Response to Reviewer 1

Manuscript: “Molecular-Level Characterization of Urban Aerosol Analogues in Controlled Atmospheric Simulations”

We thank Reviewer 1 for the detailed evaluation of our manuscript. We respond point by point below and indicate, for each point, the exact text that has been added or modified in the revised manuscript, together with its location.

Comment 1.1 - Lack of Novelty

> *“The conclusion ‘atmospheric simulation chambers as complementary tools to field studies for urban air quality assessment’ has long been recognized by the atmospheric chemistry community, so I did not find any real novelty in this work.”*

We respectfully disagree with this assessment. The novelty of our work does not reside in the use of atmospheric simulation chambers per se, but in the unique integration of controlled atmospheric chemistry with chronic biological exposure via the PolluRisk platform.

A critical clarification: all measurements and chemical characterization results presented in this manuscript were obtained in the exposure isolator, not directly in CESAM. CESAM serves exclusively as an aerosol generation and photochemical aging system. A controlled 1:10 dilution transfers the generated atmosphere to the isolator, where mice are exposed under concentrations representative of real urban environments.

We have added a sentence to the Introduction (p.2) clarifying this point of novelty: “**Furthermore, the limited number of studies that have coupled atmospheric chambers with biological exposure have generally relied on organic aerosol concentrations substantially exceeding ambient levels (e.g., Krug et al. (2018) generated a PM-focused surrogate atmosphere reaching 976 µg.m-3). The present study therefore focuses on the chemical characterization of two controlled atmospheric scenarios, rather than on a general classification of urban pollution levels.”**

We have also revised the Conclusions (p.22) to remove the sentence on atmospheric simulation chambers as complementary tools, replacing it with: “**This study provides a controlled chemical framework for separate biological exposure experiments.**

The PolluRisk platform demonstrates the feasibility of chronic biological exposure to chemically characterised urban atmospheres. Systematic investigation of emission source contributions under controlled atmospheric conditions facilitates the isolation of specific processes, which are otherwise difficult to conduct in complex ambient environments.”

Furthermore, to address the reviewer's concern about the positioning of the study, we have also streamlined the Introduction to remove extensive qualitative classifications of urban pollution levels.

In addition, we have revised the paragraph on urban pollution levels in the Introduction (p.2) to remove the lengthy classification of “highly”, “moderate”, and “low” polluted atmospheres, replacing it with a more concise statement: “**The target PM_1 concentrations were selected to overlap with concentration ranges observed in the Paris region, whereas the molecular composition and temporal variability of the generated mixtures cannot be assumed to reproduce ambient urban air in full. This chemical characterization is designed to define**

exposure conditions for biological models, not to serve as a comprehensive ambient urban air replica."

Comment 1.2 - Absence of Toxicological Data

"The second paragraph of the Introduction is very brief... More importantly, the authors did not report any toxicity or health effects, so the paper did not answer this scientific question."

We have clarified the scope of the manuscript. The present paper reports the chemical characterization of the exposure mixtures; biological and toxicological outcomes are outside its scope and are addressed separately.

We have clarified the Introduction (p.2): "**This paper reports the chemical characterization of PM1 and its organic molecular composition in the two controlled cases. We combine online measurements of particle, trace-gas, and VOC concentrations with offline UPLC/ESI-IMS-QTOFMS analysis of particle-phase extracts. The study identifies source-related and oxidation-related molecular features and estimates their volatility classes. These measurements define the chemical conditions for separate exposure experiments. Thus, the biological outcomes are outside the scope of the present paper. The associated biological and toxicological outcomes observed in murine exposure models will be reported in a companion manuscript currently in preparation.**"

We have also modified the Abstract accordingly to clarify from the outset that this study provides a chemical framework for biological exposure studies, with the health outcomes to be reported separately.

Comment 1.3 - Qualitative vs Quantitative Analyses

"The chemical analysis results were too simplified... the author primarily reported qualitative results with very few quantitative or semi-quantitative discussions."

Our analyses include several levels of quantitative data: 5 SOA markers with an internal standard, PM_1 mass concentrations and bulk chemical composition (ACSM), and 23 VOC species quantified by calibrated PTR-TOF-MS. We also provide semi-quantitative volatility classification (log C*) for all 32 identified compounds, O:C ratios, and diagnostic ratios (VOC/NOx, $[NO_3^-]/[SO_4^{2-}]$).

Following this comment and further internal review, we have clarified Section 3.2 (p.15) with a revised text that now reads: "**Quantification was attempted for five representative SOA markers (2-nitrophenol, cis-pinonic acid, MBTCA, norpinic acid, and pinic acid) using camphor-10-sulfonic acid as an internal standard, following calibration curves established for each compound (Sect. S4, Supporting Information). Atmospheric concentrations were calculated by referencing the mass identified on the filter to the specific surface area analysed and the total volume of air sampled. Sampled volumes ranged between 0.51 and 0.56 m3 for the CESAM chamber and 2.76 and 2.95 m3 for the exposure isolator. Reliable quantification in the exposure isolator was limited by the 1:10 dilution from CESAM combined with the comparatively low organic aerosol loading achieved in this campaign, particularly for the standard urban scenario (1.7 µg.m-3), which approached instrumental detection limits for individual compound quantification.**

A confirmed quantified concentration was obtained for cis-pinonic acid in CESAM under standard urban conditions (255 ± 23 ng.m-3). Comprehensive isolator-level quantification of these markers for both scenarios was not achieved in this campaign and is identified as a priority for future campaigns with higher organic loadings or increased sampling frequency (Sect. 4.3). Qualitative detection was possible for the 32 molecular features even when compound-specific quantification was not completed. However, identifications based only on m/z remain tentative and should not be interpreted as definitive structural assignments (Table 3)."

Regarding volatility estimates: these were calculated using the group contribution method of Donahue et al. (2011) via the UManSysProp platform (Section 2.4.2, p.8). We have added a caveat acknowledging methodological uncertainty: "**We note that alternative volatility estimation methods (e.g., the parametrization of Li et al., 2016) may yield different log C* estimates for the same compounds. Values reported here should therefore be considered indicative rather than absolute.**"

The dual origin of organic aerosol in the biomass burning scenario is supported by molecular marker evidence already presented in the manuscript: the detection of a levoglucosan isomer (primary wood combustion tracer) alongside nitrophenolic compounds and biogenic oxidation products (secondary photochemical markers), consistent with the increase in organic mass fraction from 17% to 40% and the shift in $[NO_3^-]/[SO_4^{2-}]$ ratio from 0.36 to 0.58.

Level 2 identification (Schymanski et al., 2014) for compounds without available reference standards is the accepted standard practice in high-resolution environmental mass spectrometry.

Comment 1.4 - Wall Loss Effects

"Have the potential effects of wall loss and photolysis of reaction products in the chamber been considered?"

This is a valid concern for extended chamber experiments. We have therefore added a new Section 4.3 (p.20) entitled "Wall effects and experimental stability", which provides a substantially expanded discussion of wall losses, heterogeneous reactions, and the empirical evidence for a quasi-steady state. This section replaces the former "Limitations" section and incorporates the previous limitations as sub-points. The full text of this new section is provided below:

"**Wall effects are an inherent consideration in extended chamber experiments. Particle wall losses have not been specifically investigated during these experiments but were characterized in previous work: polydisperse ammonium sulfate particles (submicronic) and dusts (supermicronic) were injected into CESAM, and the number size distribution was measured as a function of time to derive first-order decay rates for each size bin. These experimental loss rates were well reproduced using the parametrization of Lai and Nazaroff (2000). The aerosol lifetime in CESAM was determined to range from 10 h to 4 days depending on particle size distribution, which is therefore quite long, although not negligible for experiments lasting several days as in this study. These losses would need to be considered when performing numerical simulations, which was not the case here.**

The loss of semi-volatile species has also been investigated in previous studies and found to be highly dependent on the wall state (Suarez-Bertoa et al., 2012). A passivation of the

walls was observed when repeating the same experiment over several days, leading to a decreasing wall loss rate for the semi-volatile compounds injected. The impact of heterogeneous reactions occurring on the walls on gas-phase composition has also been investigated in CESAM; for example, the reduction of NO_2 into HONO has been observed, and identified heterogeneous wall reactions are described in an "auxiliary mechanism" (Wang et al., 2011).

Taken together, these considerations confirm that particles and semi-volatile compounds may be lost on the walls, affecting the composition of the mixture. However, this study does not aim to investigate detailed chemical processes but to generate mixtures representative of urban atmospheres. What matters here is the composition of the mixture actually reaching the exposure isolator. Considering that experiments last for several days, we expect that chamber walls progressively passivate, leading to an equilibrium between emissions, losses, and partitioning between gas-particles and walls. This is supported by the stability of the dilution ratio between CESAM and the exposure isolator throughout both experimental periods, consistent with the nominal 1:10 design specification, as well as by the day-by-day stability of the aerosol size distribution (Fig. S1a-b, Supporting Information). Such heterogeneous wall reactions have not been observed to lead to new compounds affecting SOA composition."

Concerning the photolysis of products: since the actinic flux in CESAM closely approximates the solar spectrum, photochemistry is expected to be representative of that occurring in the real atmosphere (Wang et al., 2011).

Comment 1.5 - Figure 5 and Volatility Predominance

> *"Fig. 5 does not show relative signal intensities or abundances. Concluding predominance solely based on number of detected compounds may be biased."*

This is a valid and important critique. Figure 5 visualises chemical diversity across volatility classes, not mass-weighted abundance. We have made two corrections:

In the Results (p.16) and Discussion (p.19): we replaced "revealed compound distributions predominantly within..." and "revealed compounds dominantly in..." with "**identified compounds distributed across...**" in both instances.

For the Figure 5 (p.17): we added the sentence "**. In particular, figure 5 represents the molecular diversity of the identified compounds across volatility classes and does not give information on the relative mass contributions of individual species to total organic aerosol.**"

We trust that these responses and the associated manuscript revisions address the reviewer's concerns satisfactorily.