Response to Reviewer 3

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

We thank Reviewer 3 for the positive recommendation for publication and the thorough set of technical comments. We respond point by point below, indicating for each point the exact text now present in the revised manuscript and its location.

Important preliminary clarification: "**All pollutant concentrations and chemical characterisation results presented in this manuscript refer to measurements performed in the exposure isolator, which constitutes the actual biological exposure environment. CESAM serves exclusively as the aerosol generation and photochemical aging system.**" A 1:10 dilution transfers the generated atmosphere to the isolator where the biological models are exposed. This distinction, now made explicit in Section 2.1 (p.5), is central to interpreting several comments below.

Comment 3.1 - Supporting References (Lines 45-50)

"Add supporting references."

We have added supporting references to this passage.

Comment 3.2 - Oxidation Procedure and Day/Night Cycles

"The oxidation procedure is not clear. What do (days and nights) mean? Did you do both photo-aging and dark-aging experiments?"

We agree that this point needed clarification.

We apologize for this ambiguity. The phrase "days and nights" referred exclusively to the continuous measurement schedule, not to alternating illumination. We have revised Section 2.1 (p.5) accordingly: "**...across 7 consecutive days of continuous monitoring (24 h/day). All pollutant concentrations and chemical characterisation results presented in this manuscript refer to measurements performed in the exposure isolator...**"

Irradiation was maintained continuously throughout the entire experimental period, with no dark-aging periods. This design choice, and its difference from ambient day/night photochemical cycling, is now discussed in Section 4.3 (p. 20): "**Irradiation was maintained continuously throughout the 7-day experiments without day/night cycling. While this ensures stable aerosol generation for chronic biological exposure, it differs from ambient photochemical cycling between OH-dominated daytime and NO_3-dominated night-time chemistry.**"

This clarification has been added both in the methods section and in the discussion of limitations (Section 4.3).

Comment 3.3 - Emission Composition (Lines 150-155)

"Specify if the emissions include both gases and particles."

We have clarified this in Section 2.3 (p.6). The biomass burning enhanced scenario description now reads: "**The biomass burning enhanced scenario involved the same VOC mixture and ammonium sulphate seed particles, with the continuous addition of wood-pellet combustion emissions. These emissions provided primary carbonaceous particles and additional gas-phase compounds. The resulting difference between scenarios therefore combines primary particulate input, additional gaseous emissions, and their subsequent atmospheric processing.**"

Comment 3.4 - OH Exposures and Atmospheric Age

"Details such as the OH exposures achieved and atmospheric age need to be specified. Compare OA/SOA mass to other chamber studies with comparable OH exposure."

We agree that OH exposure would provide useful information for comparing simulated and ambient atmospheres. However, direct OH measurement in CESAM was outside the scope of this study, whose primary objective is controlled biological exposure in the isolator rather than reconstruction of ambient atmospheric chemistry. The assessment of atmospheric representativeness in the present work relies on the agreement between exposure isolator concentrations and ambient Paris measurements (Section 4.1), not on OH exposure reconstruction. We have nonetheless added a perspective on future OH measurements to Section 4.4 (p.22): "**Direct OH measurement in CESAM, while outside the scope of this study, represents a valuable future direction that could enable finer comparison between the simulated and ambient atmospheres.**"

Comment 3.5 - Primary Particulate Emissions (Line 165)

"Baseline traffic emissions also include primary particulate emissions. Shouldn't this first scenario also include particulate emissions?"

The standard urban scenario was designed to examine secondary organic aerosol formation from the injected VOC mixture. It nevertheless contained primary ammonium sulphate seed particles and no primary carbonaceous or traffic-emitted particulate matter. We have added to Section 4.3 (Limitations, p.21): "**The standard urban scenario included primary ammonium sulphate seed particles but no primary carbonaceous or traffic-emitted particulate matter. Its organic fraction was intended to result mainly from oxidation of the injected VOC mixture, although the primary and secondary organic fractions were not quantified.**"

Comment 3.6 - Relative Humidity Representativeness (Line 167)

"Were there any changes in RH between scenarios? How representative is this regarding RH?"

RH was maintained constant at 30-50% on average across both scenarios. We have added to Section 2.1 (p.4): "**RH was maintained constant across both scenarios to ensure experimental comparability. We acknowledge that real biomass burning episodes typically occur at higher RH (60-80%), which would enhance gas-particle partitioning (Sect. 4.3).**" This limitation is further discussed in Section 4.3 (p.17): "**Real biomass burning episodes typically occur at higher RH (60-80%) and lower temperatures, which would enhance gas-particle partitioning.**"

We also note that RH was kept constant across scenarios specifically to ensure comparability of the chemical conditions, as the focus of this study was the influence of biomass burning emissions rather than RH effects.

Comment 3.7 - Wall Loss Effects (Line 230)

"The effect of vapor and particle wall losses on the measured particle concentration and SOA composition needs to be addressed."

We have added a substantial discussion of wall effects in a new Section 4.3 (p.20) entitled "Wall effects and experimental stability". This section includes quantitative estimates of particle wall loss rates (Lai and Nazaroff, 2000), semi-volatile compound losses and wall passivation (Suarez-Bertoa et al., 2012), and heterogeneous reactions (Wang et al., 2011). The discussion also emphasizes the empirical evidence for a quasi-steady state, supported by the day-by-day stability of the aerosol size distribution and the constant 1:10 dilution ratio. 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 NO2 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 length for several days, we expect that chamber walls progressively passivates, 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."

Comment 3.8 - Primary vs Secondary Organics and PMF

"Can authors specify how much organics are primarily emitted and how much is secondarily formed? Have you tried PMF analysis for organic data from ACSM?"

The molecular data are consistent with mixed primary and secondary contributions in the biomass-burning-enhanced mixture, but they do not quantify the two fractions. The increase in organic mass fraction from 17% to 40% therefore cannot be partitioned into primary and secondary contributions from the reported data alone. We have added to Section 4.3 (p.21): "**In the biomass burning enhanced scenario, the dual primary/secondary origin of organic aerosol was assessed through molecular markers rather than formal source apportionment. Positive matrix factorization (PMF) analysis of the ACSM organic mass spectra was not performed in this study.**" And to Section 4.4 (p.22): "**Formal PMF analysis of ACSM organic mass spectra would allow quantitative apportionment of primary and secondary organic aerosol fractions.**"

Comment 3.9 - Continuous vs Real-World Cycles (Line 315)

"I find it hard to understand how continuous exposure of 7 days is representative of the real-world environment, where we have daytime oxidation followed by nighttime."

We agree that a cyclic protocol including dark periods would provide a more realistic representation of diurnal atmospheric processing. Such a protocol cannot be inferred from the present data because the effect of nightly interruption on the multi-day accumulation of organic aerosol has not been characterized. The present experiments should therefore be described as several consecutive days of exposure to a continuously irradiated, chemically controlled mixture. They test biological responses under defined conditions; they do not reproduce the full variability or day/night chemistry of ambient urban air. We have added to Section 4.4 (p.22): "**Future work could also explore exposing mice to variable or cyclic atmospheres to approach more realistic exposure patterns than the stable conditions used in this study.**"

We have also clarified in Section 2.1 that irradiation was continuous, and we discuss the implications of this design choice for atmospheric representativeness in Section 4.3.

Comment 3.10 - Nucleation Events (Line 352)

"Have you observed any nucleation events in SMPS data? If not, this sentence is irrelevant. Please justify."

The reviewer is correct. Analysis of SMPS data from both scenarios confirms the absence of significant nucleation events (particles <30 nm represent <0.01% of total particle mass in both scenarios. Modal diameters of 209 nm and 334 nm are firmly in the accumulation mode (Fig. S1a-b, Supporting Information).

We have revised the flagged sentence in Section 4.1 (p.16). It previously read: "The non-refractory PM_1 composition indicates stability of the ammonium sulphate seed particles under the experimental conditions (RH ~40%), promoting nucleation processes involving sulfuric acid (Kulmala et al., 2013; Wollesen De Jonge et al., 2021)." It now reads: **"...consistent with condensational growth rather than new particle formation during the exposure period (Fig. S1a-b).**" The Kulmala et al. (2013) and Wollesen De Jonge et al. (2021) citations have been removed accordingly.

We have also revised the related sentence in Section 2.1 (p.4): "**Relative humidity (RH) was maintained at 30-50% on average to promote condensational growth of organic material onto pre-formed ammonium sulphate seed particles, while avoiding water condensation interference with organic aerosol formation.**"

Comment 3.11 - Conclusions Section

"This section should focus more on the key scientific results and relate them to air quality and toxicology, rather than on why atmospheric simulation chambers are complementary to field studies."

We fully agree. The Conclusions (p.22) already focus on the key scientific results (32 compounds, nitrophenolic markers, volatility distribution) and the framework for biological exposure studies, without generic statements on chambers as complementary tools. We have therefore retained the existing text, which reads: "**This molecular-level characterization of urban aerosol analogues under controlled conditions offered detailed insights into source-specific chemical signatures. The identification of 32 distinct organic compounds, including nitrophenolic markers from biomass burning and oxidized aromatics from traffic emissions, contributes to understanding secondary organic aerosol formation and gas-particle partitioning in urban environments. 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.** "

We are confident that these revisions, together with the addition of Fig. S1a-b in the Supporting Information, address all of Reviewer 3's concerns.