Reviewer's report

Title: Induction of the interleukin 6/ signal transducer and activator of transcription pathway in the lungs of mice sub-chronically exposed to mainstream tobacco smoke.

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Reviewer: Joost van Delft

Reviewer's report:

In their manuscript the authors describe an investigation on differential gene expression in lungs of mice following exposure to mainstream cigarette smoke. In general the research is conducted according to current standards, though I have several considerations with respect to the experimental design of the study.

Major:

- This deals with the design of the study. Gene expression is measured in total lung at 3 h after the last MTS exposure, and 6 and 12 weeks thereafter. Exposure to MTS affects the cellular composition of the lung (as is also shown by the authors in Table 4), and merely this change may have its effect on the gene expression profile. E.g. only after 12 weeks exposure an increase of mononuclear cells was observed, and also the IL-6/ SOCS3 effects were only present at that moment. The effects on inflammatory pathway may thus well represent the influx of mononuclear cells. The cross-contamination of lung with blood cells could have been reduced when the gene expression was conducted on the lungs after alveolar lavage, as that removes most of the infiltrated cells. Why was that not done? All these aspects must be described in the discussion.

- In figure 1, the numbering of the columns cannot be correct! 7-8 are in a separate branch of the tree, not 1-2, as is written by the authors on page 12.

Minor:

- No histopathology was conducted on the lungs. This weakens the study, as it could further substantiate the findings.

- Page 16 line 7, mentions that early upregulation of genes was found. Upregulation after 6 weeks of exposure cannot be called early, as that should be within hours or maybe a day, and requires more time-dependent analyses.

- The effect on gene expression at 3 h after the last exposure, may very well reflect the last exposure (thus is a measure of an acute effect) and not an accumulative effect of the 6 or 12 weeks exposure. The authors should discuss this.

- What is the relevance for humans, especially with respect to the observation that at 6 weeks after exposure all effects on gene expression are gone? This should be discussed.
**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests