ADDITIONAL MATERIALS AVAILABLE ON THE HEI WEBSITE

Research Report 201

Understanding the Functional Impact of VOC–Ozone Mixtures on the Chemistry of RNA in Epithelial Lung Cells

Contreras et al.

Additional Materials 1: Appendix A. Supplementary Figures

These Additional Materials were not formatted or edited by HEI. This document was part of the HEI Review Committee's review process.

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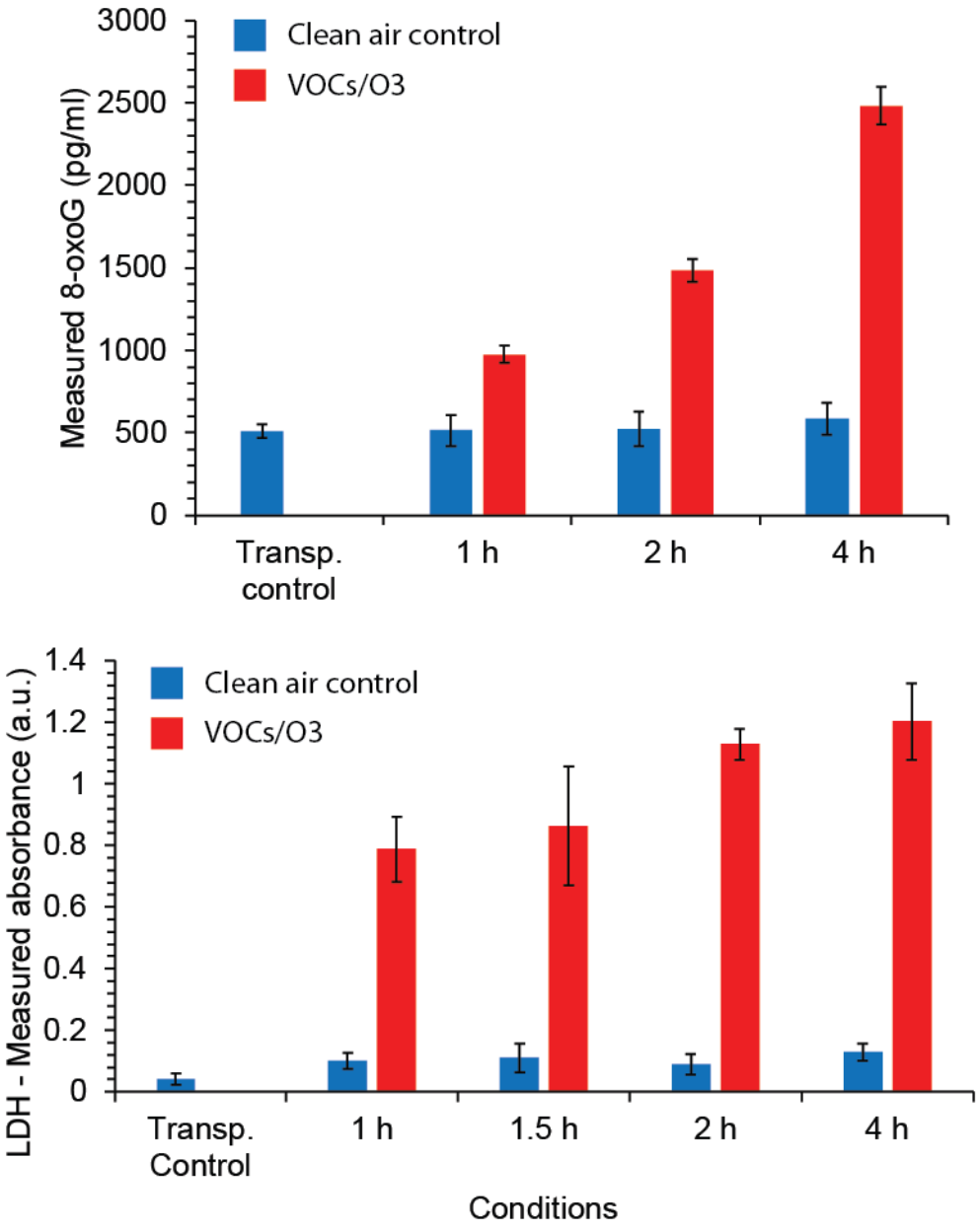
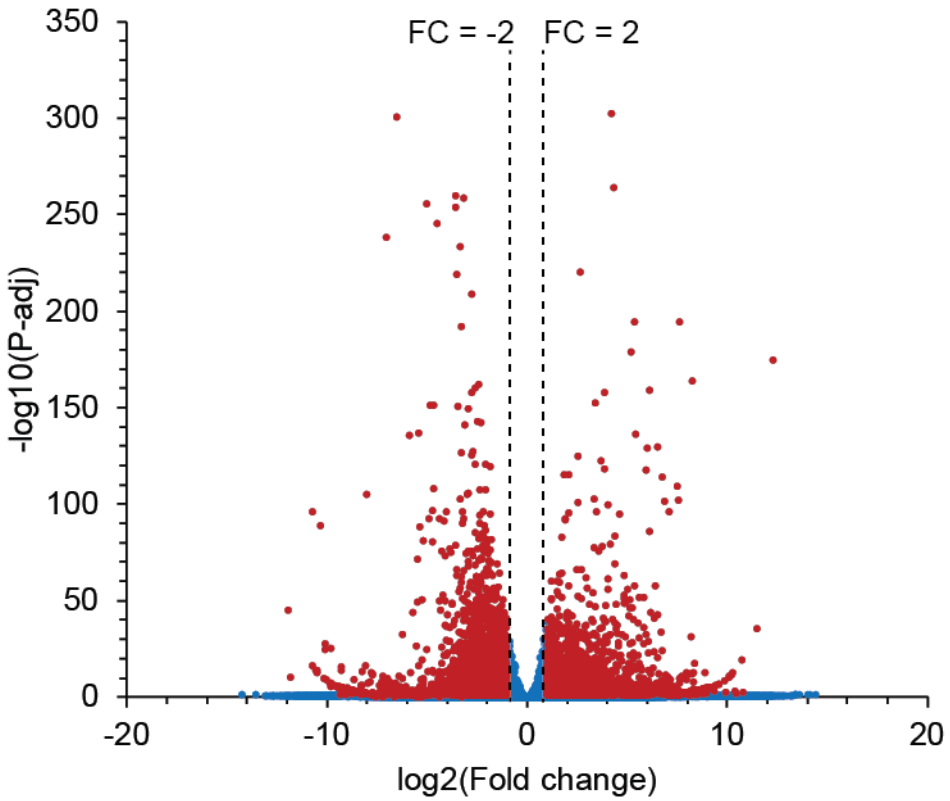
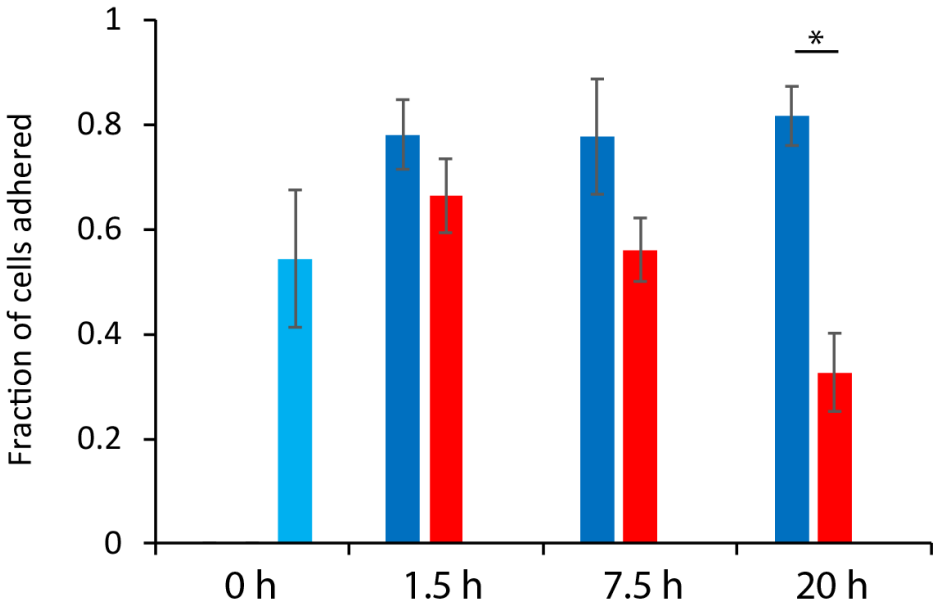
Appendix A



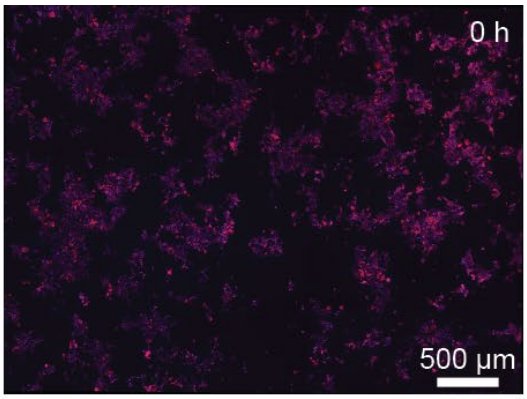
Appendix Figure A.1. Levels of biomarkers for investigation of the duration of the exposure of BEAS-2B cells to VOCs/O3 mixtures derived from 790 ppb acrolein, 670 ppb methacrolein, and 4 ppm ozone. LDH is used as a measured of measure of cell death or stress and 8-oxoG marker is used as a measure of RNA modification cause by oxidative stress conditions.



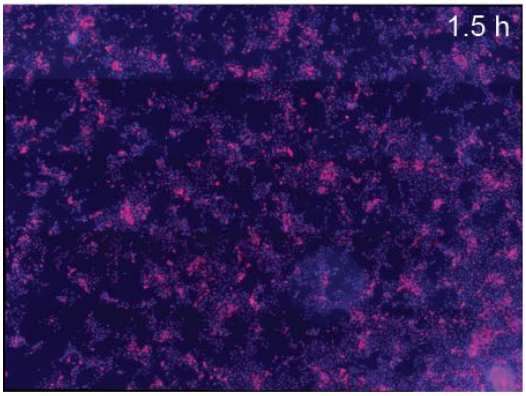
Appendix Figure A.2. Volcano plot (log10(P-adj) vs log2(Fold change)) shows transcripts enriched in RNA oxidation in the clean air control. The dots in red represent the transcripts significantly enriched with a significance of adjusted p-value < 0.05 and FC < -2 or >2. These transcripts feature the basal level of cellular oxidation even in the absence of environmental stress.

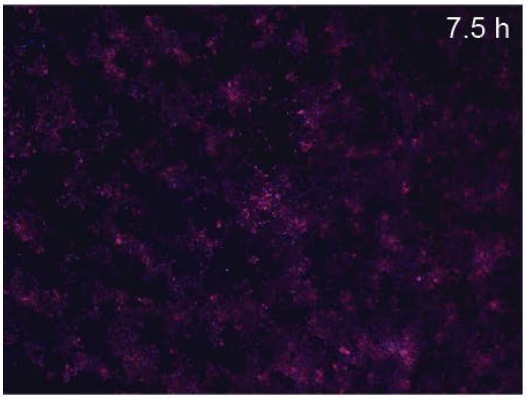


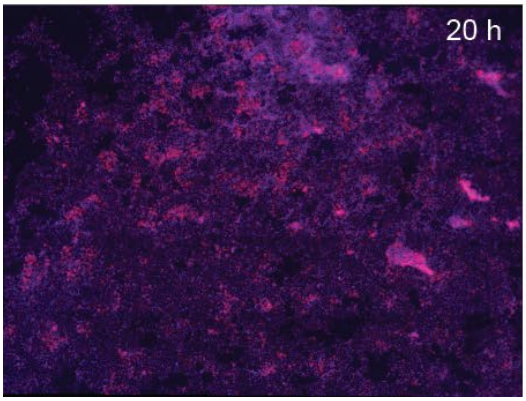
Appendix Figure A.3. Fraction of cells adhered to membrane inserts (or confluence) after exposure of BEAS-2B cells to the air pollution mixture (derived from the reaction of 790 ppb acrolein, 670 ppb methacrolein, and 4 ppm O_3) for 1.5 h. The fraction was estimated by analysis of confocal microscopy images using Fiji/Image J on three images per condition. Statistical difference was computed by T-test analysis and significance is denoted as * for p-value < 0.05, ** for p-value < 0.005, and *** for p-value < 0.0005.



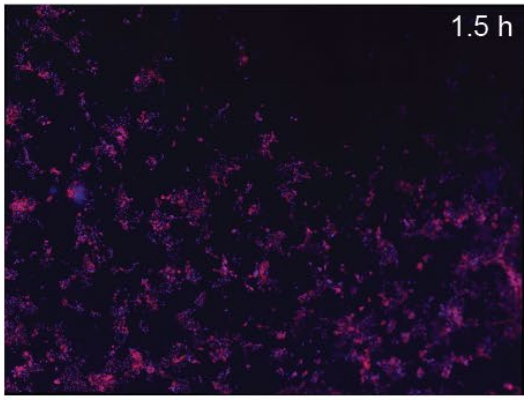
Clean air control

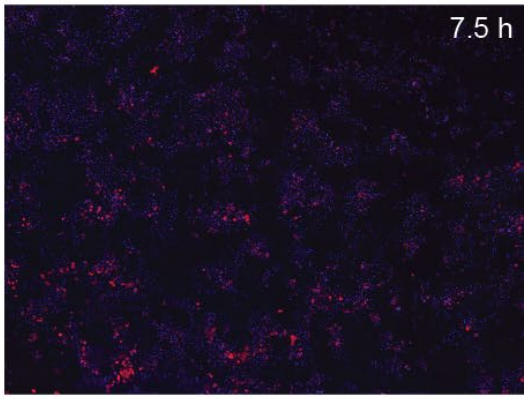


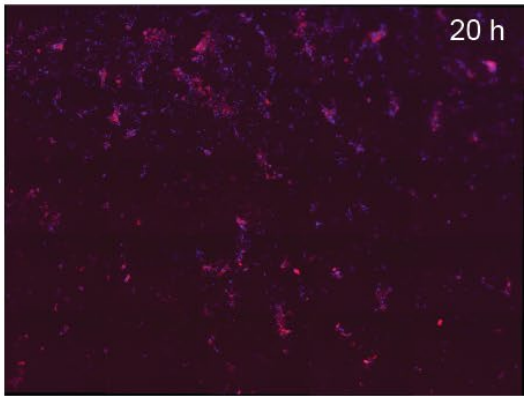




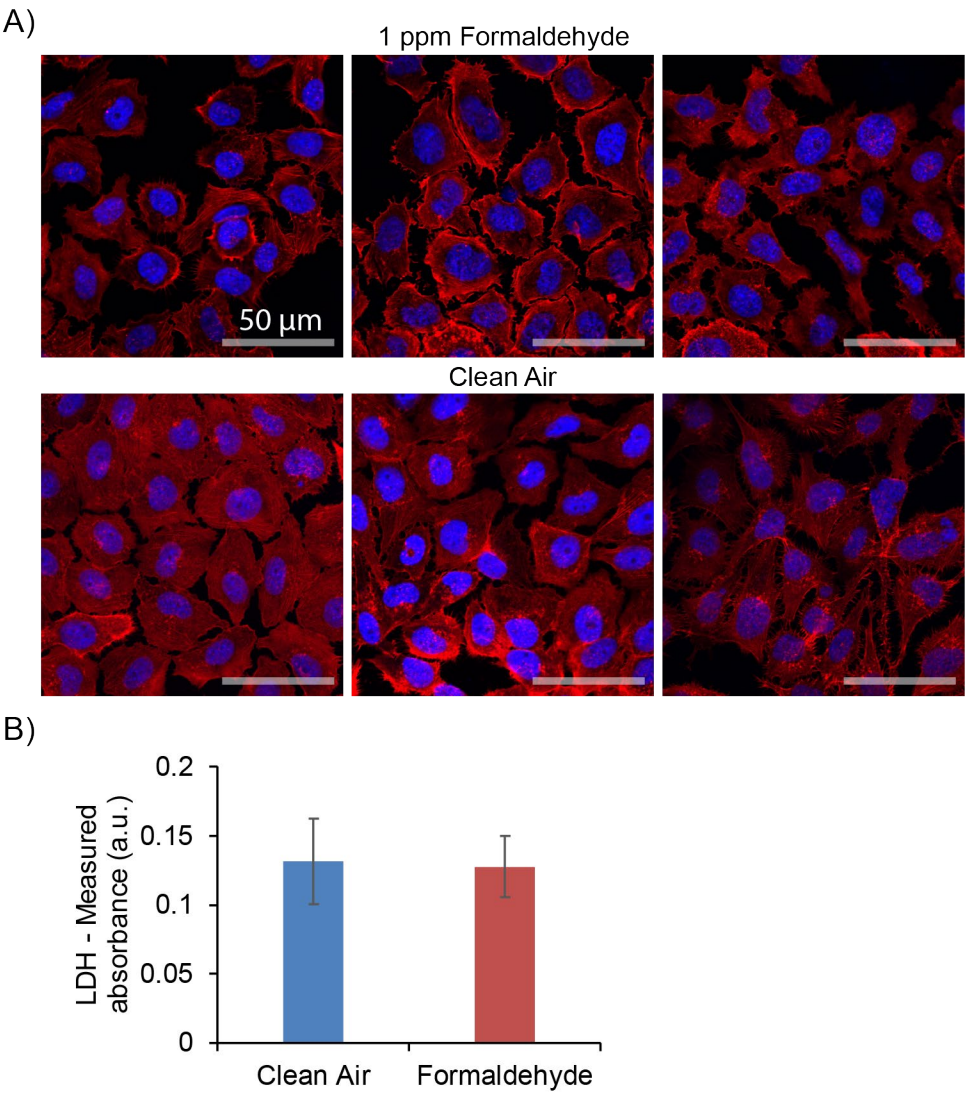
VOCs/O3







Appendix Figure A.4. Tiled images acquired by widefield fluorescent microscopy for BEAS-2B cells exposed to VOCs/O3 mixtures (derived from 790 ppb acrolein, 670 ppb methacrolein, and 4 ppm ozone) for 1.5 h. Cells exposed to clean air control proliferate closely reaching confluence in the time frame of 20 hours postexposure.



Appendix Figure A.5. BEAS-2B cells exposed to 1 ppm formaldehyde exposures. Exposures were conducted for 2 hours and cells were analyzed after 6 hours incubation post exposure. (A) Confocal fluorescent microscopy images of F-actin staining with Alexa Fluor 594 phalloidin (red) and nuclei staining with DAPI using a magnification of 63X (blue). The images are representative of three independent air exposures. Magnification of 63X. (B) LDH release in the cell media collected shortly after 2 hours of 1 ppm formaldehyde exposure.