Preliminary assessment of stable nitrogen and oxygen isotopic composition of USGS51 and USGS52 nitrous oxide reference gases and perspectives on calibration needs

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Abstract:

RATIONAL: Despite a long history and growing interest in isotopic analyses of N₂O, there is a lack of isotopically characterized N₂O isotopic reference materials (standards) to enable normalization and reporting of isotope-delta values. Here we report the isotopic characterization of two pure N₂O gas reference materials, USGS51 and USGS52, which are now available for laboratory calibration (<https://isotopes.usgs.gov/lab/referencematerials.html>).

METHODS: A total of 400 sealed borosilicate glass tubes of each N₂O reference gas were prepared from a single gas filling of a high vacuum line. We demonstrated isotopic homogeneity via dual-inlet isotope-ratio mass spectrometry. Isotopic analyses of these reference materials were obtained from eight laboratories to evaluate interlaboratory variation and provide preliminary isotopic characterization of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$, and site preference (S_P) measurements.

RESULTS: Isotopic homogeneity of USGS51 and USGS52 was demonstrated for both USGS51 and USGS52 by one-sigma standard deviations associated with the determinations of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and S_P of 0.12 mUr or better. The one-sigma standard deviations of S_P measurements of USGS51 and USGS52 reported by eight laboratories participating in the interlaboratory comparison were 1.27 and 1.78 mUr, respectively.

CONCLUSIONS: The agreement of isotope-delta values obtained in the interlaboratory comparison was not sufficient to provide reliable accurate isotope-measurement values for USGS51 and USGS52. We propose that provisional values for the isotopic composition of USGS51 and USGS52 determined at the Tokyo Institute of Technology can be adopted for normalizing and reporting sample data until further refinements are achieved through additional calibration efforts.

For a remarkably simple molecule, consisting of only two elements and three atoms, there is a surprising degree of isotopic complexity in nitrous oxide (N_2O).^[1] The two stable isotopes of nitrogen and three stable isotopes of oxygen combine to form 12 isotopic molecules or isotopocules. This coupled with the asymmetrical structure of N_2O (N-N-O) means that the N_2O molecule contains bulk ($\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{17}\text{O}$), mass independent ($\Delta^{17}\text{O}$), site specific ($^{15}\text{N}^{14}\text{N}^{16}\text{O}$ vs. $^{14}\text{N}^{15}\text{N}^{16}\text{O}$), and clumped (e.g. $^{15}\text{N}^{14}\text{N}^{18}\text{O}$) isotopic information. Strong interest in this greenhouse gas, as well as advances in technology that have lowered instrument costs and improved sample throughput, have resulted in a doubling of publications related to the isotopic composition of N_2O over the last 20 years.^[1] This has been facilitated, in part, by the fact that traditional mass spectrometric approaches to the determination of isotope ratios are now being supplemented by laser-based spectroscopy.^[2,3] Additionally, the recent development of double-focusing mass spectrometers for precise stable isotope analyses of rare isotopocules with high mass resolution and good sensitivity now enable clumped isotopic analysis of N_2O .^[4,5]

Despite the strong interest in and long history of isotopic analyses of N_2O ,^[6,7] there is a lack of isotopically characterized N_2O isotopic reference materials (standards) to enable normalization^[8] and reporting of compatible isotope-delta values (within analytical uncertainty) from different laboratories worldwide. Thus, while reported isotope-delta values for N_2O may be consistent within one laboratory, disparity among laboratories can be substantial. This was recently demonstrated by an 11 mUr range^[9] in the quantity site preference (symbol S_P), which we define as the difference in $\delta^{15}\text{N}$ values between the central, α , and outer, β , nitrogen atoms in a N_2O sample. Non-uniformity of isotopic measurements between laboratories may be a significant factor compromising global assessments of N_2O sources and sinks based on stable isotope data.^[10] Relative isotope ratios expressed as delta values are commonly reported in units of per mil (symbol ‰) that are not considered acceptable in the International System of Units.^[11] In this study, we substitute the term urey (symbol Ur) for the delta unit as suggested by Brand and Coplen^[12], such that one milliurey (0.001) equals 1 ‰. A delta value in the traditional form of -25 ‰, for example, can be expressed as -25 mUr. In addition, although the symbol S_P is commonly used in the scientific literature for site preference; we use S_P because symbols of quantities in the International System of Units (SI) should be a single character (with desired superscripts or subscripts).^[13] Furthermore, symbols of quantities should be printed in italic font.

With this article we report the isotopic characterization of two pure N₂O gas reference materials, USGS51 and USGS52, which are now available in sealed borosilicate glass tubes for laboratory calibration (<https://isotopes.usgs.gov/lab/referencematerials.html>). We demonstrated isotopic homogeneity via dual-inlet isotope-ratio mass spectrometry (DI-IRMS). Further, we present data on isotopic analyses of these isotopic reference materials from eight laboratories to evaluate interlaboratory variation and provide preliminary isotopic characterization. Ultimately, there is a need for (1) additional pure N₂O gas isotopic reference materials with a wider range of isotopic compositions, (2) additional calibration efforts to improve accuracy of the isotope values assigned to these isotopic reference materials, and (3) development of large-volume standards in an air matrix that will be of use for calibration of spectroscopic instruments for analyses of N₂O in air.

METHODS

USGS51 was prepared from Air Products (Trexlerstown, Pennsylvania, USA) VLSI grade N₂O (99.9994 %). USGS52 was prepared from Air Products USP grade N₂O. Aliquot preparation occurred within a high-vacuum glass manifold consisting of 20 0.5-L glass reservoirs, following the procedure of Coplen and Kendall.^[14] By opening appropriate manifold stopcocks, the 20 reservoirs were connected in series with a diaphragm pump so the gas could be cycled through the reservoirs and isotopically homogenized. Stopcocks were manipulated to expand the gas from each of the 20 reservoirs into a manifold having ports for 20 6-mm borosilicate glass tubes and a mercury piston for gas homogenization. Thus, 400 glass tubes containing identical aliquots of a gas were prepared from a single filling of the high vacuum line. Procedures are described in detail in Coplen and Kendall^[14] and are summarized briefly here: (1) evacuate the entire vacuum manifold, (2) fill the entire manifold with N₂O from the tank of interest to a pressure of approximately 70 kPa for a period of 5 min to treat the surface of the glass manifold, (3) evacuate N₂O, (4) refill the manifold with N₂O from the sample tank to a pressure of 70 kPa, (5) cycle the N₂O through the 20 0.5-L glass reservoirs with a diaphragm pump for 30 min to isotopically homogenize the N₂O, (6) isolate the N₂O in the 20 0.5-L glass volumes by closing stopcocks on each volume, (7) expand the N₂O in the first 0.5-L glass volume into the manifold containing 20 6-mm borosilicate glass tubes, each with a line volume of about 40 mL, (8) homogenize the N₂O by cycling the mercury piston pump for 30 min, (9) isolate the 20 stopcocks on the 40 mL volumes, (10) cryogenically condense the N₂O into each of the 20 borosilicate glass tubes, (11) seal each of the 20 tubes one-by-one with a torch, evacuating non-condensable gases with a mercury diffusion pump during sealing, and (12) repeat steps 7 through 11 for the N₂O in the other 19 0.5-L glass reservoirs. Each of the 400 glass tubes is expected to contain approximately 300 μmol of N₂O in a volume of approximately 45 mL.

To confirm isotopic homogeneity, one tube from each set of 20, and multiple tubes from a few of the sets, were analyzed for $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$ and S_p by dual-inlet isotope-ratio mass spectrometry (DI-IRMS) using an Elementar Isoprime mass spectrometer (Elementar Analysensysteme GmbH Elementar-Straße 1, 63505 Langenselbold) at Michigan State University (Table 1; Laboratory 4 in Table 2). The $\delta^{17}\text{O}$ values and clumped isotopic compositions of USGS51 and USGS52 were not evaluated. Mass-overlap corrections were performed using the approach of Toyoda and Yoshida.^[15] The $\delta^{15}\text{N}^\alpha$ value was determined from the m/z 31 to m/z 30 ratio of the NO^+ fragment ion. The $\delta^{15}\text{N}^\beta$ and S_p values are calculated based on the following equations:

$$\delta^{15}\text{N} = (\delta^{15}\text{N}^\alpha + \delta^{15}\text{N}^\beta)/2 \quad (1)$$

and

$$S_p = \delta^{15}\text{N}^\alpha - \delta^{15}\text{N}^\beta \quad (2)$$

Two glass tubes were selected at random and sent to eight laboratories for isotopic analysis (Table 2). Laboratory 1 determined the $\delta^{15}\text{N}$, $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$, $\delta^{18}\text{O}$ and S_p values within the dual inlet of a MAT252 mass spectrometer (Thermo Fisher Scientific Inc., Waltham MA USA) in comparison to a isotopically characterized laboratory tank of pure N_2O . The $\delta^{15}\text{N}$, $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$ values of the local reference gas were anchored to atmospheric N_2 (Air- N_2) scale by ammonium nitrate decomposition,^[15] whereas the $\delta^{18}\text{O}$ value was anchored to Vienna Standard Mean Ocean Water (VSMOW) standard by converting N_2O to CO_2 with graphite and platinum foil (Yoshida, unpublished).^[16] Laboratory 2 performed isotopic analyses by mid-infrared quantum cascade laser absorption spectroscopy in an air matrix with a volume fraction of approximately 60 ppm against internal reference gases previously analyzed by Laboratory 1.^[2] Laboratory 3 determined the isotopic composition of USGS51 and USGS52 using a PRECON (Thermo Fisher Scientific Inc., Waltham MA USA) ^[17] sample introduction system interfaced to Thermo Fisher Scientific Delta^{plus} XP isotope-ratio mass spectrometer. Both gases were diluted and analyzed in N_2 , at a volume fraction of N_2O of 10 ppm. Isotope-delta values were normalized using the secondary standards of Laboratory 6. Laboratory 4 determined the isotopic composition of USGS51 and USGS52 using a dual-inlet interfaced to an Elementar Isoprime mass spectrometer in comparison to a laboratory compressed tank of pure N_2O calibrated by Laboratory 1. Laboratory 5 measured the bulk $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ values, as well as $\delta^{15}\text{N}^\alpha$, $\delta^{15}\text{N}^\beta$, and S_p values by dual-inlet isotope-ratio mass spectrometry on a Thermo Finnigan Delta^{plus} XP (Thermo Fisher Scientific Inc., Waltham MA USA). An aliquot of the reference gas used in the analysis was calibrated to the Air- N_2 reference scale for determination of bulk $\delta^{15}\text{N}$, $\delta^{15}\text{N}^\alpha$, and $\delta^{15}\text{N}^\beta$ values, and to the $\delta^{18}\text{O}$ VSMOW isotope-delta scale by Laboratory

1. Laboratory 6 determined the isotopic composition of USGS51 and USGS52 in continuous flow mode using a Delta V PLUS mass spectrometer connected to a Precon and a Trace GC (Thermo Fisher Scientific Inc.). Both gases were diluted and analyzed in synthetic air or N₂, respectively, at a volume fraction of N₂O of 10 ppm.^[18] Isotope-delta values were normalized using two secondary standards differing in S_p value (-2.14 mUr and 18.93 mUr, respectively), both calibrated by Laboratory 1. Laboratory 7 measured bulk $\delta^{15}\text{N}$ by dual-inlet isotope-ratio mass spectrometry via analyses of N₂ produced by off-line conversion of N₂O and ammonium sulfate reference materials with Cu + CuO; N₂O data were normalized to an assumed $\delta^{15}\text{N}$ value of +0.43 mUr for IAEA-N1 (Table 3). Laboratory 8 evaluated the isotopic composition of the two USGS standards with the dual inlet of a Thermo Fisher Scientific MAT252 mass spectrometer in comparison to a local tank reference gas. Only Laboratory 1 characterized its laboratory standards by ammonium nitrate decomposition. All other laboratories obtained calibrations by exchange of standards with Laboratory 1.

RESULTS

Isotopic homogeneity in USGS51 and USGS52 was demonstrated both within sets of 20 tubes (12-A vs. 12-B and 15-A vs. 15-B for USGS51 and 1A-C for USGS52) and across sets of 20 glass tubes (Table 1). The one-sigma standard deviations associated with the determinations of $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}^{\alpha}$, $\delta^{15}\text{N}^{\beta}$ and S_p values across all aliquots of USGS51 was 0.06 mUr or better; that for USGS52 was 0.12 mUr or better, which suggests slightly greater heterogeneity in USGS52. For each gas aliquot the standard deviation was greater for S_p than other isotope quantities measured, but this is not surprising as the quantity S_p is calculated from two isotope ratios (Equation 2). Nonetheless, the overall repeatability for all isotope measurements was better than that typically reported for measurements by gas chromatography interfaced to a mass spectrometer or for spectroscopic instruments.^[2,19]

The one-sigma standard deviations of $\delta^{15}\text{N}$ measurements of USGS51 and USGS52 reported by eight laboratories are 0.21 and 0.25 mUr, respectively (Table 2). The one-sigma standard deviations of S_p measurements of USGS51 and USGS52 are 1.27 and 1.78 mUr, respectively (Table 2). The total range in S_p values of USGS52 was 5.1 mUr, which represents an improvement in comparison to a prior interlaboratory calibration exercise that reported an 11-mUr range.^[9] To verify accuracy in $\delta^{15}\text{N}$ Laboratory 7 converted the IAEA-N1 and IAEA-N2 ammonium sulfate standards, USGS51, USGS52 and internal laboratory N₂O standards from Laboratories 1, 4 and 8, to N₂ and performed isotopic analysis in comparison to the NSVEC N₂ secondary standard (Table 3). The results, shown as a difference in $\delta^{15}\text{N}$ between the measured and reported values cluster in two groups near 0.0 mUr (IAEA-N1, IAEA-N2, NSVEC and NINO) and approximately -0.3 mUr (USGS51, USGS52, TokyoTech and MSU-C).

DISCUSSION

Evaluation of USGS51 and USGS52 reference materials

The accurate assessment of isotope-delta values and other isotope measurements is a fundamental challenge to the isotope biogeochemistry research community. A small set of primary international measurement standards has been identified to anchor δ scales and enable comparable isotope data sets to be generated by laboratories across the globe.^[20] For N_2O these primary standards are Air- N_2 and VSMOW for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ measurements, respectively. Numerous secondary isotopic reference materials have been established that are chemically similar to various materials being analyzed (e.g. sulfate, nitrate, and hair) to permit consistency in isotopic measurements involving various sample preparation and handling protocols. While seeming inherently straightforward, accuracy continues to be surprising elusive. For example, analytical precision in the $^{13}\text{C}/^{12}\text{C}$ ratio of calcite has been reported to 3 μUr , yet uncertainty in the absolute value of the primary carbon isotope standard, NBS 19, is 2.5 mUr.^[20, 21, 22] While reducing uncertainty in the isotopic compositions of primary isotope standards continues to be an ongoing effort, it is essential that consistency of relative isotope-ratio measurements among laboratories be obtained.

The lack of secondary isotopic reference materials for N_2O measurements has meant that laboratories have had to develop internal calibration protocols or exchange standards with a laboratory that has performed calibration by ammonium nitrate decomposition or other method. The bulk $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, and $\delta^{17}\text{O}$ values of N_2O can be determined by analyzing N_2 and/or O_2 produced by decomposition or reduction of N_2O using hot copper^[23], gold tubes^[24, 25], microwave discharge^[26], or carbon rods wrapped with platinum wire at high temperature.^[27] Obtaining precise site-specific $\delta^{15}\text{N}$ values for N_2O has been challenging and calibration schemes have been based primarily on N_2O produced by decomposition of ammonium nitrate during which the α nitrogen atom is derived from nitrate and the β atom from ammonium.^[15] If the ammonium and nitrate $\delta^{15}\text{N}$ values in ammonium nitrate are known, then N_2O can be generated with known $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$ values that are anchored to the Air- N_2 nitrogen-isotope scale. Unfortunately, this reaction is difficult to reproduce, potentially explosive, and non-quantitative.^[3, 15, 28, 29] Consequently, few laboratories have used this approach for calibration. A recent detailed assessment of this calibration approach has shown that the α and β nitrogen atoms fractionate distinctly during ammonium nitrate decomposition.^[29] Given the challenge of accurately conducting ammonium nitrate decomposition, many laboratories have calibrated internal N_2O standards by exchange with previously calibrated laboratories. In particular, for $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$ determinations, and hence S_P determination, N_2O isotopic calibration has primarily been obtained by exchange with the laboratory of Naohiro Yoshida and Sakae Toyoda at Tokyo Institute of Technology.

With the introduction of USGS51 and USGS52, the first pair of N_2O secondary reference materials is now widely available as pure gases containing approximately

300 μmol of gas in glass tubes. We demonstrate isotopic homogeneity across aliquots with an uncertainty (one standard deviation) of 0.12 mUr or better for $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, $\delta^{15}\text{N}^{\alpha}$, $\delta^{15}\text{N}^{\beta}$ and S_p values (Table 1). Results from analyses of USGS51 and USGS52 by eight laboratories exhibited uncertainty that ranged from 0.21 mUr for $\delta^{15}\text{N}$ to 1.78 mUr for S_p (Table 2). These analyses were performed by three different approaches: spectroscopic (Laboratory 2), continuous flow - gas chromatography - isotope ratio mass spectrometry (Laboratory 6) and dual-inlet mass spectrometry (Laboratories 1, 3, 4, 5, 7 and 8) (Table 2). Gas chromatography may not result in complete removal of O_2 and N_2 and co-elution of these gases with N_2O may have resulted in some deterioration in reproducibility and accuracy via production of NO within the ion source. Dilution of the pure gas N_2O standards in He instead of N_2 or O_2 will likely yield better results. The reproducibility of measurement results for USGS52 is somewhat better than that of USGS51. To rule out contamination, two tests were performed. First, m/z scans of USGS51 and USGS52 were performed and compared—no measurement differences outside measurement error were observed. Second, USGS51 and USGS52 were analyzed against each other with a double focusing, dual-inlet isotope-ratio mass spectrometer having a total ion beam monitor.^[30] This instrument compares the total ion beam current of all m/z ions with the m/z 44 ion beam current. A contamination value of USGS51 of 0.01 ± 0.02 percent was measured. Thus, no contamination of USGS51 could be identified and differences in reproducibility may reflect minor differences in sample handling. The larger variability in S_p relative to other isotope ratios reflects the fact that this value is calculated from two isotope ratios and is dependent upon measurement of the NO fragment ion that is lower in abundance than the molecular ion and is affected by rearrangement in the ion source of mass spectrometers.

The uncertainty that we report (Table 2) is sufficiently large that we recognize that additional calibration efforts are needed. For comparison, the standard deviation of reported $\delta^{15}\text{N}$ values from ten laboratories participating in the isotopic characterization of the NSVEC N_2 standard was 0.05 mUr.^[31] Nonetheless, the uncertainty reported here for S_p values for USGS51 and USGS52 is smaller than the uncertainty of 4.3 mUr for an interlaboratory analysis of N_2O standards in air by mass spectrometry with a mixing volume ratio of 90 ppm.^[9] Thus, either (1) improvements in precision have occurred since the Mohn et al.^[9] study, or (2) precision is more readily obtained, particularly for mass spectrometry, using pure N_2O than for N_2O in a matrix of air at elevated concentrations. Nonetheless, we cannot be certain that the average isotope values presented in Table 2 represent accurate isotope-measurement values for USGS51 and USGS52.

Of the eight participating laboratories in the current study, only one indicated calibration of local N_2O standards by decomposition of ammonium nitrate (Laboratory 1); others were dependent upon exchange of N_2O standards and calibration data, primarily originating in the laboratory of Naohiro Yoshida at the Tokyo Institute of Technology (Laboratory 1 in Table 2). Such interlaboratory exchanges may be reflected in clustering of $\delta^{15}\text{N}$ values from several different laboratories near those reported by Laboratory 1 in Table 2. In contrast, Laboratory 7, which converted N_2O to N_2 and calibrated data against IAEA ammonium sulfate

reference materials, reported bulk $\delta^{15}\text{N}$ values approximately 0.2–0.3 mUr more negative than the Laboratory 1 values (Table 3). A similar offset of 0.39 mUr was reported for a tank of medical grade N_2O analyzed by combustion to N_2 in Laboratory 8 and by Laboratory 1.^[2] This result suggests that there may be a 0.2–0.3 mUr offset of the USGS standards in $\delta^{15}\text{N}$. Because not all analyses in this study were calibrated independently and some of the differences are not fully resolved, we conclude that additional analyses and interlaboratory exchanges are needed to obtain more accurate values for the isotopic compositions of USGS51 and USGS52. Analytical methods may include decomposition of a common ammonium nitrate reference material and analysis by multiple laboratories and/or comparison to N_2O allowed to reach isotopic equilibrium at moderate temperatures.^[4] Meanwhile, we suggest that the complete set of values reported by Laboratory 1 (Tokyo Institute of Technology, highlighted in bold type in Table 2) can be used provisionally for normalizing N_2O isotopic measurements to improve interlaboratory consistency following the reporting guidelines given below.

Reporting of stable-isotope-delta values

The following recommendations are provided for reporting stable oxygen and nitrogen isotope-delta values^[32]:

- The $\delta^{18}\text{O}$ values of all oxygen-bearing substances should be expressed relative to VSMOW-SLAP on a scale where $\delta^{18}\text{O}_{\text{SLAP}} = -55.5$ mUr exactly.^[33,34] Reporting of $\delta^{18}\text{O}$ values relative to SMOW should be discontinued.^[54]
- The $\delta^{15}\text{N}$ values of all nitrogen-bearing substances should be expressed relative to atmospheric nitrogen gas (Air N_2).^[36]
- Authors should report δ values of internationally distributed (secondary) isotopic reference materials that were assumed for normalization of data for samples of similar chemical composition, as appropriate for the measurement method. Thus, for reporting isotope-delta values of nitrous oxide samples, values assumed for USGS51 and USGS52, or other widely distributed reference gases, should be included in publications. In this manner, readers can re-normalize reported results subsequently when recommended values of reference materials are improved with new techniques.

Future directions

There is a compelling need for the N_2O isotope research community to continue calibration efforts, and this should ideally occur with support from funding agencies and in collaboration with international agencies experienced in such measurements (e.g., the IAEA). Because of the isotopic complexity of N_2O , no single calibration effort is likely to be successful, and we envision that this will be a long-term effort consisting of multiple steps. We recommend the establishment of at least

one secondary ammonium nitrate reference material that can then be used in decomposition experiments to accurately characterize S_p values. The clumped isotopic composition of pure N_2O reference materials can be evaluated by equilibration with activated alumina at moderate temperatures,^[4] and this may be a viable approach for calibration of S_p values. There is a need for common mass overlap corrections in mass spectrometric analysis ^[3,15] and we encourage publication of spreadsheets to accomplish this task, as was recently done for the isotopic analysis of water by spectroscopic approaches.^[37] As mass overlap corrections for N_2O are complicated and involve assumptions, such as equivalent isotopic fractionation during breakage of α and β nitrogen bonds during ionization, spectroscopic approaches may prove useful in evaluating the validity of such assumptions.^[1,38] There is a need for additional pure N_2O gas isotope reference materials because, although USGS51 and USGS52 differ markedly in S_p , they are similar in bulk $\delta^{15}N$ and $\delta^{18}O$. Spectroscopic instruments require large volumes of standards with a wide range of isotope values in a matrix similar to samples (typically air) and with concentrations encompassing the samples being analyzed. This is unlikely to be a realistic task for international agencies; however, isotopically characterized tanks of N_2O in purified air could be provided by a well-calibrated laboratory. The research community should not underestimate the challenge of such calibration efforts nor the need. If the full isotopic complexity of N_2O (bulk, site specific, and clumped isotope values) can be revealed, there is great potential to more accurately evaluate microbial and abiotic N_2O sources and global budgets of this important greenhouse gas.

Distribution of USGS51 and USGS52

USGS51 and USGS52 N_2O isotopic reference materials are available from:

U.S. Geological Survey
Reston Stable Isotope Laboratory
431 National Center
12201 Sunrise Valley Drive
Reston, Virginia 20192
USA
<http://isotopes.usgs.gov/>
Fax: +1 703 648 5889

Each distribution unit contains approximately 300 μmol of N_2O in a 6-mm borosilicate glass tube approximately 30 cm in length. Instructions for transferring N_2O into instrumentation are given at

<https://isotopes.usgs.gov/lab/referencematerials/USGS51-USGS52.pdf>.

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Table 1. Results of isotopic homogeneity tests of USGS51 and USGS52 nitrous oxide reference materials analyzed by dual-inlet isotope-ratio mass spectrometry at Michigan State University. Samples were selected from each batch of 20 glass tubes and include multiple tubes from some sets (A-C). Set 7 of USGS51 (italicized) was considered an outlier based on an anomalous S_p (site preference) value and was discarded. The averages across each reference material (including multiples) and one-standard deviations (excluding USGS51 set 7) are shown in bold and included in Table 2.

Name	Set	$\delta^{15}\text{N}_{\text{Air-N}_2}$ (mUr)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (mUr)	$\delta^{15}\text{N}^{\alpha}_{\text{Air-N}_2}$ (mUr)	$\delta^{15}\text{N}^{\beta}_{\text{Air-N}_2}$ (mUr)	S_p (mUr)
USGS51	1	+1.25	+42.05	+1.84	+0.65	+1.19
USGS51	2	+1.28	+42.08	+1.80	+0.75	+1.05
USGS51	3	+1.23	+42.00	+1.72	+0.72	+1.00
USGS51	4	+1.24	+42.04	+1.81	+0.66	+1.15
USGS51	5	+1.28	+42.11	+1.77	+0.77	+1.00
USGS51	6	+1.22	+42.02	+1.73	+0.69	+1.04
<i>USGS51</i>	<i>7</i>	<i>+1.28</i>	<i>+42.14</i>	<i>+2.17</i>	<i>+0.39</i>	<i>+1.78</i>
USGS51	8	+1.28	+42.12	+1.81	+0.74	+1.08
USGS51	9	+1.27	+42.08	+1.81	+0.71	+1.11
USGS51	10	+1.24	+42.06	+1.83	+0.64	+1.19
USGS51	11	+1.26	+42.07	+1.81	+0.70	+1.11
USGS51	12-A	+1.27	+42.13	+1.77	+0.75	+1.02
USGS51	12-B	+1.25	+42.01	+1.76	+0.72	+1.04
USGS51	13	+1.28	+42.07	+1.80	+0.74	+1.06
USGS51	14	+1.26	+42.05	+1.76	+0.75	+1.01
USGS51	15-A	+1.30	+42.16	+1.77	+0.82	+0.95
USGS51	15-B	+1.24	+42.04	+1.77	+0.71	+1.05
USGS51	16	+1.24	+42.01	+1.80	+0.67	+1.13
USGS51	17	+1.24	+42.03	+1.76	+0.71	+1.05
USGS51	18	+1.27	+42.08	+1.82	+0.70	+1.12
USGS51	19	+1.26	+42.02	+1.81	+0.70	+1.11
USGS51	20	+1.29	+42.12	+1.80	+0.76	+1.05
Average		+1.26 ± 0.02	+42.06 ± 0.04	+1.79 ± 0.03	+0.72 ± 0.04	+1.07 ± 0.06
USGS52	1-A	+0.41	+41.54	+12.66	-11.85	+24.51
USGS52	1-B	+0.39	+41.49	+12.67	-11.90	+24.57
USGS52	1-C	+0.37	+41.42	+12.68	-11.95	+24.63
USGS52	2	+0.45	+41.56	+12.69	-11.81	+24.50
USGS52	3	+0.40	+41.53	+12.68	-11.88	+24.56
USGS52	4	+0.39	+41.48	+12.68	-11.91	+24.59
USGS52	5	+0.40	+41.50	+12.66	-11.87	+24.53
USGS52	6	+0.44	+41.49	+12.65	-11.78	+24.42
USGS52	7	+0.40	+41.41	+12.59	-11.79	+24.38
USGS52	8	+0.41	+41.42	+12.56	-11.76	+24.33
USGS52	9	+0.42	+41.46	+12.63	-11.80	+24.43
USGS52	10	+0.40	+41.50	+12.72	-11.92	+24.65
USGS52	11	+0.40	+41.52	+12.71	-11.92	+24.63
USGS52	12	+0.39	+41.47	+12.71	-11.94	+24.65

USGS52	13	+0.39	+41.54	+12.78	-12.01	+24.79
USGS52	14	+0.42	+41.57	+12.71	-11.87	+24.58
USGS52	15	+0.38	+41.48	+12.65	-11.90	+24.54
USGS52	16	+0.43	+41.54	+12.75	-11.90	+24.65
USGS52	17	+0.41	+41.54	+12.71	-11.90	+24.61
USGS52	19	+0.38	+41.53	+12.74	-11.98	+24.71
USGS52	20	+0.40	+41.53	+12.78	-11.98	+24.76
Average		+0.40 ± 0.02	+41.50 ± 0.05	+12.69 ± 0.05	-11.89 ± 0.07	+24.57 ± 0.12

Table 2. Interlaboratory comparison of USGS51 and USGS52 N₂O isotopic measurements. Individual laboratory uncertainties are standard deviations of minimum of three repeated measurements against the laboratory reference gas and do not include any calibration uncertainties of the laboratory reference gas with respect to Air-N₂ and VSMOW.

[Laboratory codes: 1, Tokyo Institute of Technology, Yokohama, Japan; 2, Laboratory for Air Pollution & Environmental Technology, Dübendorf, Switzerland; 3, Georg-August University Göttingen, Göttingen, Germany; 4, Michigan State University, East Lansing, MI, USA; 5, Stanford University, Stanford, CA, USA; 6, Thünen Institut, Braunschweig, Germany; 7, U.S. Geological Survey, Reston, VA, USA; 8, Max-Planck-Institute for Biogeochemistry, Jena, Germany. DI-IRMS, dual-inlet isotope-ratio mass spectrometry; Spectro., spectroscopy; CF-GC-IRMS, continuous flow - gas chromatography – isotope ratio mass spectrometry; S_p , site preference. The set of values shown in bold can be used provisionally for normalizing N₂O isotopic measurements to improve interlaboratory consistency following the reporting guidelines given in the text.

Laboratory code	Name	$\delta^{15}\text{N}_{\text{Air-N}_2}$ (mUr)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (mUr)	$\delta^{15}\text{N}^{\alpha}_{\text{Air-N}_2}$ (mUr)	$\delta^{15}\text{N}^{\beta}_{\text{Air-N}_2}$ (mUr)	S_p (mUr)	Methodology
1	USGS51	+1.32 ± 0.04	+41.23 ± 0.04	+0.48 ± 0.09	+2.15 ± 0.12	−1.67	DI-IRMS
2	USGS51	+1.23 ± 0.06	+40.97 ± 0.05	+0.48 ± 0.17	+1.97 ± 0.13	−1.49 ± 0.28	Spectro.
3	USGS51	+1.25 ± 0.13	+41.44 ± 0.10	+0.34 ± 0.21	+2.15 ± 0.22	−1.82 ± 0.34	DI-IRMS
4	USGS51	+1.26 ± 0.02	+42.06 ± 0.04	+1.79 ± 0.03	+0.72 ± 0.04	+1.07 ± 0.06	DI-IRMS
5	USGS51	+1.34 ± 0.04	+41.25 ± 0.04	+0.91 ± 0.05	+1.77 ± 0.09	−0.86 ± 0.11	DI-IRMS
6	USGS51	+1.31	+41.56	−0.03	+2.66	−2.69	CF-GC-IRMS (in N ₂)
6	USGS51	+1.35	+41.36	+0.12	+2.59	−2.47	CF-GC-IRMS (in Air)
7	USGS51	+1.11 ± 0.03					DI-IRMS (N ₂ conversion)
8	USGS51	+0.68 ± 0.07	+41.73 ± 0.06				DI-IRMS
Average	USGS51	+1.21 ± 0.21	+41.45 ± 0.34	+0.59 ± 0.61	+2.00 ± 0.65	−1.42 ± 1.26	
1	USGS52	+0.44 ± 0.02	+40.64 ± 0.03	+13.52 ± 0.04	−12.64 ± 0.05	+26.15	DI-IRMS
2	USGS52	+0.16 ± 0.10	+40.19 ± 0.10	+13.60 ± 0.30	−13.29 ± 0.13	+26.89 ± 0.10	Spectro.
3	USGS52	+0.50 ± 0.09	+40.81 ± 0.07	+12.44 ± 0.12	−11.39 ± 0.18	+23.83 ± 0.25	DI-IRMS
4	USGS52	+0.40 ± 0.02	+41.5 ± 0.09	+12.69 ± 0.06	−11.89 ± 0.07	+24.57 ± 0.12	DI-IRMS

5	USGS52	$+0.49 \pm 0.04$	$+40.69 \pm 0.04$	$+14.28 \pm 0.05$	-13.31 ± 0.09	$+27.58 \pm 0.12$	DI-IRMS
6	USGS52	$+0.60$	$+40.62$	$+14.31$	-13.12	$+27.43$	CF-GC-IRMS
6	USGS52	$+0.06$	$+40.75$	$+15.05$	-13.86	$+28.91$	(in N ₂) CF-GC-IRMS
7	USGS52	$+0.14 \pm 0.04$					(in Air) DI-IRMS (N ₂ conversion)
8	USGS52	-0.15 ± 0.07	$+41.23 \pm 0.06$				DI-IRMS
Average	USGS52	$+0.29 \pm 0.25$	$+40.80 \pm 0.40$	$+13.70 \pm 0.93$	-12.78 ± 0.87	$+26.48 \pm 1.78$	

Table 3. Evaluation of bulk $\delta^{15}\text{N}$ values of USGS51, USGS52, and various other N isotopic reference materials. Measured $\delta^{15}\text{N}$ values were determined in the current study by laboratory 7, assuming a value of +0.43 mUr for IAEA-N1. TokyoTech, MSU-C and NINO are the internal laboratory pure gas N_2O standards utilized by laboratories 1, 4 and 8, respectively (Table 2).

ID	Compound	Measured $\delta^{15}\text{N}_{\text{Air}}$ (mUr)	n	Reported $\delta^{15}\text{N}_{\text{Air}}$ (mUr)	Difference in $\delta^{15}\text{N}_{\text{Air}}$ (mUr)
IAEA-N1	$(\text{NH}_4)_2\text{SO}_4$	+0.43±0.00	5	+0.43±0.07	0.00
IAEA-N2	$(\text{NH}_4)_2\text{SO}_4$	+20.44±0.03	2	+20.41±0.12	+0.03
NSVEC	N_2	-2.72±0.02	4	-2.78±0.04	+0.06
USGS51	N_2O	+1.11±0.03	4	+1.26±0.02 ¹	-0.21
USGS52	N_2O	+0.14±0.04	4	+0.40±0.02 ¹	-0.30
TokyoTech	N_2O	-2.68±0.01	2	-2.39	-0.29
MSU-C	N_2O	-1.22±0.01	2	-0.93±0.10	-0.29
NINO	N_2O	+0.28±0.01	2	+0.32±0.08	-0.04

¹ From Table 1