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Stability Assessment of Gas Mixtures Containing Monoterpenes in Varying Cylinder Materials and Treatments

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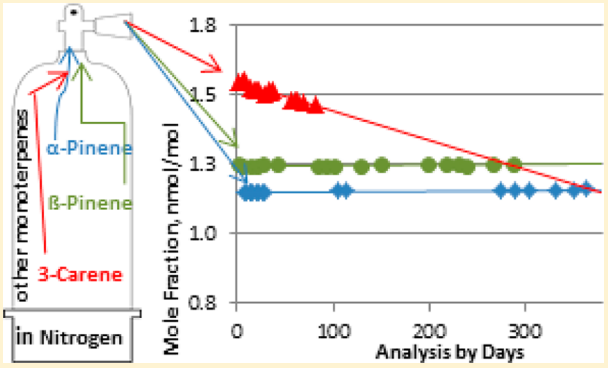
Supporting Information

ABSTRACT: Studies of climate change increasingly recognize the diverse influences exerted by monoterpenes in the atmosphere, including roles in particulates, ozone formation, and oxidizing potential. Measurements of key monoterpenes suggest atmospheric mole fractions ranging from low pmol/mol (parts-per-trillion; ppt) to nmol/mol (parts-per-billion; ppb), depending on location and compound. To accurately establish the mole fraction trends, assess the role of monoterpenes in atmospheric chemistry, and relate measurement records from many laboratories and researchers, it is essential to have good calibration standards. The feasibility of preparing well-characterized, stable gas cylinder standards for monoterpenes at the nmol/mol level was previously tested using treated (Aculife IV) aluminum gas cylinders at NIST.



Results for 4 of the 11 monoterpenes, monitored versus an internal standard of benzene, indicated stability in these treated aluminum gas cylinders for over 6 months and projected long-term (years) stability. However, the mole fraction of the key monoterpene β-pinene decreased, while the mole fractions of α-pinene, D-limonene (R-(+)-limonene), p-cymene, and camphene (a terpene not present in the initial gas mixture) increased, indicating a chemical transformation of β-pinene to these species. A similar pattern of decreasing mole fraction was observed in α-pinene where growth of D-limonene, p-cymene, and camphene has been observed in treated gas cylinders prepared with a mixture of just α-pinene and benzene as the internal standard. The current research discusses the testing of other cylinders and treatments for the potential of long-term stability of monoterpenes in a gas mixture. In this current study, a similar pattern of decreasing mole fraction, although somewhat improved short-term stability, was observed for β-pinene and α-pinene, with growth of D-limonene, p-cymene, and camphene, in nickel-plated carbon steel cylinders. β-Pinene and α-pinene showed excellent stability at over 6 months in aluminum cylinders treated with a different process (Experis) than used in the original study.

There is considerable interest in measuring the levels of monoterpenes in the atmosphere and emissions from terrestrial vegetation and forest and from dynamic enclosure studies of collected sample vegetation. Monoterpenes are an important part of atmospheric chemistry, contributing to atmospheric photochemical processes leading to the formation of secondary photo-oxidants such as ozone that lead to photochemical smog.[1,2] Production and loss of tropospheric ozone are influenced by atmospheric mole fractions of monoterpenes. Monoterpenes may also control the mole fraction of OH radicals that oxidize methane and other greenhouse gases.[3] The origins of these volatile organic compounds (VOCs) can be either biogenic (BVOC) or anthropogenic (AVOC) with the most important BVOCs being isoprene, isoprenoids, and monoterpenes.[4] Large numbers of studies have shown variations between vegetation species in strength of emissions,[5] indicating the unreliability of using a single species for estimating emissions of many species combined.[6] Biogenic emissions from tree species in the North American, European, and Mediterranean temperate systems have received the most interest.[7−10] However, tropical forest emissions are interesting because of the potential for higher monoterpene emission rates.[11−13] Eucalypts trees are some of the highest biogenic emitters and have been studied for terpene and monoterpene emissions in Australia.[14] Enclosure studies have been reported including the study of Norway spruce twigs[4] and aerobic decomposition study of orange waste.[15] Räisänen et al. reported results from emission studies of α-pinene, ß-pinene, 3-carene, sabinene, myrcene, p-cymene, and 1,8-cineole from boreal Scots pine forest samples in Finland.[16] Monoterpene emissions from plants and their effect on air quality have been studied in Las Vegas, NV,[17] and elsewhere.[18,19]

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To accurately establish mole fraction trends, assess the role of monoterpenes in atmospheric chemistry, and relate measurement records from many laboratories and researchers, it is essential to have good calibration standards. In 2005 the World Meteorological Organizations (WMO) Global Atmosphere Watch (GAW) group for Volatile Organic Compounds approached the Gas Analysis Working Group (GAWG) of the Consultative Committee on Quantity of Material (CCQM) and asked for assistance in researching and developing gas standards containing monoterpenes that would be stable for at least 10 years. The CCQM is a committee whose members represent the National Metrology Institutes (NMIs) different nations. Standards comparisons (Key Comparisons) are undertaken in the CCQM/GAWG to determine the equivalence of one NMIs standard to others. The WMO\GAW-VOC would like to have standards with a relative ±5% expanded uncertainty (95% confidence interval); therefore, an acceptable drift, at nominal mole fraction of 2 nmol/mol, would be 0.01 nmol/mol/year. There is limited literature on the development and use of terpene gas phase standards contained in cylinders or generated by other means. Batterman et al. reported the preparation of α-pinene, β-pinene, limonene, and 3-carene at (3−5) nmol/mol in humidified air and humidified nitrogen in electropolished stainless steel canisters.[20] Mole fractions of the monoterpenes decreased considerably, 19% in the humidified air-filled set, in just the first hour, and continued to decrease over the measurement period at a slower rate. Pollmann et al. reported the preparation of sesquiterpenes generated with a capillary diffusion system with the output being diluted with air.[21] Permeation devices containing pure terpenes have also been used to produce calibration standards.[16] Several studies cited the use of commercial gas standards containing monoterpenes at the (1−10) μmol/mol (parts-per-million; ppm) level that were then diluted with either nitrogen or humidified air.[4,13] Another study cited a combination of commercial gas standards and diffusion techniques to calibrate instruments.[14]

Previous standards development research at the National Institute of Standards and Technology (NIST) yielded mixed results.[22] Monoterpenes in nitrogen gas mixture standards contained in aluminum gas cylinders treated with a proprietary process, Aculife IV, by Scott Specialty Gases, Plumsteadville, PA (now Air Liquide America Specialty Gases), were only partially successful. Isoprene and the monoterpenes 1,8-cineole, myrcene, Δ-3-carene, and isoprene showed good stability for 7 months. On the basis of experience with VOC gas standards, these results should project long-term stability as degradation usually starts within days of preparation if it is to occur. However, ß-pinene, sabinene, α-terpinene, and camphor appreciably degraded over this 7 month period. The ß-pinene showed what appears to have been chemical transformation into α-pinene, D-limonene, p-cymene, and camphene, a monoterpene not included in several of the mixtures prepared and studied. Several studies document ß-pinene transforming into those four monoterpenes in the presence of oxides and elevated temperatures.[23−25] It was postulated that there would be aluminum oxide present in the treated gas cylinders and that high temperatures may not be necessary for the chemical transformation to take place. Because most of the gas mixtures studied contained almost all of the monoterpenes, it was difficult to determine if those that showed increases through suspected chemical reactions were actually stable. A mixture containing only α-pinene, camphor, and benzene as an internal standard, in the same aluminum Aculife IV cylinders, showed that both monoterpenes started degrading immediately with increasing levels of camphene and D-limonene observed over a time frame of 39 days.

Because most of the monoterpenes studied were not stable in Aculife IV aluminum gas cylinders, two alternative package systems have been studied. This Article discusses the results of this latest research.

EXPERIMENTAL SECTION

Procedures for preparing primary standard mixtures (PSMs) have been well documented and demonstrated with Standard Reference Material (SRM) 1800 non-methane hydrocarbon compounds in nitrogen, which contains 15 nonmethane hydrocarbons (NMHCs).[26−31] NIST has developed many PSMs of hydrocarbons and VOCs down to low pmol/mol (parts-per-trillion; ppt) levels.[32] These same procedures were used to prepare the terpene gas mixtures.

Cylinders. New gas cylinders were obtained commercially and used in the preparation of the nmol/mol PSMs. Aluminum (Al) gas cylinders, 20 L internal volume, were obtained from Air Products, Belgium. These cylinders were fitted with nickel-plated Ceodeux valves, and the internal walls were treated with a proprietary process (Experis) to passivate the internal cylinder walls; these cylinders will be designated "Al-Exp". Carbon steel (CS) cylinders, 10 L internal volume, were obtained from Airgas, Riverton, NJ. They had the interior cylinder walls nickel-plated (Ni-p), and then fitted with 316 stainless steel Ceodeux valves.

Regulators and Tubing. The same two-stage, high-purity, low dead volume, stainless steel regulators were used for analysis as in the previous work.[22] The regulators were processed and cleaned without using products that would contribute to terpene contamination. Silonite, Entech Instruments, Inc. in Simi Valley, CA, coated stainless steel tubing and traps were used in the cryogenic preconcentration unit and from the regulators on the cylinders to the sampling manifold to minimize adsorption/desorption of monoterpenes.

Monoterpene Reagents. The monoterpenes of interest were purchased from commercial suppliers with stated purities ranging from 90% to 99.95%. These neat liquids were analyzed for impurities using gas chromatography with flame-ionization detection (GC/FID).[22] Supporting Information Table S-1 lists the terpene impurities of interest present in each "pure" monoterpene reagent.

Cylinders of high-purity diluent nitrogen (N_2) gas obtained from Air Liquide America Specialty Gases, Plumsteadville, PA, were used to prepare the nmol/mol terpene gas standards. All N_2 gas materials were stated by their manufacturer to have a minimum purity of 99.9995% (excluding argon). The diluent nitrogen was analyzed for impurities of the monoterpenes to be studied by GC/FID coupled to a cryogenic preconcentrator. No monoterpenes (limit of detection (0.001 ± 0.001) nmol/mol)) were detected in the nitrogen diluent gas.

Weighing Apparatus. Cylinders were weighed on a Mettler SR64001 top-loading floor balance with a 64 kg capacity and 0.1 g sensitivity. The monoterpenes were weighed into individual glass capillary tubes using a Mettler UM3 ultramicro balance with a 3 g capacity and 0.1 μg resolution.[22]

Preparation of nmol/mol Primary Standard Mixtures. A minimum of five independent weighings (tare, cylinder placement, stabilization, mass recording) was made after evacuating each cylinder. The weighed liquid monoterpenes prepared in capillary tubes of 2−3 cm length were then

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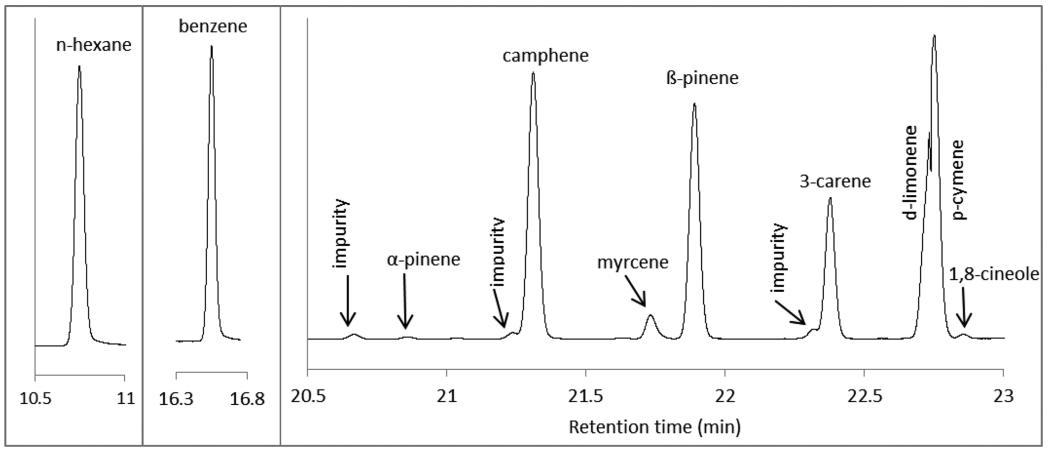

Figure 1. Typical chromatogram showing elution order of monoterpenes.

Table 1. Preparative Mole Fraction of Analytes in Gas Mixture Cylinders[a]

	Experis-treatment aluminum				nickel-plated carbon steel					
	D646508 (Al-Exp-08) (8.3 MPa)		D646507 (Al-Exp-07) (10.3 MPa)		41311187Y (CS-Ni-87Y) (14.5 MPa)		41311192Y (CS-Ni-92Y) (13.6 MPa)		41311193Y (CS-Ni-93Y) (13.3 MPa)	
analyte	x^b	$U(x)^c$	x^b	$U(x)^c$	x^b	$U(x)^c$	x^b	$U(x)^c$	x^b	$U(x)^c$
α-pinene	12.88	0.52	*0.021*	*0.002*	1.41	0.10	*0.010*	*0.001*	*0.006*	*0.001*
β-pinene	*0.015*	*0.001*	9.91	0.35	5.34	0.43	4.65	0.36	2.57	0.45
3-carene	*0.009*	*0.004*	9.56	0.52	4.10	0.54	9.07	0.53	3.33	0.36
1,8-cineole	*0.015*	*0.001*	*0.014*	*0.002*	4.48	0.42	6.63	0.37	2.31	0.47
myrcene			1.92	0.37						
D-limonene			9.32	0.47	*0.003*	*0.001*	*0.002*	*0.001*	*0.001*	*0.001*
p-cymene	*0.008*	*0.002*	10.70	0.31	*0.026*	*0.001*	*0.041*	*0.002*	*0.015*	*0.001*
camphene	*0.001*	*0.001*	9.58	0.50	*0.004*	*0.001*	*0.005*	*0.001*	*0.002*	*0.001*
n-hexane			14.19	0.57	11.51	0.58	8.82	0.56	4.60	0.53
benzene	14.74	0.60								

[a]Values displayed in italics are mole fractions of monoterpenes present as an impurity in one or more of the reagents used to prepare the mixture. [b]Preparative mole fraction of analyte in gas mixture, nmol/mol. [c]Expanded uncertainty, $k = 2$ (approximate 95% confidence interval), of preparative mole fraction, nmol/mol.

transferred into an evacuated gas cylinder. A tube containing *n*-hexane or benzene was also prepared and transferred to the cylinder to be used as an internal standard. Balance nitrogen was added followed by a final weighing. The amount-of-substance fractions (mole fractions) were calculated in mol/mol based on the mass of the added monoterpenes, internal standard, and nitrogen, taking into account the impurities in each of the neat reagents. The monoterpene gas mixtures were prepared to a nominal range of (2−10) nmol/mol in one step using the previously described technique.[22]

Chromatographic Analysis. An Agilent 7890 gas chromatograph with a flame-ionization detector (GC/FID) was used for all analyses. A 60 m × 0.32 mm capillary column with a 1.8 μm film thickness of DB-624 was used to achieve approximate baseline separation of the compounds. The column was temperature programmed from an isothermal start of 50 °C for 8 min to 220 °C at 10 °C/min. A helium column flow of 4 mL/min was used with a detector makeup flow of 26 mL/min helium. The FID was operated at 250 °C. The monoterpene standards were prepared for injection onto the column using a Nutech 3551DS cryogenic preconcentrator, collecting 100 mL of sample at a flow of 50 mL/min at −180 °C, then heating the sample trap to 150 °C and transferring to a cryofocuser at −180 °C. The cryofocuser was then heated to 220 °C, and the GC analysis ensued. The chromatograph's software package was used to integrate the peak areas. Integration in most cases was baseline to baseline. Using the chromatographic conditions described above, D-limonene and *p*-cymene coeluted when present as major components as shown in Figure 1. These peaks were resolved by integrating the first peak, D-limonene, from baseline to a drop line from the top of the peak, then multiplying by 2. The doublet was integrated as one, baseline to baseline, for a total peak area. The *p*-cymene was estimated as the difference between the total area and the estimated D-limonene area. This was, to a lesser extent, also a problem in samples where the D-limonene and *p*-cymene were present as small impurities. Retention times and peak areas for all monoterpenes were exported to a spreadsheet for further analysis.

RESULTS AND DISCUSSION

Because this study was designed to test the stability of the monoterpenes in gas mixtures, it was not critical to prepare the cylinders to the highest accuracy. However, gravimetric techniques were used to prepare the mixtures to enable the cylinders to be used as primary standards if the mixtures proved stable. The preparative mole fractions of the monoterpenes in each of the gas mixtures and their combined standard uncertainties are listed in Table 1. All analyte mole fractions listed in Table 1 in italics arise from impurities in the reagent

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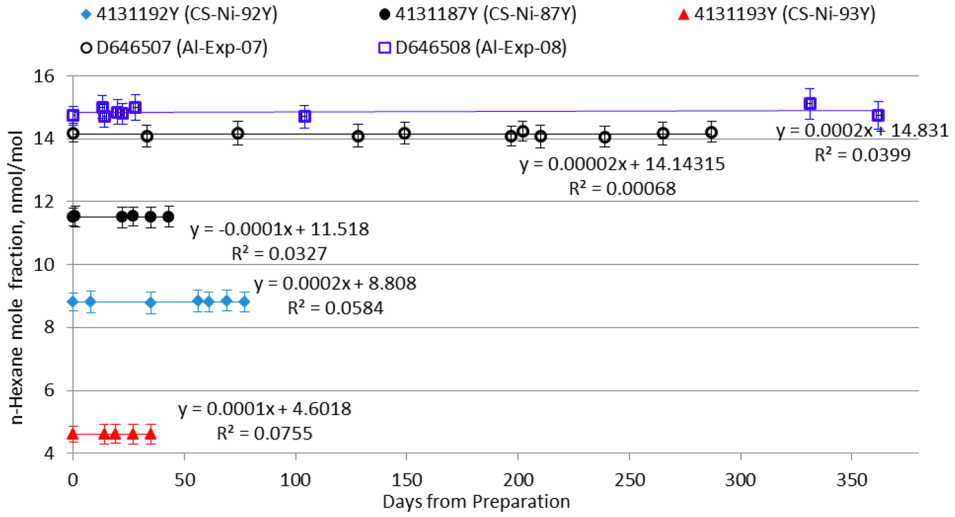

Figure 2. Stability data for benzene in D646508 and *n*-hexane in remaining standards.

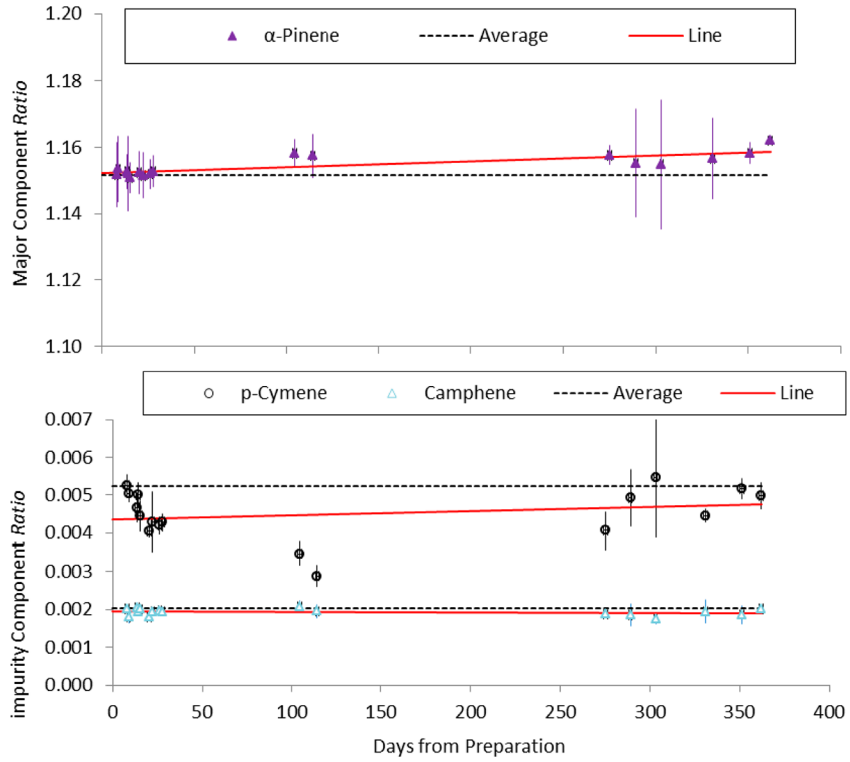

Figure 3. Monoterpene ratios in Experis-treated aluminum cylinder D646508 (Al-Exp-08).

liquids. Impurity mole fractions given for monoterpenes in each mixture were calculated from the purity analysis data (see Table S-1) and the mass data from addition of the individual "neat" monoterpenes into the cylinder. The final fill pressures, in megapascals (MPa), are listed under the cylinder number. Benzene or *n*-hexane was included in the mixtures as internal standards because both are known to be stable in gas mixtures in many types of cylinder materials.[33]

Two mixtures were prepared in the Experis-treated aluminum cylinders. Cylinder D646508 contained benzene as the internal standard and only α-pinene to test the hypothesis of possible interactions among monoterpenes present in the same mixture. Several impurities from the α-pinene component, including ß-pinene, 3-carene, *p*-cymene, and camphene, were also present at very small amounts, which should have greatly diminished their roles in possible chemical reactions with the α-pinene. Following some 30 days of analysis of cylinder D646508, a mixture of ß-pinene, myrcene, D-limonene, *p*-cymene, camphene, and *n*-hexane as the internal standard was prepared in cylinder D646507. The mixtures in these cylinders are hereafter referred to as "Al-Exp-08" and "Al-Exp-07", respectively.

Three mixtures of ß-pinene, 1,8-cineole, 3-carene, and *n*-hexane as an internal standard were later prepared in the nickel-plated carbon steel cylinders 41311187Y ("CS-Ni-87Y"), 41311192Y ("CS-Ni-92Y"), and 41311193Y ("CS-Ni-93Y"). The mixture in cylinder CS-Ni-87Y also included α-pinene.

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The benzene in Al-Exp-08 and *n*-hexane in each of the other mixtures described above was occasionally measured against one to four other standards to track stability. Figure 2 shows the linear plots of the data. An increase in the benzene in Al-Exp-08 corresponds to about 1.5% relative in a year. The *n*-hexane has remained constant within the standard uncertainty, $k = 1$ (68% confidence interval), error bars as depicted graphically. Additionally, the intercepts for each regression also are in agreement with the gravimetric mole fractions for the *n*-hexane in each mixture.

Analysis of Monoterpene Gas Mixtures in Aluminum Experis-Treated Cylinders. *Cylinder D646508 (AL-Exp-08).* The Al-Exp-08 mixture containing α-pinene, with benzene as the internal standard, was initially analyzed 8 days after preparation and then 16 times over a period of 362 days. During the early measurement sets, 6−10 consecutive sample injections of the gas mixture to the GC/FID were made on any given day. Later, measurement sets were reduced to 3−5 injections to conserve the gas mixture. The relative peak area ratios for the α-pinene, and any of the monoterpene impurities present, were calculated by dividing α-pinene peak area by the benzene peak area in the same sample injection to obtain a ratio. Camphene, *p*-cymene, and D-limonene are known to be growth products from the chemical degradation of α- and ß-pinene under certain physical conditions.[23−25] The camphene and *p*-cymene that were present in the gas mixture as impurities in the neat α-pinene were monitored for possible growth. The 3-carene and 1,8-cineole impurities in the pure α-pinene are given in Table 1 but were not monitored as they are not suspected growth products of α-pinene degradation, and the low mole fraction, <0.02 nmol/mol, is not of interest to this study. Therefore, there are no data reported in subsequent Supporting Information Table S-2 and Figure 3 for these two monoterpenes.

Data were summarized for each monoterpene as the mean within set ratio and its combined standard uncertainty (u). The u was estimated from the relative standard deviations of the mean benzene GC peak areas and the monoterpene ratio, and divided by the square root of the number of injections in the set.

For each set of injections, Supporting Information Table S-2 lists the analysis date, number of days from preparation, ratios, and the approximate 95% expanded uncertainty for the ratio, $U_{95} = t_s{}^*u$, where t_s is the student's t expansion factor of 2.78 appropriate to four degrees of freedom, rounded to 3.0 to provide a conservatively large expansion. In the limited cases where only three injections were made, the student's t factor was 4.3. (The ratio is the GC monoterpene peak area divided by the benzene peak area.) Following each set of injection results, the number of data sets, n, is given along with the summary mean, standard deviation (sd), and relative standard deviation (rsd) expressed as %; the pooled value of the U_{95}, $k = 2$, is also listed. The following rows give the least-squares regression of each monoterpene's summary data as a linear function of the number of days from preparation: ratio = intercept (i) + slope (s)*days. The last two rows of Supporting Information Table 2 address whether the s is statistically different from zero.

The mean ratio and its U_{95} uncertainty as a function of days from preparation are illustrated in Figure 3. The black dotted lines represent the initial ratio of each monoterpene (essentially the initial preparative mole fraction) and have a gradient of zero. This is the line that all future points should overlap within uncertainties if there is no increase or loss of the monoterpene. The solid red lines represent the regression-estimated linear trend line for all data points over time. The linear plots suggest that the *p*-cymene is increasing and the camphene is decreasing. However, one should keep in mind that the detection limit is (0.001 ± 0.001) nmol/mol. The mole fraction of camphene, (0.001 ± 0.001) nmol/mol given in Table 1, is calculated from the purity data and the amount added to the cylinder. This mole fraction is right at the detection limit and thus limits the ability to measure. This may explain the variations in ratios, making it difficult to discern between a flat line or growth/degradation. The same argument may also apply to the *p*-cymene, which is present at (0.008 ± 0.002) nmol/mol.

The α-pinene linear trend line yields a s of 0.000018 (0.000003) nmol/mol and an i of 1.1523 (0.0007) ratio, where the values in parentheses are standard uncertainties on the parameter estimates. The squared correlation (R^2) between the days and the ratios is 0.63; the root-mean squared error (RMSE) of the linear regression model is 0.0020 and is small relative to the sd of the ratios, 0.0032, and the pooled U_{95} of 0.0087. Because the RMSE estimates the one sd scatter of the observed ratios about the trend line (assuming that the magnitude of the differences is not dependent upon days), the relatively small value suggests that the linear regression line well describes the α-pinene mole fraction changes. Second-order regression fits were also applied; however, linear fits were the appropriate regression. The absolute value of the s for α-pinene divided by its standard uncertainty, $|s|/u(s)$, is 5.10. Assuming the distribution for the s estimate approximately follows the Student's t distribution with $n - 2 = 15$ degrees of freedom, there is little statistical probability that the nonzero value for s can be attributed to measurement imprecision. Note, however, that the estimated value for s is positive but small.

Considering the small R^2 values of 0.06 and 0.07 for *p*-cymene and camphene, respectively, and identical values of the RMSE to the standard deviations of the ratios, there is little to no evidence that the relative mole fraction of either of these monoterpenes changed over time. Additionally, D-limonene, not present as an impurity in the "neat α-pinene", is a known growth product from α-pinene, and was monitored throughout the experiment but not detected (limit of detection 0.001 nmol/mol). Although the small apparent increase in the α-pinene mole fraction is improbable, the data suggest that it is increasing; therefore, it is assumed that adsorbing on the cylinder wall is not taking place. The internal standard benzene in this mixture also appears to be increasing as depicted in Figure 2, which is also improbable. A more plausible theory is that this is a result of an artifact of the measurement system, therefore suggesting no chemical transformation or wall adsorption of α-pinene has taken place in the Al-Exp-08 mixture.

Cylinder D646507 (AL-Exp-07). The Al-Exp-07 mixture was prepared after determining that α-pinene was stable in the Experis-treated aluminum cylinders, about 4 months after the preparation of Al-Exp-08. The mixture was prepared from neat ß-pinene, 3-carene, D-limonene, *p*-cymene, camphene, myrcene, and *n*-hexane as the internal standard; it also contained measurable levels of α-pinene and 1,8-cineole, present as impurities in the neat reagents. Although previous studies indicated that benzene does not significantly interact with the monoterpenes, *n*-hexane was used as the internal standard in this and later mixtures to eliminate the possibility of an aromatic ring compound somehow interacting with the

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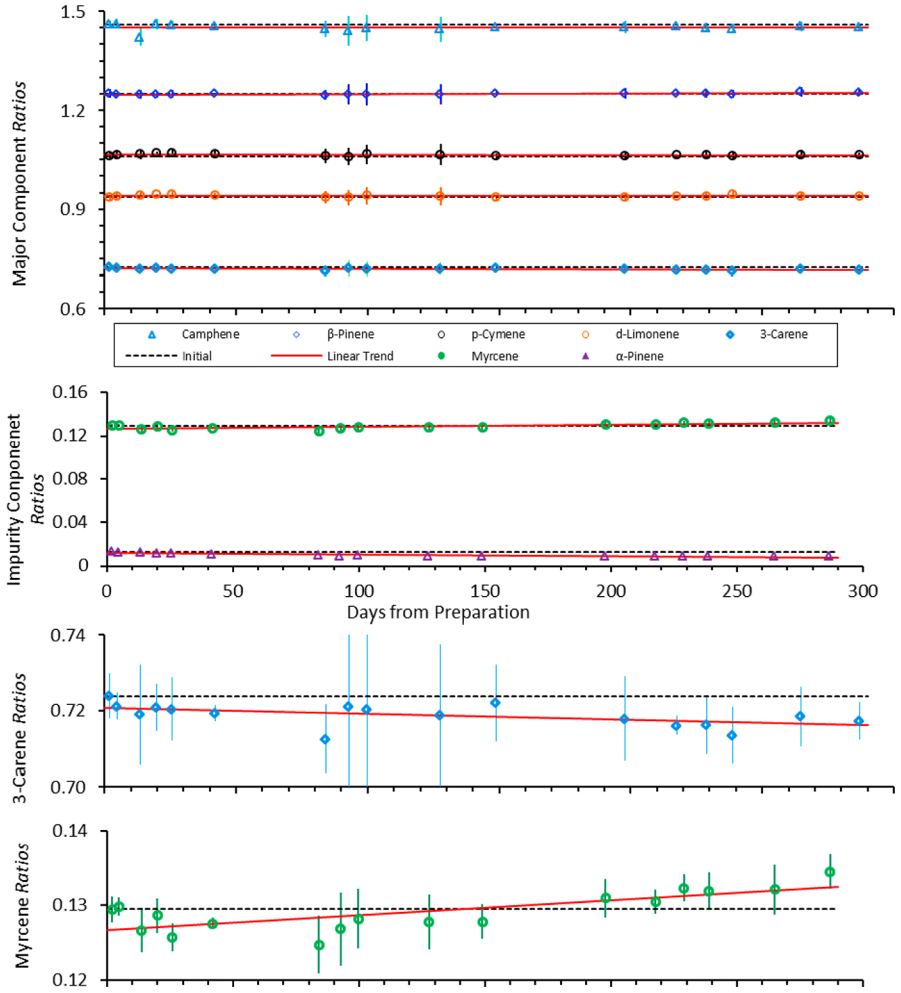

Figure 4. Monoterpene ratios in Experis-treated aluminum cylinder D646507 (Al-Exp-07).

monoterpenes. The Al-Exp-07 mixture was initially analyzed 3 days after preparation and then 13 more times over a period of 230 days. Three to six consecutive injections were made of the mixture on any given day.

Supporting Information Table S-3 summarizes the data and statistics for the Al-Exp-07 mixture, while Figure 4 displays the monoterpene to *n*-hexane ratio results, both in the same format used with the Al-Exp-08 mixture. The estimated *s* values for the monoterpenes are not statistically different from zero at the usual 0.05 significance threshold except for the impurity α-pinene. However, visual inspection of the trend for myrcene suggests that the mole fraction of this compound has slightly increased, and that α-pinene and 3-carene may be degrading. The α-pinene is present as an impurity at 0.021 μmol/mol, which is close to the limit of detection of 0.001 nmol/mol. This may magnify the difficulty in determining if it is actually decreasing as the GC software has a more difficult time accurately measuring a peak area on a very small peak. It is noted here that the myrcene is plotted with the "impurity components" due to the fact the mole fraction is nominal 2 nmol/mol as compared to nominal 14 nmol/mol for the *n*-hexane, resulting in a much smaller ratio. The myrcene and the 3-carene plots are difficult to visualize and have been expanded as shown at the bottom of Figure 4. These plots show myrcene increasing and 3-carene decreasing from their initial mole fraction. Considering the identical values for the RMSE and sd (of the mean ratio) for all of the monoterpenes in the mixture, whether major or impurity, there is little to no statistical evidence that the relative mole fraction of either of these monoterpenes has changed over time. Given the uncertainties of the individual ratio determinations, longer term measurements are required to establish whether monoterpenes are absolutely stable in Experis-treated aluminum cylinders. However, the statistics suggest that the major monoterpenes are stable for 287 days, and there is confidence that this cylinder mixture could be used as reference material with continued monitoring of the monoterpenes to *n*-hexane. We assume that the Experis-treatment chemically inactivates any metal oxides, one of the claimed driving forces in the chemical reaction of specific monoterpenes.[23−25]

Analysis of Monoterpene Gas Mixtures in Nickel-Plated Carbon Steel Cylinders. *Cylinder 41311192Y (CS-Ni-92Y).* Mixture CS-Ni-92Y was prepared from ß-pinene, 3-carene, 1,8-cineole, and *n*-hexane as the internal standard and detectable mole fractions of α-pinene, D-limonene, *p*-cymene, and camphene present as impurities in the neat reagents. CS-Ni-92Y was initially analyzed 1 day after preparation and then 15 more times over a period of 81 days. Three to six consecutive injections were made of the cylinder gas mixture on any given day. Supporting Information Table S-4 lists the summary data for ratios and statistics as for the previous mixtures. Figure 5 illustrates the mean ratios and their U_{95} uncertainties as a function of days from preparation. The linear trend lines for the three main monoterpenes in this mixture, 3-

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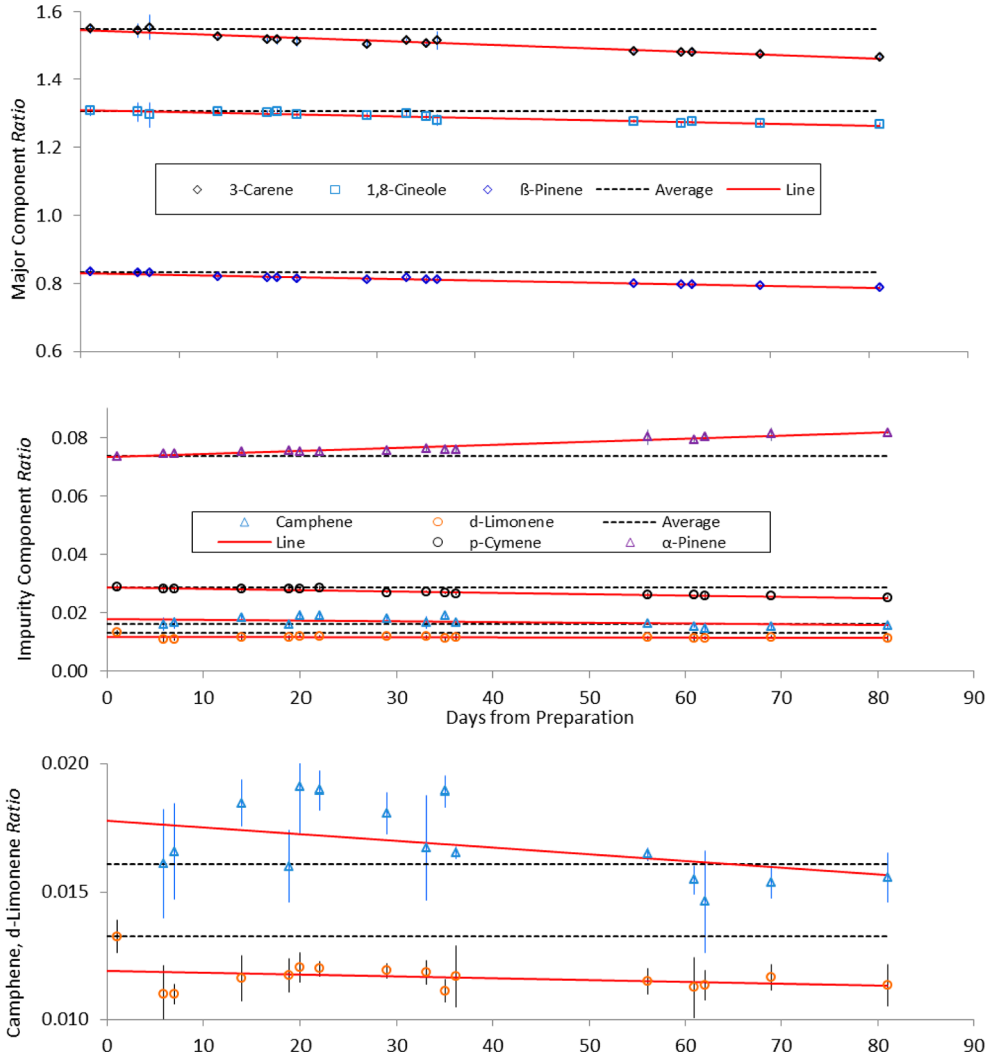

Figure 5. Monoterpene ratios in carbon steel nickel-plated cylinder 4131192Y (CS-Ni-92Y).

carene, 1,8-cineole, and ß-pinene, all indicate a downward trend, suggesting degradation in the gas mixture. The R^2 values between the days and the ratios support these visual observations: 3-carene (0.92), 1,8-cineole (0.87), and ß-pinene (0.78). The impurities in CS-Ni-92Y are shown in the lower panel of Figure 5. Camphene and D-limonene visually appear to be stable, but from an expanded plot at the bottom of Figure 5 a decreasing trend is shown. The α-pinene visually shows a growth rate and *p*-cymene a degradation rate, both supported by R^2 values of 0.94 and 0.90, respectively. These results suggest that α-pinene is the only monoterpene increasing, supporting the ß-pinene to α-pinene theory.[23−25] All other monoterpenes in this gas mixture, including the small impurities, are decreasing, which suggest that they are being adsorbed onto the cylinder wall. It is also possible, and likely, that the α-pinene is adsorbing but may not be verified until all of the ß-pinene is either adsorbed on the cylinder walls or reacted to α-pinene. The camphene, D-limonene, and *p*-cymene showed growth rates in Aculife IV aluminum cylinders containing ß-pinene.[22] On the basis of that study, it is possible that the ß-pinene is chemical transforming into these monoterpenes, and they in turn are adsorbed on the cylinder walls.

Cylinder 41311187Y (CS-Ni-87Y). Mixture CS-Ni-87Y containing ß-pinene, 1,8-cineole, 3-carene, α-pinene, and *n*-hexane internal standard was prepared 33 days after CS-Ni-92Y, and initially analyzed 1 day after preparation followed by 10 more random analyses over a period of 47 days. Three to six consecutive injections were made of the gas mixture on any given day. Supporting Information Table S-5 lists the data and statistics for mixture CS-Ni-87Y with the mean ratios and their U_{95} uncertainties as a function of days from preparation illustrated in Supporting Information Figure S-1. The linear trends for all of the major and minor impurities in this mixture indicate degradation, supported by the large R^2 values listed in Supporting Information Table S-5. While the α-pinene has a relatively small R^2 value of 0.5167 and shallow degradation trend in Figure S-1, it also suggested that the *n*-hexane-normalized α-pinene ratio decreases linearly with time as shown in the expanded plot at the bottom of Figure S-1. These results indicate that, regardless of whether chemical transformation is occurring, every monoterpene in this mixture is likely being adsorbed on the cylinder walls.

Cylinder 41311193Y (CS-NI-93Y). A third mixture, CS-Ni-93Y, was prepared to include ß-pinene, 3-carene, 1,8-cineole, and internal standard *n*-hexane 11 days after the preparation of CS-Ni-87Y. It was initially analyzed 6 days after preparation and

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Table 2. Summary of Mole Fraction, Slope (s), and Rate Calculation for Monoterpenes in Standards

compound	statistics	D646508 (AL-Exp-08)	D646507 (AL-Exp-07)	41311187Y (CS-Ni-87Y)	41311192Y (CS-Ni-92Y)	41311193Y (CS-Ni-93Y)
α-pinene	mole fraction, nmol/mol[a]	12.88 ± 0.26	*0.021*	1.41 ± 0.10	*0.010*	*0.006*
	s (slope)	0.000018	*−0.00001*	−0.000040	*0.00010*	*0.00014*
	i (intercept)	1.1523	*0.0116*	0.1755	*0.0733*	*0.01136*
	rate (nmol/mol/yr)	0.072 ± 0.064	*−0.095*	-0.117	*0.005*	*0.027*
	projected shelf-life, years	3.6		0.9		
β-pinene	mole fraction, nmol/mol[a]		9.91 ± 0.35	5.34 ± 0.43	4.56 ± 0.36	2.57 ± 0.45
	s (slope)		0.00002	−0.00090	−0.00054	−0.00350
	i (intercept)		1.2476	0.6635	0.8304	0.9030
	rate (nmol/mol/yr)		0.058	−2.646	−1.083	−3.638
	projected shelf-life, years		6.0	0.2	0.3	0.1
3-carene	mole fraction, nmol/mol[a]		9.56 ± 0.52	4.10 ± 0.54	9.07 ± 0.53	3.33 ± 0.36
	s (slope)		−0.00002	−0.00072	−0.00105	−0.00884
	i (intercept)		0.7208	0.3292	1.5467	0.7502
	rate (nmol/mol/yr)		−0.097	−3.275	−2.249	−14.332
	projected shelf-life, years		5.4	0.2	0.2	0.0
1,8-cineole	mole fraction, nmol/mol[a]			4.48 ± 0.42	6.63 ± 0.37	2.31 ± 0.47
	s (slope)			−0.00261	−0.00055	−0.00324
	i (intercept)			0.5531	1.3083	0.5123
	rate (nmol/mol/yr)			−7.722	−1.018	−5.336
	projected shelf-life, years			0.1	0.4	0.1
D-limonene	mole fraction, nmol/mol[a]		9.32 ± 0.47	*0.003*	*0.002*	*0.001*
	s (slope)		0.000000	*−0.000020*	*−0.000010*	*0.000000*
	i (intercept)		0.9391	*0.0022*	*0.0119*	*0.0012*
	rate (nmol/mol/yr)		0.000	*−0.010*	*−0.001*	*0.000*
	projected shelf-life, years		infinity			
p-cymene	mole fraction, nmol/mol[a]	*0.008*	10.70 ± 0.31	*0.026*	*0.041*	*0.015*
	s (slope)	*0.00000*	−0.00001	*−0.00001*	*−0.00005*	*0.00001*
	i (intercept)	*0.0044*	1.0653	*0.0035*	*0.0287*	*0.0043*
	rate (nmol/mol/yr)	*0.001*	**−0.037**	*−0.027*	*−0.023*	*0.013*
	projected shelf-life, years		8.4			
camphene	mole fraction, nmol/mol[a]	*0.001*	9.58 ± 0.50	*0.004*	*0.005*	*0.002*
	s (slope)	*0.00000*	0.00000	*−0.00002*	*−0.00001*	*0.00002*
	i (intercept)	*0.0020*	1.4510	*0.0066*	*0.0168*	*0.0029*
	rate (nmol/mol/yr)	*0.000*	0.000	*−0.004*	*−0.001*	*0.005*
	projected shelf-life, years		Infinity			
myrcene	mole fraction, nmol/mol[a]		1.92 ± 0.37			
	s (slope)		0.00002			
	i (intercept)		0.1267			
	rate (nmol/mol/yr)		0.111			
	projected shelf-life, years		3.3			
benzene or n-hexane	mole fraction, nmol/mol[a]	14.74 ± 0.60	14.19 ± 0.57	11.51 ± 0.58	8.82 ± 0.56	4.60 ± 0.53
	s (slope)	0.00002	0.00002	0.0001	0.0002	0.0001
	i (intercept)	14.831	14.1432	4.602	8.808	4.602
	rate (nmol/mol/yr)	0.073 ± 0.040	0.007 ± 0.038	0.037 ± 0.030	0.073 ± 0.030	0.037 ± 0.030
	projected shelf-life, years	8.3	81	14.5	7.7	14.5

[a]Expanded uncertainty of k = 2; 95% confidence interval.

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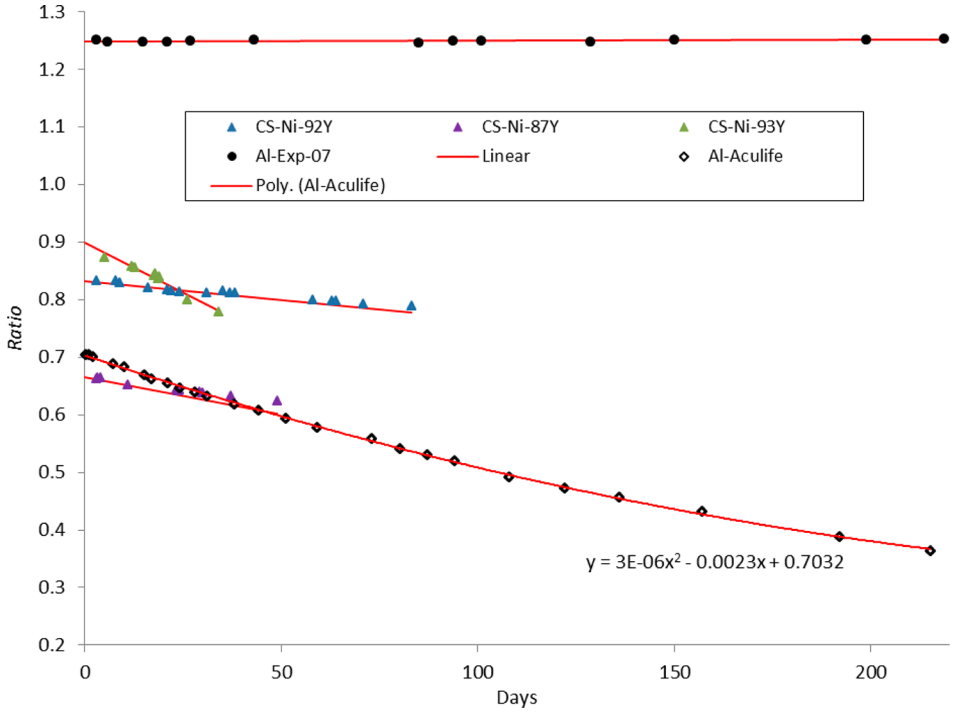

Figure 6. Ratios for β-pinene in CS-Ni-92Y, CS-Ni-87Y, CS-Ni-93Y, Al-Exp-07, and Al-Aculife IV cylinders.

then nine more times randomly over a period of 35 days. The data and statistics are summarized in Supporting Information Table S-6. The linear trends of the ratios and their U_{95} uncertainties as a function of days from preparation are shown in Supporting Information Figure S-2. As was seen in CS-Ni-92Y and CS-Ni-87Y, the summary results and linear trend lines for ß-pinene, 3-carene, and 1,8-cineole suggest that the *n*-hexane-normalized ratios decrease linearly with time. However, unlike mixture CS-Ni-87Y, the monoterpene impurities of α-pinene, *p*-cymene, and camphene show linear growth changes with time, while the D-limonene is unchanged, noting that it is an impurity in this mixture that is right at the limit of detection (0.001 nmol/mol) and very difficult to measure. These results suggest that the ß-pinene chemical transformation is occurring in this particular CS-Ni cylinder. Even though all of the CS-Ni cylinders are essential the same, the nickel plating may not be consistent from cylinder to cylinder, which can result in different adsorption rates.

Rates of Change in Monoterpene Mixtures. Another tool to assist in analysis of the data is to calculate a rate of change (rate) via the following equation:

$$\text{rate} = ((365.25 * MF_{\text{terpene}}) * s)/i \quad (1)$$

where 365.25 is days/yr and MF_{terpene} is the initial mole fraction of the terpene in the gas mixture as calculated by gravimetry, and *s* is the slope and *i* the intercept from the linear regression. Table 2 contains, for each monoterpene, the gravimetric mole fraction, *s*, *i*, the calculated rate change (rate), and the projected time in years before the total rate change exceeds the 95% confidence interval of the uncertainty in the mole fraction. The rates for benzene and *n*-hexane give projected shelf-lives of ≥7.7 years for all mixtures, showing good stability of the internal standard. Assuming a constant linear rate, the α-pinene in Al-Exp-08 calculates out to (0.072 ± 0.064) nmol/mol/yr. This projects 7.2 years of shelf-life before the uncertainty, ±0.52 nmol/mol, in the gravimetric mole fraction of the α-pinene is exceeded. In mixture Al-Exp-07, the projected years are myrcene 3.3, 3-carene 5.4, ß-pinene 6.0, *p*-cymene 8.4, D-limonene 65, and camphene 143. To put these projected shelf-lives in perspective, typical shelf-life for a nonreactive NIST Standard Reference Material (SRM) ranges from 4 to 8 years. Expirations of 2−4 years are typical for reactive specie SRMs such as nitric oxide (NO) and sulfur dioxide (SO_2).

The rates and projected shelf-life for the monoterpenes in the nickel-plated carbon steel cylinders demonstrate that these containers are not suitable for monoterpene gas mixtures for extended storage times. None of the projected shelf-life data showed more than 0.9 years of stability within uncertainty limits of the original mole fraction of the monoterpenes, with most projections at 0.1−0.3 years.

Comparison of ß-Pinene Results in All Cylinder Types. It appears that α- and ß-pinene are the least stable monoterpenes in a gas mixture. Observed stability of α- and ß-pinene in any of the cylinder types studied would suggest that the other monoterpenes would also demonstrate stability. Figure 6 shows the initial ratio and linear trend lines for the cylinder data from this research, as well as that from the previous aluminum Aculife IV (Al-Aculife IV) treated cylinder studied at NIST and showing a second-order polynomial regression.[22] The polynomial regression, and the plot in Figure 6, would indicate that the chemical transformation (and/or adsorption to the cylinder walls) of ß-pinene to other monoterpenes will eventually slow. The ß-pinene shows losses in the CS-Ni-*x* and Al-Aculife IV cylinders, while demonstrating stability for 1 year in the Al-Experis-treated cylinder. Additionally, the ß-pinene estimated value for the *s* is negative and significantly different in each of the three CS-Ni-*x* cylinders as is the rate of change: −0.117 nmol/mol/yr for CS-Ni-87Y, −1.083 nmol/mol/yr for CS-Ni-92Y, and −3.638 nmol/mol/year for CS-Ni-93Y. With time these trend lines may actually become second-order polynomials, as with the aluminum Aculife IV cylinder, as there are less ß-pinene molecules and

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active cylinder sites available for adsorption or transformation, and the negative rates will decrease. These results suggest that the nickel treatment applied to the cylinder walls is not consistent from cylinder to cylinder. We theorize that the nickel coating may not be thick enough, leaving uncovered active sites available for ß-pinene molecule adsorption. The stable results in the proprietary Experis-treated aluminum cylinder, D646507 (Al-Exp-07) rate of 0.058 nmol/mol/yr, suggest that the coating is close to 100% complete and may actually prevent aluminum oxide from permeating into the cylinder and gas mixture.

CONCLUSIONS

Results of this research and literature suggesting chemical reactions of monoterpenes indicate that a key to developing a cylinder/treatment package for stable monoterpene gas mixtures requires that oxides be blocked from permeating into the cylinder and gas mixture. The presence or absence of oxides in the cylinders was not confirmed, but it may be possible to develop a test or analytical method to check for their presence. One of the two packages tested, nickel-plated carbon steel, failed to provide a suitable container for a stable gas mixture of the key monoterpenes studied. However, this research indicates that a very promising cylinder package, an aluminum cylinder with a proprietary Experis-treatment, has been identified. We speculate that the Experis-treatment is efficient in actually blocking oxides from permeating into the gas mixture. Major monoterpenes of interest, in particular α- and ß-pinene, have been stable, individually, in nitrogen for 362 and 287 days, respectively, within uncertainties, in this container package, with a projected stability of 3−10+ years. While a gas mixture of monoterpenes has shown good stability in two cylinders studied, additional mixtures in the aluminum Experis-treatment package must be studied to ensure consistency from cylinder to cylinder. Additionally, neither of the gas mixtures in the aluminum Experis cylinder contained both α-and ß-pinene together. Research of additional monoterpene gas mixtures in this package that contain both pinenes is currently under way to assess their consistency when combined. Currently, however, several calibration gas mixtures that contain either the α-pinene or the ß-pinene (but not together) could be provided to the WMO/GAW-VOC and the community in general, to calibrate the instrumentation used to obtain atmospheric measurements. Only future research, where both of the pinenes included in a single gas mixture result in stability, will allow for a single calibration mixture containing many monoterpenes.

ASSOCIATED CONTENT

Supporting Information

Table S-1: Mole fraction of impurities in pure terpene reagents. Table S-2: Monoterpene statistics in aluminum B-treatment cylinder in D646508. Table S-3: Monoterpene statistics in aluminum B-treatment cylinder in D646507. Table S-4: Monoterpene statistics in carbon steel nickel-plated cylinder 4131192Y. Table S-5: Monoterpene statistics in carbon steel nickel-plated cylinder 4131187Y. Table S-6: Monoterpene statistics in carbon steel nickel-plated cylinder 4131193Y. Figure S-1: Monoterpene ratios in carbon steel nickel-plated cylinder 4131187Y. Panel a: α-pinene, β-pinene, 1,8-cineole, and 3-carene. Panel b: camphene, D-limonene, p-cymene. Figure S-2: Monoterpene ratios in carbon steel nickel-plated cylinder 4131193Y. Panel a: β-pinene, 1,8-cineole, and 3-carene. Panel b: α-pinene, camphene, D-limonene, p-cymene. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Haagen-Smit, A. J. *Ind. Eng. Chem.* **1952**, *44*, 1342−1346.
(2) Plass-Düelmer, C.; Michl, K.; Ruf, R.; Berresheim, H. *J. Chromatogr.* **2002**, *953*, 175−197.
(3) Atkinson, R.; Arey, J. *Chem. Rev.* **2003**, *103*, 4605−4638.
(4) Grabmer, W.; Kreuzwieser, J.; Wisthaler, A.; Cojocariu, C.; Graus, M.; Rennenberg, H.; Steigner, D.; Steinbrecher, R.; Hansel, A. *Atmos. Environ.* **2006**, *40*, S128−S137.
(5) Guenther, A. *Reactive Hydrocarbons in the Atmosphere*; Academic Press: San Diego, CA, 1999; pp 97−118.
(6) Kesselmeier, J.; Staudt, M. *J. Atmos. Chem.* **1999**, *33*, 23−88.
(7) Smiatek, G.; Steinbrecher, R. *Atmos. Environ.* **2006**, *40*, S166.
(8) Steinbrecher R. Ph.D. Thesis, University of Munchen, Germany, 1989.
(9) Schürmann, W.; Ziegler, H.; Kotzias, D.; Schönwitz, R.; Steinbrecher, R. *Naturwissenschaften* **1993**, *80*, 276−278.
(10) Steinbrecher, R.; Hauff, K.; Hakola, H.; Rössler, J. Biogenic voc emissions and photochemistry in boreal regions of europe. Air Pollution Research Report no. 70, 1999.
(11) Rasmussen, R.; Khalil, M. *J. Geophys. Res.* **1988**, *D2*, 1417−1421.
(12) Kuhn, U.; Rottenberger, S.; Biesenthal, T.; Wolf, A.; Schebeske, G.; Ciccioli, P.; Brancaleoni, E.; Frattoni, M.; Tavares, R.; Kesselmeier, J. *Global Change Biol.* **2004**, *10*, 663−682.
(13) Baker, B.; Bai, J.; Johnson, C.; Cai, Z.; Li, Q.; Wang, Y.; Guenther, A.; Greenberg, J.; Klinger, L.; Geron, C.; Rasmussen, R. *Atmos. Environ.* **2005**, *39*, 381−390.
(14) Winters, A.; Adams, M.; Bleby, T.; Rennenberg, H.; Steigner, D.; Steinbrecher, R.; Kreuzwieser, J. *Atmos. Environ.* **2009**, *43*, 3035−3043.
(15) Wang, X.; Wu, T. *Environ. Sci. Technol.* **2008**, *42*, 3265−3270.
(16) Räisänen, T.; Ryyppö, A.; Kellomäki, S. *Agric. For. Meteorol.* **2009**, *149*, 808−819.
(17) Papiez, M.; Potosnak, M.; Goliff, W.; Guenther, A.; Matsunaga, S.; Stockwell, R. *Atmos. Environ.* **2009**, *43*, 4109−4123.
(18) Steinbrecher, R.; Smiatek, G.; Köble, R.; Seufert, G.; Theloke, J.; Hauff, K.; Ciccioli, P.; Vautard, R.; Curci, G. *Atmos. Environ.* **2009**, *43*, 1380−1391.
(19) Holzke, C.; Dindorf, T.; Kesselmeier, J.; Kuhn, U.; Koppmann, R. *J. Atmos. Chem.* **2006**, *55*, 81−102.

(20) Batterman, S.; Zhang, G.; Baumann, M. *Atmos. Environ.* **1998**, *32*, 1647−1655.
(21) Pollmann, J.; Ortega, J.; Helmig, D. *Environ. Sci. Technol.* **2005**, *39*, 9620−9629.
(22) Rhoderick, G. *Anal Bioanal. Chem.* **2010**, *398*, 1417−1425.
(23) Foletto, E.; Valentini, A.; Probst, L.; Porto, L. *Lat. Am. Appl. Res.* **2002**.
(24) Allahverdiev, A.; Gündüz, G.; Murzin, D. *Ind. Eng. Chem. Res.* **1998**, *37*, 2373−2377.
(25) Findik, S.; Gündüz, G. *J. Am. Oil Chem. Soc.* **1997**, *74*, 114−1151.
(26) Rhoderick, G.; Zielinski, W.; Miller, W. *Environ. Sci. Technol.* **1993**, *27*, 2849−2854.
(27) Rhoderick, G.; Dorko, W. *Environ. Sci. Technol.* **2004**, *38*, 2685−2692.
(28) Rhoderick, G. *Environ. Sci. Technol.* **1995**, *29*, 2797−2800.
(29) Rhoderick, G. *Fresenius' J. Anal. Chem.* **1997**, *359*, 477−483.
(30) NIST (1993) SRM 1800 − Non-methane Hydrocarbon Compounds in N_2. Certificate accessible through http://ts.nist.gov/MeasurementServices/ReferenceMaterials/index.cfm.
(31) NIST (2004) SRM 1800b − Eighteen Non-methane Hydrocarbon Compounds in N_2. Certificate accessible through http://ts.nist.gov/MeasurementServices/ReferenceMaterials/index.cfm.
(32) Rhoderick, G.; Duewer, D.; Ning, L.; DeSirant, K. *J. Anal. Chem.* **2010**, *82*, 859−867.
(33) Rhoderick, G. *Anal. Bioanal. Chem.* **2005**, *383*, 98−106.

dx.doi.org/10.1021/ac400324v | *Anal. Chem.* 2013, 85, 4675−4685