Responses to reviewers

Reviewer comments are in ***black italic type***. Author responses are labelled with [R] and author actions with [A]. Line numbers in the responses correspond to the **revised manuscript with track-changes**. Modifications to the manuscript are in blue.

Reviewer 1

This manuscript characterizes the particulate emissions and associated oxidative potential (both acellular DTT and cellular macrophage ROS) generated from the indoor burning of Arabian incense (Bakhoor). Characterizing regionally significant indoor combustion sources is an important endeavor, and the authors provide a valuable dataset bridging size-resolved emission factors with toxicological endpoints. However, to fully realize the potential of this work, several methodological assumptions and analytical choices require further clarification and refinement. Addressing these points will strengthen the mechanistic insights and ensure the robustness of the conclusions prior to publication.

[R0] We thank the reviewer for the valuable feedback and constructive suggestions. Detailed responses are given below.

Major comments

1.The authors report impressive peak particle generation rates of (6-7) × 10^{11} particles min^{-1} g^{-1}. Given these exceptionally high concentrations within a 125 L chamber volume , treating particle decay primarily as a first-order wall loss process might underestimate the effects of size-dependent coagulation. Rapid coagulation in such dense plumes could significantly alter the observed nucleation and ultrafine modes over the residence time. The manuscript would benefit from a discussion on how coagulation dynamics were accounted for or why they are considered negligible here.

[R1] Thanks for this important comment. We agree that particle-particle coagulation can influence ultrafine particle dynamics under high number concentrations. We have revised the manuscript to clarify this point and added a representative coagulation timescale estimate for our chamber conditions.

The chamber volume was 125 L and the outlet flow rate was 20 L min^{-1}, giving a flow-through residence time of 6.25 min. To evaluate whether coagulation could substantially modify the observed ultrafine mode over this residence time, we estimated representative same-size coagulation rates for 20 and 100 nm particles. For particles in size bin i colliding with particles in size bin j, the first-order coagulation loss

contribution of particle i can be written as (Hinds and Zhu, 2022; Seinfeld and Pandis, 2016):

$$k_{\mathrm{coag},i\leftarrow j} = \beta_{ij} N_j$$

where β_{ij} is the Brownian coagulation kernel and N_j is the particle number concentration in size bin j. The Brownian coagulation kernels were calculated using the Fuchs transition-regime formulation. The particle diffusion coefficient was first calculated from the Stokes-Einstein equation with the Cunningham slip correction:

$$D_p = \frac{k_B T C_c}{3\pi\mu d_p}$$

where k_B is the Boltzmann constant, T is temperature, C_c is the Cunningham slip correction factor, μ is the dynamic viscosity of air, and d_p is particle diameter. The Brownian coagulation kernel was then calculated as:

$$\beta_{ij} = \frac{4\pi(D_i + D_j)(r_i + r_j)}{\dfrac{r_i + r_j}{r_i + r_j + \sqrt{\delta_i^2 + \delta_j^2}} + \dfrac{4(D_i + D_j)}{\sqrt{\bar{c}_i^2 + \bar{c}_j^2}(r_i + r_j)}}$$

where r_i and r_j are particle radii, D_i and D_j are particle diffusion coefficients, $\bar{c}_i$ and $\bar{c}_j$ are mean thermal speeds, and δ_i and δ_j are the Fuchs correction lengths. The calculation used $T = 298\mathrm{K}$, $\mu = 1.85 \times 10^{-5}$ kg m^{-1} s^{-1}, an air mean free path of approximately 65 nm for the Cunningham slip correction, and an effective particle density of 1 g cm^{-3} for estimating particle thermal speed.

The representative intermediate values were:

Particle diameter (nm)	C_c	D_i (m^2 s^{-1})	$\bar{c}_i$ (m s^{-1})	l_i (nm)	δ_i (nm)
20	11.37	1.34×10^{-8}	1.58	68	16
100	2.86	6.75×10^{-10}	0.14	12	6.5

For same-size collisions, the calculated Brownian coagulation kernel was:

$$\beta_{20,20} \approx 2.35 \times 10^{-9}\ \mathrm{cm^3\ s^{-1}}$$

for 20 nm particles and:

$$\beta_{100,100} \approx 1.43 \times 10^{-9}\ \mathrm{cm^3\ s^{-1}}$$

Using the burning-period-averaged UFP number concentration estimated from Fig. 1A:

$$N_{\mathrm{UFP,avg}} \approx 1.0 \times 10^{6}\ \mathrm{cm^{-3}}$$

these representative kernels give:

$$k_{\mathrm{coag},20} = \beta_{20,20} N_{\mathrm{UFP,avg}} \times 60 = (2.35 \times 10^{-9})(1.0 \times 10^{6}) \times 60 \approx 0.141\ \mathrm{min^{-1}}$$

and:

$$k_{\mathrm{coag},100} = \beta_{100,100} N_{\mathrm{UFP,avg}} \times 60 = (1.43 \times 10^{-9})(1.0 \times 10^{6}) \times 60 \approx 0.086\ \mathrm{min^{-1}}$$

The corresponding coagulation timescales are approximately 7.1 min for 20 nm particles and 11.6 min for 100 nm particles. These values are comparable to or longer than the chamber residence time of 6.25 min. This estimate suggests that coagulation may influence the smaller end of the UFP mode and shift some particles toward larger diameters, but it is unlikely to dominate the burning-period-averaged particle number estimate. Because coagulation reduces particle number, the reported UFP number emission rates likely represent conservative estimates. We have revised the manuscript to clarify this point.

[A1] We have added the following discussion in Section 3.1:

"Because Bakhoor burning generated high particle number concentrations in the chamber, particle-particle coagulation may contribute to the evolution of the measured size distribution. To evaluate its potential influence under our experimental conditions, representative same-size Brownian coagulation kernels were calculated for 20 and 100 nm particles using the Fuchs transition-regime formulation. The estimated kernels were 2.35×10^{-9} and 1.43×10^{-9} cm^3 s^{-1}, respectively (Hinds and Zhu, 2022; Seinfeld and Pandis, 2016). With the burning-period-averaged UFP number concentration of approximately 1.0×10^6 cm^{-3}, these kernels correspond to coagulation timescales of approximately 7.1 and 11.6 min for 20 and 100 nm particles, respectively. These values are comparable to or longer than the chamber flow-through residence time of 6.25 min, suggesting that coagulation may influence the smaller end of the UFP mode but is unlikely to dominate the burning-period-averaged particle number estimate. Since coagulation reduces particle number and shifts particles toward larger diameters, the reported UFP number emission rates may represent conservative estimates."

2.The 24-hour particle exposure duration for the DCFH-DA assay in MH-S alveolar macrophages provides a useful integrated response but may introduce confounding factors. Since the authors note that high doses approach cytotoxicity, extended exposure to complex combustion mixtures might inadvertently capture terminal cell death signaling rather than a direct, chemically induced oxidative response. Restricting the AUC analysis strictly to verified non-cytotoxic dose ranges would strengthen the attribution of the fluorescence signal to oxidative stress.

[R2] We agree that the DCFH fluorescence response after 24 h particle exposure should be interpreted together with cell viability, especially at high particle doses. In the revised analysis, we therefore used the MTT results to define a non-cytotoxic low-dose range and recalculated the DCFH AUC within this range. Specifically, we used cell viability greater than 80% of the untreated control as a conservative criterion for defining the non-cytotoxic dose range. This threshold is stricter than the 70% viability cutoff commonly used in ISO 10993-5-based cytotoxicity assessment (International Organization for Standardization, 2009). Based on the MTT assay, the three lowest loading levels, 0.0001×, 0.001×, and 0.01×, were selected for the

restricted AUC analysis. Higher loading levels were excluded to minimize potential confounding from cytotoxicity-associated processes or response saturation. We recalculated the DCFH AUC over the common low-dose range of 0.0001× to 0.01×. Because the DCFH response was reported as fold change relative to untreated controls, the baseline control value of 1 was subtracted before integration so that the restricted AUC reflected particle-induced fluorescence above the untreated control. Since this restricted range included three dose points, trapezoidal integration over log10-transformed particle loading was used rather than refitting the Hill equation over a limited number of points. For the fresh total-particle samples, the restricted AUC values for the three Bakhoor samples were 0.249, 0.241, and 0.215, with an average of 0.235 ± 0.018. The corresponding value for the protocol-matched sidestream cigarette-smoke reference was 0.140. Thus, even when the analysis was restricted to the MTT-supported low-dose range, Bakhoor-burning particles still produced a stronger intracellular oxidative-stress response than sidestream cigarette-smoke particles under the same assay conditions. This result supports the attribution of the low-dose DCFH signal to oxidative-stress responses occurring before substantial viability loss. We have revised the manuscript to clarify that the higher-dose DCFH responses are retained as part of the full dose-response characterization, but the oxidative-stress interpretation is now supported by the restricted low-dose AUC analysis.

[A2] We have revised Section 3.5 as follows: "Because high particle doses can reduce cell viability, DCFH fluorescence at the upper end of the dose-response curve may include contributions from cytotoxicity-associated processes. We therefore used the MTT assay to define a non-cytotoxic low-dose range, with cell viability greater than 80% of the untreated control as a conservative criterion. This threshold is stricter than the 70% viability cutoff commonly used in ISO 10993-5-based cytotoxicity assessment (International Organization for Standardization, 2009). The three lowest loading levels, 0.0001×, 0.001×, and 0.01×, were selected for the low-dose AUC analysis. The DCFH AUC over this range was calculated using baseline-subtracted DCFH fold changes. Because this range included three dose points, trapezoidal integration over log10-transformed particle loading was used. For fresh total-particle samples, the low-dose AUC values for the three Bakhoor samples were 0.249, 0.241, and 0.215, compared with 0.140 for the protocol-matched sidestream cigarette-smoke reference. These results indicate that Bakhoor-burning particles induced measurable intracellular oxidative-stress responses under low-dose exposure conditions before substantial viability loss. The higher-dose responses were retained as part of the full dose-response characterization but were interpreted in light of the MTT viability results."

3.When evaluating macrophage responses to combustion products derived from raw wood and botanical additives, it is important to rule out external biological confounders. As a standard QA/QC measure, please detail any endotoxin screening performed on the filter samples, extraction media, or charcoal blanks.

Environmental wood samples can sometimes harbor endotoxins that strongly activate macrophage oxidative bursts, so confirming their absence would solidify the chemical basis of the observed DCFH response.

[R3] We agree that endotoxin is a relevant QA/QC consideration for macrophage-based assays involving particles derived from wood or botanical materials. Endotoxin, including lipopolysaccharide (LPS), can activate macrophages and induce ROS-related inflammatory responses (Sanlioglu et al., 2001; Maitra et al., 2009). Endotoxin-specific quantification, such as the Limulus amebocyte lysate (LAL) assay or recombinant Factor C (rFC) analysis, was not included in the present analysis. We have now stated this explicitly in the revised manuscript.

The DCFH assay in this work was designed to evaluate the integrated macrophage oxidative-stress response to particles emitted from the practical Bakhoor-burning configuration used in this study, in which Bakhoor was heated on smoldering charcoal. The cells were therefore exposed to thermally processed emitted particles collected on PTFE filters, rather than to raw wood or botanical materials directly suspended in the culture medium. Dry-heat treatment at 170–250 °C has been shown to reduce lipopolysaccharide activity (Tsuji and Harrison, 1978). In our setup, the charcoal temperature reached approximately 300 °C (Figure S6), and this high-temperature processing may have reduced the potential contribution of raw-material-associated endotoxin to the cellular assay. However, residual particle-associated endotoxin cannot be fully excluded without endotoxin-specific quantification. If residual endotoxin associated with wood-derived or botanical-derived material remained in the emitted particles, it would represent part of the particle mixture generated under practical Bakhoor-burning conditions, rather than an external contaminant introduced during sample handling.

For the cellular assay, blank PTFE filter controls processed under the same immersion, vortexing, and exposure protocol were included as procedural controls to account for filter handling and filter-related background. DCFH responses were reported as fold changes relative to untreated control cells. Because charcoal was part of the practical Bakhoor-burning configuration, no additional charcoal-only subtraction was applied to the cellular response; the DCFH measurements represent the response to the full emitted particle mixture generated under this real-use condition.

We have revised the manuscript to clarify that the absence of endotoxin-specific quantification mainly limits detailed attribution of the macrophage response drivers, rather than the observation that Bakhoor-burning particles induced intracellular oxidative-stress responses under the exposure protocol used here. The comparison between Bakhoor-burning particles and the protocol-matched sidestream cigarette-smoke reference remains informative because both were evaluated using the same cellular exposure protocol and

the same approach for background control and response normalization. Future studies focused on mechanistic source attribution should include endotoxin-specific QA/QC of particle-loaded filters, blank filters, exposure media, and source blanks.

[A3] We have added the following discussion in Section 3.5: "An additional consideration for the macrophage assay is the potential contribution of endotoxin, because lipopolysaccharide can activate macrophage inflammatory and ROS-related responses (Sanlioglu et al., 2001; Maitra et al., 2009). Endotoxin-specific quantification, such as the Limulus amebocyte lysate assay or recombinant Factor C analysis (Lee et al., 2004), was not included in this study. However, the cells were exposed to particles emitted from Bakhoor heated on smoldering charcoal, rather than to raw wood or botanical materials directly suspended in culture medium. Dry-heat treatment at 170–250 °C has been shown to reduce lipopolysaccharide activity (Tsuji and Harrison, 1978). In our setup, the charcoal temperature reached approximately 300 °C (Figure S6), and this high-temperature processing may have reduced potential contributions from raw-material-associated endotoxin. Residual particle-associated endotoxin nevertheless cannot be fully excluded without endotoxin-specific quantification. If such material remained associated with the emitted particles, it would be part of the particle mixture generated under the real-use Bakhoor-burning condition, rather than an external contaminant introduced during sample handling. The absence of endotoxin-specific quantification mainly limits detailed attribution of the macrophage response drivers, rather than the observation that Bakhoor-burning particles induced a DCFH response under the exposure protocol used here."

4.The accelerated aging protocol exposes filter-bound particles to 1500 ppb of ozone for 30 minutes to simulate extensive indoor exposure. While methodologically convenient for delivering controlled oxidant doses, this static filter setup may introduce heterogeneous reaction artifacts not fully representative of typical indoor aging. Furthermore, indoor processing of volatile and semi-volatile organics is often heavily influenced by OH and NO3 radicals. Expanding the discussion to acknowledge the limitations of this high-concentration, single-oxidant proxy would provide a more balanced context.

[R4] Thanks for the suggestion. We agree that the accelerated ozone-aging protocol should be interpreted with a clearly defined scope. The purpose of this experiment was to isolate O_3-driven processing under controlled and reproducible conditions and to test whether Bakhoor-burning particles show measurable changes in composition and oxidative potential after oxidant exposure. We have revised the manuscript to clarify the rationale for using O_3, to distinguish this controlled O_3 sensitivity analysis from OH- or NO_3-driven indoor aging, and to identify mixed-oxidant aging as an important direction for future work.

The 1500 ppb O_3 exposure for 30 min corresponds to an integrated ozone dose of 750 ppb h, equivalent to approximately 150 h at 5 ppb O_3. This equivalence is used as an exposure-dose reference, not as evidence that the filter-bound accelerated protocol fully reproduces indoor suspended-particle aging. Similar filter-based O_3 exposure of particle-loaded samples has been used previously to evaluate changes in aerosol oxidative potential, including for biomass-burning organic aerosol (Wong et al., 2019), supporting its use as a practical approach for assessing O_3 sensitivity. O_3 was selected because it is widely regarded as an important indoor oxidant, particularly for heterogeneous and surface chemistry. It is commonly introduced indoors through air exchange, and can be delivered as a stable and controllable oxidant dose for reproducible particle-aging experiments (Waring and Wells, 2015; Weschler and Carslaw, 2018).

We have also expanded the discussion of limitations. Compared with suspended particles, filter-bound particles no longer evolve in the same gas-particle partitioning environment, and their exposure to O_3 may be influenced by particle loading on the filter and contact with the filter substrate. In addition, indoor OH and NO_3 chemistry can occur but is more condition-dependent than the controlled O_3 exposure used here. Indoor OH can be generated through ozonolysis and photolysis-related processes, whereas NO_3 chemistry becomes more relevant under NO_2/O_3-rich conditions (Waring and Wells, 2015; Abbatt and Wang, 2020; Zannoni et al., 2022; Dewald et al., 2023). Therefore, OH- and NO_3-driven processing should be regarded as additional indoor aging pathways that were not simulated by the present filter-bound O_3 exposure. These revisions clarify the scope of the aging experiment while retaining its value as a controlled test of O_3-induced changes in Bakhoor-burning particles. Detailed studies using suspended particles and mixed-oxidant systems will be incorporated in future work to evaluate the relative roles of O_3, OH, and NO_3 in more realistic indoor aging scenarios.

[A4] We have added the following discussion in Section 2.3:

"Similar filter-based O_3 exposure of particle-loaded samples has been used previously to evaluate changes in aerosol oxidative potential, including for biomass-burning organic aerosol (Wong et al., 2019). This filter-bound O_3 treatment provides a practical approach to probe the O_3 sensitivity of Bakhoor-burning particles. O_3 was selected because it is widely regarded as an important indoor oxidant, particularly for heterogeneous and surface chemistry. It is commonly introduced indoors through air exchange, and can be delivered as a stable and controllable oxidant dose for reproducible particle-aging experiments (Waring and Wells, 2015; Weschler and Carslaw, 2018). Indoor particles may experience O_3 exposure both while airborne and after deposition onto indoor surfaces; deposited particles can persist longer and remain exposed to oxidants. Accordingly, this experiment should be interpreted as a controlled test of the post-emission O_3 responsiveness of Bakhoor-burning particle-phase material, rather than as a full simulation of suspended-

particle aging in indoor air. Compared with suspended particles, filter-bound particles no longer evolve within the same gas-particle partitioning environment, and their exposure to O_3 may be influenced by particle loading and contact with the filter substrate. In addition, indoor OH and NO_3 chemistry can occur but is more condition-dependent than the controlled O_3 exposure used here. Indoor OH can be generated through ozonolysis and photolysis-related processes, whereas NO_3 chemistry becomes more relevant under NO_2/O_3-rich conditions (Waring and Wells, 2015; Abbatt and Wang, 2020; Zannoni et al., 2022; Dewald et al., 2023). Therefore, OH- and NO_3-driven processing should be regarded as additional indoor aging pathways that were not simulated by the present filter-bound O_3 exposure and should be evaluated in future suspended-particle or mixed-oxidant studies."

5. The reliance on positive mode electrospray ionization (ESI+) for the UHPLC-HRMS analysis is well-suited for certain compound classes but may introduce a detection bias. ESI+ preferentially ionizes basic and nitrogen-containing compounds, potentially suppressing the detection of highly oxygenated or acidic organic species that are often strong drivers of redox activity. Discussing how this analytical choice might affect the comparison between the nitrogen-rich cigarette smoke and the Bakhoor emissions would improve the transparency of the structure-activity correlations.

[R5] Thanks for the suggestion. We used UHPLC-HRMS in ESI+ mode because this mode provides useful coverage of semi-polar, readily ionizable, and nitrogen-containing organic compounds in complex combustion-aerosol extracts. For an untargeted source comparison, robust sample-to-sample interpretation requires sufficient signal intensity and reliable molecular-feature extraction. During preliminary method evaluation across the present samples, ESI− produced approximately one to two orders of magnitude lower total ion signal than ESI+ and yielded fewer molecular features with sufficient signal-to-noise ratios for reliable formula assignment. ESI+ was therefore selected as the primary and more robust analytical window for the present untargeted source comparison.

The UHPLC-HRMS analysis was intended to compare source-dependent molecular fingerprints rather than to provide an exhaustive quantitative inventory of particle-phase organics. For this purpose, we compared the two sources using relative ESI+ feature intensities, i.e., the signal contribution of each assigned feature normalized to the total assigned ESI+ signal within the same sample. This normalization emphasizes differences in the distribution of detected molecular features between cigarette-smoke and Bakhoor-burning particles, rather than differences in total extracted signal or absolute ion abundance. Because all samples were analyzed using the same extraction, chromatographic, ionization, and data-processing workflow, the observed contrast provides an internally consistent comparison of the two sources within the ESI+-detected feature space. Within this analytical window, sidestream cigarette-smoke particles were strongly enriched

in CHN/CHON formulas, whereas Bakhoor-burning particles were dominated by CHO formulas.

We agree that electrospray ionization polarity is analyte-dependent and that ESI+ and ESI− provide complementary molecular coverage (Liigand et al., 2017; Patriarca et al., 2018). Thus, the relative ESI+ feature intensities support source-to-source comparison within the same analytical workflow, but they remain ESI-response-weighted signals and do not remove compound-class-dependent ionization bias or provide a complete quantitative inventory of particle-phase organics. We have therefore revised the manuscript to clarify that the molecular patterns reported here represent ESI+ resolved source fingerprints of the particle extracts. The stronger CHN/CHON contribution detected in cigarette smoke may reflect both real source differences and favorable ESI+ response of nitrogen-containing compounds, while acidic or highly oxygenated CHO species may be less efficiently represented under ESI+ conditions. This distinction does not preclude comparison between cigarette-smoke and Bakhoor-burning particles within the ESI+-detected feature space, but it constrains the scope of compositional interpretation.

We also revised the structure-activity discussion to avoid over-attribution. Correlations between OP_{DTTm} and molecular descriptors such as O, H, AImod, and elemental-class metrics are now interpreted as empirical associations within the measured ESI+ molecular feature space. These associations are useful for identifying molecular patterns linked to higher DTT activity across the present samples, but they should not be interpreted as quantitative apportionment of the full particle-phase organic mixture or as evidence for the absence of redox-active species outside the ESI+ analytical window. The DTT and cellular assays provide integrated oxidative-response endpoints that are not limited to ESI+-detectable compounds. Future work using paired ESI+ and optimized ESI− analyses would be needed to more fully resolve acidic, highly oxygenated, nitrogen-containing, and other ionizable contributors to oxidative potential.

[A5] We have added the following discussion in Section 3.4:

“Bakhoor burning particles were dominated by CHO formulas (~65% of assigned species), whereas cigarette smoke particles were relatively less oxygenated and strongly enriched in nitrogen-containing formulas (CHN+CHON ≈ 84%) (Figure S9). These comparisons were based on relative ESI+ feature intensities, calculated as the signal contribution of each assigned feature normalized to the total assigned ESI+ signal within the same sample. This normalization emphasizes differences in the distribution of detected molecular features between Bakhoor-burning and cigarette-smoke particles, rather than differences in total extracted signal or absolute ion abundance. Because all samples were analyzed using the same extraction, chromatographic, ionization, and data-processing workflow, the observed contrast provides an internally consistent ESI+-resolved source fingerprint comparison. Electrospray ionization

polarity is analyte-dependent, and positive and negative modes provide complementary molecular coverage (Liigand et al., 2017; Patriarca et al., 2018). Therefore, the stronger CHN/CHON contribution detected in cigarette smoke may reflect both real source differences and favorable ESI+ response of nitrogen-containing compounds, while acidic or highly oxygenated CHO species may be less efficiently represented under ESI+ conditions. Accordingly, these molecular patterns should be interpreted as ESI+-resolved source fingerprints rather than a complete quantitative inventory of particle-phase organics."

We have also revised the structure–activity discussion as follows:

And "Consistent with these ESI+ resolved source fingerprints, Bakhoor-burning particles were dominated by CHO formulas and only a minority met the aromatic-like criterion (AImod≥ 0.5; Figure S11), whereas a substantially larger fraction of cigarette-derived CHN/CHON formulas were aromatic-like (Figure S11). Within the ESI+ molecular feature space, this pattern is consistent with the higher OP_{DTTm} observed for cigarette-smoke particles and with the positive association between OP_{DTTm} and ESI+ detected nitrogen-containing aromatic-like formulas. However, these correlations are interpreted as empirical structure–activity associations within the measured ESI+ molecular feature space, rather than as quantitative apportionment of the full particle-phase organic mixture or as evidence for the absence of redox-active species outside this analytical window."

6.Line 176-184: the authors mentioned that they performed MTT assay to check the cell viability. But there is not too much discussion about this cytotoxicity index. It is recommended to amend this result to the DTT and ROS data to show the oxidative stress effects of Bakhoor burning aerosol holistically

[R6] Thanks for this helpful suggestion. We have expanded Section 3.5 to discuss the MTT-based cell viability results together with the DTT and DCFH data. As shown in Figure S13, MTT viability was largely maintained at lower particle mass loadings, including the low-dose range used for the DCFH AUC analysis described in [R2]. At higher particle mass loadings, MTT viability decreased markedly, indicating that the upper exposure range was accompanied by reduced cellular metabolic activity. This cytotoxicity trend complements the DCFH response by supporting the interpretation that DCFH responses in the low-dose range occurred before substantial viability loss, whereas responses at higher loadings may be influenced by reduced cellular metabolic activity.

We have revised Section 3.5 to discuss these endpoints as complementary biological and chemical indicators, consistent with PM toxicology studies that evaluate acellular oxidative potential, intracellular

oxidative stress, and MTT-based cytotoxicity together (Lionetto et al., 2021). In the revised text, OP_{DTT}^{m} characterizes acellular redox activity, DCFH fluorescence characterizes intracellular oxidative-stress responses, and MTT viability identifies the exposure range associated with the loss of cellular metabolic activity.

[A6] As noted in [A2], we revised Section 3.5 to include the MTT-defined low-dose range and the low-dose DCFH AUC analysis. We also expanded the discussion immediately after the high-dose DCFH plateau sentence as follows:

"MTT viability further showed a dose-dependent reduction in cellular metabolic activity at higher particle mass loadings (Figure S13). Cell viability was largely maintained at the lower loadings used for the low-dose DCFH AUC analysis, whereas higher loadings led to a marked reduction in viability. This result supports the interpretation that DCFH responses in the low-dose range occurred before substantial viability loss, whereas responses at higher loadings may be influenced by reduced cellular metabolic activity. Such combined interpretation of acellular oxidative potential, intracellular oxidative-stress response, and MTT-based cytotoxicity is consistent with prior PM toxicology studies using complementary chemical and cellular endpoints (Lionetto et al., 2021)."

Minor comments

Line 100: Assuming a uniform effective particle density of 1 g cm⁻³ across all mobility diameters is a helpful simplification, but may not fully capture the complex morphology of fresh combustion soot and organics. The authors should consider adding a brief discussion in terms of the potential uncertainties. Also, any references for using this density?

[R7] Thanks for this comment. We have added references and a brief clarification for the assumed effective particle density. The value of 1 g cm^{-3} was used to convert size-resolved number distributions to mass concentrations, consistent with previous indoor particle and combustion-emission studies that estimated particle mass from size distributions using unit density (Wallace, 2006; Klosterköther et al., 2021). This value is also close to the reported density of incense smoke particles, approximately 1.1 g cm^{-3} (Ji et al., 2010). We have also noted that fresh combustion aerosols may have source- and size-dependent effective densities because of differences in morphology and composition.

[A7] We have revised Section 2.1 as follows:

"Particle number size distributions from the SEMS and OPC were converted to mass concentrations assuming spherical particles with an effective particle density of 1 g cm^{-3}. This value has been used in previous indoor particle and combustion-emission studies to estimate particle mass from size distributions (Wallace, 2006; Klosterköther et al., 2021) and is close to the reported density of incense smoke particles, approximately 1.1 g cm^{-3} (Ji et al., 2010). Fresh combustion aerosols may have source- and size-dependent effective densities because of differences in morphology and composition."

Line 121: Wondering why these two ozone levels were selected

[R8] Thanks for this comment. We have clarified the rationale for selecting the two ozone levels in Section 2.3. Both treatments were conducted for 30 min to keep filter handling and other time-dependent processes consistent, while varying the cumulative O_3 exposure through the O_3 concentration. The 200 and 1500 ppb O_3 conditions correspond to integrated exposures of 100 and 750 ppb h, respectively, equivalent to approximately 20 and 150 h at a representative indoor O_3 level of 5 ppb, which is consistent with reported central tendencies of average indoor ozone concentrations (Nazaroff and Weschler, 2022).

The lower exposure was selected to approximate airborne residence-scale O_3 processing, consistent with reported residential air-exchange-based particle residence times on the order of hours to about one day (Zhao and Liu, 2020; Salthammer, 2011). The higher exposure was selected to probe more extended O_3 exposure relevant to deposited indoor particle reservoirs, which can remain on indoor surfaces and undergo heterogeneous or multiphase chemical processing after removal from the airborne phase (Abbatt and Wang, 2020; Ault et al., 2020). We have added this explanation while retaining the original rationale that the accelerated protocol was selected for practicality, reproducibility, and isolation of ozone-driven processing.

[A8] We have revised Section 2.3 as follows:

"The lower exposure was selected to approximate airborne residence-scale O_3 processing, consistent with reported residential air-exchange-based particle residence times on the order of hours to about one day (Zhao and Liu, 2020; Salthammer, 2011). The higher exposure was selected to probe more extended O_3 exposure relevant to deposited indoor particle reservoirs, which can remain on indoor surfaces and undergo heterogeneous or multiphase chemical processing after removal from the airborne phase (Abbatt and Wang, 2020; Ault et al., 2020)."

Line 148: Water extraction is performed via 30 minutes of sonication. Please specify if the water bath temperature was controlled (e.g., using ice to prevent heating), as elevated temperatures during sonication can sometimes degrade reactive oxygen species or alter the DTT response.

[R9] We have clarified the extraction condition in Section 2.5. The 30 min water extraction by sonication was performed in an ice-water bath to minimize temperature increase during sonication. In addition, as described in the manuscript, we compared sonication with vortex shaking to evaluate possible extraction-related artifacts. The DTT consumption rates showed no significant difference between the two extraction methods, indicating that sonication did not measurably alter the DTT response under the conditions used here.

[A9] We have revised Section 2.5 as follows: "Briefly, PM-loaded PTFE filter samples were extracted in deionized water by 30 minutes of sonication in an ice-water bath to minimize heating during extraction. Given the potential for radical formation or temperature-related artifacts during sonication, a comparison between sonication and vortex shaking was conducted. The results showed no significant difference in DTT consumption, indicating that sonication did not introduce measurable artifacts."

Page 7: For the operational separation of the hexane-soluble fraction , it would be reassuring to mention whether steps were taken to ensure the solvent extraction did not structurally alter the microphysical porosity of the remaining wood residue , which could independently affect its subsequent combustion behavior.

[R10] Thanks for this comment. We agree that solvent extraction could, in principle, alter the physical structure of the remaining wood residue and thereby affect its subsequent combustion behavior. We have revised Section 3.2 to clarify how this possibility was evaluated. The hexane separation was used operationally to remove the hexane-soluble fraction (HSF) from the Bakhoor material. We did not directly measure the pore structure of the wood residue after extraction. Instead, we used the HSF-reconstitution experiment as a functional control for possible extraction-induced changes in the residue. In this experiment, the extracted HSF was recombined with the corresponding wood residue at the original mass fraction and then burned under the same conditions. The reconstituted material reproduced particle mass emission rates similar to those of the original Bakhoor sample (Figure S3). This result suggests that any extraction-induced changes to the residual wood matrix did not dominate the observed emission behavior. We have revised the text to present the HSF as an operationally defined fraction and to clarify the role of the reconstitution experiment.

[A10] We have revised Section 3.2 as follows: "Because solvent extraction could, in principle, alter the physical structure of the remaining wood residue and independently influence combustion behavior, we conducted a reconstitution experiment as a functional control. In this experiment, the extracted HSF was recombined with the corresponding wood residue at the appropriate mass fractions and burned under the same conditions. The reconstituted material reproduced particle mass emission rates similar to those of the original samples (Figure S3), suggesting that extraction-induced changes to the residual wood matrix did

not dominate the observed emission differences. We therefore interpret the HSF as an operationally defined fraction that plays a major role in particle formation during Bakhoor burning."

Page 8: Please clarify in the text or figure captions if the reported DTT consumption rates for the Bakhoor samples (approx. 32 pmol min⁻¹ μg⁻¹) explicitly subtract the background activity generated from the charcoal-only blanks.

[R11] Thanks for this comment. We have clarified in Section 3.3 and in the Figure 3 caption that the reported OP_{DTT}^{m} values for Bakhoor-burning particles were not corrected by subtracting the charcoal-only burn. This is because the DTT assay in this study was intended to characterize particles emitted under the common real-use scenario in which Bakhoor is heated on smoldering charcoal. Therefore, the reported OP_{DTT}^{m} values represent the oxidative potential of the full real-use emitted particle mixture. As described in Section 2.5, procedural background correction for the DTT assay was based on blank PTFE filters processed using the same extraction and assay procedure. We have revised the text and figure caption to make this distinction explicit.

[A11] We have revised Section 3.3 as follows:

"Fresh Bakhoor extracts exhibited an average OP_{DTT}^{m} of approximately 32 pmol $\mathrm{min^{-1}}$ $\mu\mathrm{g^{-1}}$ (Figure 3A). This value was not corrected by subtracting the charcoal-only burn and therefore represents the exposure-relevant oxidative potential of the full particle mixture emitted under the common real-use scenario in which Bakhoor is heated on smoldering charcoal."

Page 10: The direct comparison of cellular responses to naphthalene SOA is interesting but slightly abrupt. A sentence explaining why this specific single-precursor SOA is compared here would be helpful.

[R12] Thanks for this comment. We have added a sentence to clarify why naphthalene-derived SOA was used as a reference. The comparison was not intended to imply that Bakhoor-burning particles and naphthalene SOA have similar sources or composition. Rather, naphthalene-derived SOA was included because it is a well-studied anthropogenic aromatic SOA system and has been reported to induce strong macrophage DCFH responses using a comparable cellular assay framework (Tuet et al., 2017). We have revised Section 3.5 to make this reference role explicit.

[A12] We have revised Section 3.5 as follows:

"Bakhoor burning particles elicited stronger DCFH fluorescence responses than sidestream cigarette smoke particles across the tested dose range (Figure 5B). The AUC metric ranged from 0.51 to 0.62 for Bakhoor samples, higher than 0.41 for cigarette smoke. Naphthalene-derived SOA was used as an external cellular-response reference because it is a well-studied anthropogenic aromatic SOA system that has been shown to induce strong macrophage DCFH responses under a comparable assay framework (Tuet et al., 2017). The magnitude of the response induced by Bakhoor burning particles was comparable to that reported for naphthalene-derived secondary organic aerosol."

Line 358: change the title of section 4 to "Atmospheric implications"

[R13] [A13] We have revised the title of Section 4 from "Conclusions and atmospheric implications" to "Atmospheric implications" accordingly.

Line 404: please rephrase this sentence

[R14] Thanks for the suggestion. We have rephrased this sentence for clarity.

[A14] We have revised the sentence in Section 4 as follows: At the user level, wiping Bakhoor pieces prior to burning may partially remove surface-associated HSF and reduce emissions, although the extent of emission reduction will likely depend on product composition and user practice and should be evaluated under realistic operating conditions.

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