

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 $(6-7) \times 10^{11}$ particles $\text{min}^{-1} \text{g}^{-1}$. 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):

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

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:

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

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 $T = 298\text{K}$, $\mu = 1.85 \times 10^{-5} \text{kg m}^{-1} \text{s}^{-1}$, 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 ($\text{m}^2 \text{s}^{-1}$)	$\bar{c}_i$ (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:

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

for 20 nm particles and:

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

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

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

these representative kernels give:

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

and:

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

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 Bakhooor 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 $1.43 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, respectively (Hinds and Zhu, 2022; Seinfeld and Pandis, 2016). With the burning-period-averaged UFP number concentration of approximately $1.0 \times 10^6 \text{ cm}^{-3}$, 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, $0.0001\times$, $0.001\times$, and $0.01\times$, 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 $0.0001\times$ to $0.01\times$. 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, $0.0001\times$, $0.001\times$, and $0.01\times$, 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 O₃-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 O₃, to distinguish this controlled O₃ sensitivity analysis from OH- or NO₃-driven indoor aging, and to identify mixed-oxidant aging as an important direction for future work.

The 1500 ppb O₃ exposure for 30 min corresponds to an integrated ozone dose of 750 ppb h, equivalent to approximately 150 h at 5 ppb O₃. 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 O₃ 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 O₃ sensitivity. O₃ 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 O₃ may be influenced by particle loading on the filter and contact with the filter substrate. In addition, indoor OH and NO₃ chemistry can occur but is more condition-dependent than the controlled O₃ exposure used here. Indoor OH can be generated through ozonolysis and photolysis-related processes, whereas NO₃ chemistry becomes more relevant under NO₂/O₃-rich conditions (Waring and Wells, 2015; Abbatt and Wang, 2020; Zannoni et al., 2022; Dewald et al., 2023). Therefore, OH- and NO₃-driven processing should be regarded as additional indoor aging pathways that were not simulated by the present filter-bound O₃ exposure. These revisions clarify the scope of the aging experiment while retaining its value as a controlled test of O₃-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 O₃, OH, and NO₃ in more realistic indoor aging scenarios.

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

“Similar filter-based O₃ 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 O₃ treatment provides a practical approach to probe the O₃ sensitivity of Bakhoor-burning particles. O₃ 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 O₃ 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 O₃ 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 O₃ may be influenced by particle loading and contact with the filter substrate. In addition, indoor OH and NO₃ chemistry can occur but is more condition-dependent than the controlled O₃ exposure used here. Indoor OH can be generated through ozonolysis and photolysis-related processes, whereas NO₃ chemistry becomes more relevant under NO₂/O₃-rich conditions (Waring and Wells, 2015; Abbatt and Wang, 2020; Zannoni et al., 2022; Dewald et al., 2023). Therefore, OH- and NO₃-driven processing should be regarded as additional indoor aging pathways that were not simulated by the present filter-bound O₃ 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, Almod, 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 $\approx$ 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 ($AI_{mod} \geq 0.5$; 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_{DTM} observed for cigarette-smoke particles and with the positive association between OP_{DTM} 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_{MTT}^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; Klosterkötter 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; Klosterk  ther 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. $32 \text{ pmol min}^{-1} \mu\text{g}^{-1}$) 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 $32 \text{ pmol min}^{-1} \mu\text{g}^{-1}$ (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.](#)

Reviewer 2

I was slightly surprised to see this in ACPD, given the strong focus on indoor air. Apart from that, this manuscript tells a coherent and interesting story, albeit with some methodological concerns that should be addressed before publication. Please find my more detailed comments below.

[R0] We thank the reviewer for the positive assessment and constructive comments. We understand the concern regarding the indoor-air focus of the manuscript. We view the study as relevant to ACPD because it addresses indoor combustion aerosol emissions, particle oxidative potential, and ozone-driven chemical processing, all of which are connected to atmospheric chemistry in indoor environments. Detailed responses to the methodological comments are provided below.

Specific comments

- *Page 2, line 58 and following: while it is true that Arabian incense is much less characterized, than e.g. solid fuel burning, the literature isn't quite as sparse as these sentences suggest. I would suggest adding here at least the study by Cohen et al. (2013) as a previous example of a cell tox study, and potentially some of the epidemiological evidence cited within.*

[R1] Thanks for this helpful suggestion. We agree that the previous wording may have understated the existing literature on Arabian incense smoke. We have revised the Introduction to better acknowledge prior health-oriented studies, including Cohen et al. (2013), which reported chamber measurements of UAE incense smoke and inflammatory responses in exposed human lung cells, and Al-Rawas et al. (2009), which provided epidemiological evidence related to domestic bakhour exposure and respiratory symptoms.

We have also refined the framing of the knowledge gap. Prior work has established health-relevant concern for Bakhour and related incense smoke. The present study builds on this literature by focusing on particle-based metrics that remain less constrained for practical Bakhour burning, including size-resolved particle emissions, the relative contributions of the Bakhour material and ignition charcoal, the role of product composition in controlling emission strength, and acellular and cellular oxidative responses before and after ozone exposure.

[A1] We have revised the relevant Introduction paragraph as follows:

“Previous studies have provided evidence that Bakhour and related incense smoke can have adverse respiratory and toxicological effects. Epidemiological work has linked domestic bakhour use with respiratory symptoms. For example, Al-Rawas et al. (2009) reported that Arabian incense burning was a common trigger of wheezing among asthmatic children in Oman. Cohen et al. (2013) characterized particles

and gases emitted from United Arab Emirates (UAE) incense burning under indoor chamber conditions and reported inflammatory responses in exposed human lung cells, providing an important prior hazard-assessment study of Arabian incense smoke. The chemical complexity of Bakhoor further complicates source interpretation. Elsayed et al. (2016) reported that raw, unburned Bakhoor materials contain a complex mixture of organic constituents, including nitrogen-containing and other additive-related species. However, the composition of particles emitted during Bakhoor burning can differ substantially from the raw-material profile because thermochemical processing during heating and pyrolysis can transform the material and generate new condensable products that partition to the particle phase. Dalibalta et al. (2015) identified compounds in Bakhoor smoke classified as carcinogenic, toxic, and/or respiratory irritants, while Alarifi et al. (2004) observed structural changes in pneumocytes following repeated animal exposure to Bakhoor smoke. Together, these studies establish a health-relevant basis for concern. However, the particle-level basis for assessing exposure from practical Bakhoor burning remains insufficiently constrained, including how emissions vary with source configuration and product composition, and how the emitted particles express oxidative potential before and after indoor-relevant aging. These gaps motivate a quantitative particle-based evaluation of Bakhoor burning.”

- ***Section 2.2: Your chamber is quite small and the reached concentrations are rather high. Since nucleation and growth are dynamic phenomena and depend on a multitude of factors, I am wondering on how much the your observed values are affected by e.g. the chosen dilution rate (which will affect nucleation, condensation and coagulation)...how realistic is this when compared to room air?***

[R2] Thanks for this important comment. We agree that chamber operating conditions can affect aerosol microphysics during indoor combustion. The selected dilution condition may influence vapor residence time, vapor supersaturation, particle formation, condensational growth, and particle-particle coagulation. We have therefore revised the manuscript to clarify how the measured size distributions should be interpreted relative to room air, and we provide below a timescale-based analysis for post-formation condensational uptake and coagulation under our chamber condition.

In our setup, the chamber volume was 125 L and the outlet flow rate was 20 L min⁻¹, giving a dilution rate of 0.16 min⁻¹, equivalent to an air exchange rate of 9.6 h⁻¹. The corresponding flow-through residence time was 6.25 min. This configuration combines a high source-to-volume ratio with flow-through dilution and is most relevant to a near-source indoor burning condition shortly after ignition, when vapor and particle loadings are elevated. Many residential environments have lower air exchange rates, commonly on the order of 0.05–1 h⁻¹ (Zhao and Liu, 2020; Salthammer, 2011), but they also have much larger volumes and different mixing, deposition, surface-loss, and gas-particle partitioning conditions than the chamber. Therefore, our chamber results should not be interpreted as direct room-averaged particle concentrations or

room-scale aerosol distributions. Instead, they are best viewed as controlled near-source source-term measurements from the practical Bakhoor-on-charcoal burning configuration.

For vapor-driven particle formation and condensational growth, the relevant quantity is the vapor history, including vapor residence time, supersaturation, and the amount of condensable material available for particle formation and growth. This dependence can be described conceptually by a simplified chamber vapor balance:

$$\frac{dC_v}{dt} = \frac{E_v}{V} - (\lambda + k_{\text{wall},v} + CS)C_v$$

where C_v is the condensable vapor concentration, E_v is the vapor emission rate, V is the chamber volume, $\lambda = Q/V$ is the dilution rate, $k_{\text{wall},v}$ is the vapor wall-loss rate, and CS is the condensation sink to particles. This simplified expression follows the standard aerosol-dynamic treatment of dilution, vapor wall loss, and first-order vapor uptake by particles (Seinfeld and Pandis, 2016; Zhang et al., 2014; Tuovinen et al., 2021). The purpose of introducing this vapor-balance expression is to show explicitly why the selected dilution rate can influence particle formation and growth: dilution and vapor wall loss lower condensable-vapor concentrations, whereas particle uptake removes vapors through condensation once particles have formed. This expression therefore provides the physical basis for the timescale comparison below and helps define how the measured size distributions should be interpreted under the selected near-source flow-through condition. Similar dilution sensitivity has been reported for combustion aerosols, including diesel exhaust, wood smoke, and biomass-burning plumes (Lipsky and Robinson, 2006; Hodshire et al., 2019).

Because the SEMS measured particles with mobility diameters of 10 nm and larger, the observed UFP mode represents particles after initial formation and early growth rather than the initial molecular-cluster nucleation stage itself. The present measurements therefore do not provide an independent constraint on true nucleation rates. We use the vapor-balance framework to explain the expected dilution sensitivity of particle formation, while the quantitative estimate below focuses on post-formation condensational uptake by the observed particle population. Using the burning-period-averaged UFP number concentration of approximately $1.0 \times 10^6 \text{ cm}^{-3}$, particle diameters of 30–100 nm, and a representative organic-vapor diffusivity of $D_v \sim 0.05 \text{ cm}^2 \text{ s}^{-1}$, the condensation sink,

$$CS \sim 2\pi D_v \sum_i d_{p,i} N_i$$

is on the order of 10^{-1} to 1 s^{-1} , corresponding to vapor uptake timescales of seconds to tens of seconds. This estimated condensation-sink rate is much larger than the bulk dilution rate of $2.7 \times 10^{-3} \text{ s}^{-1}$, and the corresponding vapor-uptake timescale is shorter than the 6.25 min chamber residence time. Thus, post-formation condensational uptake was rapid relative to both bulk dilution and chamber residence time under the selected chamber condition.

For coagulation, the relevant quantity is the particle-number history during chamber residence. The first-order coagulation loss contribution for particles in size bin i colliding with particles in size bin j can be written as (Hinds and Zhu, 2022; Seinfeld and Pandis, 2016):

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

where β_{ij} is the Brownian coagulation kernel and N_j is the particle number concentration in size bin j . Using the Fuchs transition-regime formulation, the representative same-size Brownian coagulation kernels for 20 and 100 nm particles were 2.35×10^{-9} and $1.43 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1}$, respectively (Hinds and Zhu, 2022; Seinfeld and Pandis, 2016). Using the burning-period-averaged UFP number concentration, with $N_{\text{UFP}} \approx 1.0 \times 10^6 \text{ cm}^{-3}$, as a representative collision-partner concentration, these kernels correspond to first-order coagulation rates of 0.141 and 0.086 min^{-1} , and coagulation timescales of approximately 7.1 and 11.6 min. These rates are lower than the chamber dilution rate of 0.16 min^{-1} , and the corresponding coagulation timescales are comparable to or longer than the chamber residence time of 6.25 min. This comparison provides a size-dependent timescale for interpreting particle-number evolution under the selected chamber condition and indicates that coagulation may modify the smallest-particle number concentration and modal evolution, but is unlikely to dominate the burning-period-averaged emission estimates.

Taken together, our measurements should be interpreted as a controlled near-source source-term characterization of practical Bakhooor burning, with the measured size distributions representing near-source emission aerosol populations rather than room-averaged particle distributions. The timescale analysis indicates that near-source particle formation and early growth are linked to high vapor loading shortly after ignition, whereas condensational adjustment after particle formation is rapid relative to the chamber residence time and coagulation provides a secondary modification under the present conditions. For room-scale applications, these measured emission rates and size distributions can be used as source inputs, with subsequent evolution governed by room volume, ventilation, deposition, surface losses, and possible semivolatile evaporation or gas-particle repartitioning. Users of our results are not required to re-simulate the near-source nucleation and early condensational growth that produced the reported size distributions, while recognizing that coagulation may still be considered in room-scale models if predicted particle number concentrations remain high.

[A2] We have added the following clarification to Section 2.2:

“Particle formation, growth, and early size evolution were interpreted in the context of the controlled flow-through chamber configuration. The chamber volume was 125 L and the outlet flow rate was 20 L min^{-1} , giving a dilution rate of 0.16 min^{-1} , equivalent to an air exchange rate of 9.6 h^{-1} , and a flow-through residence time of 6.25 min. Because this configuration combines a high source-to-volume ratio with flow-through dilution, the measured size distributions should not be interpreted as direct room-averaged particle

concentrations or room-scale aerosol distributions. Instead, they are best viewed as controlled near-source source-term measurements from the practical Bakhoor-on-charcoal burning configuration. Under this source-term interpretation, the measured size distributions include the outcome of near-source particle formation and early growth. For room-scale applications, these measured emission rates and size distributions can be used as source inputs, whereas subsequent evolution will depend on room volume, ventilation, mixing, deposition, surface losses, coagulation, and possible semivolatile evaporation or gas-particle repartitioning.”

- ***Section 2.3: I am surprised that the ozone aging was done with the already collected filters, as the aging behavior is likely to differ from those of suspended particles. Could you please comment on this?***

[R3] Thanks for this important comment. The O₃ exposure experiment was designed to evaluate the O₃ sensitivity of emitted Bakhoor particle-phase material, using changes in molecular composition and oxidative potential as indicators of post-emission chemical aging. Similar filter-based O₃ 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). We agree that suspended-particle aging can differ from filter-bound treatment because filter-bound particles no longer evolve in the same gas-particle partitioning environment, and their exposure to O₃ may be influenced by particle loading and contact with the filter substrate. We have therefore clarified this limitation in the revised manuscript while retaining the interpretation that the filter-bound treatment provides a controlled measure of the O₃ sensitivity of the collected particle phase rather than a complete simulation of suspended-particle indoor aging.

Indoor particles may experience O₃ exposure both during their airborne lifetime and after deposition onto indoor surfaces. The airborne residence time of fine particles is typically limited by ventilation and deposition processes, whereas deposited particles can persist on surfaces and remain exposed to oxidants over much longer timescales. The present filter-based protocol therefore probes the post-emission O₃ sensitivity of the collected particle phase, rather than the full time evolution of suspended smoke. We have clarified this distinction in the revised manuscript.

[A3] We have added the following discussion in Section 2.3:

“Similar filter-based O₃ 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 O₃ treatment provides a practical approach to probe the O₃ sensitivity of Bakhoor-burning particles. O₃ 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 O₃ 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 O₃ 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 O₃ may be influenced by particle loading and contact with the filter substrate.”

- ***Page 4, line 131: Did you use ice (or some other method) to control the water bath temperature (for avoiding sample degradation)?***

[R4] We have clarified in Sections 2.5 and 2.6 that sonication was performed in an ice-water bath to minimize sonication-induced heating. In Section 2.5, we also retained the comparison between sonication and vortex shaking, which showed no significant difference in DTT consumption, indicating that sonication did not introduce measurable artifacts in the DTT assay.

[A4] We have revised Section 2.5 as follows: “Filter samples were extracted in acetonitrile via 30-minute sonication in an ice-water bath to minimize sonication-induced heating and potential thermal degradation, followed by filtration through a 0.22 µm PTFE syringe filter.”

- ***Page 5, line 135: Why did you decide to use only positive mode? Are you expecting few polar compounds, or do you think those do not matter for toxicity?***

[R5] Thank you for the comment. ESI+ was selected as a consistent UHPLC-HRMS analytical window for untargeted molecular profiling of semi-polar, readily ionizable, and nitrogen-containing organic compounds in complex combustion-aerosol extracts. This window was useful for the present source comparison because the within-study sidestream cigarette-smoke reference was strongly enriched in CHN/CHON formulas, whereas Bakhoor-burning particles were dominated by CHO formulas in the ESI+ dataset. It was also useful for interpreting the DTT results because nitrogen-containing aromatic-like formulas detected in ESI+ showed positive associations with OP_{DTT}^m , especially at N:C $\approx$ 0.10–0.25.

We agree that ESI+ does not provide a complete inventory of particle-phase organics. Electrospray

ionization polarity is analyte-dependent, and ESI⁺ and ESI[−] provide complementary molecular coverage (Liigand et al., 2017; Patriarca et al., 2018). In particular, acidic or highly oxygenated CHO species may be less efficiently represented under ESI⁺ conditions, although they may still contribute to oxidative potential or cellular responses. We have therefore revised the manuscript to clarify that the molecular patterns reported here represent the ESI⁺-detectable fraction of the particle extracts. Accordingly, the molecular-formula correlations are interpreted as associations within this analytical window, rather than as evidence for the absence or toxicological irrelevance of compounds outside this window. The DTT and cellular assays provide integrated oxidative-response endpoints that are not limited to the ESI⁺-detectable compounds.

[A5] We have clarified the rationale and limitation of using ESI⁺ mode in Section 2.4 and 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 $\approx$ 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.”

And “Consistent with ESI⁺ resolved source fingerprints, Bakhoor-burning particles were dominated by CHO formulas and only a minority met the aromatic-like criterion ($AI_{mod} \geq 0.5$; 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_{DTM} observed for cigarette-smoke particles and with the positive association between OP_{DTM} 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.”

- ***Section 2.5: The DTT assay can exhibit a non-linear mass dependence (Charrier, 2016). Did you adjust the mass loadings to be roughly the same? Or do you think this is not relevant in your case due to the specific particle composition?***

[R6] Thank you for raising this important point. We agree that mass-normalized DTT activity can depend on the PM mass introduced into the assay. Charrier et al. (2016) demonstrated this bias mainly for ambient PM samples in which soluble Cu and Mn made substantial contributions to the DTT response, while also noting that other DTT-active species could in principle exhibit non-linear concentration-response behavior. In their experiments, the PM mass introduced into the DTT assay varied over a much wider range than in the present study, reaching approximately 40-fold across their tested samples and about 20-fold within an individual concentration-response experiment.

In the present study, the mass loadings were not intentionally adjusted to an identical value, but the collected PM masses for the main Bakhoor-burning and cigarette-smoke samples used in the DTT comparison varied within about a factor of four before extraction. All samples were processed using the same extraction and DTT reaction protocol, and the DTT consumption rate was determined from the linear decrease in residual DTT over the incubation period. In addition, our Chelex experiments showed negligible changes in OP_{DTTm} after metal chelation, indicating that dissolved transition metals made a minimal contribution to the DTT response in our samples. Therefore, the specific Cu/Mn-driven mass-normalization bias demonstrated by Charrier et al. (2016) is unlikely to dominate the source-level comparison in this study, although potential loading dependence from other DTT-active species cannot be fully excluded.

[A6] We have revised Section 2.5 by adding the following clarifications:

“For the main Bakhoor-burning and cigarette-smoke samples used in the DTT comparison, the collected PM masses varied within about a factor of four before extraction.”

“The DTT consumption rate was determined from the linear decrease in residual DTT over the incubation period.”

“Because mass-normalized DTT activity can depend on particle loading and on the concentration-response behavior of the dominant DTT-active species (Charrier et al., 2016), mass-normalized DTT oxidative-potential comparisons were interpreted with this potential loading-related uncertainty in mind. However, the Chelex tests showed negligible changes in $OP_{DTT,m}^{WS}$ after metal chelation, suggesting that the specific

Cu/Mn-driven mass-normalization bias reported by Charrier et al. (2016) is unlikely to dominate the source-level comparison in this study.”

- ***Page 5. Line 157-158: Did you filter the suspensions after the reaction? Otherwise, given the tendency of quartz fiber filters to disintegrate, I'd be surprised if there were no interference with the optical measurement. Or were these measurements done with the PTFE filters? Please clarify***

[R7] Thank you for the comment. The DTT measurements were conducted using particles collected on PTFE filters, not quartz fiber filters. For the water-soluble oxidative potential (OP^{WS-DTT}) measurement, the aqueous extract was filtered before the DTT reaction. For the total oxidative potential ($OP^{Total-DTT}$) measurement, the suspension was not filtered after extraction because this measurement was intended to include particle-associated or insoluble DTT-active components. Blank PTFE filters processed using the same protocol were used for background correction. We have clarified this point in Section 2.5.

[A7] We have revised Section 2.5 by adding the following clarification:

“Briefly, **PM-loaded PTFE filters** were extracted in deionized water by 30 minutes of sonication in an ice-water bath to minimize heating during extraction.” and “This ensured that DTT-active species associated with insoluble particles suspended in the extract or still attached to the filter surface were also included in the measurement (Fang et al., 2017; Gao et al., 2017). **Blank PTFE filters processed using the same protocol were used for background correction.**”

- ***Section 2.6: have you performed any positive controls for your cell exposure experiments?***

[R8] Thank you for the comment. The cellular exposure experiments were designed primarily to compare oxidative-stress responses across particle sources and aging conditions, including fresh and ozone-aged Bakhoor-burning particles and sidestream cigarette smoke as a protocol-matched indoor combustion reference. Thus, the cellular assay was interpreted using a source-level particle comparator and its aged counterpart under the same exposure protocol, rather than a single chemical oxidant. We acknowledge that a chemical positive control, such as H_2O_2 or tert-butyl hydroperoxide, can be useful for verifying assay responsiveness, but such a control was not included in the same exposure batch. The cellular-response comparison was supported by untreated cell controls, blank PTFE filter controls processed using the same protocol, dose-response measurements across particle loadings, and the accompanying MTT viability assay. We have clarified this point in Section 2.6.

[A8] We have revised Section 2.6 by adding the following clarification:

“The cellular exposure experiments were designed to compare oxidative-stress responses across particle sources and aging conditions, with sidestream cigarette smoke included as a protocol-matched indoor combustion reference; no separate chemical positive control for intracellular ROS generation was included in the same exposure batch.”

- ***Figure 1: there is a clear discontinuity in your size distribution data (1a & 1b). Any idea what happened there?***

[R9] The discontinuity near 500 nm is an instrumental switching artifact caused by the transition between the two sizing instruments used in this study. Particles below 500 nm were measured by the SEMS, whereas particles above 500 nm were measured by the OPC. We therefore do not interpret this discontinuity as a real particle mode or physical feature of the emitted aerosol size distribution. We have clarified this in the Figure 1 caption.

[A9] We have clarified this in the Figure 1 caption:

“The apparent discontinuity near 500 nm in panels A and B reflects the use of two instruments: particles below 500 nm were measured by the SEMS, whereas particles above 500 nm were measured by the OPC. This discontinuity is therefore not interpreted as a physical feature of the emitted aerosol size distribution.”

- ***Page 7, line 203-205: given that your reference emissions are usually low due to the lack of active puffing, I think the “benchmark” framing is slightly misleading as it makes the incense smoke look worse in comparison (substantially higher than an unusually low value). At the minimum, you should state some values for active smoking condition to enable the reader to better put the observed values into context.***

[R10] Thank you for this helpful comment. We apologize for the ambiguity in the original wording. In the original version, Hu et al. (2023) was cited after the statement that the cigarette reference was generated without active puffing. However, Hu et al. (2023) mainly provides a broader mainstream-versus-sidestream tobacco-smoke comparison rather than a scaling factor for active-puffing effects on sidestream particle-mass emissions.

To address this point more directly, we added literature values comparing sidestream particle mass under puffing and free-burn conditions. Previous measurements showed that sidestream particulate matter under puffing conditions remained within approximately 0.84–0.97 times the free-burn value (Browne et al., 1980). Sidestream smoke is also a major component of environmental tobacco smoke; Pieraccini et al. (2008) reported that environmental tobacco smoke consists mainly of sidestream smoke, with sidestream

smoke contributing approximately 85% and exhaled mainstream smoke contributing approximately 11%. These values indicate that although our cigarette reference was generated without active puffing, it is not expected to represent an unusually low sidestream particle-mass reference.

We have changed “sidestream cigarette benchmark” to “smoldering sidestream cigarette reference” and revised Section 3.1 accordingly. The revised text preserves the same-protocol comparison showing that Bakhoor burning produced substantially higher particle mass emission rates than the smoldering sidestream cigarette reference, while adding quantitative context for the effect of active puffing on sidestream particulate matter.

[A10] We have revised Section 3.1 as follows: “ On this basis, Bakhoor burning produced particle mass emission rates of 670-1690 $\mu\text{g min}^{-1} \text{ g}^{-1}$, substantially higher than the same-protocol smoldering sidestream cigarette smoke reference in this study ($\sim 250 \mu\text{g min}^{-1} \text{ g}^{-1}$). The cigarette-smoke comparison was based on a controlled same-protocol smoldering condition without active puffing. Previous measurements comparing sidestream particulate matter under puffing and free-burn conditions showed that sidestream particulate matter under puffing conditions remained within approximately 0.84–0.97 times the free-burn value (Browne et al., 1980).”

- ***Page 7, line 217-218: see my previous comment: UFP concentrations likely also depend on additional parameters (e.g. the dilution rate changing coagulation & nucleation behavior).***

[R11] Thank you for the comment. We agree, consistent with our response to the earlier chamber-condition comment, that dilution rate and residence time can influence nucleation, condensational growth, and coagulation during the early evolution of freshly emitted UFPs. In the sentence cited by the reviewer, our statement was referring specifically to the comparison between charcoal-only burning and Bakhoor burning on charcoal conducted under the same chamber configuration. Charcoal-only burning produced only a short-lived nucleation-mode signal, whereas Bakhoor burning on charcoal sustained elevated particle concentrations and extended to larger particle diameters.

We have revised the wording to clarify that this matched comparison indicates an additional source-side contribution from Bakhoor material, rather than attributing the observed particle formation to the ignition charcoal alone.

[A11] We have revised Section 3.2 as follows:

“Figure 2A shows that charcoal-only burning produces a short-lived nucleation-mode signal immediately after ignition that decays rapidly, whereas Bakhoor burning on charcoal sustains elevated particle concentrations and extends to larger particle diameters. [This side-by-side comparison was conducted under the same chamber flow-through configuration and indicates that the sustained particle formation observed during Bakhoor burning cannot be explained by the charcoal ignition source alone. Instead, it is consistent with additional particle-forming material released from Bakhoor.](#)”

- ***Page 8, line 255: was the mass determined gravimetrically or from the particle counters? In the latter case, I'd expect a rather large uncertainty due to the assumptions regarding the particle densities, which would impact the comparison with literature values.***

[R12] The PM mass used for calculating OP_{DTM} was determined gravimetrically from the difference between the pre- and post-sampling masses of the PTFE filters. Therefore, the density assumption does not affect the mass-normalized DTT values or their comparison with literature OP_{DTM} values. We have clarified this point in Section 2.5.

[A12] We have revised Section 2.5 by adding the following sentence:

[“The PM mass used to calculate mass-normalized DTT oxidative potential was determined gravimetrically from the difference between the pre- and post-sampling masses of the PTFE filters.”](#)

- ***Page 9, line 269-270: while I think that it is fine to focus on ozone only, recent research indicates that OH chemistry is also relevant indoors (e.g. Zannoni, 2022).***

[R13] Thank you for the suggestions. We agree that indoor oxidative aging is not limited to ozone and that OH chemistry can also be relevant indoors. In this study, ozone aging was used as a controlled single-oxidant probe to evaluate whether Bakhoor-burning particles show measurable changes in composition and oxidative potential after O_3 exposure, rather than to represent the full range of indoor oxidant chemistry.

We have revised the relevant discussion in Section 2.3 to clarify this scope and to acknowledge other indoor oxidants, including OH radicals generated through O_3 -initiated chemistry in occupied environments (Zannoni et al., 2022), and NO_3 radicals under NO_2/O_3 -rich conditions.

[A13] We have revised Section 2.3 as follows:

[“In addition, indoor OH and \$NO_3\$ chemistry can occur but is more condition-dependent than the controlled](#)

O₃ exposure used here. Indoor OH can be generated through ozonolysis and photolysis-related processes, whereas NO₃ chemistry becomes more relevant under NO₂/O₃-rich conditions (Waring and Wells, 2015; Abbatt and Wang, 2020; Zannoni et al., 2022; Dewald et al., 2023). Therefore, OH- and NO₃-driven processing should be regarded as additional indoor aging pathways that were not simulated by the present filter-bound O₃ exposure and should be evaluated in future suspended-particle or mixed-oxidant studies.”

- **Page 9, line 274: is resuspension actually likely in your case, given that your particles are predominantly rather small?**

[R14] Thank you for the comment. We agree that the wording should be clarified. Our original statement was intended to refer to deposited material on real indoor surfaces, where later surface disturbance, human activity, air movement, or cleaning can influence the fate of deposited particles. Indoor particle resuspension from surfaces is a documented process under occupant activity and surface-disturbance conditions (Ferro et al., 2004; Benabed and Boulbair, 2022). However, this indoor surface process should be distinguished from the PM-loaded filters used in our O₃-aging experiment.

In our O₃-aging experiment, PM-loaded PTFE filters were exposed to a low continuous O₃ flow through the filter holder, without mechanical disturbance or reverse flow. The O₃ exposure flow rate was 0.5 L min⁻¹, corresponding to an estimated filter face velocity of approximately 0.48 cm s⁻¹ for a 47 mm filter. Prior filter re-entrainment and reaerosolization studies provide a useful scale for the airflow conditions required to release particles from loaded filters. For example, Qian et al. (1997a) tested particle re-entrainment from loaded fibrous filters at air velocities up to 500 cm s⁻¹; this velocity was nearly 100 times their particle-loading filtration velocity of 6.6 cm s⁻¹. In comparison, the face velocity in our O₃-aging experiment was approximately three orders of magnitude lower than their maximum re-entrainment velocity. In a related N95-filter study, Qian et al. (1997b) reported that reaerosolization of particles smaller than 1 μm was 0.025% or less even at a re-entrainment air velocity of 300 cm s⁻¹, a condition intended to simulate violent sneezing or coughing. These comparisons suggest that particle release from the PM-loaded filters is unlikely to be important under the low-flow, undisturbed filter-holder conditions used here.

[A14] We have revised Section 3.3 as follows:

“Moreover, the high indoor surface-to-volume ratio promotes rapid particle deposition to surfaces (Abbatt and Wang, 2020), creating [surface reservoirs](#) that are not removed by ventilation and can persist for days. [These deposited materials can continue to undergo chemical processing on indoor surfaces and may remain relevant to exposure if later disturbed or transferred by human activity, air movement, or cleaning.](#)”

- ***Section 3.6: Again, please motivate why you chose to only look at positive mode***

[R15] [A15] Thank you for the comment. We agree that the rationale and limitation of using positive ionization mode should be clearly stated wherever the molecular-formula results are interpreted. This point has been addressed in the revisions described in our response to [R5]. Specifically, we added text to explain why ESI+ mode was used and clarified that the molecular-formula distributions and OP_{DTM} correlations are interpreted within the ESI+-detectable molecular fraction, rather than as a complete inventory of particle-phase organics.

- ***As you describe in the supplement, the assumption of spherical particles for LDSA calculation is likely not that appropriate (given that for your source, the particles are likely not spherical). Do you have any estimate regarding the LDSA uncertainty that is introduced this way? Either way, I think this caveat should be mentioned also in the main text.***

[R16] Thank you for the suggestion. We agree that particle morphology can introduce uncertainty into the LDSA estimate, particularly for combustion-derived particles that may be nonspherical or partially agglomerated. The LDSA calculation in this study used the measured size distributions and regional deposition fractions, with particle surface area estimated assuming spherical particles. The effective density used for converting size distributions to particle mass was 1 g cm⁻³, close to the reported density of incense smoke particles (~1.1 g cm⁻³; Ji et al., 2010), suggesting that density-related uncertainty is likely modest. Previous aerosol studies have shown that particle morphology can affect the relationship between particle size, projected surface area, and geometric surface area, particularly for agglomerated particles (Decarlo et al., 2004; Ku and Maynard, 2005; Levin et al., 2016).

Because morphology-resolved scanning electron microscopy/transmission electron microscopy (SEM/TEM) or Brunauer–Emmett–Teller (BET) surface-area measurements were not available for the present samples, we cannot quantify or apply a sample-specific morphology correction. We have therefore added this caveat to the main text.

[A16] We have revised the main text as follows: “Because particles at intermediate diameters dominate the emitted size distribution and the integrated surface-area dose (Figure S14), the LDSA ratio integrated over the measured particle size range is approximately 4 for both the Tb and Al regions (Figure 6C). **This LDSA calculation assumes spherical particles when converting measured size distributions to particle surface area. It should be noted that nonspherical or agglomerated combustion particles may introduce a morphology-dependent surface-area uncertainty (Decarlo et al., 2004; Ku and Maynard, 2005; Levin et al., 2016).**”

Technical comments

- **Page 6, line 195: I don't think that "enriched" is really the right word here.**

[R17] [A17] Thank you for the suggestions. We have replaced "enriched" with more precise wording. "The ultrafine-mode peak was approximately 1.4 times the secondary peak, [indicating a large contribution of ultrafine particles \(UFPs, <100 nm\) to the total particle number.](#)"

- **I would suggest to use superscript (e.g. WS and tot) to distinguish between $OP_{DTT,m}$ for water-soluble and total OP , respectively (as you already do in one of your figures). Using the same abbreviation for both makes the text harder to parse for the reader.**

[R18] [A18] Thank you for the suggestion. We agree that using distinct notation improves readability and have revised the notation throughout the text accordingly.

- **Figure 3C is currently not referred to, I would guess it's meant to be in page 9, line 279?**

[R19] [A19] Thank you for noting this. We have added the missing reference to Figure 3C in the sentence comparing Bakhoo OP_{DTTm} with literature values.

"Across fresh and O_3 -aged samples, the median OP_{DTTm} of Bakhoo water extracts fell within the broad range reported for laboratory combustion-derived PM and overlapped values reported for field PM ([Figure 3C](#)), although many field studies report total OP_{DTTm} , which is typically higher than the water-soluble fraction."

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