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# Suitability of selected free-gas and dissolved-gas sampling containers for carbon isotopic analysis

P. Eby<sup>1\*</sup>, J. J. Gibson<sup>1,2</sup> and Y. Yi<sup>1,2</sup>

<sup>1</sup>Alberta Innovates Technology Futures, 3-4476 Markham Street, Victoria, BC V8Z 7X8, Canada

<sup>2</sup>Department of Geography, University of Victoria, PO Box 1700 STN CSC, Victoria, BC V8W 2Y2, Canada

**RATIONALE:** Storage trials were conducted for 2 to 3 months using a hydrocarbon and carbon dioxide gas mixture with known carbon isotopic composition to simulate typical hold times for gas samples prior to isotopic analysis. A range of containers (both pierced and unpierced) was periodically sampled to test for  $\delta^{13}\text{C}$  isotopic fractionation.

**METHODS:** Seventeen containers were tested for free-gas storage (20°C, 1 atm pressure) and 7 containers were tested for dissolved-gas storage, the latter prepared by bubbling free gas through tap water until saturated (20°C, 1 atm) and then preserved to avoid biological activity by acidifying to pH 2 with phosphoric acid and stored in the dark at 5°C. Samples were extracted using valves or by piercing septa, and then introduced into an isotope ratio mass spectrometer for compound-specific  $\delta^{13}\text{C}$  measurements.

**RESULTS:** For free gas, stainless steel canisters and crimp-top glass serum bottles with butyl septa were most effective at preventing isotopic fractionation (pierced and unpierced), whereas silicone and PTFE-butyl septa allowed significant isotopic fractionation. FlexFoil and Tedlar bags were found to be effective only for storage of up to 1 month. For dissolved gas, crimp-top glass serum bottles with butyl septa were again effective, whereas silicone and PTFE-butyl were not. FlexFoil bags were reliable for up to 2 months.

**CONCLUSIONS:** Our results suggest a range of preferred containers as well as several that did not perform very well for isotopic analysis. Overall, the results help establish better QA/QC procedures to avoid isotopic fractionation when storing environmental gas samples. Recommended containers for air transportation include steel canisters and glass serum bottles with butyl septa (pierced and unpierced). Copyright © 2015 John Wiley & Sons, Ltd.

Appropriate storage of samples is a key step in making reliable stable isotope measurements, but is frequently overlooked. It is generally acknowledged that gas containers should be gas tight, non-diffusive and non-reactive to avoid loss of sample, which can be accompanied by isotopic fractionation. Furthermore, the appropriateness of a specific container can depend on the analyte in question – effectively holding some compounds but not others. Previous studies have examined the efficacy of free-gas containers for sample storage prior to gas concentration analyses including effects such as piercing of septa and air transportation.<sup>[1–3]</sup> Limited work has been conducted to date on  $\delta^{13}\text{C}$  fractionation effects in free-gas mixtures, with much of the work focused on  $\text{CO}_2$ .<sup>[4–9]</sup> Dissolved-gas containers were discussed by Weimer and Lee<sup>[10]</sup> although no study to our knowledge has yet examined isotopic effects.

This study tested the effectiveness of multiple containers (steel canisters, gas sample bags, crimp-top glass serum bottles, and threaded glass vials) and septa types and thicknesses (butyl rubber, silicone rubber, polytetrafluoroethylene (PTFE)) to store samples containing a mixture of carbon dioxide ( $\text{CO}_2$ ), methane, ethane, propane, and butane while preserving  $\delta^{13}\text{C}$  isotopic signatures. Both free-gas and

dissolved-gas samples were considered. In addition, the effects of air travel, piercing of septa by needles, and preservation (for dissolved-gas samples) were explored.

## EXPERIMENTAL

A selection of industry-standard sample containers for both free gas (10 types) and dissolved gas (7 types) was gathered, and charged to 1 atmosphere with a standard gas containing known concentrations of methane, ethane, propane, i-butane, n-butane, and  $\text{CO}_2$  (Table 1). The reproducibility for both free gas and dissolved gas is estimated as the standard deviation of repeat measurements (Table 1).

Dissolved-gas samples were prepared by bubbling this gas mixture through tap water that had been left to equilibrate with air (20°C, 1 atmosphere). Once saturated, as confirmed by baseline measurements, the water was added to containers. To ensure that no biological activity would change the isotopic composition of the dissolved gases, the samples were stored in the dark at 5°C. Samples were also acidified to pH 2 with phosphoric acid to provide additional preservation.<sup>[11]</sup> The pH change affected the  $\text{CO}_2$  solubility, but the  $\delta^{13}\text{C}$  value for dissolved  $\text{CO}_2$  is already made unreliable by fractionation when coming out of solution.<sup>[12]</sup> As a comparison, some samples were stored with no preservative action taken.

\* Correspondence to: P. Eby, Alberta Innovates Technology Futures, 3-4476 Markham Street, Victoria, BC V8Z 7X8, Canada.  
E-mail: peby@uvic.ca

**Table 1.** Gas standard composition

Component	Abbreviation	% <sup>a</sup>	$\delta^{13}\text{C}$ value (‰)	Reproducibility <sup>b</sup> (%)	
				Free gas	Dissolved gas
Methane	C1	83	-42.4	0.09	0.13
Ethane	C2	5	-30.1	0.14	0.13
Propane	C3	3	-33.1	0.13	0.26
i-Butane	iC4	2	-30.2	0.19	0.53
n-Butane	nC4	2	-33.5	0.18	0.39
Carbon dioxide	CO2	5	-32.0	0.19	0.28

<sup>a</sup>% in free gas.  
<sup>b</sup>standard deviation of repeats.

Tables 2 (free gas) and 3 (dissolved gas) show the various sample containers used. Figures 1 and 2 show images of sample containers and septa used in this study. All septa that included a Teflon layer were utilized with the Teflon layer in direct contact with the sample.

Some containers with septa were also included twice to test for possible effects of piercing of the septa. Each pierced septum was perforated twice with a B-D Precision Glide 21 gauge disposable needle. Data for some containers is available only for pierced septa, due to the impracticality of

loading a sample without using needles. Dissolved-gas samples were all prepared with unpierced septa, except for thick butyl septa (D04) for which piercing was required to insert the septum into the bottle.

Sufficient numbers were prepared to allow measurement of a set of samples at approximately 0.5, 1, 2 and 3 months. Each data point required a separate container, except for free gas in canisters and in bags. Because they have valves, sub-samples for multiple time points can be collected from these containers without affecting the integrity of the remaining sample.

**Table 2.** Containers used for free gas

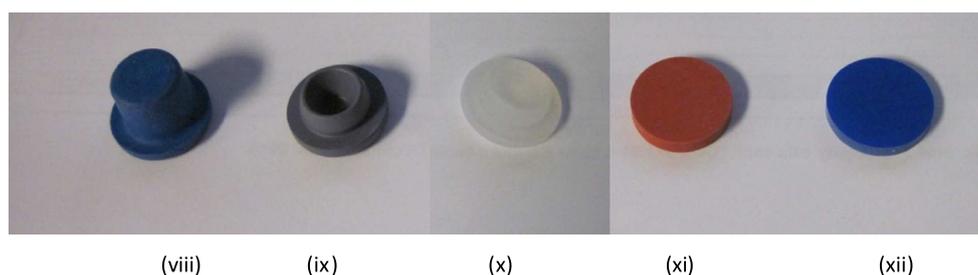
Container	Size	Brand	Part #	Septa material	Min thickness (mm)	Pierced	Brand	Septa Part #	
F01	Silcosteel canister (i)	400 mL	Restek	24193	none				
F02	Tedlar bag (ii)	1 L	SKC	232-01	butyl rubber	2	N		
F03	Tedlar bag (ii)	1 L	SKC	232-01	butyl rubber	2	Y		
F04	FlexFoil Plus bag (iii)	1 L	SKC	252-01	silicone rubber	3	N		
F05	FlexFoil Plus bag (iii)	1 L	SKC	252-01	silicone rubber	3	Y		
F06	Vacutainer (iv)	16 mL	Tyco Healthcare	301819	butyl rubber	6	Y		
F07	Exetainer (v)	12 mL	Labco	938W	butyl rubber	3	Y		
F08	EPA vial (vi)	40 mL	Fisher Scientific	360993496	PTFE-silicone	3	Y		
F09	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	butyl rubber (viii)	13	N	Bellco Glass	2048-11800
F10	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	butyl rubber (viii)	13	Y	Bellco Glass	2048-11800
F11	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	butyl rubber (ix)	3	N	Chrom Spec	C714396M
F12	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	butyl rubber (ix)	3	Y	Chrom Spec	C714396M
F13	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	silicone rubber (x)	3	N	Sigma Aldrich	27235-U
F14	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	silicone rubber (x)	3	Y	Sigma Aldrich	27235-U
F15	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	PTFE-butyl rubber (xi)	3.1	N	Sigma Aldrich	27233
F16	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	PTFE-butyl rubber (xi)	3.1	Y	Sigma Aldrich	27233
F17	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	PTFE-silicone (xii)	2.7	N	Sigma Aldrich	508608

**Table 3.** Containers used for dissolved gas

	Container	Size	Brand	Part #	Septa material	Min thickness (mm)	Pierced	Brand	Part #
D01	Tedlar bag (ii)	1 L	SKC	232-01	butyl rubber	2	N		
D02	FlexFoil Plus bag (iii)	1 L	SKC	252-01	silicone rubber	3	N		
D03	EPA vial (vi)	40 mL	Fisher Scientific	360993496	PTFE-silicone	3	N		
D04	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	butyl rubber (viii)	13	Y	Bellco Glass	2048-11800
D05	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	butyl rubber (ix)	3	N	Chrom Spec	C714396M
D06	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	silicone rubber (x)	3	N	Sigma Aldrich	27235-U
D07	Serum bottle crimp top (vii)	100 mL	Wheaton	223747	PTFE-butyl rubber (xi)	3.1	N	Sigma Aldrich	27233



**Figure 1.** Containers tested in this study, including (i) steel canister (Restek, State College, PA, USA), (ii) Tedlar bag (SKC, Eighty Four, PA, USA), (iii) FlexFoil bag (SKC), (iv) Vacutainer (Tyco Healthcare, Mansfield, MA, USA), (v) Exetainer (Labco, Lampeter, UK) with screw-top lid, (vi) EPA vial (Fisher Scientific, Pittsburgh, PA, USA) with screw-top lid, and (vii) glass serum bottle (Wheaton, Millville, NJ, USA) with crimp-top lid.



**Figure 2.** Septa used on crimp-top serum bottles, including (viii) 13-mm butyl rubber (Bellco Glass, Vineland, NJ, USA), (ix) 3-mm butyl rubber (Chromatographic Specialties, Brockville, ON, CA), (x) 3-mm silicone rubber (Sigma Aldrich, St. Louis, MO, USA), (xi) 3.1-mm PTFE-butyl rubber (Sigma Aldrich), and (xii) 2.7-mm PTFE-silicone (Sigma Aldrich).

Additional free gas samples were prepared to test for any measurable effect of air transportation. This trial was designed to answer speculation that errors could be introduced during flight due to the change in air pressure or temperature. These samples were shipped by FedEx air from Victoria to Calgary, and back to Victoria, before measurement (a round trip of approximately 1400 km). However, there is no information

available as to whether the samples travelled in a pressurized or unpressurized cabin, or what temperatures they were subjected to during transport.

All measurements were performed on a MAT 253 isotope ratio mass spectrometer, interfaced to a Trace GC Ultra gas chromatograph, a GC IsoLink and a ConFlo IV interface (all from Thermo Fisher Scientific, Waltham, MA, USA). On

each analysis day, multiple measurements of the standard gas mix described in Table 1 were made such that all isotopic ratios could be reported as deviations from this starting point, or delta over baseline.<sup>[13]</sup>

$$\Delta^{13}\text{C} = \delta^{13}\text{C}_{\text{sample}} - \delta^{13}\text{C}_{\text{reference}}$$

The standard gas mix, kept in a Praxair T sized high-pressure cylinder, was assumed to be isotopically stable over the time span of these experiments. The reproducibility for replicate free-gas measurements was better than  $\pm 0.2\%$  (Table 1).

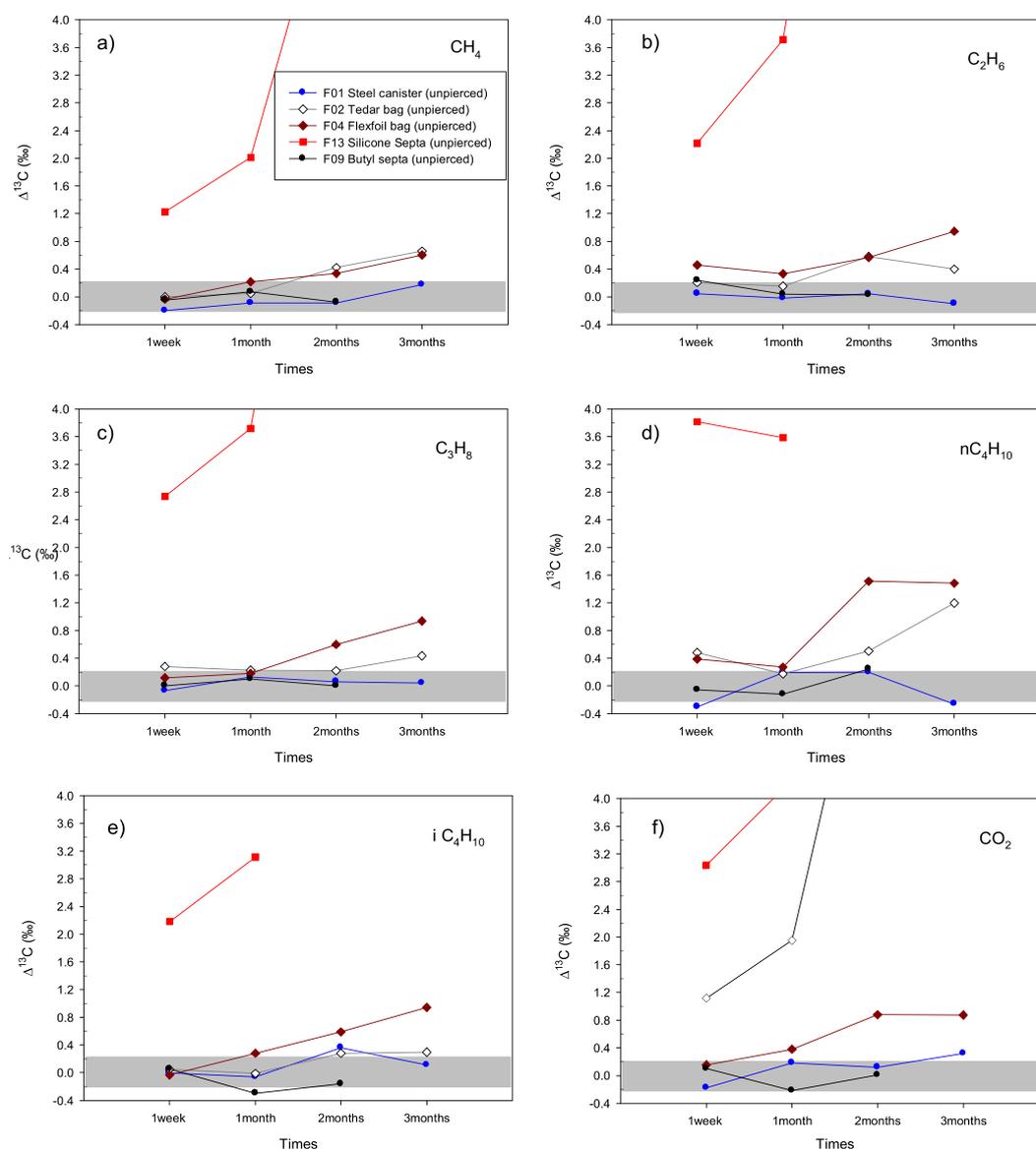
Dissolved-gas samples were measured by introducing a headspace of 30 mL helium. After agitating and allowing a minimum of 5 min for equilibration, the headspace could then be sampled and introduced as a gas sample.

The reproducibility of dissolved-gas measurements varied by component, but was typically less than  $\pm 0.5\%$  (Table 1).

Gas samples (and headspace of dissolved samples) were introduced into the mass spectrometer by flushing a six-port sampling valve with an appropriately sized sample loop. This fed into a 60 m GSQ porous layer open tube column (0.32 mm ID; Agilent Technologies, Santa Clara, CA, USA). The column was temperature programmed to allow measurement of C1 to C4 components.

## RESULTS

The results are tabulated in Appendices A and B. Key results are plotted and described below.

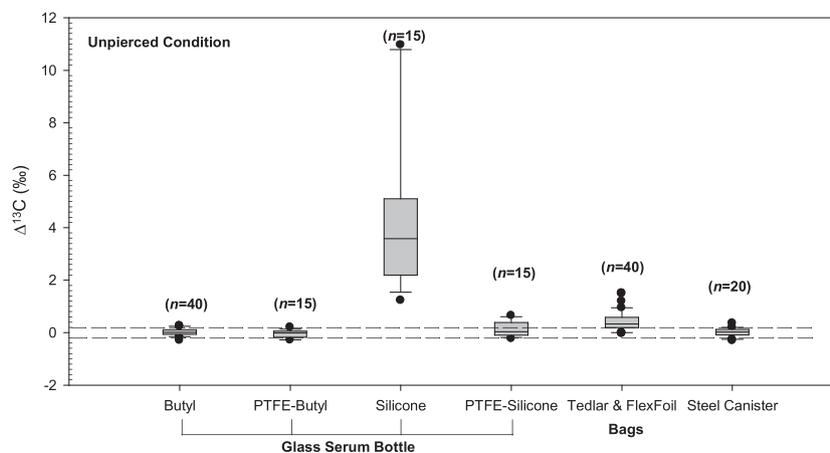


**Figure 3.**  $\Delta^{13}\text{C}$  of gas samples stored in various types of container without piercing over a period of 3 months. The gas isotope measurements include  $\Delta^{13}\text{C}$  for (a) methane ( $\text{CH}_4$ ), (b) ethane ( $\text{C}_2\text{H}_6$ ), (c) propane ( $\text{C}_3\text{H}_8$ ), and (d) n-butane ( $\text{nC}_4\text{H}_{10}$ ), i-butane ( $\text{iC}_4\text{H}_{10}$ ), and carbon dioxide ( $\text{CO}_2$ ). The shading indicates the accuracy of the free gas technique,  $\pm 0.2\%$ .

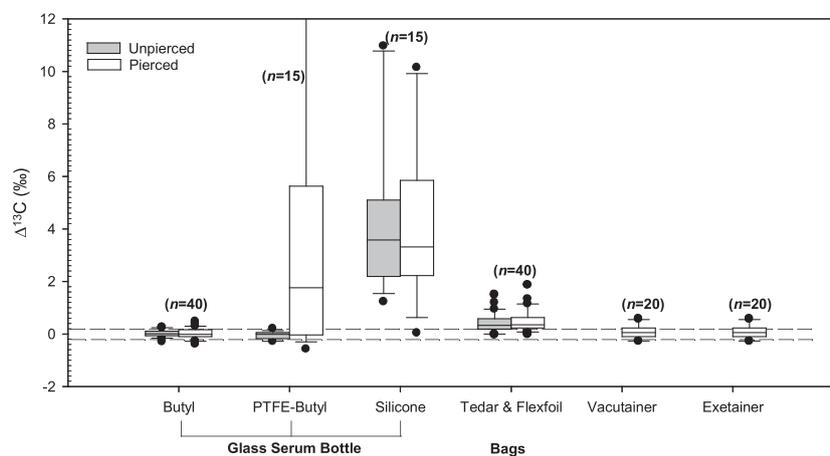
### Free-gas containers without piercing

Piercing of septa is a common practice with some containers either during collection or during the process of taking a sample from the container. We first examined the effectiveness of storing gas samples for isotope analysis in containers that did not have pierced septa. These containers included: glass serum bottles with several types of lids (F09-butyl-thick, F11-butyl-thin, F13-silicone and F15-PTFE-butyl), steel canister (F01), Tedlar bags (F02), and FlexFoil bags (F04). Isotopic results are shown for methane (Fig. 3(a)), ethane (Fig. 3(b)), propane (Fig. 3(c)), n-butane (Fig. 3(d)), i-butane (Fig. 3(e)) and CO<sub>2</sub> (Fig. 3(f)) over the 3-month trial. Note that for glass serum bottles, results are only shown for silicone (F13) and thick butyl rubber septa (F09); thin butyl (F11) and PTFE-butyl septa (F15) behaved similarly to F09. In general for gas components, <sup>13</sup>C was found to become more enriched over time (see Fig. 3). This drift is attributed mainly to preferential

escape of the light isotopic species of gas molecules compared with the heavy isotopic species by diffusion from the containers over time. There is likely to be little in the way of an isotopic effect related to ambient air as gas concentrations are very low (by orders of magnitude) compared with the reference gas. For interest, we note that the isotopic composition of methane in outside air is expected to be depleted in <sup>13</sup>C relative to the gas standard (e.g. methane in air at the earth's surface is close to -47.1 ‰<sup>[14]</sup>) and the δ<sup>13</sup>C value in the higher order gases (ethane, propane, butane) in outside air is expected to be similar to that of the gas standard (in the range -27 ‰ to -33 ‰ for propane and n-butane in urban environments<sup>[15]</sup>). In terms of efficacy, the steel canister (F01) and glass serum bottles with butyl rubber septa (F09 and F11) were found to be the most effective for storing all the gases, with only minor fractionation beyond 0.2 ‰ noted for the δ<sup>13</sup>C value of n-butane (Fig. 3(d)). Tedlar and FlexFoil bags (F02 and F04, respectively) tended to be effective within



**Figure 4.** Comparative summary of  $\Delta^{13}\text{C}$  in C1 to C4 gases for storage in glass serum bottles, bags, steel canister and EPA vial, all under unpierced conditions. Dashed lines indicate the upper and lower limits of accuracy of the analytical technique and n refers to number of analyses over the 3-month trial.



**Figure 5.** Comparative summary of  $\Delta^{13}\text{C}$  in C1 to C4 gases for storage in selected containers under both pierced and unpierced conditions. Dashed lines indicate the accuracy of the free-gas technique and n refers to number of analyses over the 3-month trial.

0.2 ‰ for methane for storage times up to 1 month but substantial changes were noted for longer storage times and higher order gases. Tedlar bags (F02) were found to allow significant  $\delta^{13}\text{C}$  fractionation of  $\text{CO}_2$ . The glass serum bottle with silicone septa (F13) was found to be entirely unreliable for all gases and storage times.

In an effort to simplify the visual comparison of various containers, we created box plots integrating the results from C1 to C4 gases for each container or septum type (Fig. 4). Note that  $\text{CO}_2$  was not included as more variable results were generally obtained. Note also that good performance for free-gas storage is considered to be within about 0.2 ‰ for all gas species (see dashed line, Fig. 4). Overall, it is clear that silicone septa (F13) are the least effective for storage, and that Tedlar (F02) and FlexFoil bags (F04) tend to allow more significant fractionation than ideal containers such as steel canister (F01) or glass serum bottles with butyl/PTFE-butyl septa (F9, F11, F15) (Fig. 4). PTFE-silicone septa (F17) were also found to be better than silicone alone, but less than ideal for C1 to C4 (Fig. 4) and poor for  $\text{CO}_2$  (not shown).

### Free-gas containers with pierced septa

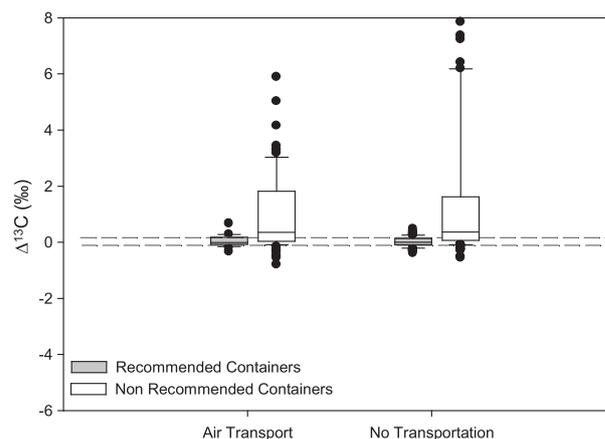
Six containers with pierced septa were also examined, four of which were tested with both pierced and unpierced septa. The other two, Vacutainers (F06) and Exetainers (F07), would be difficult to routinely use without piercing to introduce the sample. Box plots comparing the performance of the various containers over the 3-month trial are shown in Fig. 5. Overall, we see that the Vacutainer (F06) and Exetainer (F07) performed well, as did butyl rubber septa (F10 and F12). However, several containers show poorer performance when pierced including silicone (F14) and PTFE-butyl septa (F16). While Tedlar (F03) and FlexFoil bags (F05) did not appear to worsen after piercing, they do show slight fractionation compared with ideal containers particularly after the first month. This is a sign that the bags may be semi-permeable to the lighter isotopic species of gases.

### Effect of air transport in free-gas containers

The effect of air transport on free-gas samples was tested for a variety of containers. Based on our results we can recommend several types of containers that are less affected by air transport including: steel canister (F01) and glass serum bottles, both pierced and unpierced with thin and thick butyl septa (F9, F10, F11, F12). As shown in Fig. 6, these containers perform better than non-recommended containers (which include the remaining 12 types).

### Dissolved-gas containers

In general dissolved gas was found to be more difficult to store without fractionation, which is attributed to biological activity especially when samples were not preserved. Isotopic results are shown for methane (Fig. 7(a)), ethane (Fig. 7(b)), propane (Fig. 7(c)), n-butane (Fig. 7(d)), i-butane (Fig. 7(e)) and  $\text{CO}_2$  (Fig. 7(f)). Similar to the free-gas experiments, the gases were generally found to become more  $^{13}\text{C}$  enriched over time, which is also attributed to preferential escape of the light isotopic species of the various gases by diffusion from the containers, or to preferential consumption through biological processes.



**Figure 6.** Summary of  $\Delta^{13}\text{C}$  after air transportation. The results are also categorized into recommended container (F01 – steel canister; F09 – glass serum bottle with unpierced thick butyl lid; F10 – glass serum bottle with thick pierced butyl lid; F11 – glass serum bottle with unpierced thin butyl lid; F12 – glass serum bottle with thin pierced butyl lid) and non-recommended containers (other 12 types of containers tested in this study), to compare the effect of air transportation.

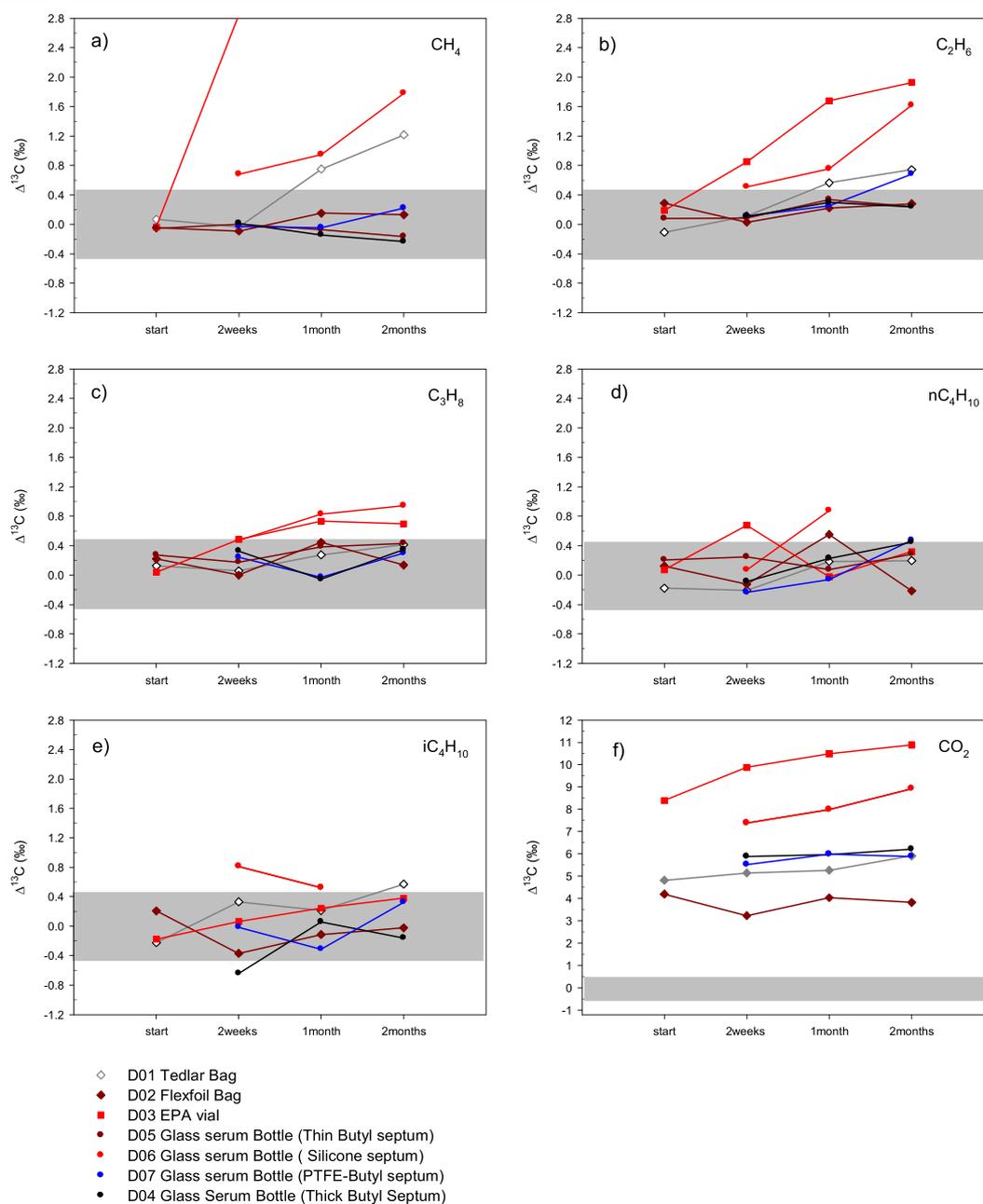
In terms of efficacy, glass serum bottles with thick and thin butyl septa (D04 and D05) and FlexFoil bags (D02) performed well over 2-month storage periods, particularly for methane whose  $\delta^{13}\text{C}$  value remained within approx.  $\pm 0.2$  ‰ of that of the gas standard (Fig. 7(a)). Higher order gases were found to be more difficult to store (Figs. 7(b)–7(e)). The best containers for storage of higher order gases were again glass serum bottles with butyl septa (D04 and D05) and FlexFoil bags (D02) although more variability in  $\delta^{13}\text{C}$  values was noted than when they were used for free-gas storage ( $\sim 0.5$  ‰; Fig. 8).

Containers that did not perform well included EPA vials (D03), glass serum bottles with silicone or PTFE-silicone septa (D06 and D07) and Tedlar bags (D01) (Fig. 8). No containers performed well for the analysis of  $\text{CO}_2$  (Fig. 7(f)) as this is strongly influenced by acidification and by carbonate equilibria in solution.<sup>[12]</sup> The effect of preservatives on dissolved gas stored in glass serum bottles, shown to be a good container for storing free-gas samples, is shown in Fig. 9.

## DISCUSSION

Some free-gas containers such as the steel canister (F01) performed very well, showing no drift in  $\delta^{13}\text{C}$  values over time and no adverse effect from air transportation. The glass serum bottle with thick and thin butyl rubber septa (F09, F10, F11, F12) showed equally good results, both pierced and unpierced. In fact, the values for both the steel canister and the serum bottle with thick butyl septa remained within 0.2 ‰ of that of the gas standard even after an extended period of 11 months.

The results for the Vacutainer (F06) and Exetainer (F07) are essentially consistent with that of butyl rubber septa. Storage of the gas components appears effective, but the pierced septa may be showing small fractionations from air transport. It



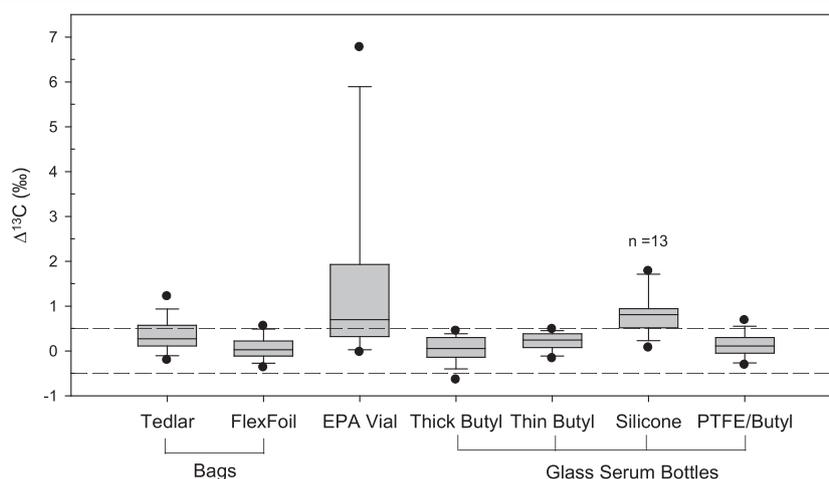
**Figure 7.**  $\Delta^{13}\text{C}$  of dissolved-gas samples stored in various types of containers over a period of 2 months. The gas isotope measurements include  $\Delta^{13}\text{C}$  for (a) methane ( $\text{CH}_4$ ), (b) ethane ( $\text{C}_2\text{H}_6$ ), (c) propane ( $\text{C}_3\text{H}_8$ ), (d) n-butane ( $\text{nC}_4\text{H}_{10}$ ), (e) i-butane, and (f) carbon dioxide ( $\text{CO}_2$ ). The shading indicates the accuracy of the dissolved-gas technique,  $\pm 0.5$  ‰.

could be that the holes left by needle piercing allow leaking and fractionation if the butyl rubber is too thin: the results for F15 versus F16 (PTFE-butyl rubber septa, unpierced and pierced; see also Fig. 5) clearly show that this can happen for thin septa. Based on preservation of nitrous oxide concentrations, Glatzel and Well<sup>[1]</sup> concluded that Exetainers performed better than crimp-top vials at preservation under simulated air transport conditions, although they used natural rubber septa in contrast to the butyl rubber septa used in our trials. Butyl rubber used here (either bromobutyl (F11 and F12) or chlorobutyl (F09 and F10), which have equivalent permeability properties) may be up to ten-fold less

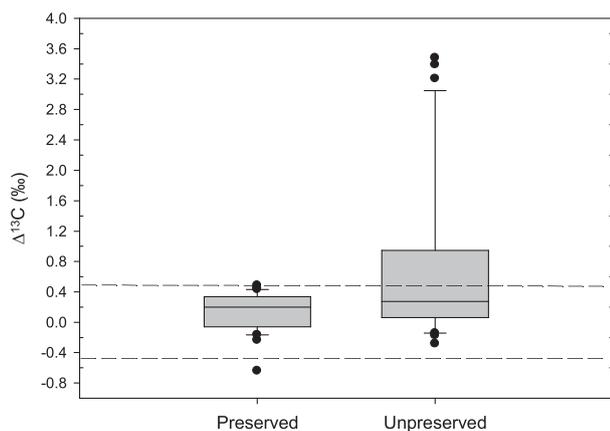
permeable than natural rubber.<sup>[16]</sup> We classify Exetainers (F08) as non-recommended containers due to fractionation of up to 0.5 ‰ in our air transport trials.

Containers using silicone septa performed poorly. The EPA vial (F08) with a pierced PTFE-silicone septum, and serum vials with pierced or unpierced pure silicone septa (F13 and F14) or with unpierced PTFE-silicone septa (F17), all quickly showed large fractionations, although the effect is not as severe for F17.

The fact that F15, which is the butyl rubber analogue to F17, performed well suggests that the PTFE layer in these septa is somewhat permeable to the target gases, and that



**Figure 8.** Comparative summary of  $\Delta^{13}\text{C}$  in C1 to C4 dissolved gases for storage in glass serum bottles, bags, and EPA vial. Dashed lines indicate the accuracy of the dissolved-gas technique ( $\pm 0.5$  ‰). Note the number of analyses  $n = 15$  over the 3-month trial except where specified.



**Figure 9.** Comparison of  $\Delta^{13}\text{C}$  in preserved and unpreserved pairs of samples collected in glass serum bottles with thick (D04) and thin (D05) butyl lids. Data shown are for methane, ethane, propane and n-butane. Range indicates the accuracy of the dissolved-gas technique.

the rubber layer behind it is serving an important role. Butyl rubber seems effective while silicone rubber does not, and adding PTFE does not completely negate the shortcomings of silicone rubber.

The two types of gas bags tested, Tedlar (F02 and F03) and FlexFoil (F04 and F05), behaved similarly with one exception: the Tedlar bags showed a very distinctive fractionation in  $\text{CO}_2$  that worsened over time (see Fig. 3). Otherwise, both bags were able to effectively store samples for up to 1 month – after which a small isotopic drift became evident. Air transport shows no effect, and for both types of bags piercing of the septa shows no distinctive difference.

However, the type of septa used in these bags gives rise to other questions. The Tedlar bags contain butyl septa, while the FlexFoil bags contain silicone septa. Tests on other silicone septa (F13 and F14) showed large fractionations, so why is that not seen for the FlexFoil bag? Could it be that the

compositions of the septa are somehow different, or does the physical structure of the bag limit interaction of the gas with the septa?

In some regards, the results for dissolved-gas samples mirror those of the free-gas samples. Both thin and thick butyl rubber septa on crimp-top serum bottles worked very well (D04, D05), showing no discernable fractionation after 2 months, while silicone rubber septa (D06) allowed fractionation of all hydrocarbon components.

The serum bottle with a PTFE-butyl rubber septum (D07) performed well, but the EPA vial (D03) with a PTFE-silicone rubber septum did not: it shows an increasing fractionation with decreasing carbon number. This supports the conclusion that PTFE allows diffusion of the light gases, and that it is the rubber behind it that provides the real containment.

Comparing dissolved-gas data from the gas sampling bags, the FlexFoil bag (D02) appears superior to the Tedlar bag (D01). After 2 weeks, the Tedlar bag begins to show fractionation, while the FlexFoil bag provides effective storage to at least 2 months. As with the free-gas results, the silicone septum in the FlexFoil bag does not seem to adversely affect its performance.

## CONCLUSIONS

While the results of these tests are not necessarily applicable to other products or manufacturers, there are some general points that can be gleaned.

For concentrations comparable with the gas standard, butyl rubber can provide an effective storage barrier, although thinner septa can be compromised by piercing with a needle. Silicone rubber should be avoided, as it allows significant fractionation. A PTFE layer on a septum does not appear to adequately overcome any shortcomings of the rubber to which it is attached.

The steel canister tested also worked very well. A FlexFoil bag was effective only up to 1 month, and the Tedlar bag showed significant fractionation of  $\text{CO}_2$ .

For dissolved-gas samples, butyl rubber again appears to be the preferred material. Silicone rubber allows fractionation, and PTFE layers do not remove this problem. FlexFoil bags are a reliable alternative, with little fractionation and with light-blocking foil which limits biological activity. The tests also demonstrate the fractionation possible when dissolved-gas samples are left unpreserved.

Future work will include storage trials at lower gas concentrations, further testing of air transport effects using longer flights while recording temperature and pressure conditions, use of larger needles for piercing septa, and assessment of specific factors (temperature, pH, light) that contribute to biological activity in dissolved-gas samples.

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Appendix A. Results from free-gas container trials. Shaded values indicate runs with minimal ( $\leq 0.2$  ‰) isotopic fractionation.

Free-gas Container type	Component	$\Delta^{13}\text{C}$ (‰)				
		Transport	1 week	1 month	2 months	3 months
F01 Canister	C1	-0.2	-0.2	-0.1	-0.1	0.2
	CO2	0.0	-0.2	0.2	0.1	0.3
	C2	0.1	0.0	0.0	0.0	-0.1
	C3	0.0	-0.1	0.1	0.1	0.0
	iC4	-0.3	0.0	-0.1	0.4	0.1
	nC4	-0.1	-0.3	0.2	0.2	-0.3
F02 Tedlar bag unpierced	C1	0.0	0.0	0.0	0.4	0.7
	CO2	1.2	1.1	2.0	7.3	9.2
	C2	0.3	0.2	0.2	0.6	0.4
	C3	0.1	0.3	0.2	0.2	0.4
	iC4	0.0	0.0	0.0	0.3	0.3
	nC4	0.3	0.5	0.2	0.5	1.2
F03 Tedlar bag pierced	C1	0.0	0.3	0.4	1.0	1.9
	CO2	1.1	1.6	2.1	6.2	8.4
	C2	0.3	0.3	0.2	0.5	0.8
	C3	0.0	0.3	0.4	0.3	0.5
	iC4	-0.4	0.2	0.3	0.6	0.5
	nC4	0.2	0.1	0.3	0.3	0.6
F04 FlexFoil bag unpierced	C1	0.2	0.0	0.2	0.3	0.6
	CO2	0.1	0.2	0.4	0.9	0.9
	C2	0.3	0.5	0.3	0.6	0.9
	C3	0.2	0.1	0.2	0.6	0.9
	iC4	0.0	0.0	0.3	0.6	0.9
	nC4	0.3	0.4	0.3	1.5	1.5
F05 FlexFoil bag pierced	C1	0.0	0.0	0.1	0.3	0.7
	CO2	0.4	0.2	0.0	0.9	1.0
	C2	0.2	0.2	0.2	0.6	1.1
	C3	0.3	0.2	0.3	0.6	1.1
	iC4	0.2	0.0	0.1	0.4	1.3
	nC4	0.2	0.1	0.4	1.0	1.9
F06 Vacutainer pierced	C1	-0.1	-0.1	-0.1	0.0	0.0
	CO2	-0.5	-0.2	0.3	-0.1	-0.5
	C2	0.6	0.1	0.1	0.1	0.2
	C3	0.4	-0.3	-0.1	-0.2	0.1
	iC4	0.4	-0.1	0.0	0.1	0.6
	nC4	-0.2	-0.3	0.4	0.3	0.6
F07 Exetainer pierced	C1	-0.1	0.0	-0.2	-0.3	0.0
	CO2	-0.8	-0.3	0.0	0.4	0.3
	C2	0.5	0.1	-0.1	-0.1	0.2
	C3	0.2	0.2	-0.1	0.0	0.1
	iC4	-0.3	0.0	-0.1	0.4	0.3
	nC4	0.1	-0.1	0.0	0.4	0.1
F08 EPA vial pierced	C1	5.0	45.5	65.2		
	CO2	4.1	15.5	16.1		
	C2	2.9	16.0	7.8		
	C3	2.3	9.1	4.7		
	iC4	1.7	5.1	8.9		
	nC4	3.2	5.7	1.9		

## Appendix A. (Continued)

Free Gas		$\Delta^{13}\text{C}$ (‰)				
Container Type	Component	Transport	1 week	1 month	2 months	3 months
F09 Thick Butyl unpierced	C1	-0.1	0.0	0.1	-0.1	
	CO2	0.3	0.1	-0.2	0.0	
	C2	0.3	0.2	0.0	0.0	
	C3	0.3	0.0	0.1	0.0	
	iC4	0.1	0.1	-0.3	-0.2	
	nC4	0.1	-0.1	-0.1	0.2	
F10 Thick Butyl pierced	C1	-0.1	-0.2	0.0	-0.3	0.0
	CO2	0.1	0.1	0.1	-0.2	-0.1
	C2	0.2	0.0	0.0	-0.1	-0.1
	C3	-0.1	0.0	0.0	-0.3	0.1
	iC4	0.0	-0.1	-0.2	-0.4	0.2
	nC4	-0.1	0.0	-0.2	0.0	0.4
F11 Thin Butyl unpierced	C1	-0.1	-0.1	-0.2	-0.1	
	CO2	0.4	0.4	0.0	-0.1	
	C2	0.2	0.2	0.1	0.1	
	C3	-0.1	-0.1	0.0	0.0	
	iC4	0.1	0.1	0.2	-0.1	
	nC4	0.3	0.3	0.0	0.2	
F12 Thin Butyl pierced	C1	-0.1	0.0	0.0	-0.3	0.5
	CO2	-0.4	0.0	0.1	0.2	0.1
	C2	0.7	0.3	-0.1	0.0	0.2
	C3	0.2	0.2	0.2	0.1	0.3
	iC4	0.0	0.2	-0.1	-0.1	0.3
	nC4	-0.1	-0.1	-0.1	0.3	0.3
F13 Silicone unpierced	C1	1.1	1.2	2.0	6.4	
	CO2	1.7		4.3	13.6	
	C2	2.4	2.2	3.7	11.0	
	C3	2.0	2.7	3.7	10.5	
	iC4	2.2	2.2	3.1		
	nC4	3.4	3.8	3.6		
F14 Silicone pierced	C1	1.3	1.5	3.3	6.1	
	CO2	2.4	3.4	7.2	13.3	
	C2	2.4	2.3	5.2	9.6	
	C3	2.0	2.5	5.6	10.1	
	iC4	2.0	2.2	4.1		
	nC4	3.3	2.7	0.0		
F15 PTFE-butyl unpierced	C1	-0.1	-0.1	-0.2	-0.1	
	CO2	-0.6	-0.3	0.3	0.4	
	C2	0.6	0.0	0.0	0.0	
	C3	0.0	-0.2	0.1	-0.1	
	iC4	-0.1	0.2	0.1	-0.3	
	nC4	0.0	-0.3	0.1	0.0	
F16 PTFE-butyl pierced	C1	5.9	13.0	47.9	0.0	
	CO2	0.4	2.2	8.2	0.6	
	C2	2.3	4.0	13.6	-0.1	
	C3	1.0	1.8	5.6	-0.1	
	iC4	0.7	0.9	3.9	-0.6	
	nC4	0.4	0.8	3.6	0.2	
F17 PTFE - silicone unpierced	C1		0.0	0.3	0.6	
	CO2		0.1	1.4	3.7	
	C2		0.0	0.0	0.4	
	C3		-0.1	0.2	0.4	
	iC4		-0.2	-0.2	-0.1	
	nC4		-0.1	0.1	0.6	

Appendix B. Results from dissolved-gas container trials. Shaded values indicate runs with minimal ( $\leq 0.5$  ‰) isotopic fractionation.

Dissolved gas Container type	Component	$\Delta^{13}\text{C}$ (‰)			
		Start	2 weeks	1 month	2 months
D01	C1	0.1	0.0	0.8	1.2
Tedlar Bag	CO2	4.8	5.1	5.3	5.9
	C2	-0.1	0.1	0.6	0.7
	C3	0.1	0.1	0.3	0.4
	iC4	-0.2	0.3	0.2	0.6
	nC4	-0.2	-0.2	0.2	0.2
D01	C1		1.6	2.4	13.9
Tedlar Bag unpreserved	CO2		3.6	4.5	1.2
	C2		0.3	0.5	4.5
	C3		0.0	0.2	0.4
	iC4		-0.4	-0.1	-0.3
	nC4		-0.1	-0.2	0.5
D02	C1	0.0	-0.1	0.2	0.1
FlexFoil Bag	CO2	4.2	3.2	4.0	3.8
	C2	0.3	0.0	0.2	0.3
	C3	0.2	0.0	0.4	0.1
	iC4	0.2	-0.4	-0.1	0.0
	nC4	0.1	-0.1	0.6	-0.2
D02	C1		0.3	0.3	0.1
FlexFoil Bag unpreserved	CO2		2.5	2.6	2.6
	C2		0.1	0.1	0.1
	C3		0.0	0.0	0.0
	iC4		0.0	0.1	0.2
	nC4		-0.3	0.0	0.1
D03	C1	0.0	2.8	5.3	6.8
EPA Vial	CO2	8.4	9.9	10.5	10.9
	C2	0.2	0.8	1.7	1.9
	C3	0.0	0.5	0.7	0.7
	iC4	-0.2	0.1	0.2	0.4
	nC4	0.1	0.7	0.0	0.3
D04	C1		0.0	-0.1	-0.2
Thick Butyl	CO2		5.9	6.0	6.2
	C2		0.1	0.3	0.2
	C3		0.3	-0.1	0.3
	iC4		-0.6	0.1	-0.2
	nC4		-0.1	0.2	0.4
D05	C1	-0.1	0.0	-0.1	-0.2
Thin Butyl	CO2	7.9	7.8	9.1	8.3
	C2	0.1	0.1	0.3	0.2
	C3	0.3	0.2	0.4	0.4
	iC4	0.2	0.5	0.4	0.3
	nC4	0.2	0.2	0.1	0.3
D06	C1		0.7	0.9	1.8
Silicone	CO2		7.4	8.0	8.9
	C2		0.5	0.8	1.6
	C3		0.5	0.8	0.9
	iC4		0.8	0.5	
	nC4		0.1	0.9	
D07	C1		0.0	0.0	0.2
PTFE – butyl	CO2		5.5	6.0	5.9
	C2		0.1	0.3	0.7
	C3		0.2	0.0	0.3
	iC4		0.0	-0.3	0.3
	nC4		-0.2	-0.1	0.5