Supplementary Information

Toxicological Responses of α-Pinene-Derived Secondary Organic Aerosol and its Molecular Tracers in Human Lung Cell Lines

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	24 Hour Treatment	48 Hour Treatment
IC50	912.0	230.7
logIC50	2.960	2.363

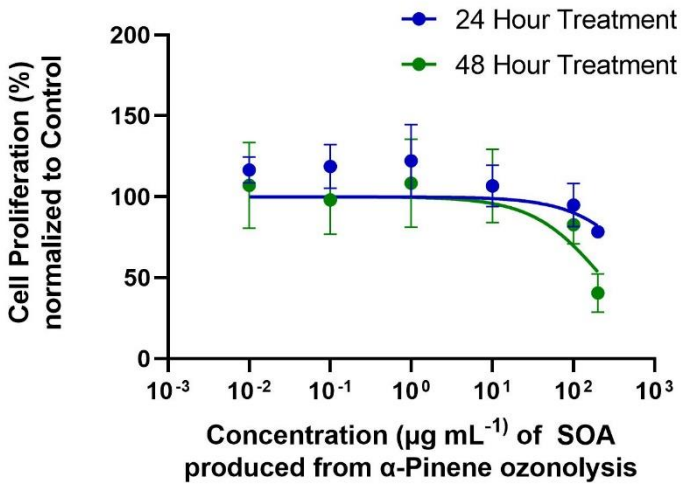


Figure S1. Inhibitory concentration-50 (IC_{50}) of SOA produced from a-pinene ozonolysis was found to be 912 and 230 µg mL^{-1} for 48 and 24 hours, respectively. $logIC_{50}$ was found to be 2.96 and 2.363 at the two different treatment time points in BEAS-2B cells. IC_{50} was calculated using GraphPad Prism (Version 8.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

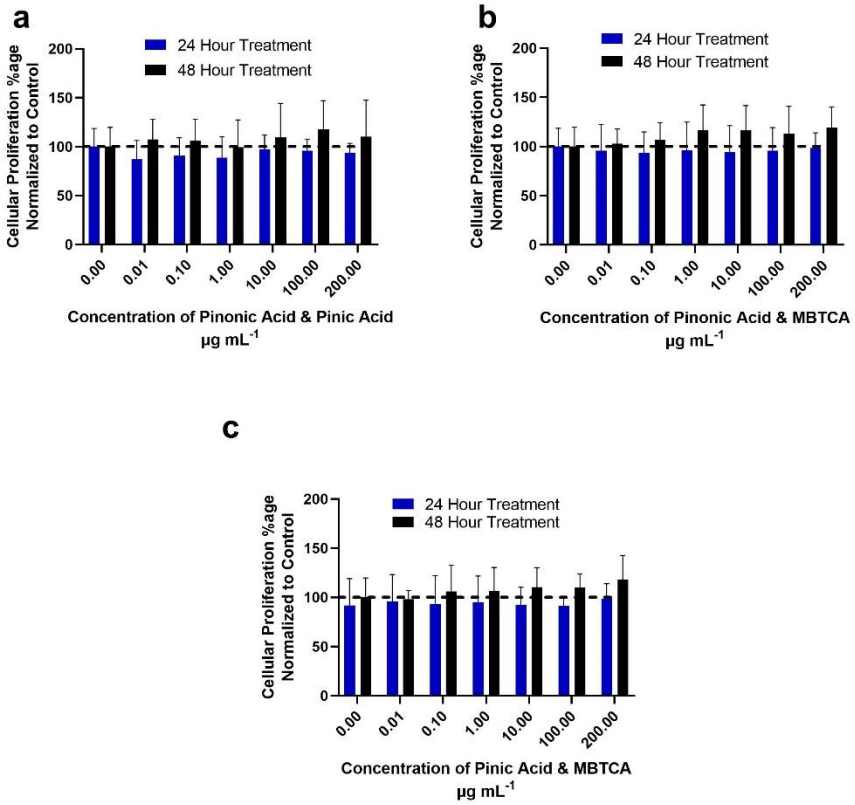


Figure S2. The cellular proliferation (in %) for BEAS-2B cells treated with equimolar mixtures of two selected α-pinene SOA molecular tracers in increasing concentration for 24 (blue bars) and 48 (black bars) hours: (a) pinonic and pinic acids; (b) pinonic acid and MBTCA; and (c) pinic acid and MBTCA.

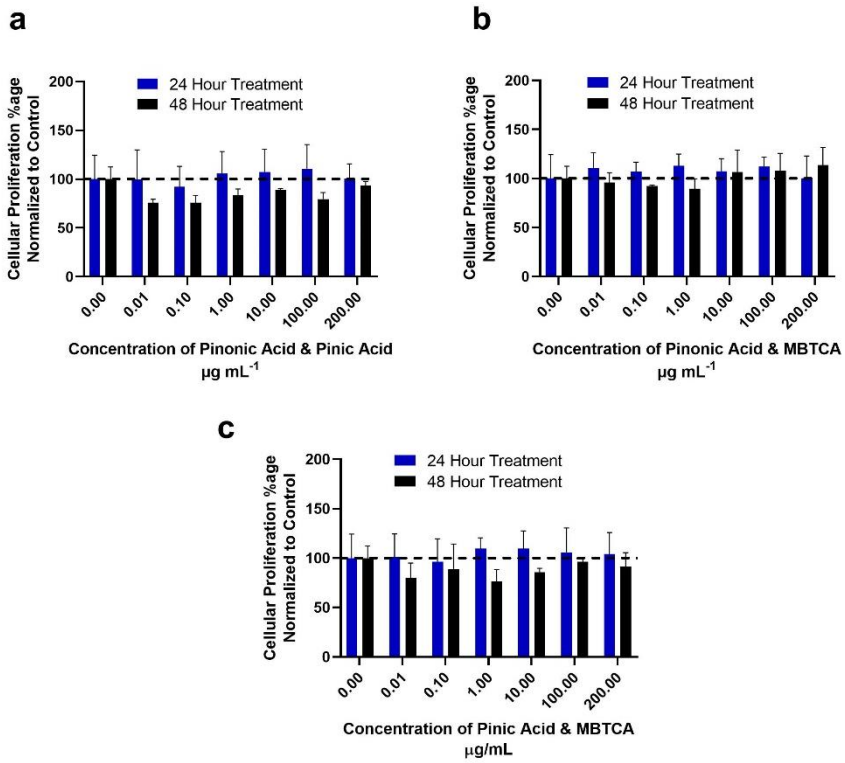


Figure S3. The cellular proliferation (in %) of A549 cell lines when treated with equimolar mixtures of two selected α-pinene SOA molecular tracers in increasing concentration for 24 (blue bars) and 48 (black bars) hours: (a) pinonic and pinic acids; (b) pinonic acid and MBTCA; and (c) pinic acid and MBTCA

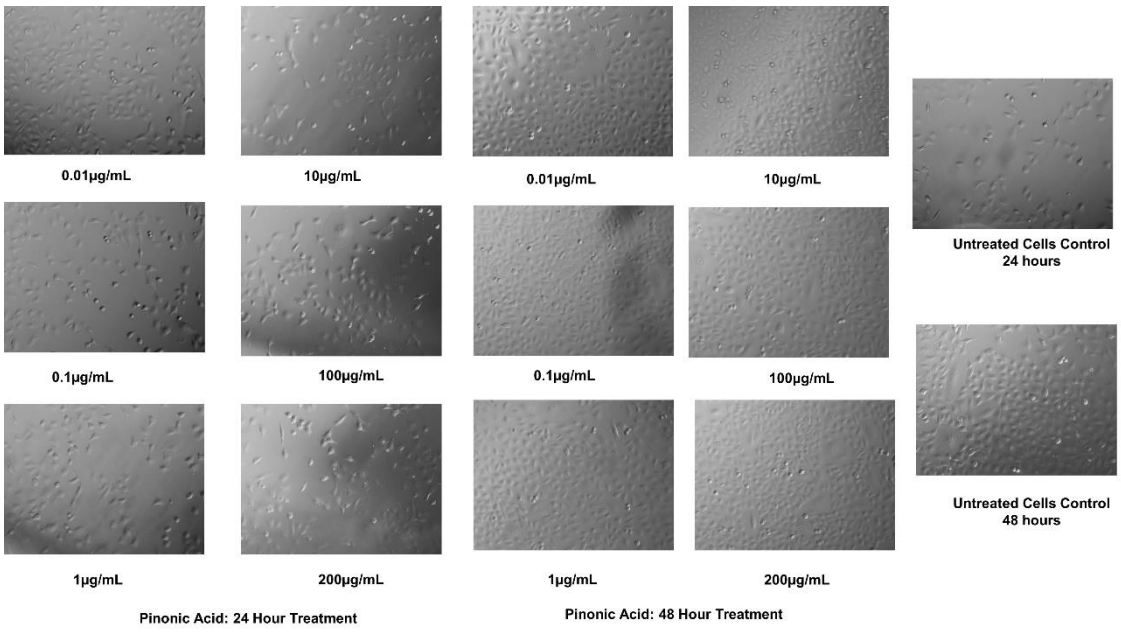


Figure S4. Inverted phase microscopy (Nikon Eclipse T1-SAM, Japan) images of A549 cells treated with increasing concentrations of pinonic acid at x100 magnification. The micrographs are scaled at 600 µm x 800 µm

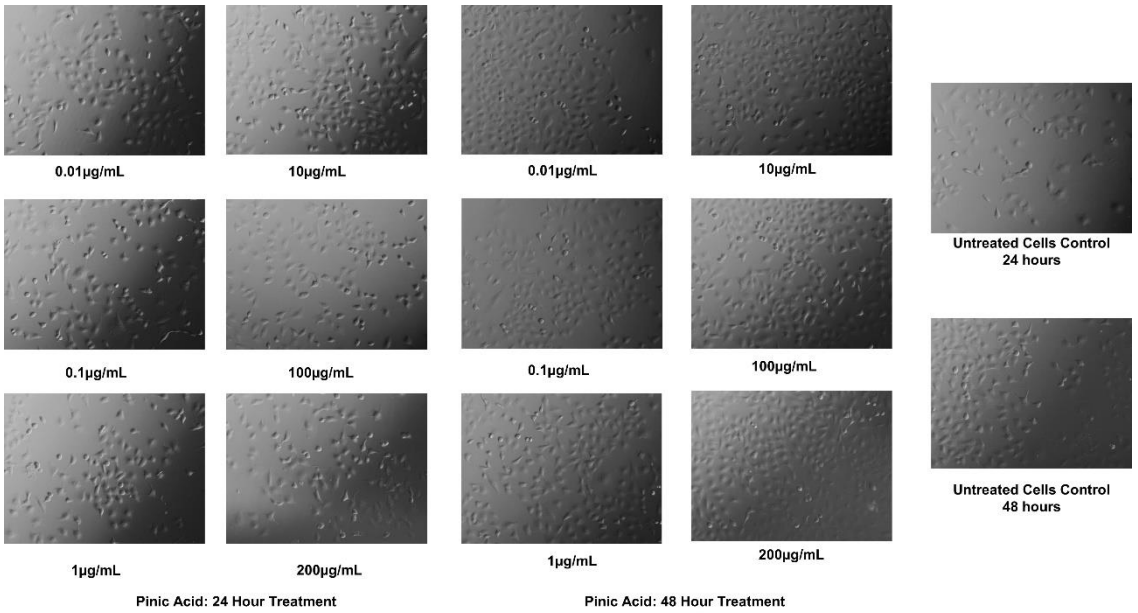


Figure S5. Inverted phase microscopy (Nikon Eclipse T1-SAM, Japan) images of A549 cells treated with increasing concentrations of pinic acid at x100 magnification. The micrographs are scaled at 600 µm x 800 µm

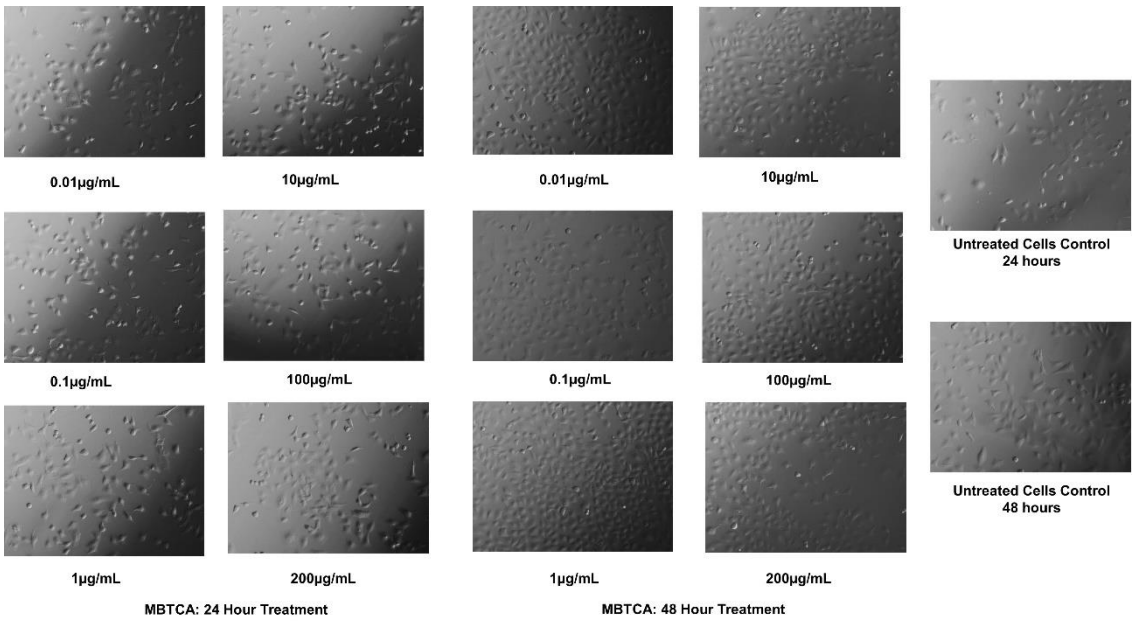


Figure S6. Inverted phase microscopy (Nikon Eclipse T1-SAM, Japan) images of A549 cells treated with increasing concentrations of MBTCA at x100 magnification. The micrographs are scaled at 600 µm x 800 µm

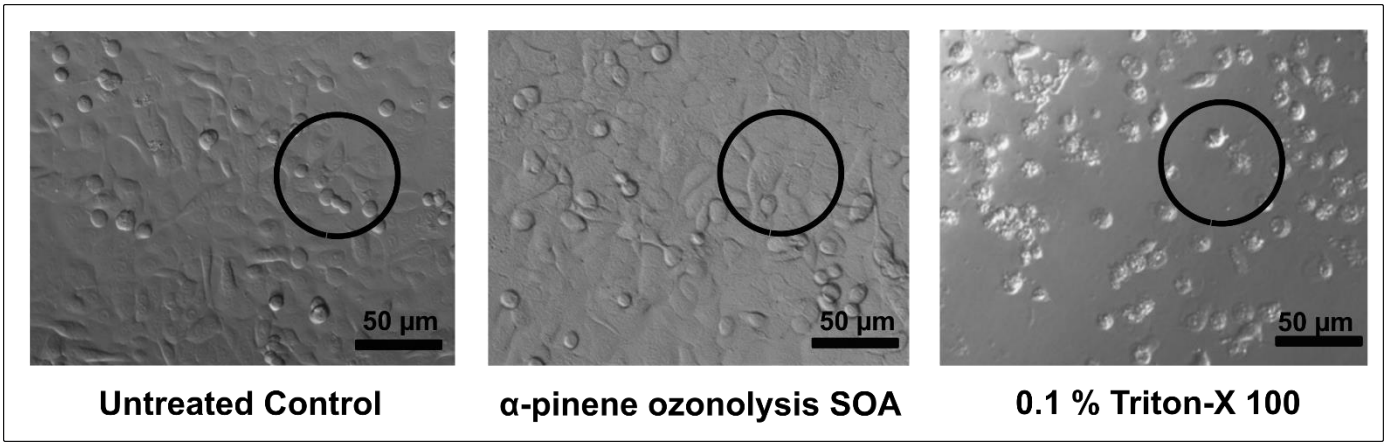


Figure S7: The phase contrast microscopy micrograph of A549 cells at 24 hours. The micrographs depict untreated control cells have similar morphology to α-pinene ozonolysis generated SOA treated A549. The positive control for cytotoxicity (Triton-X 100 treated cells) show visible signs of cellular degradation.

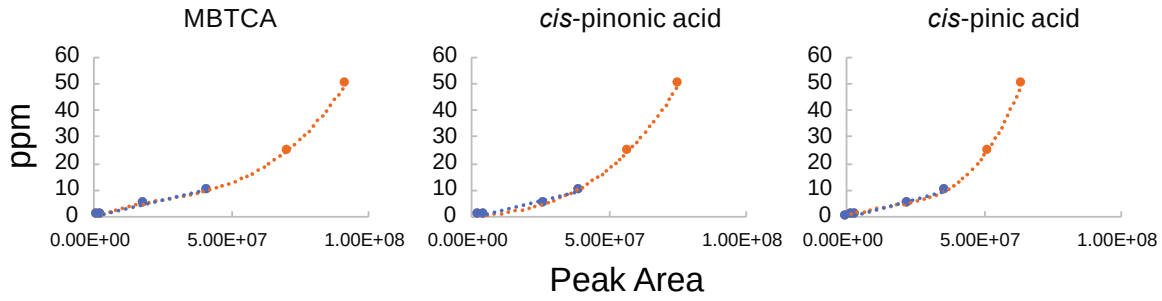


Figure S8. Six-point calibration curves for MBTCA, *cis*-pinonic acid and *cis*-pinic acid generated by RPLC/ESI-HR-QTOFMS negative ion mode analysis. Note that the data points within the linear range and the linear fit are shown in blue while the non-linear data points and the polynomial fit are shown in orange.

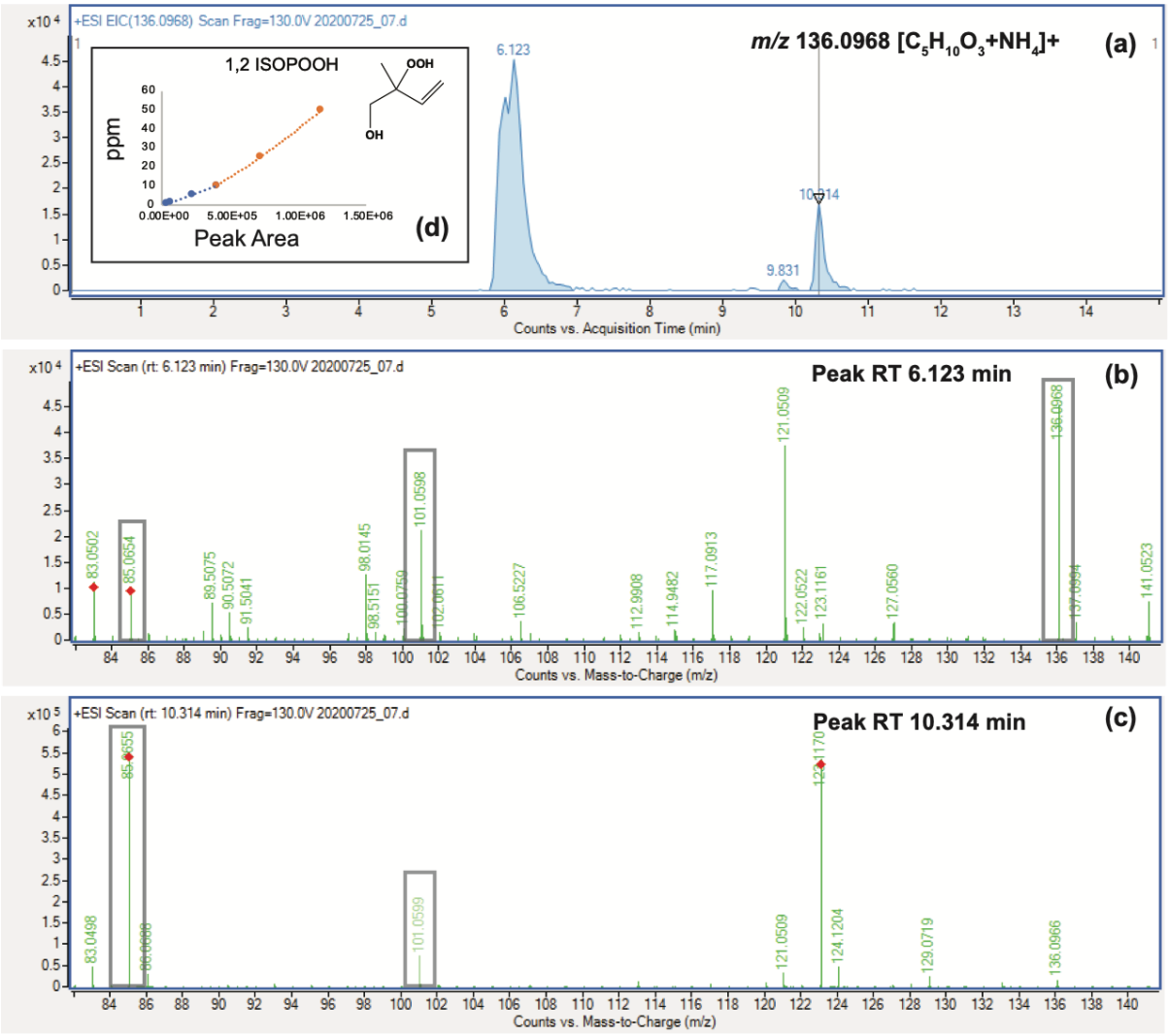
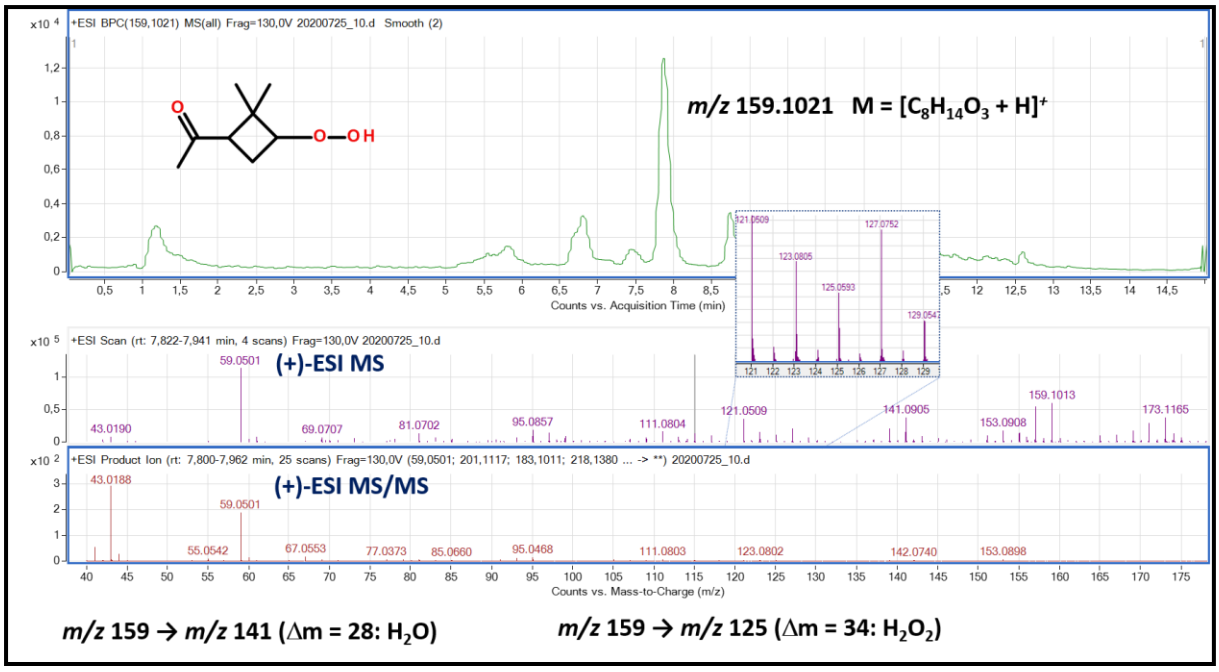
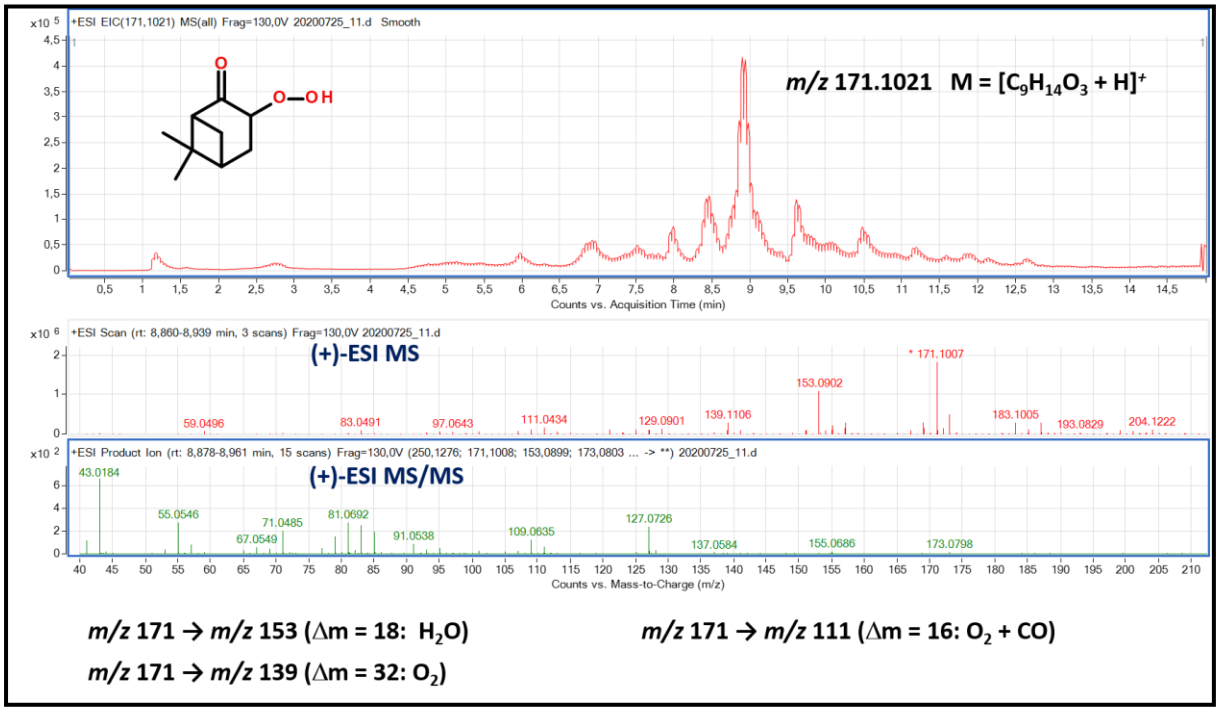


Figure S9. RPLC/ESI-HR-QTOFMS positive ion mode analysis of the 1,2-ISOPOOH standard demonstrates how organic hydroperoxides are detected and break down during our ESI-HR-Q-TOFMS analyses: (a) the extracted ion chromatogram (EIC) of $[M+NH_4]^+$ ion at mass-to-charge (*m/z*) 136 for 1,2-ISOPOOH; (b) the mass spectrum for chromatographic peak with the retention time (RT) of 6.123 min; (c) the mass spectrum for chromatographic peak with the RT of 10.314 min; (d) the six-point calibration curve for 1,2-ISOPOOH in the concentration range between 0.5 and 50 ppm. Consistent with our previous study, the $[M+H]^+$ molecular ion was not seen in the full MS scan given the -OOH group being a unfavorable protonation site within ESI. Instead, the dehydrated molecular ion $[M + H – H_2O]^+$ (*m/z* 101) was observed. The presence of the $[M+NH_4]^+$ ion resulted from background NH_4^+ contamination in our system. Therefore, the neutral loss of 35 u (i.e., NH_3 + H_2O loss from the $[M+NH_4]^+$ ion) may also contribute to the observed fragment ion at *m/z* 101. The fragment ion observed at *m/z* 85 can be explained by the neutral loss of 51 u (i.e., NH_3 + H_2O_2 loss of the $[M+NH_4]^+$ ion).

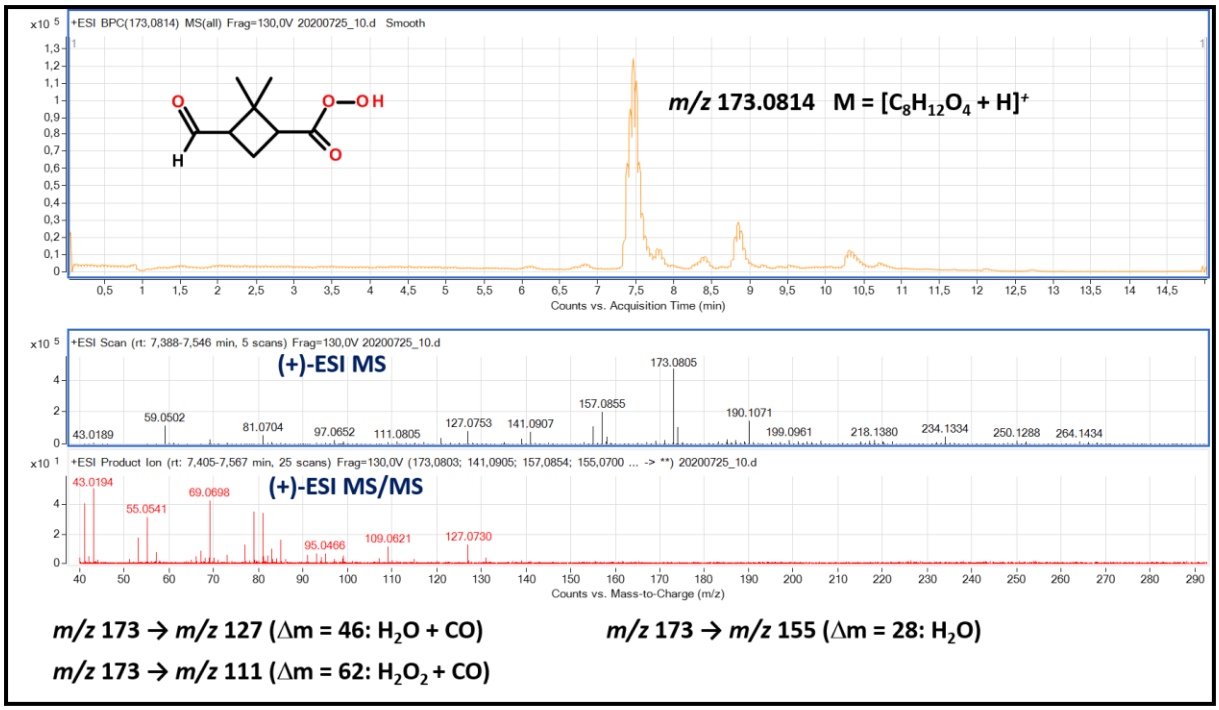
A)


+ESI BPC(159,1021) MS(all) Frag=130,0V 20200725_10.d Smooth (2)
m/z 159.1021 M = [C8H14O3 + H]+
Counts vs. Acquisition Time (min)
+ESI Scan (rt: 7,822-7,941 min, 4 scans) Frag=130,0V 20200725_10.d
(+)-ESI MS
+ESI Product Ion (rt: 7,800-7,962 min, 25 scans) Frag=130,0V (59,0501; 201,1117; 183,1011; 218,1380 ... -> **) 20200725_10.d
(+)-ESI MS/MS
Counts vs. Mass-to-Charge (m/z)
m/z 159 → m/z 141 (Δm = 28: H2O)
m/z 159 → m/z 125 (Δm = 34: H2O2)

B)


+ESI EIC(171,1021) MS(all) Frag=130,0V 20200725_11.d Smooth
m/z 171.1021 M = [C9H14O3 + H]+
Counts vs. Acquisition Time (min)
+ESI Scan (rt: 8,860-8,939 min, 3 scans) Frag=130,0V 20200725_11.d
(+)-ESI MS
+ESI Product Ion (rt: 8,878-8,961 min, 15 scans) Frag=130,0V (250,1276; 171,1008; 153,0899; 173,0803 ... -> **) 20200725_11.d
(+)-ESI MS/MS
Counts vs. Mass-to-Charge (m/z)
m/z 171 → m/z 153 (Δm = 18: H2O)
m/z 171 → m/z 111 (Δm = 16: O2 + CO)
m/z 171 → m/z 139 (Δm = 32: O2)

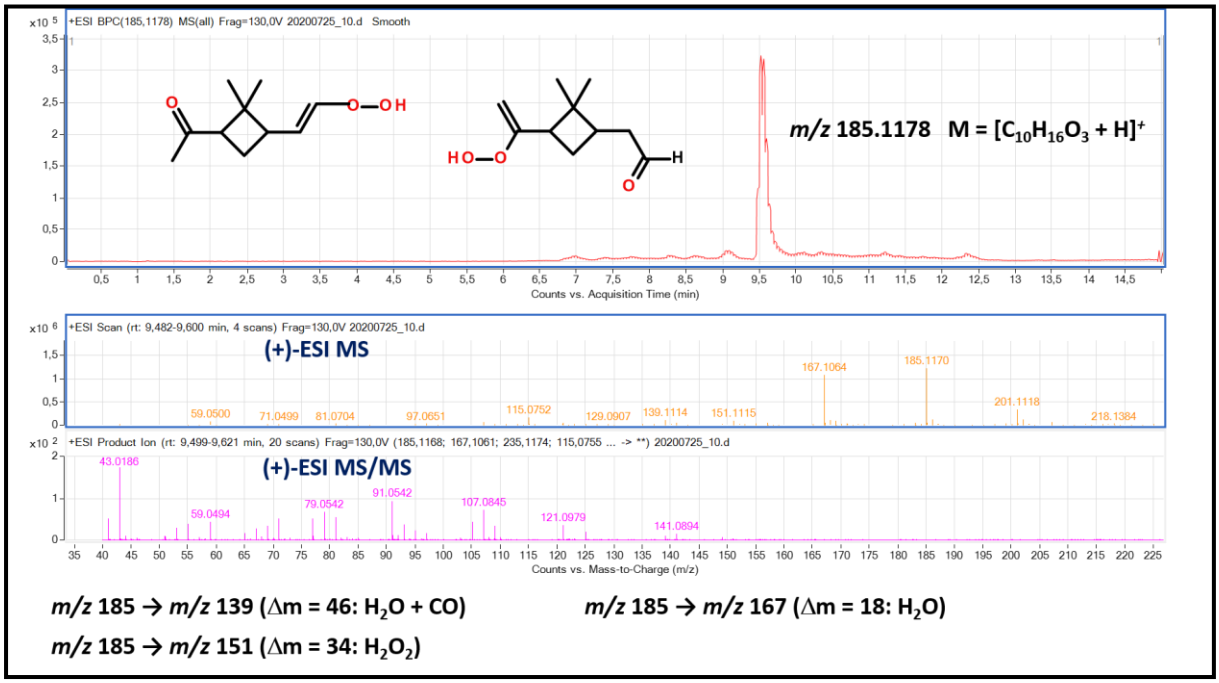
C)



m/z* 173 → *m/z* 127 (Δm = 46: H_2O + CO)** * *m/z* 173 → *m/z* 155 (Δm = 28: H_2O)**

***m/z* 173 → *m/z* 111 (Δm = 62: H_2O_2 + CO)**

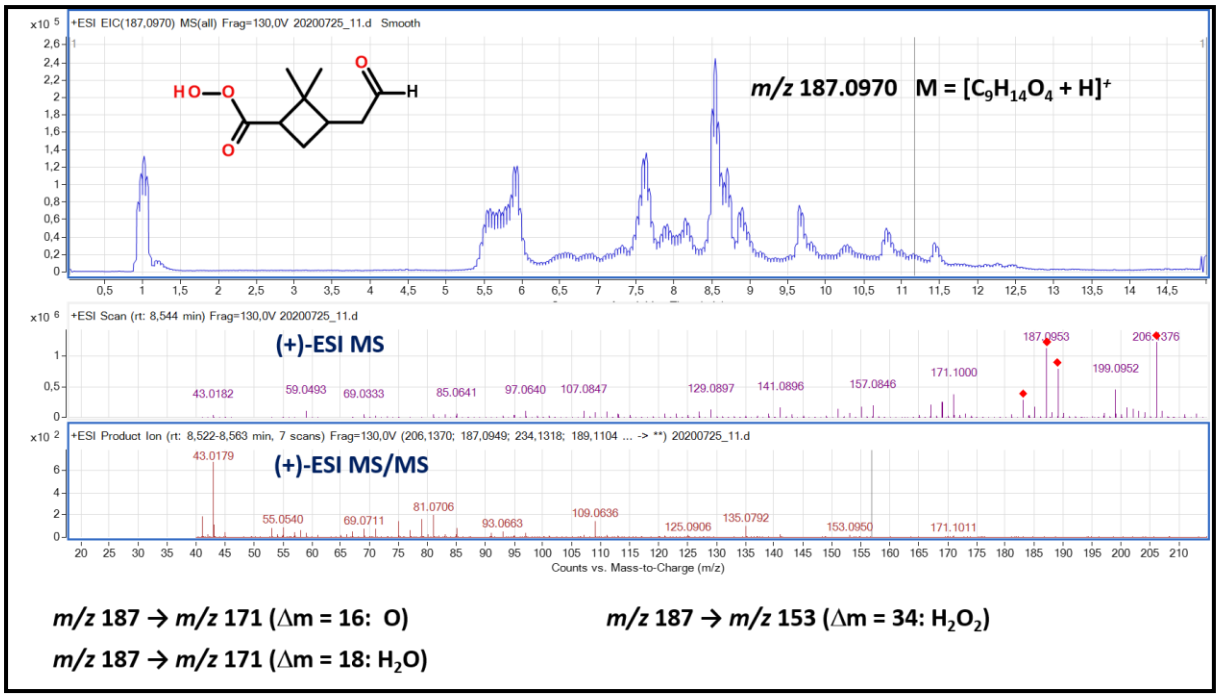
D)



m/z* 185 → *m/z* 139 (Δm = 46: H_2O + CO)** * *m/z* 185 → *m/z* 167 (Δm = 18: H_2O)**

***m/z* 185 → *m/z* 151 (Δm = 34: H_2O_2)**

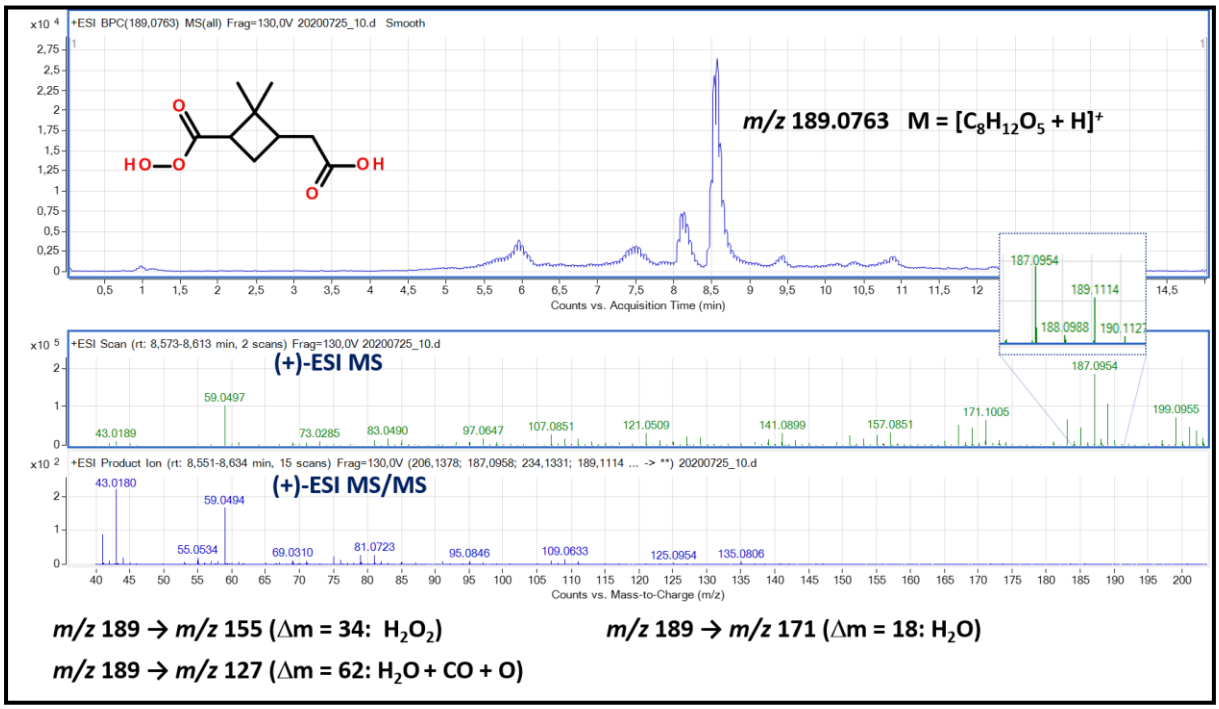
E)



***m/z* 187 → *m/z* 171 (Δm = 16: O)** ***m/z* 187 → *m/z* 153 (Δm = 34: H_2O_2)**

***m/z* 187 → *m/z* 171 (Δm = 18: H_2O)**

F)



***m/z* 189 → *m/z* 155 (Δm = 34: H_2O_2)** ***m/z* 189 → *m/z* 171 (Δm = 18: H_2O)**

***m/z* 189 → *m/z* 127 (Δm = 62: H_2O + CO + O)**

G)

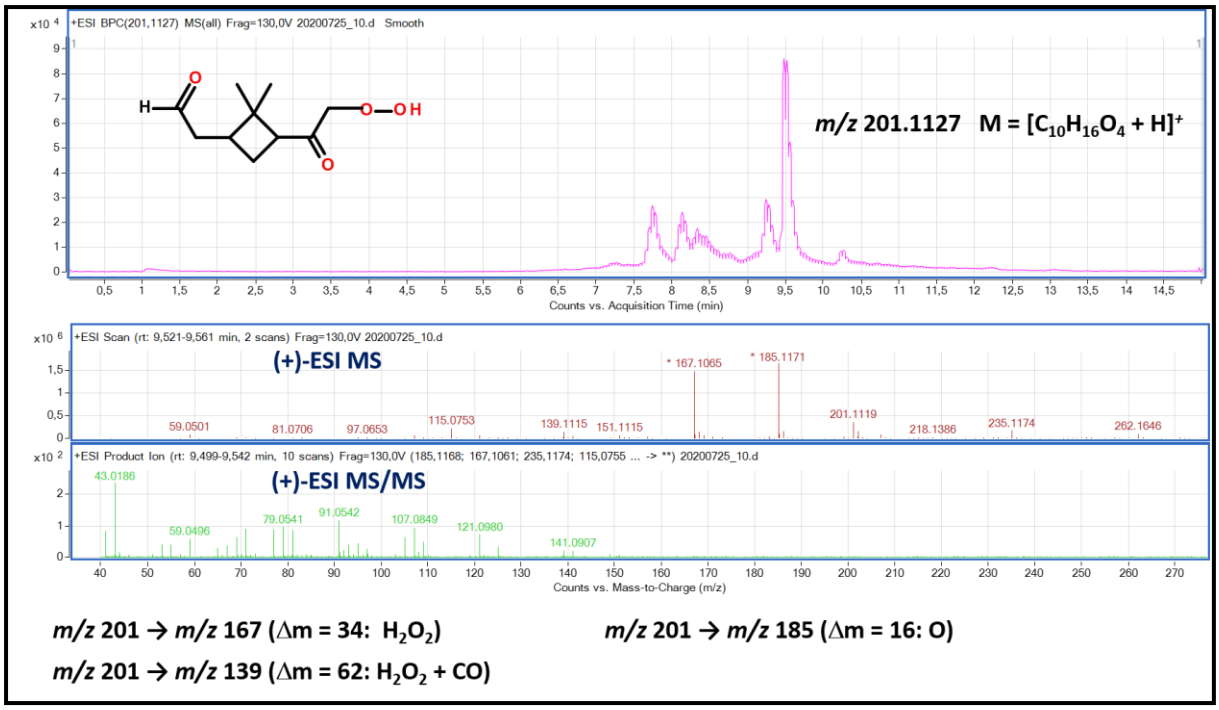


Figure S10. RPLC/ESI-HR-QTOFMS positive ion mode analysis data of the PAM-generated SOA from α-pinene ozonolysis revealed structures of organic hydroperoxides present during the exposures. Seven of these structures were tentatively identified through the accurate mass measurements and tandem mass spectra (MS/MS) spectra (figures A-G). Note that the first panel of each figure shows the extracted ion chromatogram for the $[M + H]^+$ ion associated with each organic hydroperoxide. The second panel is the positive electrospray mass spectrum for the $[M + H]^+$ ion and the third panel are the fragment ion mass spectrum (MS/MS) for the $[M + H]^+$ ion. .

Table S1. Comparison of previous toxicological studies associated with α-pinene SOA with the current study. Note that the fresh α-pinene SOA is generated through ozonolysis alone, while aged SOA is α-pinene SOA heterogeneously reacted with OH radicals.

α-Pinene SOA system studied	Model used	Response Type Studied	Toxicological End Point	Conclusions	Consistent with current Study	Reference
Pinonic Acid Pinic Acid MBTCA Fresh α-pinene	A549 BEAS-2B	Cytotoxicity Oxidative Stress 24 & 48 hours 0.01, 0.1, 1, 10, 100, 200ug/mL	MTT Assay Calcein-AM/PI Staining H_2DCFDA	α-pinene SOA at 200ug/mL induced high time-dependent cell death due to increased ROS	N/A	This Study
Fresh α-pinene	BEAS-2B	Lung Inflammatory response	IL-8 and Cytotoxicity	No significant change in IL-8 No toxicity	No	1
α-pinene+ NOx+ SOx	Macrophages	Lung Macrophage response	Cytotoxicty IL-6, IL-8, and TNF-a Phagocytic Activity Wound Heal Assay	Decreased phagocytic activity	N/A	2
NOx+ NH_3 α -pinene; SOx + NOx+ NH_3 α -pinene;	Apo E−/−) mice	Short term Cardiopulmonary response 7 days 250–300 mg/m3	gene expression of TBARS HO-1, ET-1 MMP-9	SO_2: Increased expression HO-1, MMP-9, and ET-1 No SO_2: Decreased expression	N/A	3
NOx+ α -pinene; SOx + NOx+ α -pinene;	Sprague-Dawley rats ApoE−/− mice	Short term Cardiopulmonary response 200 μg m^{-3} 7 days	gene expression of TBARS HO-1, ET-1 MMP-9	Revealed limited biological response	N/A	4
NOx+ α-pinene Fresh and Aged α-pinene	DTT acellular	Oxidative potential response	Oxidative stress	Negligible or no response	Yes	5
NOx+ aged α-pinene Aged and Fresh α-pinene	Murine alveolar macrophages 0.1-10ug	Pro-inflammatory response and oxidative stress response	ROS/RNS production and levels of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)	Similar inflammatory response to all three conditions Negligible or no response	Yes	6
Fresh & aged α-Pinene SOA	A549 cells ALI exposure system 0-14μg	Cellular Viability	LDH Assay	More decreased viability in aged than fresh sample	Yes	7
Fresh, aged & NOx α-Pinene SOA	A549 cells ALI exposure system	Cellular Viability Oxidative stress Superoxide	WST-1 assay H_2DCFDA ROS-Superoxide Detection Assay	No difference between NOx and NOx free system	Yes	8
Fresh α-Pinene SOA	BEAS-2B Cell Lines U937 Cell Lines	Cellular Viability Gene Expression (RT-PCR) 0.1, 1, or 10 μg/mL 3, 6 and 24 hours	GAPDH Ratio to: HMOX IL-8 ARE Activity	No significant change in viability Slightly increased HMOX activity IL-8 increase at 3 hours which is normalized at 24 hours No change in ARE activity	Yes-consistent results at similar dosage	9

TBARS: thiobarbituric acid reactive substance, (HO)-1: heme-oxygenase, (ET)-1: endothelin, (MMP)-9: matrix metalloproteinase, (TNF-α): tumor necrosis factor-α, (IL-6): interleukin-6, (IL-8): interleukin-8 and antioxidant response element (ARE)

References

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5. Tuet, W. Y.; Chen, Y.; Xu, L.; Fok, S.; Gao, D.; Weber, R. J.; Ng, N. L., Chemical oxidative potential of secondary organic aerosol (SOA) generated from the photooxidation of biogenic and anthropogenic volatile organic compounds. *Atmospheric Chemistry & Physics* **2017,** *17*, 839.
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