Electronic Supporting Information

Effect of Secondary Organic Aerosol from Isoprene-Derived Hydroxyhydroperoxides on the Expression of Oxidative Stress Response Genes in Human Bronchial Epithelial Cells

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Generation and Chemical Characterization of Isoprene-Derived SOA and ISOPOOH-Derived SOA

Isoprene-derived SOA used for cellular exposure were generated by photochemically oxidizing a mixture of acidified sulfate seed aerosol, isoprene, and NO injected into an outdoor smog chamber facility. The generation and chemical characterization of the isoprene-derived SOA used for the resuspension exposures in this study has been described in detail as part of a previous study.[1]

ISOPOOH-derived SOA were generated in a 10-m^3 flexible Teflon indoor chamber at the University of North Carolina, as recently described by Riva et al.[2] Experiments were performed at room temperature (25°C) under dark and low relative humidity (RH) (<5%) conditions. Prior to each experiment, the chamber was flushed for at least 24 hrs to replace at least five volumes of chamber air to ensure particle-free conditions and O_3 and VOC concentrations were below detection limits. Aerosol size distributions were measured continuously using a differential

mobility analyzer (DMA; BMI model 2002) coupled to a mixing condensation particle counter (MCPC; BMI model 1710). The O_3 concentration was monitored over the course of the experiment using a UV photometric analyzer (Model 49P. Thermo-Environmental). Temperature and RH inside the chamber were continuously monitored using an OM-62 temperature RH data logger (OMEGA Engineering, Inc.).

A neutral ammonium sulfate ($(NH_4)_2SO_4$) seed aerosol solution containing 0.06 M $(NH_4)_2SO_4$ was atomized into the chamber until the total aerosol mass concentration in the chamber was ~ 80 μg m^{-3}. Because 90% of ISOPOOH + OH yields IEPOX, neutral $(NH_4)_2SO_4$ seed was used to prevent the reactive uptake of IEPOX and allow the 10% of hydroperoxides to condense onto pre-existing aerosol.[3] Following aerosol injection, 300 ppb of 1,2-ISOPOOH, synthesized in house as described by Riva et al.,[2] was injected into the chamber by passing high-purity N_2 gas through a manifold heated to ~70°C at 2 L min^{-1} for 10 mins then at 5 L min^{-1} for 80 mins. O_3 was introduced into the chamber using an O_3 generator (model L21, Pacific ozone) followed by a continuous injection of tetramethylethylene (TME). The ozonolysyis of TME formed the OH radicals needed for the oxidation of ISOPOOH. A summary of the experimental conditions is given in Table S1. Neutral $(NH_4)_2SO_4$ seed aerosol only experiments served as controls. Following the 1.5-hr injection of TME, aerosol samples were collected onto Teflon membrane filters (47 mm diameter, 1.0 μm pore size; Pall Life Science). Exact mass loadings on the filters were calculated from total air volume sampled and average mass concentrations of aerosol during the sampling period. A density correction of 1.6 g cm^{-3} [4] and 1.25 g cm^{-3} [5] was applied to convert the measured volume concentrations to mass concentrations for the $(NH_4)_2SO_4$ seed and SOA growth.

DTT methods

Description of the DTT assay has been previously described in detail. [6-7] Briefly, DTT and DTNB stock solutions were made by adding DTT standard (powder form) (Sigma-Aldrich) or DTNB standard (Sigma-Aldrich) to an aqueous buffer solution containing 0.05 mol L^{-1} potassium phosphate monobasic-sodium hydroxide (KH_2PO_4, pH 7.4, Fisher Scientific) and 1 mM ethylenediaminetetraacetic acid (EDTA, Sigma Aldrich). A stock solution of 1,4-naphthoquinone (1,4-NQ) was made by dissolving 0.5 mg of 1,4-NQ in 0.5 mL dimethyl sulfoxide (DMSO). Volumes of stock solutions were added to additional aqueous buffer to make working solutions.

ISOPOOH-derived SOA extracts were prepared by sonicating chamber filters in high-purity methanol (LC/MS CHROMASOLV, Sigma-Aldrich). Three separate filters were used (n=3) and extracts were concentrated by drying under a gentle stream of nitrogen. Each ISOPOOH-derived SOA extract was combined with buffer and 0.05 mM DTT working solution and incubated at 37°C for 30 min. Reactions were quenched with the addition of a 1 mM DTNB working solution. A DTT calibration curve was generated by varying DTT volumes with buffer solution. A calibration curve of 1,4-NQ was generated by varying volumes of 1,4-NQ with a set amount of DTT. The consumption of DTT was measured by the absorbance of 5-thio-2-nitrobenzoic acid (TNB), formed by the oxidation of residual DTT with DTNB, at 412 nm using a UV–Visible Spectrophotometer (Hitachi U-3300 dual beam spectrophotometer) [7-8]. Dilution effects were taken into account by correcting the absorbance measurements for sample volume.

ROS generation potential was expressed as DTT activity (nmol of DTT consumed/min/μg sample) and the normalized index of oxidant generation (NIOG) for comparison with previously published studies [7]. As demonstrated by Rattanavaraha et al. [7], an index of oxidant generation (IOG) was calculated according to the following equation [7]:

$$IOG = \frac{Abs_0 - Abs'}{Abs_0} \times \frac{100}{T \times M}$$

where T is reaction time (min), M is sample mass (μg), Abs_0 and Abs' are initial absorbance and absorbance at time T, respectively. The NIOG calculation normalizes activity with respect to a 1,4-NQ standard as follows:

$$NIOG_{sample} = \frac{IOG_{sample}}{IOG_{1,4-NQ}}$$

Table S1. Summary of experimental condition for ISOPOOH oxidation experiments and control experiments.

Experiment	Precursor Concentration (ppb)	Target O_3 (ppm)	TME	Initial Seed (μg m^{-3})	SOA Growth (μg m^{-3})	sampling volume (m^3)	mass collected (μg)
ISOPOOH	300	1.5	yes	76.45	47.76	2.80	133.71
ISOPOOH	300	1.5	yes	76.99	52.44	2.66	139.76
ISOPOOH	300	1.5	yes	77.28	54.34	3.04	165.47
Seed only	-	-	no	587.54	-	0.45	267

Table S2. Gene symbols and full names of 84 oxidative stress-associated genes and housekeeping genes included in RT² Profiler™ PCR Array Human Oxidative Stress Pathway Plus (PAHS-065Y).

#	Gene Symbol	Full Name
1	*ALB*	Albumin
2	*ALOX12*	Arachidonate 12-lipoxygenase
3	*AOX1*	Aldehyde oxidase 1
4	*APOE*	Apolipoprotein E
5	*ATOX1*	ATX1 antioxidant protein 1 homolog (yeast)
6	*BNIP3*	BCL2/adenovirus E1B 19kDa interacting protein 3
7	*CAT*	Catalase
8	*CCL5*	Chemokine (C-C motif) ligand 5
9	*CCS*	Copper chaperone for superoxide dismutase
10	*CYBB*	Cytochrome b-245, beta polypeptide
11	*CYGB*	Cytoglobin
12	*DHCR24*	24-dehydrocholesterol reductase
13	*DUOX1*	Dual oxidase 1
14	*DUOX2*	Dual oxidase 2

15	*DUSP1*	Dual specificity phosphatase 1
16	*EPHX2*	Epoxide hydrolase 2, cytoplasmic
17	*EPX*	Eosinophil peroxidase
18	*FOXM1*	Forkhead box M1
19	*FTH1*	Ferritin, heavy polypeptide 1
20	*GCLC*	Glutamate-cysteine ligase, catalytic subunit
21	*GPX1*	Glutathione peroxidase 1
22	*GPX2*	Glutathione peroxidase 2 (gastrointestinal)
23	*GPX3*	Glutathione peroxidase 3 (plasma)
24	*GPX4*	Glutathione peroxidase 4 (phospholipid hydroperoxidase)
25	*GPX5*	Glutathione peroxidase 5 (epididymal androgen-related protein)
26	*GSR*	Glutathione reductase
27	*GSS*	Glutathione synthetase
28	*GSTP1*	Glutathione S-transferase pi 1
29	*GSTZ1*	Glutathione transferase zeta 1
30	*HSPA1A*	Heat shock 70kDa protein 1A
31	*KRT1*	Keratin 1
32	*LPO*	Lactoperoxidase
33	*MB*	Myoglobin
34	*MBL2*	Mannose-binding lectin (protein C) 2, soluble
35	*MPO*	Myeloperoxidase
36	*MPV17*	MpV17 mitochondrial inner membrane protein
37	*MSRA*	Methionine sulfoxide reductase A
38	*MT3*	Metallothionein 3
39	*NCF1*	Neutrophil cytosolic factor 1
40	*NCF2*	Neutrophil cytosolic factor 2
41	*NOS2*	Nitric oxide synthase 2, inducible
42	*NOX4*	NADPH oxidase 4
43	*NOX5*	NADPH oxidase, EF-hand calcium binding domain 5
44	*NUDT1*	Nudix (nucleoside diphosphate linked moiety X)-type motif 1
45	*PDLIM1*	PDZ and LIM domain 1
46	*PRDX1*	Peroxiredoxin 1
47	*PRDX2*	Peroxiredoxin 2
48	*PRDX3*	Peroxiredoxin 3
49	*PRDX4*	Peroxiredoxin 4
50	*PRDX5*	Peroxiredoxin 5
51	*PRDX6*	Peroxiredoxin 6
52	*PRNP*	Prion protein
53	*PTGS1*	Prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)
54	*PTGS2*	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
55	*RNF7*	Ring finger protein 7
56	*VIMP*	Selenoprotein S

57	*SEPP1*	Selenoprotein P, plasma, 1
58	*SFTPD*	Surfactant protein D
59	*SIRT2*	Sirtuin 2
60	*SOD1*	Superoxide dismutase 1, soluble
61	*SOD2*	Superoxide dismutase 2, mitochondrial
62	*SOD3*	Superoxide dismutase 3, extracellular
63	*SQSTM1*	Sequestosome 1
64	*SRXN1*	Sulfiredoxin 1
65	*TPO*	Thyroid peroxidase
66	*TTN*	Titin
67	*TXNRD2*	Thioredoxin reductase 2
68	*UCP2*	Uncoupling protein 2 (mitochondrial, proton carrier)
69	*AKR1C2*	Aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III)
70	*BAG2*	BCL2-associated athanogene 2
71	*FHL2*	Four and a half LIM domains 2
72	*GCLM*	Glutamate-cysteine ligase, modifier subunit
73	*GLA*	Galactosidase, alpha
74	*HMOX1*	Heme oxygenase (decycling) 1
75	*HSP90AA1*	Heat shock protein 90kDa alpha (cytosolic), class A member 1
76	*LHPP*	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase
77	*NCOA7*	Nuclear receptor coactivator 7
78	*NQO1*	NAD(P)H dehydrogenase, quinone 1
79	*PTGR1*	Prostaglandin reductase 1
80	*SLC7A11*	Solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11
81	*SPINK1*	Serine peptidase inhibitor, Kazal type 1
82	*TRAPPC6A*	Trafficking protein particle complex 6A
83	*TXN*	Thioredoxin
84	*TXNRD1*	Thioredoxin reductase 1
Housekeeping Genes	*ACTB*	Actin, beta
	B2M	Beta-2-microglobulin
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
	HPRT1	Hypoxanthine phosphoribosyltransferase 1
	RPLP0	Ribosomal protein, large, P0

Table S3. List of genes identified with significant expression fold-changes ($p<0.05$) upon exposure to ISOPOOH-derived SOA constituents. False Discovery Rate (FDR) adjusted p-value: 0.05/84=0.0005.

Gene Symbol	Fold Regulation	p-value	NRF2-associated Genes	<FDR
AKR1C2	20.21	0.000003		*
ALOX12	1.83	0.189227		
ATOX1	1.57	0.000203		*
BAG2	2.48	0.000181		*
BNIP3	1.50	0.015483		
DUOX2	1.69	0.013911		
DUSP1	10.01	0.000012		*
EPX	2.02	0.002065		
FHL2	1.93	0.000212		*
FTH1	6.71	0.000019	+	*
GCLC	5.45	0.000627	+	
GCLM	14.12	0.000008	+	*
GLA	4.28	0.000019		*
GPX2	4.99	0.004839	+	
GPX3	1.88	0.002074		
GSR	2.66	0.002702	+	
GSTP1	1.70	0.000867	+	
HMOX1	123.64	0.00002	+	*
HSP90AA1	3.01	0.000014		*
HSPA1A	14.03	0.000441		*
NCF1	2.85	0.04451		
NQO1	6.02	0.000002	+	*
PRDX1	3.82	0.000009	+	*
PRDX4	1.56	0.009166		
PRDX6	2.15	0.000045		*
PRNP	1.85	0.000066		*
PTGS1	1.87	0.011846		
PTGS2	3.02	0.001327		
RNF7	1.87	0.002062		
SLC7A11	9.54	0.000008		*
SOD1	2.50	0.000203	+	*
SOD2	3.05	0.000002	+	*
SOD3	1.96	0.001269	+	
SQSTM1	8.67	0.00001	+	*
SRXN1	8.52	0.000000		*
TXN	3.48	0.000078	+	*
TXNRD1	8.71	0.001145	+	

UCP2	2.44	0.01224		
VIMP	3.60	0.000002		*
CAT	-1.78	0.005005	+	
MSRA	-2.13	0.000138		*

Table S4. List of genes identified with significant expression fold-changes ($p<0.05$) upon exposure to ISOPOOH-derived SOA constituents. False Discovery Rate (FDR) adjusted p-value: 0.05/84=0.0005.

Gene Name	Fold Regulation	p-value	NRF2-associated Genes	<FDR
AKR1C2	2.20	0.013377		
ATOX1	1.56	0.024189		
BAG2	2.00	0.00763		
CAT	1.58	0.006833	+	
DHCR24	1.58	0.001557		
FTH1	2.28	0.008796	+	
GCLC	2.59	0.003719	+	
GCLM	2.89	0.001271	+	
GLA	1.74	0.034903		
GPX1	1.54	0.014517		
GPX2	5.95	0.000725	+	
GPX3	1.87	0.000538		
GSR	1.69	0.027743	+	
GSTP1	1.63	0.041316	+	
HMOX1	2.00	0.016293	+	
HSP90AA1	2.09	0.000988		
HSPA1A	1.59	0.001239		
NQO1	4.96	0.003367	+	
PRDX1	2.19	0.007017	+	
PRDX3	1.61	0.047334		
PRDX6	1.50	0.023451		
PTGS1	2.02	0.025923		
RNF7	1.78	0.040105		
SIRT2	1.64	0.000194		*
SLC7A11	3.43	0.000005		*
SQSTM1	2.36	0.000085	+	*
SRXN1	2.72	0.000015		*
TXN	2.23	0.016836	+	
TXNRD1	4.88	0.003985	+	
TXNRD2	1.79	0.000992		
VIMP	1.56	0.001192		
DUOX1	-2.01	0.011647		

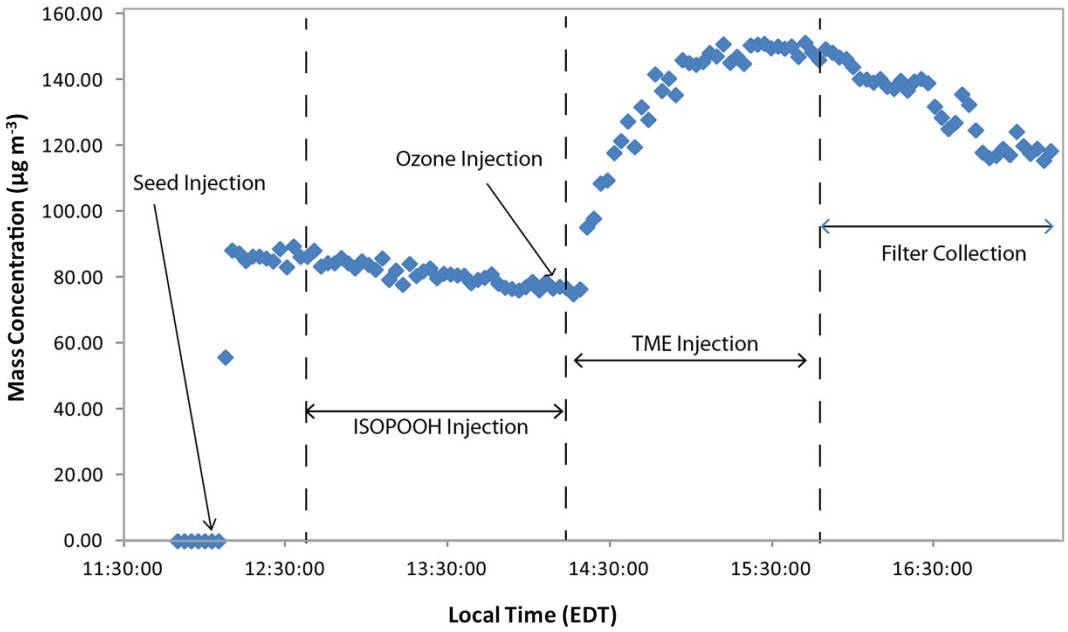

Fig. S1. Time profile of measured aerosol mass concentrations during ISOPOOH oxidation experiments.

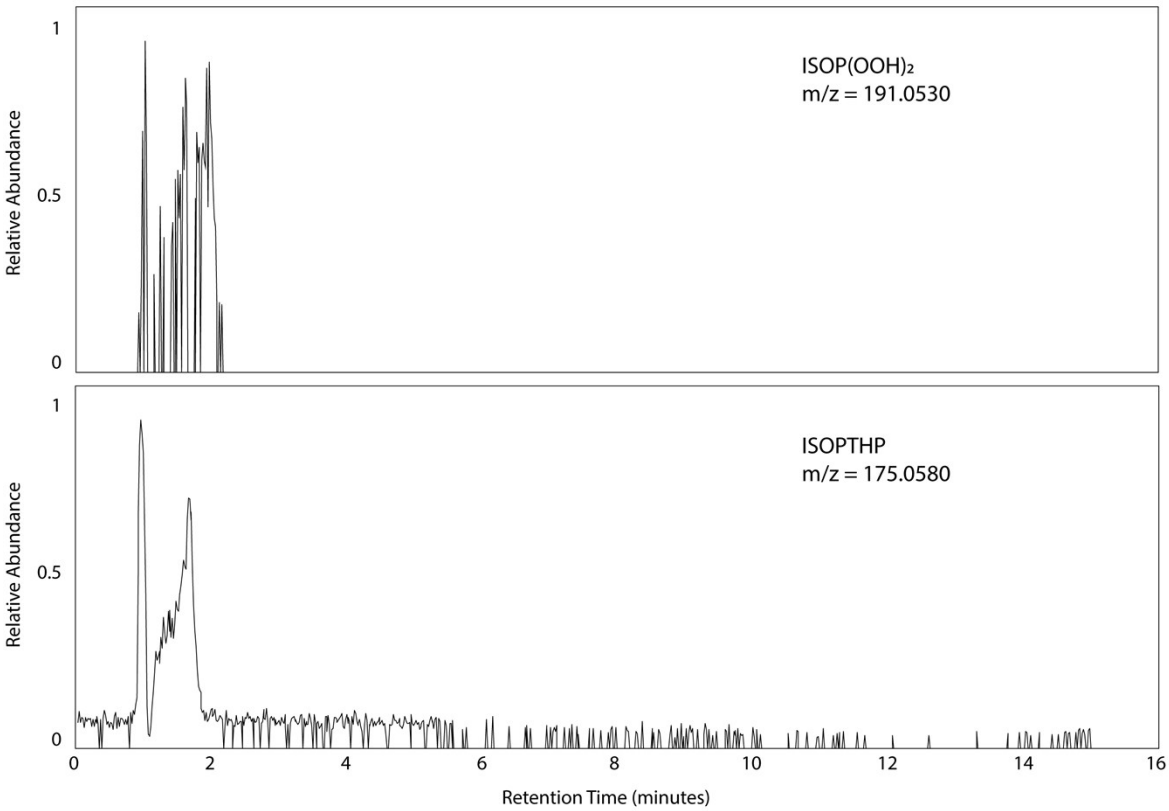

Fig. S2. UPLC/(+)ESI-HR-QTOFMS extracted ion chromatograms (EICs) at *m/z* 191.0530 and 175.0580 corresponding to $ISOP(OOH)_2$ and ISOPTHP SOA constituents, respectively. Filter

extracts were analyzed in positive ion mode which affects sensitivity but still shows the presence of hydroperoxides while not showing its large abundance.

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