg ~ 50 ng/mg, with an exposure of 10microgram/ml PM this reflects an exposure of 0.5 ng of endotoxin, however the inflammatory reponse is much more enhanced compared to the reaction of 0.1 microgram = 100ng/ml). This would suggest that endotoxin might not be the major component or that other components or synergistic effects of components might play a role. Therefore conclusions should be rephrased.

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests