Toxicant Formation in Dabbing: The Terpene Story

Supporting Information

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Experimental Setups

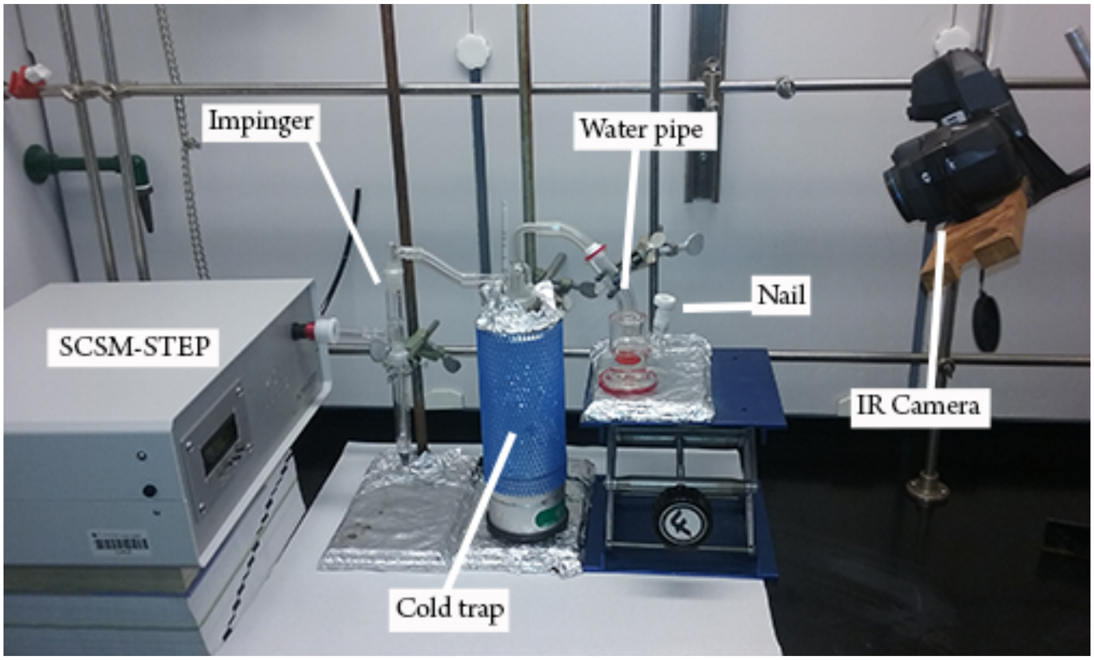

Figure S1: The experimental setup used in all NMR experiments. Conditions were exactly replicated in all experiments, using the same height in the lab jack, camera and SCSM. Tubing between the water pipe, cold trap, impinger and SCSM was the same length every experiment. Photograph courtesy of J.M.A. Copyright 2017.

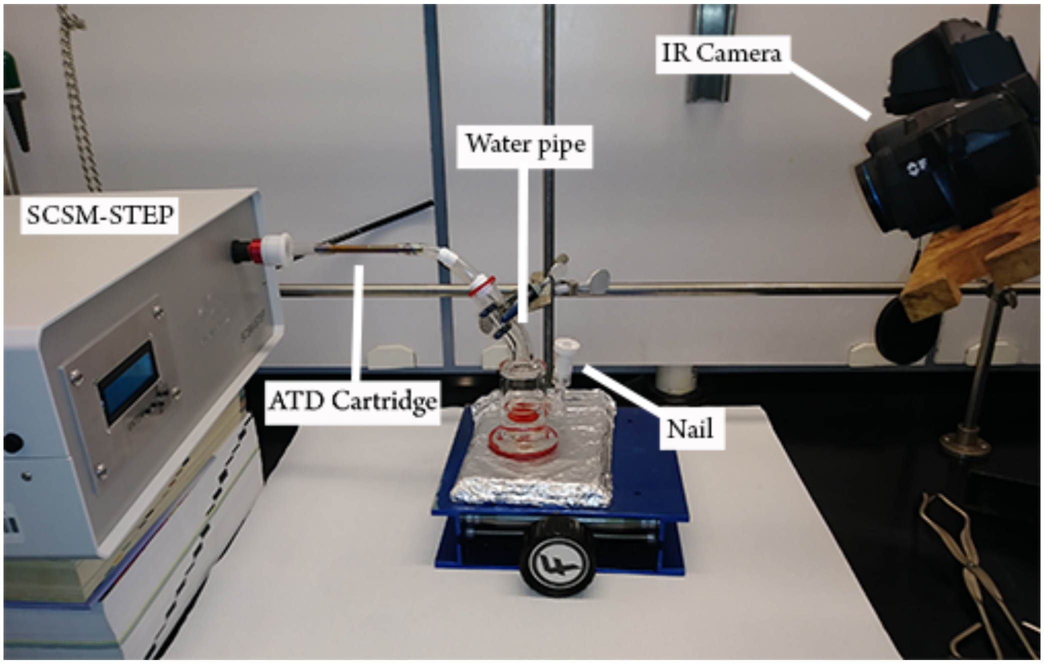

Figure S2: Experimental setup used in ATD cartridge sample collection. Photograph courtesy of J.M.A. Copyright 2017.

ATD-GC-MS Conditions

A sample of one 338 mL in 10s draw was collected onto an adsorption/thermal desorption (ATD) cartridge as showing in Figure S2. The ATD cartridge contains 100 mg of 35/60 mesh Tenax TA and 200 mg of 60/80 mesh Carbograph 1 TD (Camsco Inc., Houston, TX). Each ATD sample cartridge was thermally desorbed using a TurboMatrix 650 ATD unit (PerkinElmer, Waltham, MA). Each cartridge was automatically added with 20 ng of fluorobenzene, 18.6 ng of toluene-d8, 21.7 ng of 4-bromofluorobenzene, and 20.3 ng of 1,2-dichlorobenzene-d4 as the internal standards. The ATD unit thermally desorbed each ATD cartridge for 10 min at 285 °C with a He desorption flow of 40 mL/min and split flow of 10 mL/min, the desorption stream was trapped at –10 °C on an intermediate "Tenax® trap". Thermal desorption of the intermediate trap occurred at 295 °C and 25 psi constant pressure He, on a split flow of 12 mL/min for 4 min. Through a 1 m long and 0.25 mm i.d. deactivated fused silica transfer line (235 °C), the un-split portion of the stream was passed on to a 30 m length, 0.25 mm i.d., and 1.4 µm film thickness Rxi-624Sil MS (Restek Inc., Bellefonte, PA) capillary GC column mounted in an Agilent (Santa Clara, CA) 7890A GC. This was interfaced to an Agilent 5975C MS operated in electron impact ionization mode. The GC oven temperature was hold at 40 °C for 2 min, programing to 100 °C at 10 °C /min, then programing to 280 °C at 12 °C/min , and then at 15 °C/min to 220 °C. The MS scan range was 34 to 300 amu. The electron multiplier voltage was 1525 V.

Temperature Measurements

Limonene, linalool and Fire OG terpenes were only tested once each at the highest TR chosen for the NMR experiments. Temperatures used in each of these experiments are shown in Table S1.

	Limonene		Linalool		Fire OG	
	T_i	T_f	T_i	T_f	T_i	T_f
Hit 1	549	498	558	503	565	510
Hit 2	550	503	557	500	556	500
Hit 3	550	500	552	502	552	501
Hit 4	547	502	556	501	559	500
Hit 5	549	500	556	506	555	503
Average	549	501	556	502	557	503
St. Dev.	1.22	1.95	2.28	2.30	4.93	4.21

Table S1: Temperatures in °C used for each individual hit, shown with their averages and standard deviations.

The myrcene study consisted of dabbing it at four different TRs to capture the full array of temperatures used by those that practice dabbing.

Myrcene experiments for each TR were done in triplicate. The five T_i and T_f for each dab taken in each experiment were averaged. Data for all temperatures used are shown in Tables S3 though S6. The average of the standard deviations of each T_i and T_f for all 12 experiments is 2.4 °C, indicating the experiments were done consistently. The average of the T_i and T_f of the four TR replicates was taken and a median temperature (T_m) was calculated. Table S2 shows the total average T_i and T_f for each TR. M1- M12 are the abbreviations used for each individual myrcene experiment (four TRs x three replicates).

T_m	T_i	T_f
526	551 ± 0.8	500 ± 2.4
455	477 ± 0.1	434 ± 0.8
403	421 ± 0.2	386 ± 0.8
322	336 ± 1.4	309 ± 2.7

Table S2: T_m, T_i, and T_f values for each TR in °C.

	T_m = 526 °C					
	M1		M2		M3	
	T_i	T_f	T_i	T_f	T_i	T_f
Hit 1	552	500	550	501	555	496
Hit 2	549	500	555	503	550	498
Hit 3	550	507	550	495	554	500
Hit 4	551	504	552	499	552	501
Hit 5	552	503	550	494	551	499
Average	551	503	551	498	552	499
St. Dev.	1.30	2.95	2.19	3.85	2.07	1.92

Table S3: Temperature values used for each myrcene hit at T_m = 526 °C. The average T_i and T_f values for M1, M2 and M3 were themselves averaged to get the total average T_i and T_f for the T_m = 526 °C TR, shown in Table S2.

	T_m = 455 °C					
	M4		M5		M6	
	T_i	T_f	T_i	T_f	T_i	T_f
Hit 1	477	436	477	434	476	430
Hit 2	476	432	476	432	476	432
Hit 3	475	433	477	436	478	434
Hit 4	478	437	476	434	475	434
Hit 5	477	434	478	439	478	437
Average	477	434	477	435	477	433
St. Dev.	1.14	2.07	0.84	2.65	1.34	2.61

Table S4: Temperature values used for each myrcene hit at T_m = 455 °C. The average T_i and T_f values for M4, M5 and M6 were themselves averaged to get the total average T_i and T_f for the T_m = 455 °C TR, shown in Table S2.

	T_m = 405 °C					
	M7		M8		M9	
	T_i	T_f	T_i	T_f	T_i	T_f
Hit 1	420	385	421	384	420	383
Hit 2	421	388	420	387	422	386
Hit 3	422	388	420	385	420	383
Hit 4	419	386	420	384	421	387
Hit 5	423	386	422	387	421	386
Average	421	387	421	385	421	385
St. Dev.	1.58	1.34	0.89	1.52	0.84	1.87

Table S5: Temperature values used for each myrcene hit at T_m = 405 °C. The average T_i and T_f values for M7, M8 and M9 were themselves averaged to get the total average T_i and T_f for the T_m = 405 °C TR, shown in Table S2.

	T_m = 322 °C					
	M10		M11		M12	
	T_i	T_f	T_i	T_f	T_i	T_f
Hit 1	337	311	335	307	335	308
Hit 2	336	309	336	309	334	306
Hit 3	326	291	344	319	337	311
Hit 4	337	310	336	311	337	308
Hit 5	337	309	336	311	338	309
Average	335	306	337	311	336	308
St. Dev.	4.83	8.43	3.71	4.56	1.64	1.82

Table S6: Temperature values used for each myrcene hit at T_m = 322 °C. The average T_i and T_f values for M7, M8 and M9 were themselves averaged to get the total average T_i and T_f for the T_m = 322 °C TR, shown in Table S2.

NMR Conditions

All myrcene samples were run at 1024 scans, 6.7 second repetition rate, 30-degree flip angle with 64 k data point acquisition on a Bruker Avance III 600 MHz NMR spectrometer. Spectra were processed with 0.3 Hz of line broadening with a final data size of 64 k real data points. Fire OG terpenes, limonene and linalool were run under the same conditions but with 256 scans. Analyte assignments of benzene and methacrolein were performed by spiking of authentic standards. Integral measurements for quantitative-NMR were done using Global Spectral Deconvolution (GSD) from MestreLab software.

Toxicant Levels Generated in Myrcene NMR Experiments

Levels by Experiment			
T_m (°C)	Experiment	Benzene (ng)	Methacrolein (ng)
526	M1	432	4569
	M2	362	4279
	M3	457	4804
455	M4	*ND*	2470
	M5	*ND*	2392
	M6	*ND*	2397
403	M7	*ND*	1405
	M8	*ND*	979
	M9	*ND*	1340
322	M10	*ND*	*ND*
	M11	*ND*	*ND*
	M12	*ND*	*ND*

Table S7: Benzene and methacrolein levels determined in the NMR tube for each experiment.

Table S7 shows the initial data used for calculations. Averages of each triplicate value were taken, standard deviation calculated, and confidence interval found at 95 % confidence level.

The amount of toxicant generated per mg of myrcene administered in the dab (75 μL) was calculated from the values in Table S8, using the density of myrcene. The amount of toxicant generated per mg of limonene, linalool and Fire OG were calculated analogously using their density, results of which are shown in Table 1 in the main body of the report.

Knowing that an average mass of a dab is 40 mg, and assuming that all terpenes will degrade to form similar levels of toxicants as myrcene, the amount of toxicant per mg of myrcene formed is multiplied by the mass of terpenes in a 40 mg dab of BHO, 2.36 mg. This mass of toxicant formed per dab is then divided by the volume of the draw (338 mL) to give a concentration of toxicant in the air that would be inhaled.

Products Identified in ATD-GC-MS Experiment

Retention time (min)	Product	Match Quality	CAS Number
1.242	2-methylpropene	90	115-11-7
1.297	1,3-butadiene	91	106-99-0
1.348	acetaldehyde	72	75-07-0
1.929	isoprene	95	78-79-5
2.1	acetone	80	67-64-1
2.3	cyclopentadiene	97	542-92-7
2.868	2-methyl propanal	83	78-84-2
3.019	methacrolein	94	78-85-3
3.148	2,3-dimethyl-2-butene	90	563-79-1
3.287	1,3-hexadiene	93	592-48-3
3.439	methyl vinyl ketone	80	78-94-4
3.51	3-methyl furan	91	930-27-8
3.881	2-methyl-1,3-pentadiene	94	1118-58-7
3.993	2-methyl-1,3-cyclopentadiene	92	3727-31-9
4.481	1,3-cyclohexadiene	93	592-57-4
4.893	2-ethylacrolein	94	922-63-4
6.406	1-methyl-1,4-cyclohexadiene	94	4313-57-9
6.732	1,3,5-cycloheptatriene	95	544-25-2
14.797	naphthalene	95	91-20-3
15.691	4-isopropyl benzaldehyde	93	122-03-2
15.884	3,7-dimethyl-2,6-octadienal	97	5392-40-5
16.123	4-isopropenyl-1-cyclohexene-1-carbaldehyde	98	2111-75-3
16.307	1-methyl naphthalene	95	90-12-0
16.549	2-methyl naphthalene	96	91-57-6
22.281	(E,E)-7,11,15-trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	70	70901-63-2
22.511	(E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene	83	77898-97-6

Table S8: Products identified in a myrcene dab sample taken at the second highest TR of T_m = ca. 450 °C (T_i = 470 °C and T_f = 430 °C) using ATD-GC-MS. Products highlighted in red were also identified in the air blank.

Product Identification by Spiking

A sample from the highest TR, M1, was spiked with low concentration methacrolein and benzene standards to verify the presence of these in the spectra. All methacrolein peaks were identified (Figures S3- S6), as well as the singular benzene peak (Figure S7). The methacrolein standard was spiked twice to fully verify its existence amongst overlapping peaks.

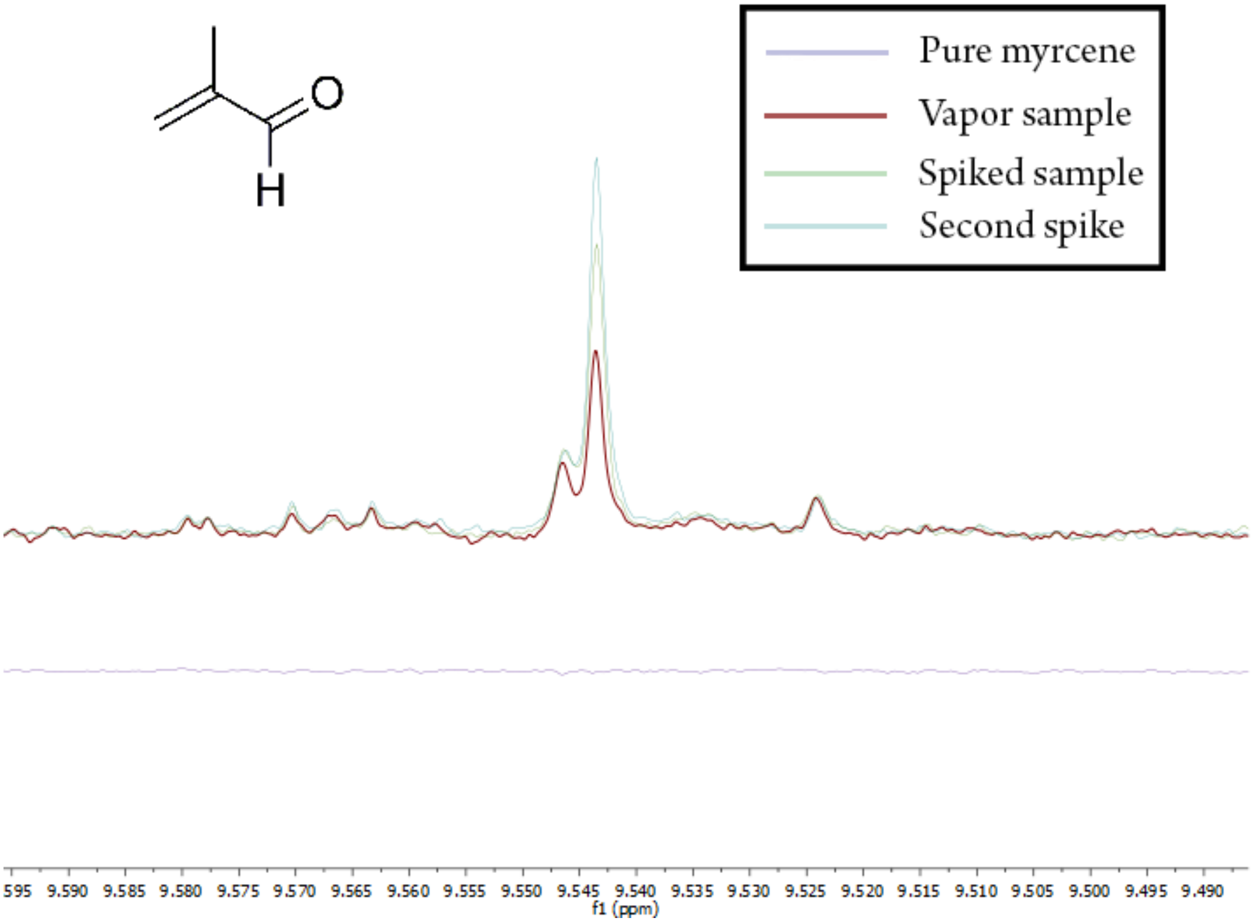

Figure S3: Overlay in the aldehyde proton region of methacrolein (9.54 ppm) displaying a pure myrcene sample, a vapor sample, the same vapor sample spiked with pure methacrolein, and a second spike with pure methacrolein showing a rise in intensity of this aldehyde signal.

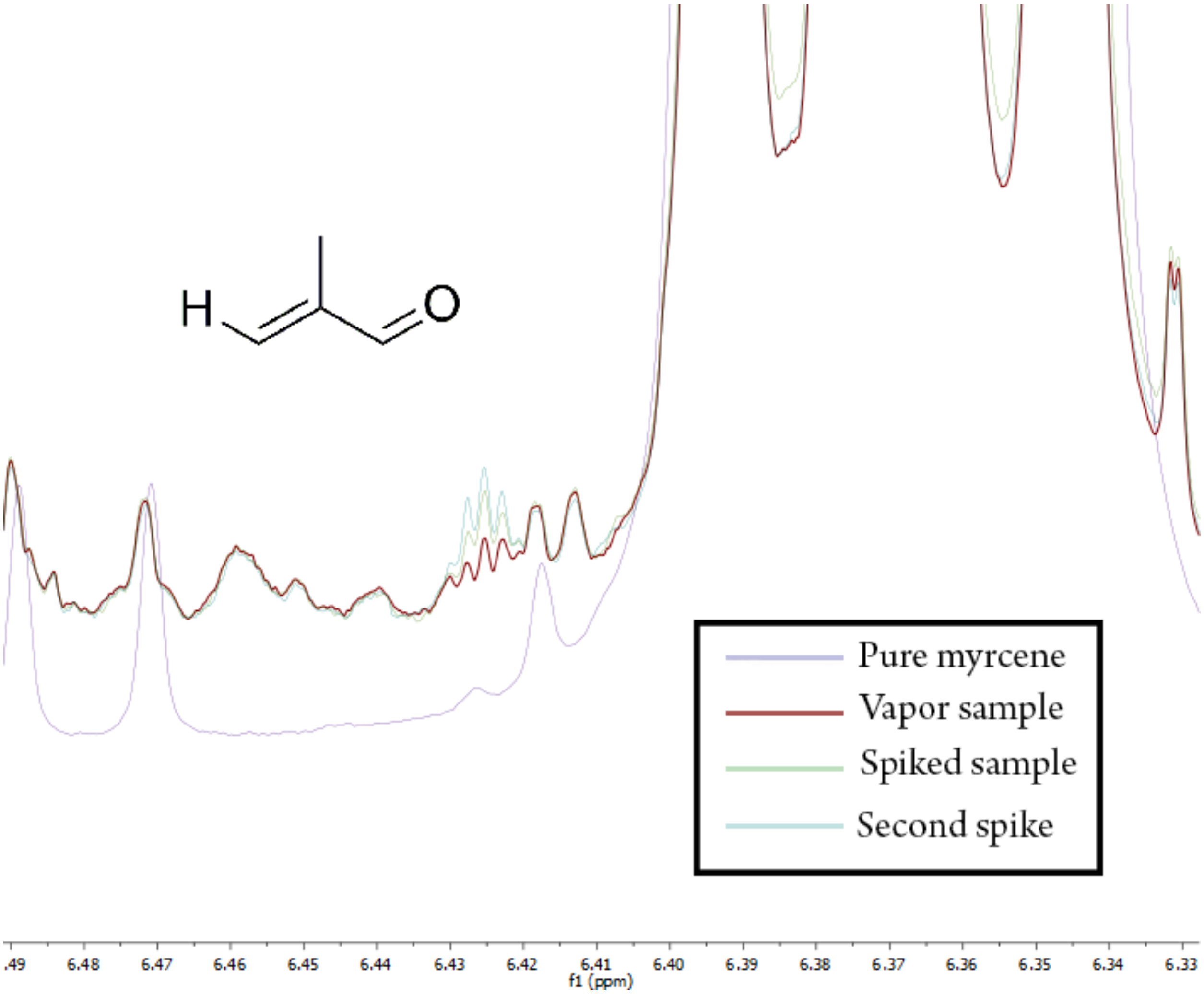

Figure S4: Overlay in the alkene proton region of methacrolein (6.43 ppm) displaying a pure myrcene sample, a vapor sample, the same vapor sample spiked with pure methacrolein, and a second spike with pure methacrolein showing a rise in intensity of this alkene signal.

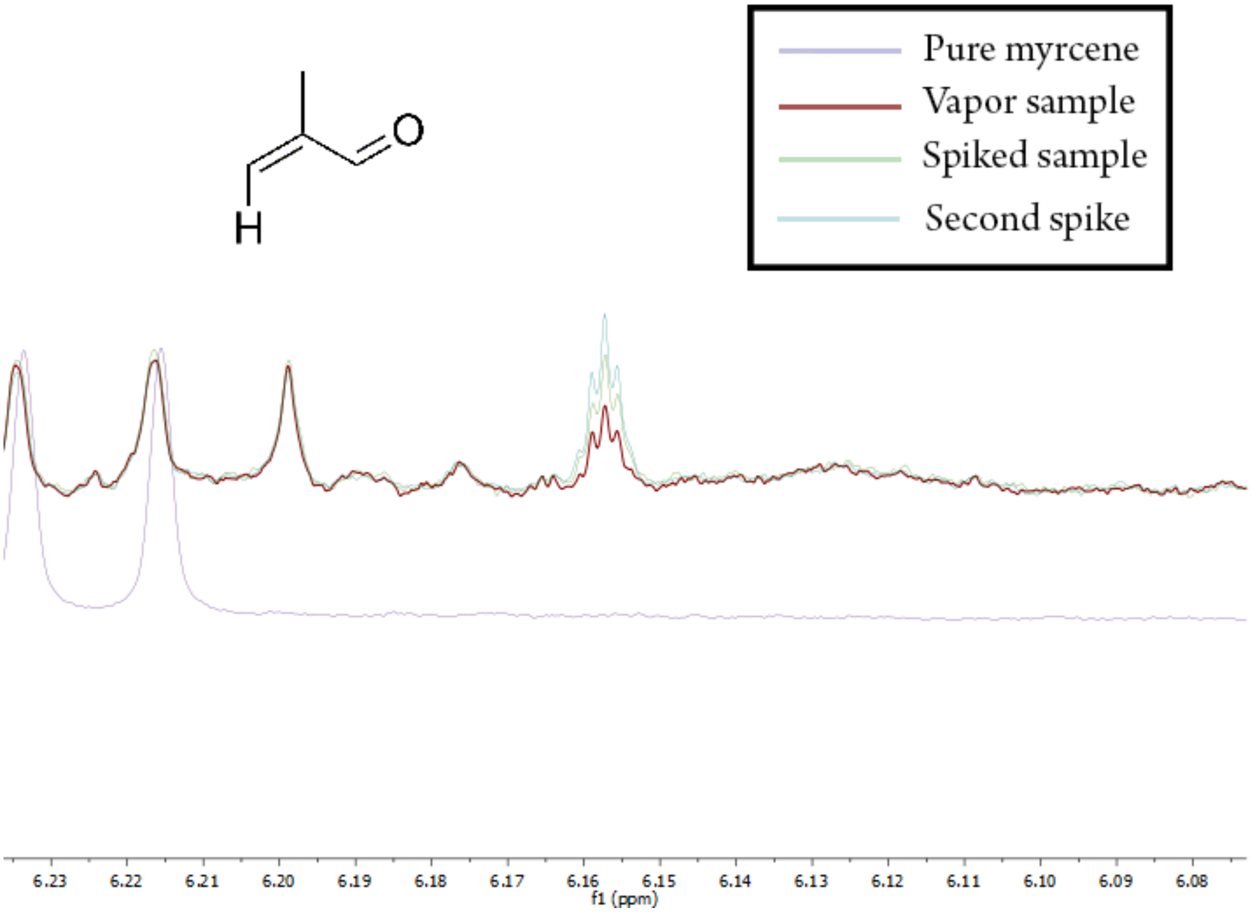

Figure S5: Overlay in the alkene proton region of methacrolein (6.16 ppm) displaying a pure myrcene sample, a vapor sample, the same vapor sample spiked with pure methacrolein, and a second spike with pure methacrolein showing a rise in intensity of this alkene signal.

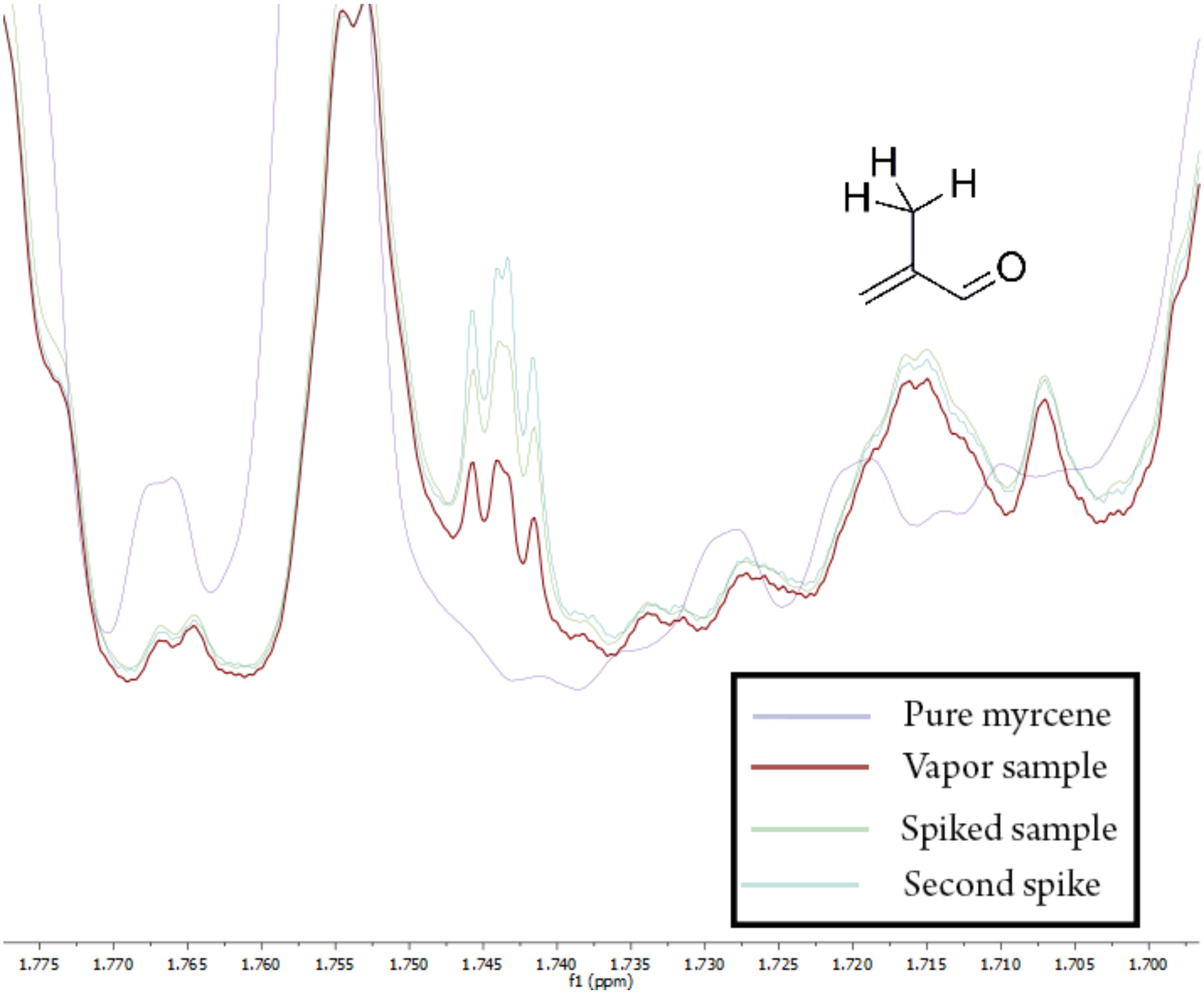

Figure S6: Overlay in the methyl proton region of methacrolein (1.74 ppm) displaying a pure myrcene sample, a vapor sample, the same vapor sample spiked with pure methacrolein, and a second spike with pure methacrolein showing a rise in intensity of this methyl signal.

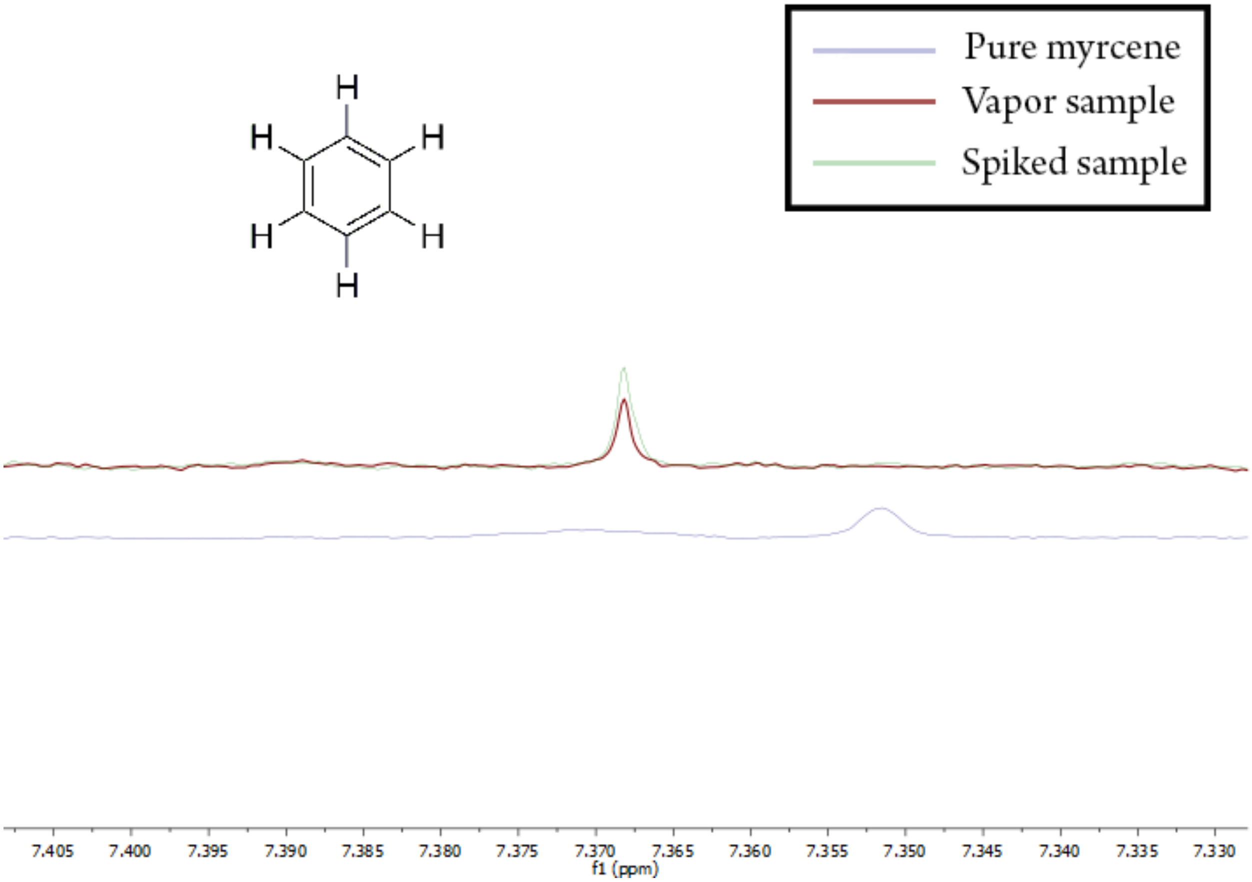

Figure S7: Overlay in the benzene proton region (7.37 ppm) displaying a pure myrcene sample, a vapor sample, the same vapor sample spiked with pure benzene showing a rise in intensity of the benzene proton signal.

Sample Chromatograms and Mass Spectra of Select Degradants

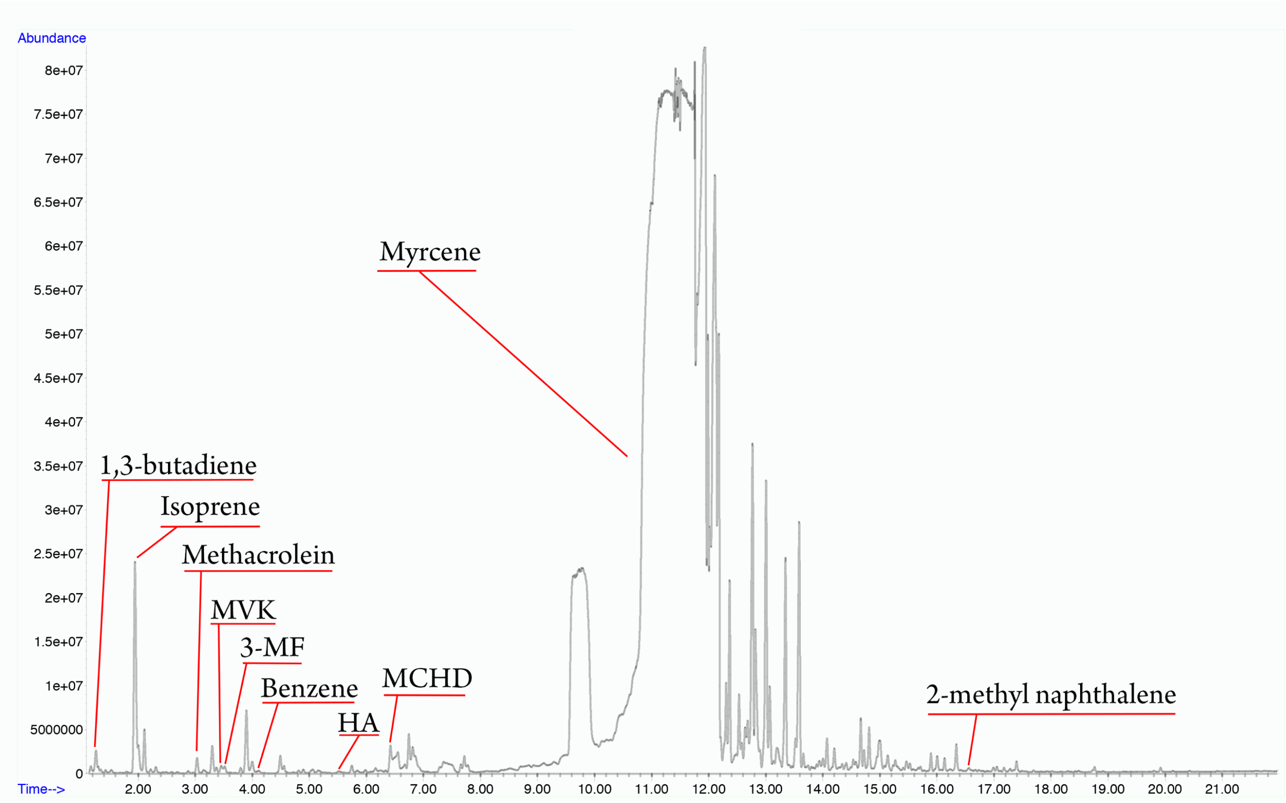

Figure S8: A sample chromatogram from a high-temperature myrcene dabbing sample collected using ATD-GC-MS. Highlighted peaks include: 1,3-butadiene, isoprene, methacrolein, methyl vinyl ketone (MVK), 3-methylfuran (3-MF), benzene, hydroxyacetone (HA), 1-methyl-1,4-cyclohexadiene (MCHD), myrcene, and 2-methylnaphthalene.

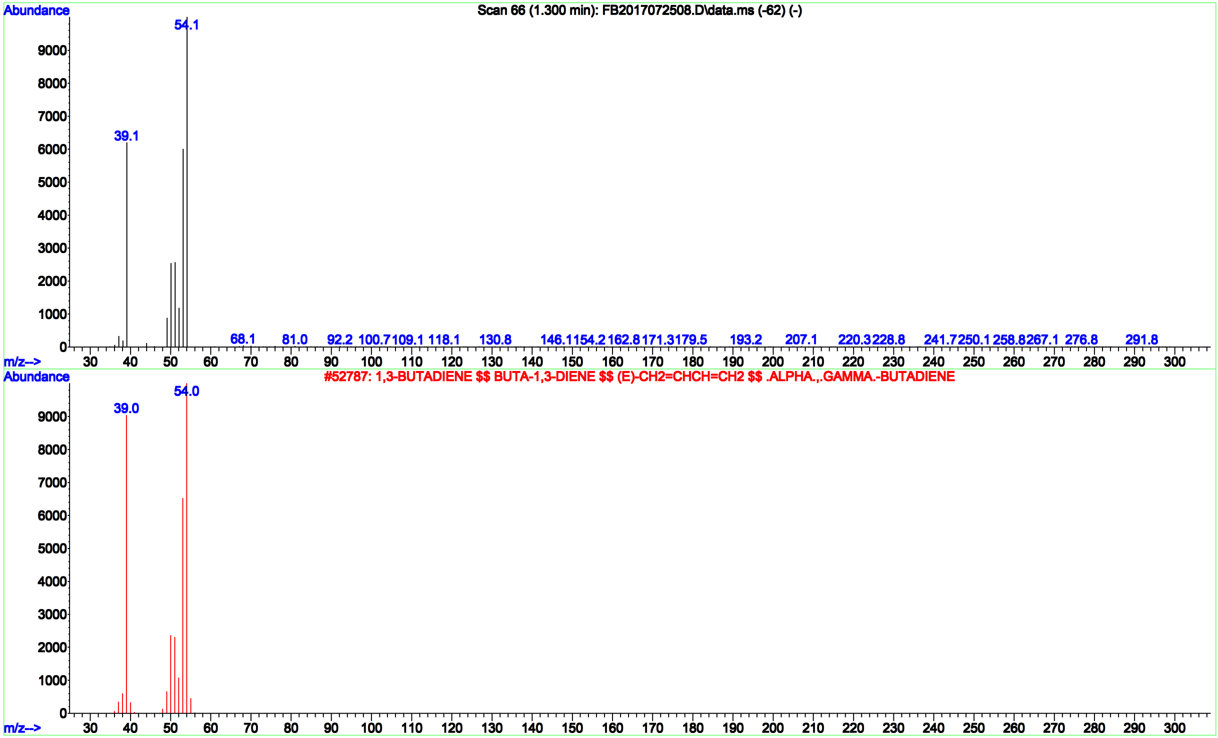

Figure S9: Mass spectrum of 1,3-butadiene (top, grey) compared to NIST library mass spectrum (bottom, red).

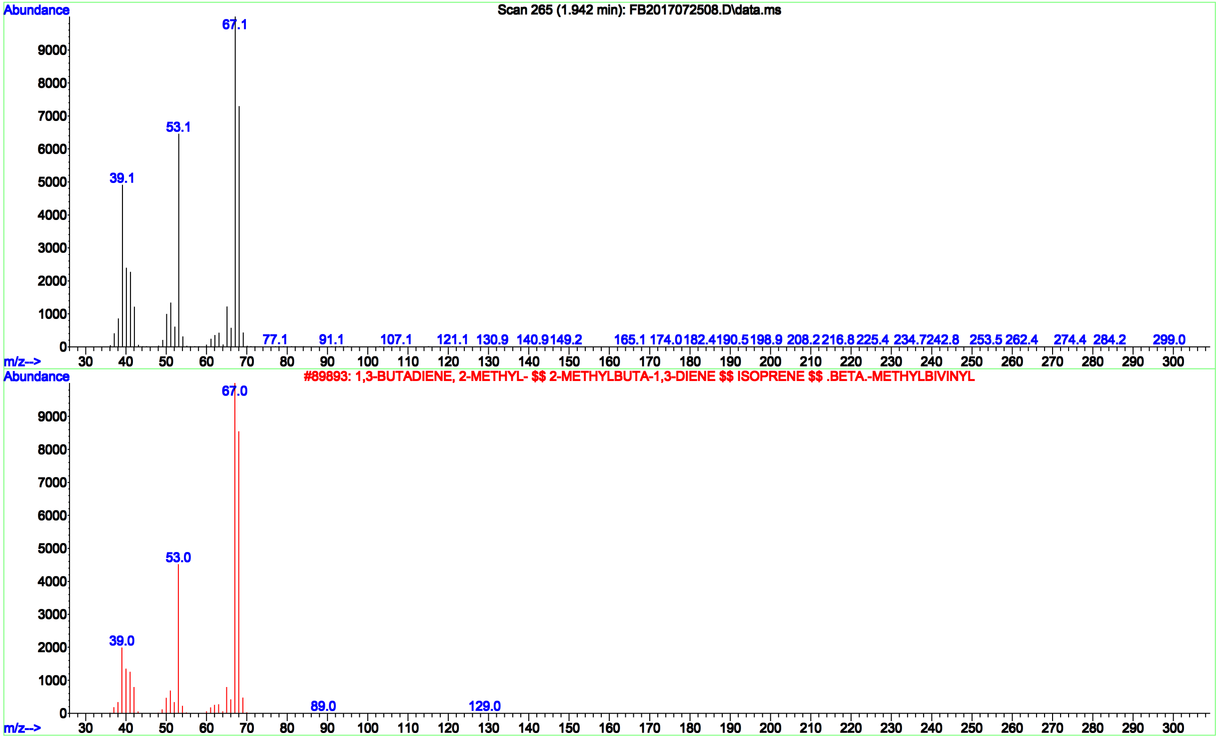

Figure S10: Mass spectrum of isoprene (top, grey) compared to NIST library mass spectrum (bottom, red).

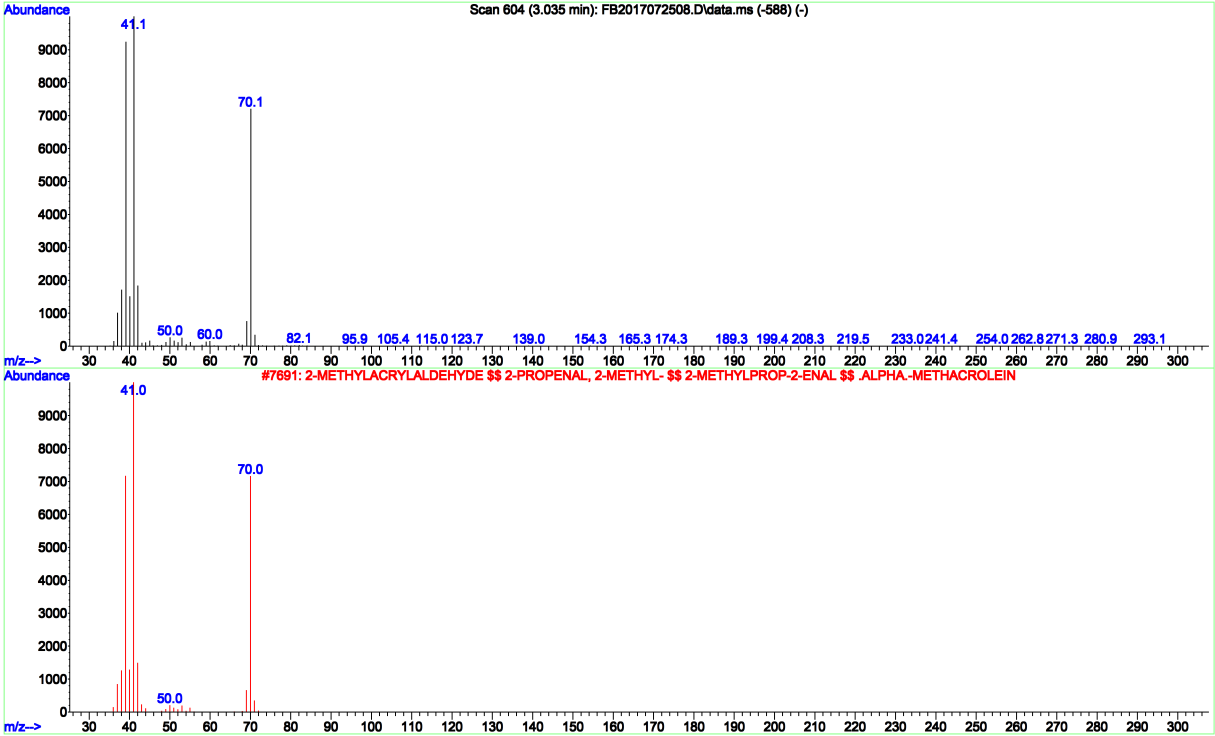

Figure S11: Mass spectrum of methacrolein (top, grey) compared to NIST library mass spectrum (bottom, red).

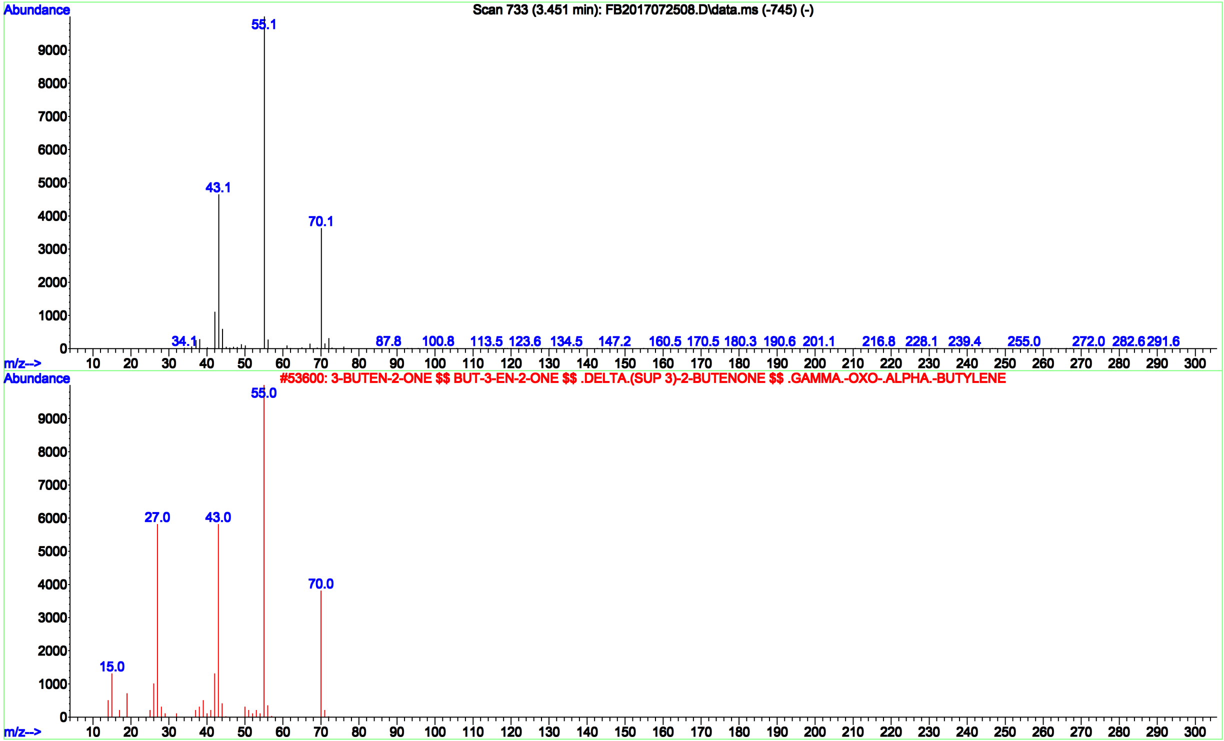

Figure S12: Mass spectrum of methyl vinyl ketone (top, grey) compared to NIST library mass spectrum (bottom, red).

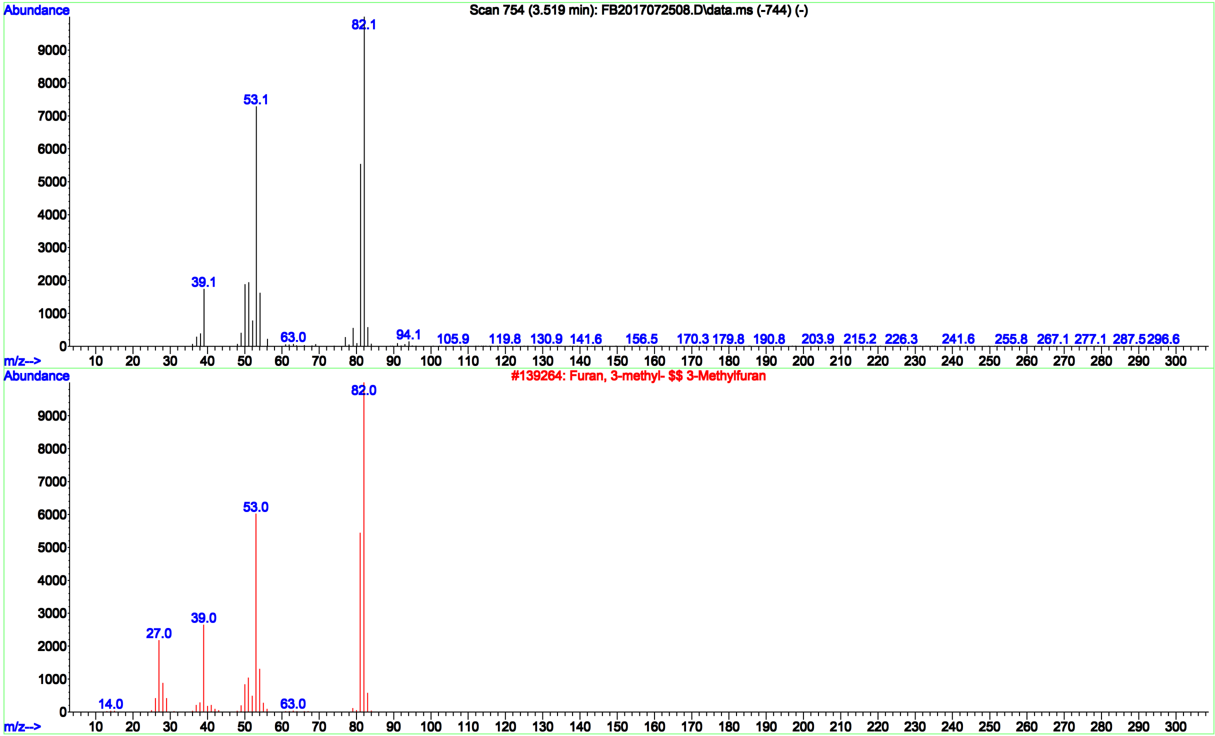

Figure S13: Mass spectrum of 3-methyl furan (top, grey) compared to NIST library mass spectrum (bottom, red).

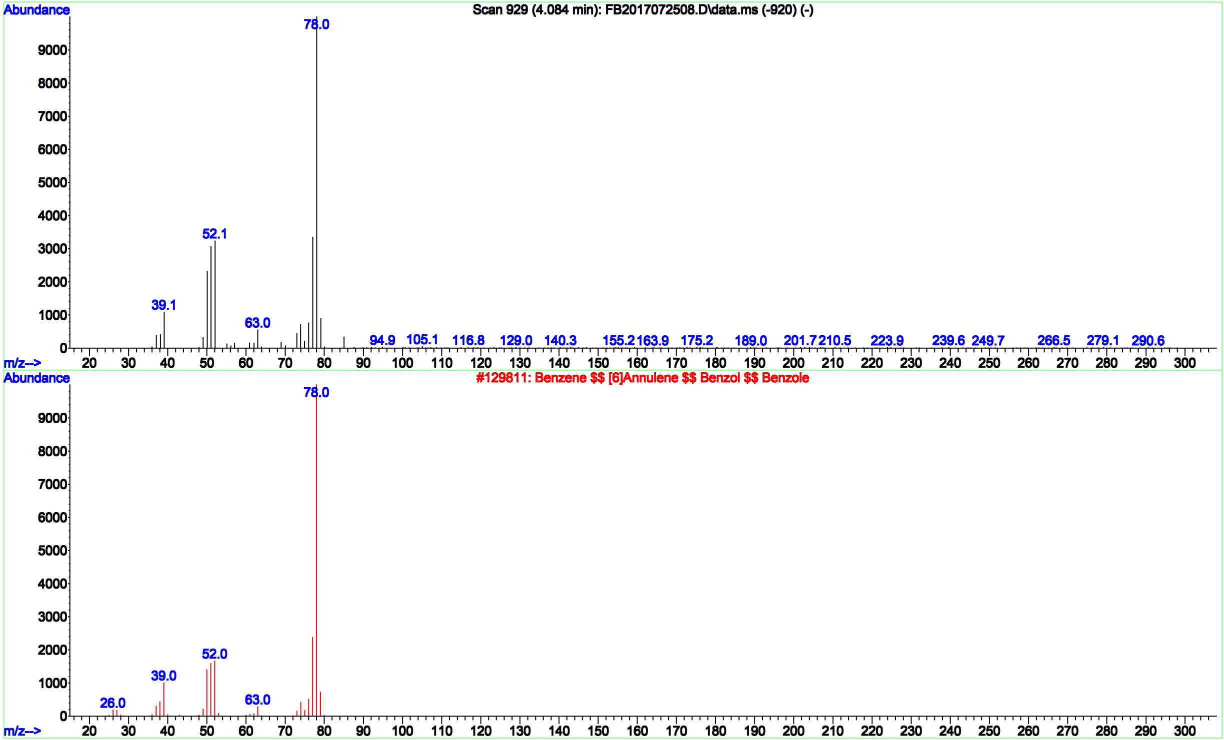

Figure S14: Mass spectrum of benzene (top, grey) compared to NIST library mass spectrum (bottom, red).

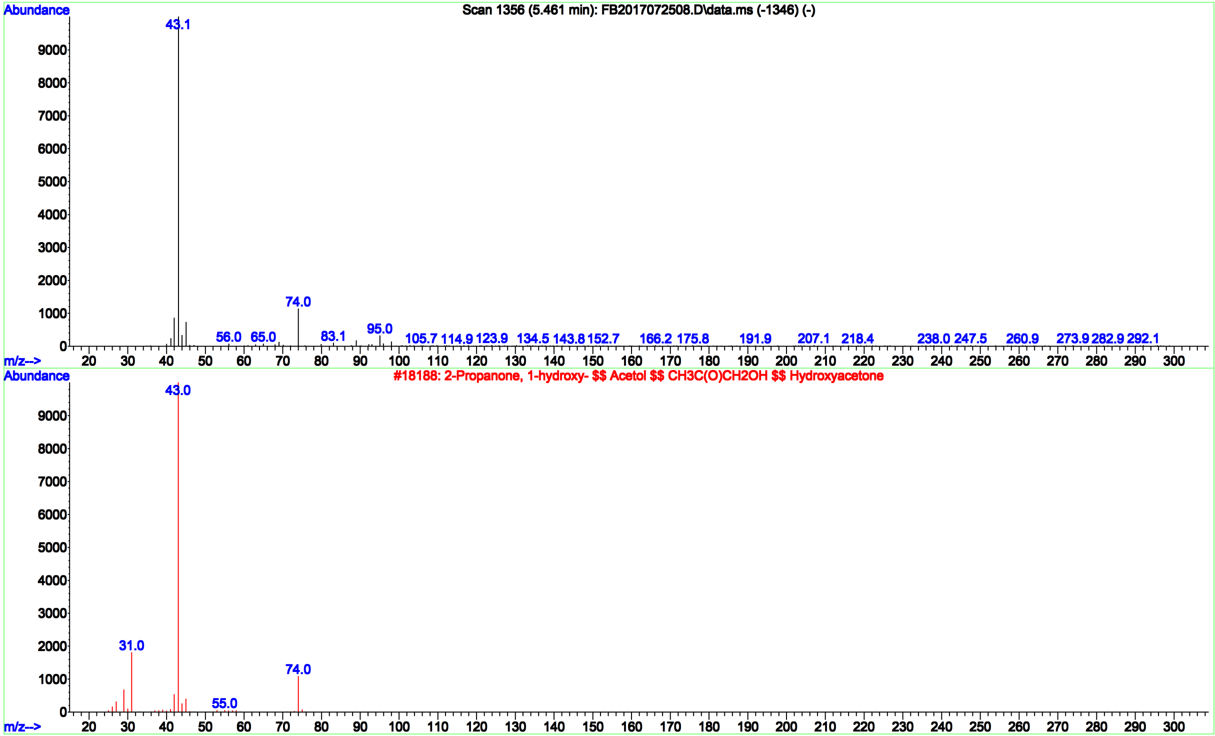

Figure S15: Mass spectrum of hydroxyacetone (top, grey) compared to NIST library mass spectrum (bottom, red).

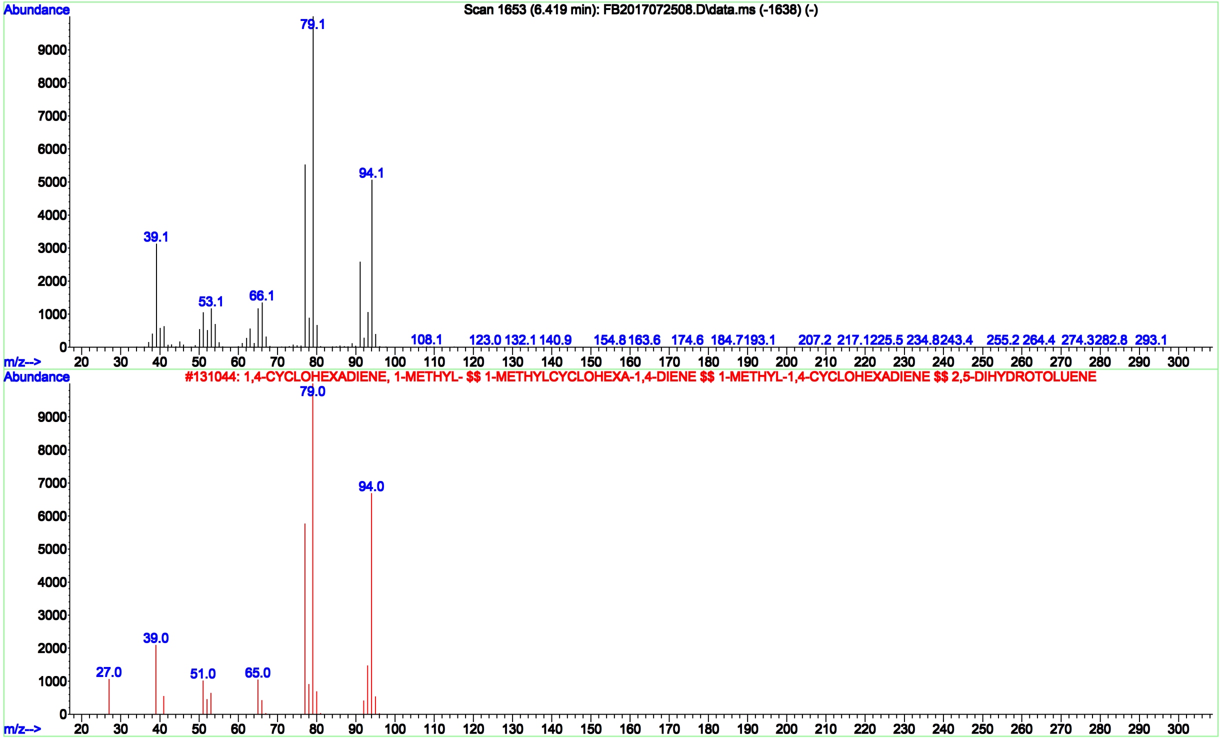

Figure S16: Mass spectrum of 1-methyl-1,4-cyclohexadiene (top, grey) compared to NIST library mass spectrum (bottom, red).

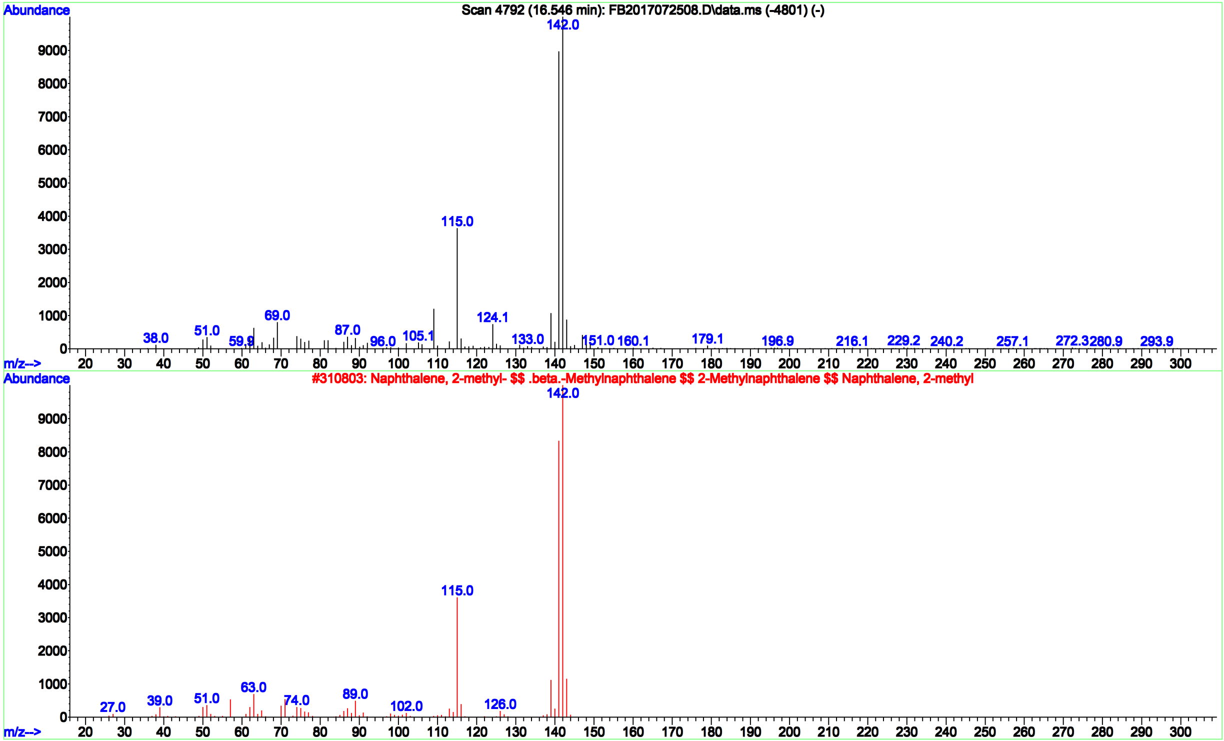

Figure S17: Mass spectrum of 2-methylnaphthalene (top, grey) compared to NIST library mass spectrum (bottom, red).