Supporting Information

Exposure of Lung Epithelial Cells to Photochemically- Aged Secondary Organic Aerosol Shows Increased Toxic Effects

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Section A. MATERIALS AND METHODS

SOA generation from the OFR. SOA with controlled aging times were produced in a 13L aluminum cylindered OFR.[1, 2] Ozone was produced outside the OFR by irradiating 0.3 L min^{-1} high purity O_2 with a mercury lamp (78−2046−07, BHK Inc., CA, USA). A 4−9 L min^{-1} humidified N_2 was directed into the OFR as carrier gas. A Nafion membrane humidifier (Perma Pure LIC, NJ, USA) was used to adjust the relative humidity (RH, 35%-38%). The average residence time in the OFR was between 100 and 200 s. The OFR employs two 254 nm low pressure 12" long Hg UV lamps (82−934−08, BHK Inc., CA, USA) which are mounted in Teflon-coated quartz cylindrical sleeves to allow peak emission at λ= 254 nm. The OH radical concentration was controlled by adjusting the UV light intensity using a dimmer. Systematic chemical kinetics modeling was previously performed to study the radical chemistry in OFRs, as a function of H_2O and NOx mixing ratios, UV photon flux and OH reactivity. The aging estimation of the produced SOA is calculated using a model constrained by the following measured parameters: ozone concentration ratios before and after the reactor, residence time and external reactivity of OH radicals.[3, 4] The ratio of the ozone and OH radical (6.9×10^4 – 4.3×10^5) were kept within atmospherically relevant range.

The ozone removal efficiency was obtained before the exposure studies. During the exposure, the flow rate is constant. Thus, the efficiency test was conducted for different initial ozone concentrations. The ozone concentration in the flow OFR outflow ranged between 15 and 61 ppm. After the ozone scrubber, the ozone concentration ranged between 102 and 388 ppb. The ozone scrubbing material is Carulite 200 catalyst which consists of manganese dioxide and copper oxide catalyst. Figure S1 below represents ozone removal efficiency. Still, the ozone does not affect viability (about 2% reduction in viability compared to the incubator control).

Oxidative Potential: detection of organic peroxide. Total organic peroxides were determined using the method introduced by Mutzel *et. al.* [5] after collecting particles on Teflon filters (0.45 μm pore size). The filters were cut into four quarters, two were used as blank and two for peroxide determination. The filters were extracted with 3mL of ultrapure water using a vortex for 15 min. The water solution containing the extracted components was filtered (syringe filter, Teflon, 0.22 μm pore size) and the resulting filtrate was acidified with acetic acid to pH 3. Then, oxygen was removed by flowing nitrogen into the liquid (with capping) for 5 min. Potassium Iodide (KI, 30 mg, 0.18 mM) was added before gasification to the peroxide test tubes and not to

the blank tubes. After 1h, absorbance was measured with UV-VIS spectrometer. Absorption at λ=351 nm was used for the analysis. The peroxide content was evaluated with H_2O_2 calibration curve[6] ranging from 6 to 100 μM, prepared freshly for each experiment.

Cell Culture and Exposure System. Cells were grown in RPMI-1640 (Gibco, Thermofisher Scientific, USA), supplemented with 2mM Glutamine, 10% Fetal Bovine Serum (FBS) and 5 μg mL^{-1} Penicillin Streptomycin (Biological Industries, Beit Ha-Emek Israel).

Cells were exposed in the CULTEX RFS system.[7, 8] Briefly, 24 h prior to the exposure, cells were seeded on Corning Trans-well inserts with micro-porous membrane (growth area ~12 mm^2, 0.4 μm pore size, Corning Transwell, USA) with an optimized density of 3×10^5 cells mL^{-1}. Before exposure, the cell medium was removed from the apical and basolateral sides. The exposure medium was supplemented with HEPES without FBS. Exposure times varied between 1 to 6 hours. After exposure, all inserts were post-incubated for 24 hours (at 37°C, 5% CO_2) and then tested for cell viability and gene expression analysis and compared to an incubator control. For ROS measurements, only 4 h post incubation time was given since ROS are short lived. The experiments were performed in triplicates and were repeated twice with different cell passages. The maximal exposure time and flow parameters were validated by measuring cell survival under clean flow. The gas flow rates through the Trans-wells and the main outlet were adjusted to 10 ml min^{-1} and 1 L min^{-1}, respectively.

As positive control, cells were exposed to copper sulfate particles generated by atomizing copper sulfate solutions (0.03 to 3 gr L^{-1}) using a constant output atomizer (TSI). In addition, cells were exposed to OFR-atmosphere that passed through HEPA filter as negative control.

Optimizations of CULTEX RFS System. To determine the possible range of exposure times, A549 cells were exposed to clean air for up to 24 hours. Exposure to clean air for up to six hours did not cause significant changes in cell viability while more significant changes were observed after 8 hours exposure (Figure S4A). A significant decrease in cell viability was observed in positive control experiments of exposure to copper sulfate, consistent with previous studies.[8, 9]

Efficient particle deposition was achieved with a unipolar electro deposition device (EDD).[10, 11] The EDD voltage was optimized for SOA particle deposition by measuring the particle size distribution downstream of the CULTEX chambers. Applying the deposition voltage (between -100 and -300 V, Figure S4B), decreased cells survival compared to the incubator

control, without significant changes between the various voltages applied. (Figure S4C). Thus, the optimal voltage for the SOA exposure experiments was set to -300 Volts.

Reactive Oxygen Species (ROS) assay. ROS generation was evaluated 4 hours after exposure to fresh and aged naphthalene SOA. Cells exposed to naphthalene SOA or filtered air were treated with 20 µM of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) for 20 min at 37^{o}C. Fluorescence was measured at ex/em 485/528 nm. Data is expressed as mean ± SD. The difference between groups was evaluated using t-test, and considered significant at $p < 0.05$

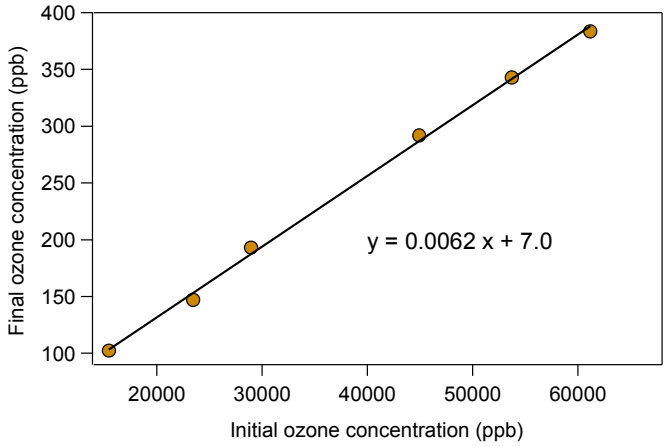


Figure S1. Ozone removal efficiency

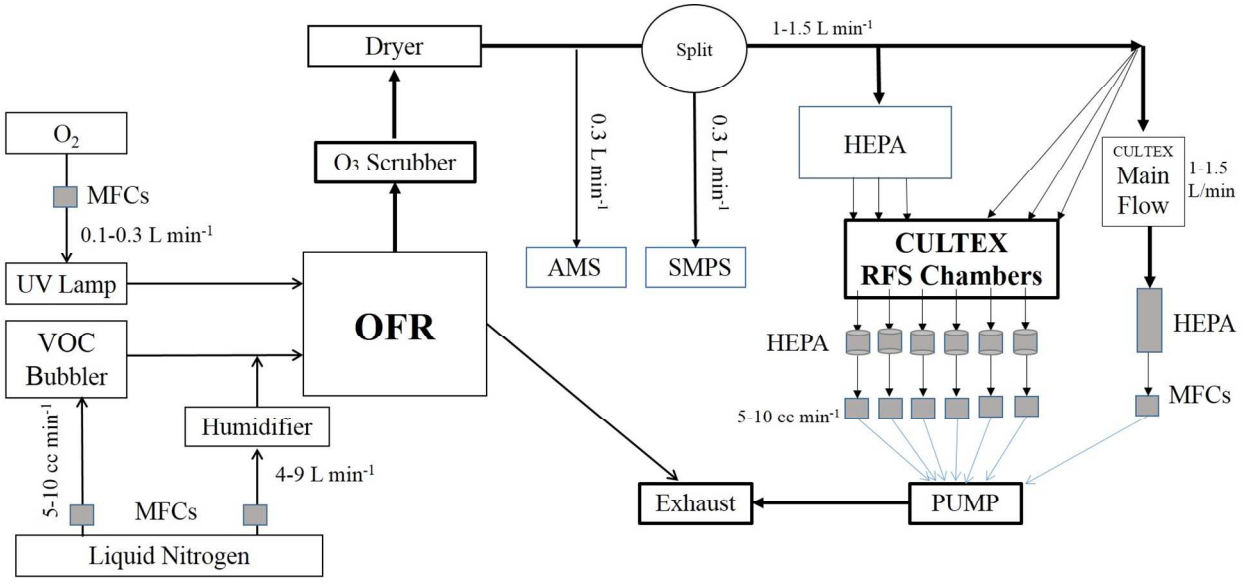


Figure S2. Schematic of the experimental system. The SOA are produced in the OFR, which is supplied by ozone, humidified nitrogen and precursor VOC. The flow is scrubbed partially from ozone and dried after the OFR. The isokinetic split enables a real-time sampling by SMPS and AMS during the exposure of the cells at the air liquid interface. OFR, oxidation flow reactor; VOC, volatile organic compounds; SMPS, scanning mobility particle sizer; AMS, HR-ToF-AMS.

Section B. RESULTS

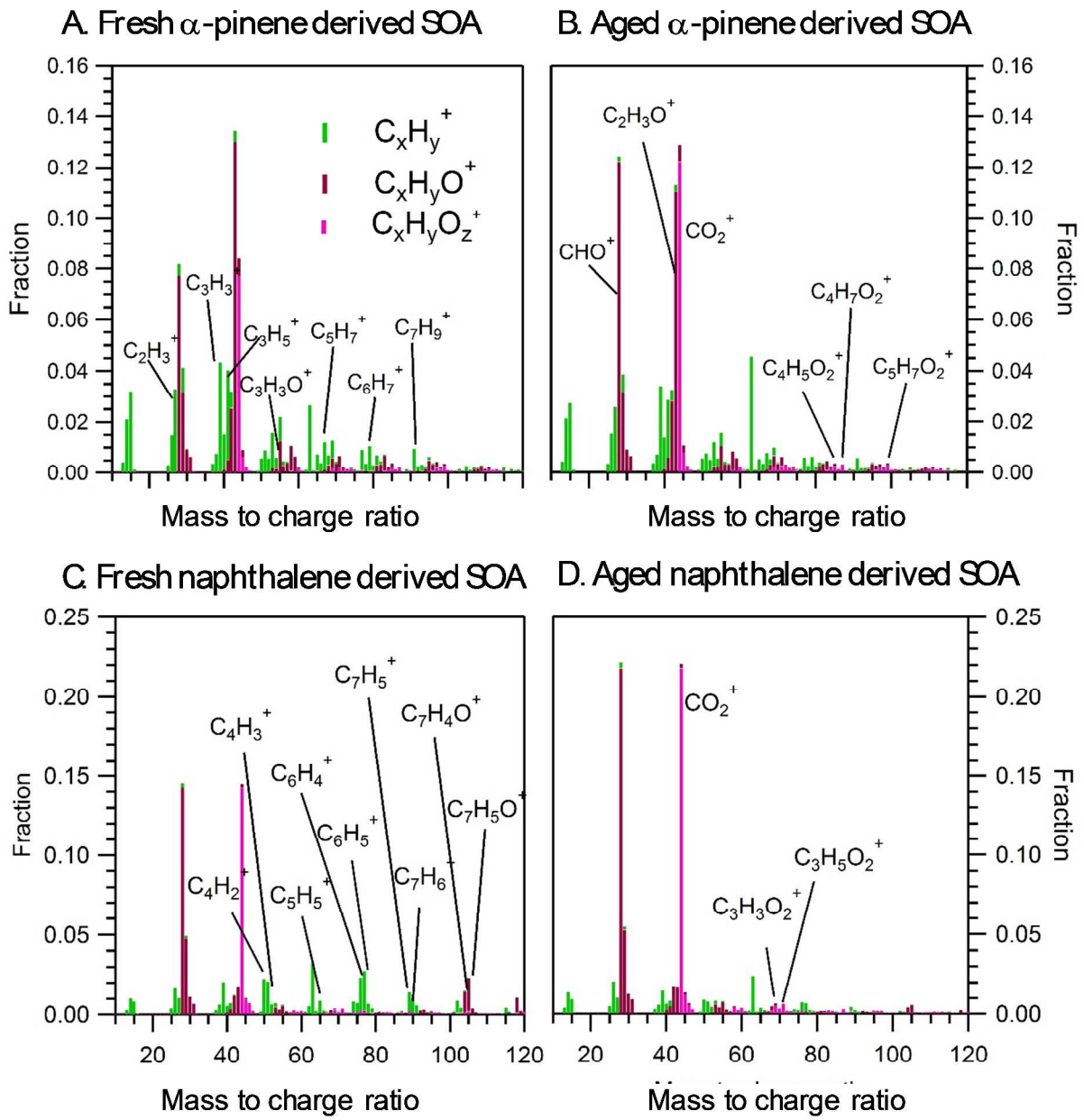


Figure S3. Mass spectra of (A) Fresh α-pinene SOA (2.3±0.5 days of aging) (B) Aged α-pinene SOA (9.8±0.5 days of aging) (C) Fresh naphthalene SOA (2.2±0.3 days of aging) and (D) Aged naphthalene SOA (11.1±1.1 days of aging), obtained by HR-TOF-AMS.

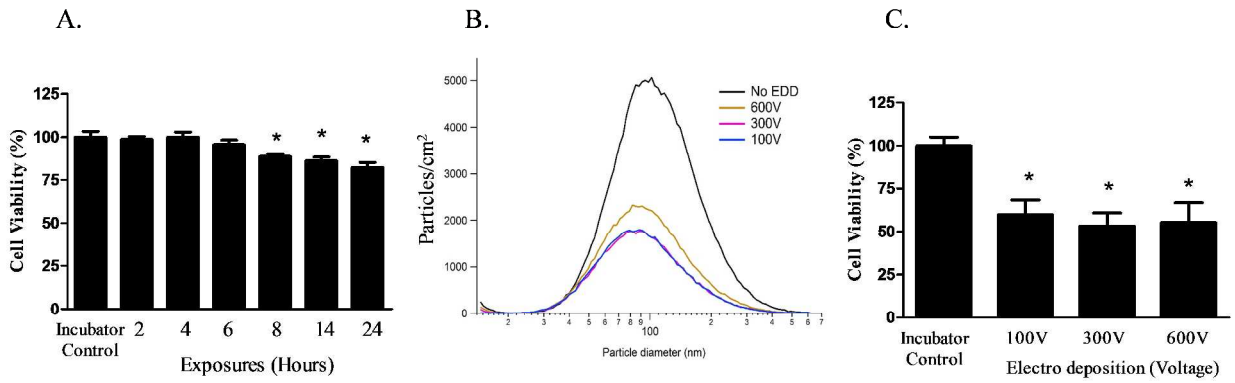


Figure S4. Optimization of system parameters. (A) Cell viability of lung cells after exposure to clean air at the indicated time points (2, 4, 6, 8, 14 and 24 hours). WST-1 assay was performed as detailed in the material and method section. Experiments were performed in triplicates and repeated twice. $P<0.05$ statistically significant from the control. (B) Size distribution of SOA nanoparticles dependent on EDD Voltage (-100, -300, -600 Volt and no Voltage). C- Cell viability of lung cells after exposure to SOA with different electro deposition voltage (-100, -300, -600 Volt and no Voltage). Experiments were performed in triplicates and repeated twice. $P<0.05$ statistically significant from the control.

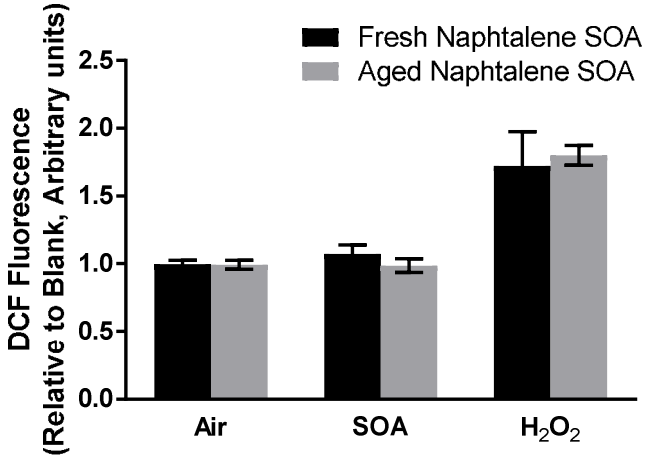
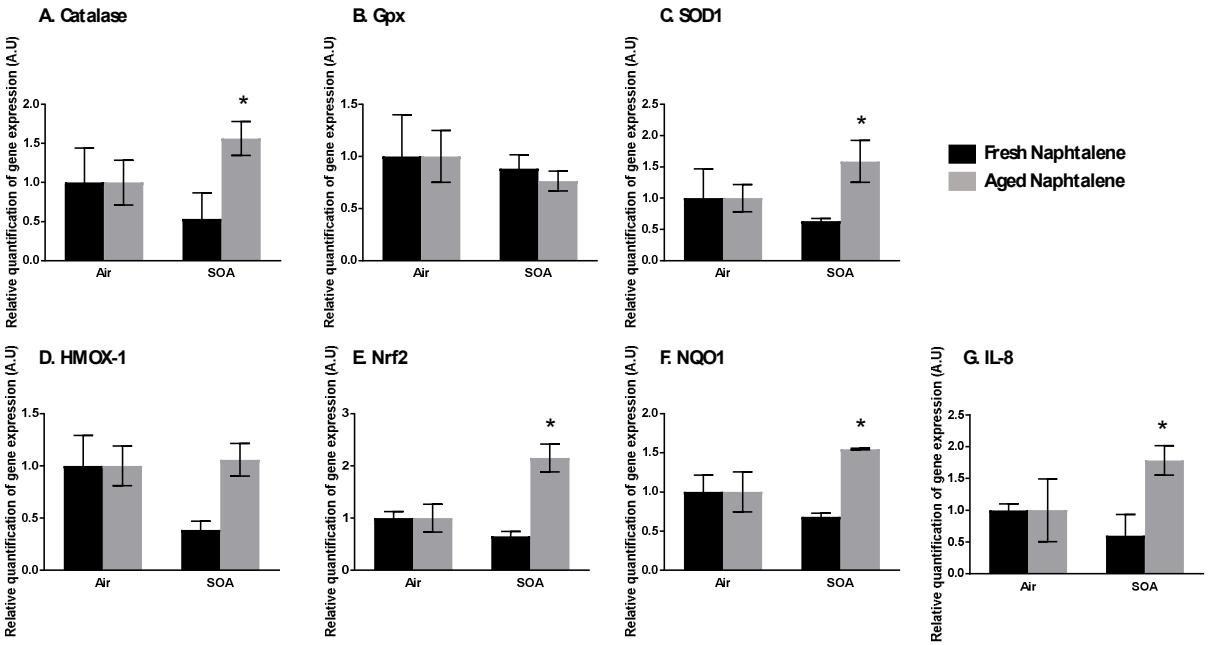


Figure S5. ROS in naphthalene SOA (fresh and aged) particles**.** ROS levels measured by H2DCF-DA as described in material and method section. DCF fluorescence quantification 4 hours after the exposure. The data represent mean ± SD. These experiments were performed in triplicate and were repeated three time.



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142 **Figure S6.** Expression of protective genes related to Nrf-2 signaling after exposure to
143 naphthalene (fresh and aged) SOA. A549 human lung epithelial cells exposed to SOA and air
144 were subjected to qPCR of (A) catalase, (B) Gpx, (C) SOD1, (D) HMOX-1, (E) Nrf2, (F) NQO1
145 and (G) Il-8. Values are expressed as fold change of gene expression compared to a calibrator
146 (endogenous controls, HPRT and β-Actin). Data represents two independent experiments,
147 means ± SD; $n = 3$ in each experiment; * significantly higher at $p < 0.05$ than their controls.

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TABLES

Table S1. List of genes and assays ID

Assay ID	Gene Symbol	Gene Name	Species	Amplicon Length
Hs01110250_m1	HMOX1	heme oxygenase 1	Human	82
Hs00156308_m1	CAT	Catalase	Human	68
Hs00829989_gH	GPX1	glutathione peroxidase 1	Human	76
Hs00533490_m1	SOD1	superoxide dismutase 1	Human	60
Hs00232352_m1	NFE2L2	nuclear factor; erythroid 2 like 2	Human	59
Hs99999903_m1	ACTB	actin beta	Human	171
Hs99999909_m1	HPRT1	hypoxanthine phosphoribosyltransferase 1	Human	100
Hs01045994_m1	NQO1	NAD(P)H quinone dehydrogenase 1	Human	77
Hs00174128_m1	TNF	tumor necrosis factor	Human	80
Hs00174131_m1	IL6	interleukin 6	Human	95
Hs00174103_m1	CXCL8	C-X-C motif chemokine ligand 8	Human	101

Table S2. Density of naphthalene and α-pinene derived fresh and aged SOA.

SOA	α-pinene SOA 2.3±0.5 days	α-pinene 9.8±0.5 days	SOA naphthalene SOA 2.2±0.3 days	aphthalene 1.0±1.1 days	SC
Density (gr cm^{-3})	1.136±0.018	1.226±0.024	1.285±0.001	1.350±0.016	

Table S3. Chemical composition of naphthalene and α-pinene-derived fresh and aged SOA.

Elemental Ratio	α-pinene SOA 2.3±0.5 days	α-pinene SOA 9.8±0.5 days	naphthalene SOA 2.2±0.3 days	naphthalene SOA 11.0±1.1 days
H:C	1.59±0.01	1.46±0.01	1.03±0.01	1.04±0.01
O:C	0.51±0.01	0.69±0.04	0.72±0.02	1.17±0.02

Table S4. Exponential parameters $y = y_o + Ae^{Ro \cdot x}$ for reduced viability after exposure to fresh and aged α-pinene and naphthalene derived SOA.

SOA	α-pinene SOA 2.3±0.5 days	α-pinene SOA 9.8±0.5 days	naphthalene SOA 2.2±0.3 days	naphthalene SOA 11.0±1.1 days
y_0	73.5 ± 1.200	48.200 ± 5.400	58.000 ± 8.500	43.300 ± 21.200
A	26.3 ± 2.000	50.900 ± 5.400	40.600 ± 8.900	53.700 ± 19.700
R_0	-0.468 ± 0.087	-0.179 ± 0.048	-0.118 ± 0.070	-0.109 ± 0.088
R-Square	0.976	0.974	0.973	0.910

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