Author’s response to reviews

Title: Cardiovascular health effects following exposure of human volunteers during fire extinction exercises

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Response to reviewer comments:

Below are point-by-point responses to comments from the reviewers. Our responses starts with "RE:..." The line numbers refer to the copy with track changes.
Reviewer reports:

Reviewer #1: Comments on the manuscript entitled "Cardiovascular health effects following exposure of human volunteers during fire extinction exercises"

In this analysis, the authors measured 1-hydroxypyrene (1-OHP), a urinary metabolite of polycyclic aromatic hydrocarbons (PAHs), at different exposure scenarios to assess whether exposure during firefighting activities was associated with biomarkers of cardiovascular effects in young volunteers training to become firefighters. Using linear mixed effects analyses, the authors found that while significant PAH (1-OHP) exposure and changes in biomarkers of cardiovascular effects separately occurred during fire extinction exercises, there was no associations between 1-OHP and measured cardiovascular outcomes among these volunteers. While it is not clear to me, given the existing literature, whether these findings carry any major implications for on-duty (active) firefighters, the topics (PM/smoke/PAH exposure and cardiovascular health among firefighters) are very important. The paper is well written and the methods seem appropriate.

However, a couple of minor issues should be further addressed, which are listed as follows in no order of importance.

I thought the main focus of the paper was to examine the relationship between acute smoke exposures (PM/PAH) and markers of cardiovascular effects in firefighting scenarios. But in its current form, the associations between 1-OHP and measured cardiovascular endpoints seemed lost in both the results and the discussion. A bit more highlights of these associations in both the results and discussions sections of the main paper would be useful.

RE: We have revised the discussion and included results from a parallel assessment of the skin exposure to PAH (lines 296-304 and some text moved from lines 305-308 to 281-285). Furthermore, we address the lack of association between 1-OHP and cardiovascular risk markers (lines 285-288).

It is unclear why age and body temperature were not adjusted for in the regression models.

RE: we did not adjust for body temperature, since we did not collect this information in the main study. We only assessed body temperature at a later visit. We did not adjust for age because the young conscripts had almost the same age (21.0 +/- 1.3 years, table 1).
As indicated by the authors, the DustTrak used could measure PM of various size fractions (i.e. PM1, PM2.5, PM4, PM10, PMtotal), but the report only referred to PM without indicating which size fraction(s).

RE: We have reported the PMtotal mass concentration in the paper (clarified in lines 219 and 316 of the main document and in the supplement).

I wonder if a single measurement is the standard practice in blood pressure measurements. I believe most studies rely on average of two or three measurements.

RE: due to logistic restrictions on time, we had to prioritize the time spent on each biomarker, and we prioritized the rather time consuming EndoPAT measurements of cardiovascular effects. Therefore, we only had time to measure blood pressure once.

Reviewer #2: This was a very well written article on the topic of where and how much firefighters are exposed to PM. I noted a few issues that need to be addressed.

You are using statistical significance testing but do not state alpha levels or consider issues of multiple testing. Either add those in or use alternative metrics (e.g., precision, patterns of association) to judge importance of effect estimates.

RE: Alpha levels are now stated. Many of the assessed cardiovascular endpoints are highly correlated, since they are derived from the same measurements. Therefore, correction for multiple testing would not be meaningful. We have therefore chosen not to do so, and this is now mentioned in the section on statistical analyses (lines 209-210).

Line 221, can you translate 50,000-250,000 particles/cm2 to ug/m3?

RE: We cannot translate the particle number concentration to mass concentration without several assumptions of the physico-chemical characteristics, which really makes the estimate highly uncertain. We have not done that.

Table 2: effects are either significant or they are not, they should not be ranked by p-value

RE: We think the study should be of interest to both toxicologists and epidemiologists. Toxicologists typically feel more comfortable with p-values than confidence intervals. We have kept the p-values as they are not confusing to the reader.
Table 3: SP and DP have significant p-values, but the actual effect and standard error do not appear to be so. typo?

RE: The table contains the SD (not standard error). The p-values for SP and DP are correct. We have clarified that the results are mean and SD (line 650).

Generally tables and figures should stand on their own so acronyms used should be defined in footnotes.

RE: We have clarified the abbreviations in the tables/figures.

Reviewer #3: Thank you for the opportunity to review the manuscript "Cardiovascular health effects following exposure of human volunteers during fire extinction exercises." This manuscript addresses an important concern about the health impacts of firefighting on personnel regularly exposed to strenuous activities and smoke.

The manuscript is well written, results are clearly displayed and authors used appropriate statistical methods. My main comment is that I struggled to understand the aim of the study. More specifically, the aim of the study is presented as "to assess whether firefighting activities... were associated with cardiovascular effects". However, a significant effort is being placed on understanding the exposure to PM and PAH to trainees which would indicate the aim to be the effect of smoke and not firefighting activities on personnel. In ether case firefighting activities and smoke exposure seem to be perfectly confounded so it may be both. I recommend more clarity and discussion of the limitations with respect to the confounded effects of the simultaneous exposure to physical activity and smoke. It is quite possible that it was not feasible to separate the effects of firefighting activities from the effects of smoke but such limitation could be discussed.

RE: the firefighting activity encompasses smoke exposure. Thus, smoke exposure is not a confounder. However, the association between smoke exposure and CVD endpoints is confounded by heat and physical exercise. Thus, we have stated in the conclusion that the CVD responses are due to complex effect of PM exposure, physical exhaustion and heat (lines 383-385). In the discussion, we have not highlighted that the effect of smoke exposure on CVD endpoints cannot be separated from heat and physical exhaustion (lines 288-291).
Second major comment is the lack of blood endpoints. I would suggest discussing the lack of blood endpoints in the study of cardiovascular health effects as a limitation.

RE: we have now included a short description of the results from a parallel assessment of biomarkers of genotoxicity and inflammation in the discussion (lines 296-304).

Minor comments

Line 56-57. Is there a reference that could be added to support the statement?
RE: we have modified the statement slightly and added a reference (lines 56-57).

Line 61. Is exposure to PM and chemicals among the factors that contribute to excess mortality?
RE: we have added “smoke” to the list of compounds that contribute to the excess mortality (line 61).

Line 89. Replace was with were.
RE: corrected (line 89)

Line 139. I found the use of term "exposure" to be confusing since trainees were using PPEs. I would suggest another term to be used here.
RE: we understand the comment, but on the other hand, we do assess the personal exposure in the inhalation zone, and the PAH exposure by 1-OHP, so we have chosen to keep the term "exposure".

Line 148. There is a jump from PM to PAH here that is not explained well. Is PAH used as a marker/biomarker of PM and exposure to smoke?
RE: we have clarified that 1-OHP is a marker of PAH, whereas PAH is a marker of PM and smoke exposure (lines 148-150)

Have the authors tried to use HF and LF normalized by the heart rate?
RE: No, we have kept it simple and used the raw HF and LF results.

Line 302. Discussion takes an unexpected focus on ultrafine particles that are not previously discussed.

RE: we have deleted the text on ultrafine particles (lines 323-327).