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An interlaboratory comparison for the quantification of aqueous dimethylsulfide

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Abstract

An interlaboratory comparison (ILC) was conducted to evaluate the proficiency of multiple laboratories to quantify dimethylsulfide (DMS) in aqueous solution. Ten participating laboratories were each supplied with blind duplicate test solutions containing dimethylsulfoniopropionate hydrochloride (DMSP HCl) dissolved in acidified artificial seawater. The test solutions were prepared by the coordinating laboratory from a DMSP HCl reference material that was synthesized and purity certified for this purpose. A concentration range was specified for the test solutions and the participating laboratories were requested to dilute them as required for their analytical procedure, together with the addition of excess alkali under gas-tight conditions to convert the DMSP to DMS. Twenty-two DMS concentrations and their estimated expanded measurement uncertainties (95% confidence level) were received from the laboratories. With two exceptions, the within-laboratory variability was 5% or less and the between-laboratory variability was ~ 25%. The magnitude of expanded measurement uncertainties reported from all participants ranged from 1% to 33% relative to the result. The information gained from this pilot ILC indicated the need for further test sample distribution studies of this type so that participating laboratories can identify systematic errors in their analysis procedures and realistically evaluate their measurement uncertainty. The outcome of ILC studies provides insights into the comparability of data in the global surface seawater DMS database.

At the fifth International Symposium on the Biological and Environmental Chemistry of DMS(P) and Related Compounds which was held in October 2010 at Goa, India, it was resolved to address the present lack of knowledge about the comparability of seawater dimethylsulfide (DMS) data. The global surface seawater DMS database (http://saga.pmel.noaa.gov/dms/) was initiated and is maintained by scientists at the US National Oceanographic and Atmospheric Administration Pacific Marine Environmental Laboratory, and is a valuable resource for climate modelers (Lana et al. 2011). The accurate quantification of DMS in surface seawater is important because the concentration present is directly related to the sea-to-air flux, which can ultimately contribute to the formation of cloud condensation nuclei (Charlson 1993). The DMS database now contains > 50,000 data points and is the third largest trace gas database after carbon dioxide and nitrous oxide. Until recently, the DMS database has largely been populated with data gathered by established methods such as 'Purge-and-Trap' gas chromatography (Bell et al. 2012).

New non-chromatographic analytical techniques such as equilibrator inlet proton transfer reaction mass spectrometry (EI-PTR-MS) (Kameyama et al. 2009) and chemical ionization mass spectrometry (CI-MS) (Saltzman et al. 2009) are now being used to generate surface seawater DMS concentration data. These are high-frequency analytical techniques that can generate large amounts of data, which is expected to transform and numerically dominate the DMS database over the coming years. Because a wide range of analytical techniques are presently being used to generate DMS data, it is important at this time to assess the comparability of the various measurement techniques. This is where an interlaboratory comparison (ILC) is valuable because it can bring together a number of laboratories that use a wide variety of measurement techniques and calibration procedures to obtain results that

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can mutually benefit all participants. Intercalibration in marine science is recognized as a relevant and essential tool, but its implementation is not simple (Cutter 2013) and this may be why there are few reports of seawater DMS intercalibration exercises. These studies can provide insights into systematic error or bias introduced by a particular step in the analysis procedure. For example, one of the few reported DMS ILC studies between only two laboratories identified the quantitative differences that can occur between filtered and nonfiltered seawater pretreatment procedures (Turner et al. 1990).

A further issue identified at the Goa meeting was the lack of an internationally recognized dimethylsulfoniopropionate hydrochloride (DMSP HCl) reference material of certified purity. DMSP HCl is nonvolatile, readily soluble, and reacts in alkaline aqueous solution to liberate an equimolar amount of DMS that can be used for calibration purposes when contained in a gas-tight vessel (Dacey and Blough 1987). When DMSP HCl is of unknown or questionable purity, it can be a significant source of error when comparing results and collating data from different sources. Bell et al. (2012) noted the significant differences in purity that can occur in different batch preparations of DMSP HCl and recommend that a material of known purity be produced and made readily available to the DMS(P) research community.

Reported here are the results of a pilot ILC for the quantification of DMS in aqueous solution using test samples that were prepared from a certified reference material (CRM) of DMSP HCl. The synthesis and certification of this material and the coordination of the ILC were conducted by the National Measurement Institute (NMI), Australia. Technical assistance relating to preparation of test samples and logistics of the ILC was provided by an international advisory group.

Materials and procedures

Interlaboratory comparison study protocol

An invitation letter was issued to a wide number of international laboratories on 15 Nov 2012. The letter outlined the proposed study that was to be conducted during 2013. Twelve laboratories indicated that they would like to take part in the study; these being from Australia, Canada, Japan, New Zealand, UK, and USA. Each laboratory was assigned a confidential code number. One laboratory wished to report two separate results using two different techniques so it was assigned two code numbers. On 27 Feb 2013, two test samples were dispatched to each laboratory along with a letter of instructions, which included a fax-back form to acknowledge receipt of samples and their condition on arrival. The following specific information and instructions were given: "these samples contain DMSP in acidified artificial seawater within the concentration range 1-10 μmole L^{-1}; dilute the samples in aqueous solution to an extent that is considered appropriate for the method of analysis employed; make the samples alkaline and quantify DMS in each solution using the normal test method; report a single result for each sample; report results as the micro-molar DMS concentration; report results applying the limit of reporting of the method used for analysis; report the associated expanded measurement uncertainty to no more than two significant figures (e.g., 5.30 ± 0.53 μmole L^{-1}); report the basis of the uncertainty estimates (e.g., uncertainty budget, repeatability precision, long term result variability); and return the completed results sheet no later than 27 May 2013." An Excel spreadsheet for the electronic reporting of results was emailed to all participants. An interim report tabling results and reported uncertainties was emailed to all participants on 3 Jun 2013 and a final detailed report was issued on 1 Aug 2013 (NMI 2013).

Preparation of the interlaboratory comparison test samples

A bulk solution of acidified saline water was prepared from ~ 280 g NaCl (BDH Anal R grade reagent) and 37 mL concentrated HCl, which were added to 8 L of 0.22 μm filtered MilliQ water. A DMSP HCl stock solution was prepared by taking ~ 24 mg of a DMSP HCl CRM (molecular weight 170.7 g mol^{-1}, NMI Collection No. WR002) and diluting it to 100 mL with acidified water. Sixteen milliliters of the DMSP HCl stock solution was added to the 8 L acidified saline water to produce a bulk test sample DMSP HCl concentration of 430.2 μg L^{-1}. This solution was calculated to liberate 2.52 μmol L^{-1} of DMS when made alkaline by the laboratories participating in the ILC. After the bulk solution was prepared, it was sealed and mixed using a magnetic stirrer for approximately two hours before dispensing it into sixty amber glass bottles of 110 mL capacity. The bottles were randomly separated into blind duplicate test samples that were identified as S1 and S2, with an identifying bottle number. The samples were prepared on 11 Feb and kept at 4°C before dispatch on 27 Feb. They were packed into Styrofoam-insulated boxes but were not refrigerated during transit.

Homogeneity testing of the test samples and determination of a reference value

Six bottles were selected at random from each of the identical S1 and S2 sample sets to assess their homogeneity and to assign a reference concentration. All samples were prepared for analysis by transferring 5 mL of each into 20 mL headspace vials using a positive displacement pipette (Eppendorf Xstream). Each sample was spiked with 10 μL of 1.155 mmol L^{-1} hexa-deuterated DMSP hydrochloride (DMSP-d_6.HCl, NMI collection number WR003) that was prepared in 0.22 μm filtered MilliQ water. Each sample was then made alkaline by the addition of one pellet (~0.2 g) of NaOH to the headspace vial, which was immediately crimp capped. The micro-molar concentration of DMS released from each sample via the base-catalyzed elimination reaction of DMSP was determined using static headspace gas chromatography isotope dilution mass spectrometry (HS-GC-IDMS) in scan mode over a narrow range from 25-150 mass units. Each vial was equilibrated at 85°C for 15 min with shaking in the headspace oven before sampling the headspace volume. DMS-d_6 was used as a stable isotope internal standard, which was generated from DMSP-d_6.HCl via the base-catalyzed reaction with NaOH. The responses from ions *m/z* 62, 63, and 64 were extracted, inte-

grated, and summed to determine the DMS response, whereas the responses from ions *m/z* 68, 69, and 70 were extracted, integrated, and summed to determine the DMS-d_6 response.

Six matrix matched calibration standards were produced from a 0.266 mmol L^{-1} DMSP HCl stock solution prepared in 0.22 μm filtered MilliQ water using the NMI CRM. To prepare these standards 10-100 μL (n = 6) of the stock solution was spiked into 5 mL of 3.5% w:v NaCl to produce standards that ranged from 0.53 to 5.31 μmol L^{-1}. The six calibration standards and a reagent blank were spiked with 10 μL of the 1.155 mmol L^{-1} DMSP-d_6.HCl internal standard solution, as was added to the samples. DMS was identified according to its GC retention time match to the calibration standards and by its characteristic 70 eV mass spectrum against a DMS reference mass spectrum. A G1888A headspace unit attached to a 5890N GC and 5973N MS (Agilent Technologies) were used to carry out the HS-GC-IDMS analysis. A 30 m long, 250 μm diameter DB-624 (6% cyanopropyl-phenyl, 94% dimethyl-polysiloxane) bonded phase capillary column of 1.4 μm film thickness was used in the GC with a temperature program of 40°C for 5 min, 16°C min^{-1} ramp to 120°C giving a total run time of 10 min. The GC was operated in constant pressure mode with an initial column flow rate of 1.3 mL min^{-1}, which gave a DMS elution time of ~3.3 min. A sample introduction split ratio of 15:1 was applied giving a total flow rate of 23.9 mL min^{-1}.

Stability of DMSP in the test samples

The stability of DMSP in two randomly selected test samples was assessed in conjunction with the ILC schedule, and for up to 91 days after its completion. This stability study measured the DMS released from samples S1/21 and S2/16, which were maintained at room temperature and 4°C, respectively, using the static HS-GC-IDMS method. The DMS released from these two test samples was measured on four occasions between 11 Feb and 26 Aug 2013.

Assessment

Preparation of a DMSP hydrochloride certified reference material and its deuterated analogue

To conduct the ILC, it was necessary to prepare test samples that contained a traceable concentration of DMSP HCl. There was no known commercial supplier of a DMSP HCl reference material with a certified purity; therefore, to fulfill this need a DMSP HCl CRM (170.7 g mol^{-1}, CAS number: 4337-33-1) was produced and identified as NMI CRM Collection No. WR002. Its hexa-deuterated analogue DMSP-d_6.HCl was also synthesized to provide an internal standard for the analysis of test samples at the NMI; it was identified as Collection No. WR003. The reagents DMS, DMS-d_6 ($C_2{}^2H_6S$), and acrylic acid were purchased from Sigma-Aldrich Chemical Co. The DMS was of stated purity ≥ 99% and the DMS-d_6 was 99% ^2H atom enriched.

Procedures reported for the synthesis of DMSP have specified reaction of DMS with acrylic acid in either toluene or dichloromethane over periods from 20 min to 4 d (Chambers et al. 1987; Howard and Russell 1995; Smith et al. 1999). Due to uncertainty in the conditions required to synthesize DMSP, a study was conducted where DMS was reacted with acrylic acid in toluene or dichloromethane or ethyl acetate for periods of 4 h and 3 d. The results of this study (Table 1) showed that toluene provided the best yield of DMSP (93%) in the shorter 4-h period. Accordingly, the DMSP synthesis was conducted by reacting excess DMS with acrylic acid in dry toluene for 4 h at room temperature followed by the addition of ethereal HCl to precipitate the hydrochloride salt. This product was subsequently purified by recrystallization from methanol/diethyl ether (Chambers et al. 1987); however, it yielded an unwanted impurity of DMSP methyl ester from reaction with methanol. This impurity was considered likely to undergo base-catalyzed elimination to liberate DMS in the same manner as DMSP. It was therefore necessary to use an alternate recrystallization solvent to prevent its formation, so acetonitrile/water was used for this purpose (Fig. 1). The recrystallized DMSP HCl was found to be free of organic impurities; however, it was particularly hygroscopic, and it was not until after 26 days under ambient conditions that a constant weight was achieved for the DMSP HCl as the monohydrate. Its confirmation of identity as DMSP HCl monohydrate was achieved by electrospray interface (ESI) LC-MS, nuclear magnetic resonance (NMR) spectroscopy and elemental microanalysis: ESI LC-MS (+ ve) *m/z* 135 ($M^+ + H$). ^1H NMR spectrum (D_2O) δ 2.97 (6H, s, CH_3), 3.02 (2H, t, J = 6.9 Hz, CH_2), 3.57 (2H, t, J = 6.9 Hz, CH_2). ^{13}C NMR spectrum (D_2O) δ 25.1 (CH_3), 28.6 (CH_2), 38.8 (CH_2), 173.7 (C = O) ppm.

Table 1. Comparison of reaction time and solvent for the synthesis of DMSP from DMS and acrylic acid.

Solvent	Reaction time	Yield (%)
Dichloromethane	4 h	78%
	3 d	74%
Toluene	4 h	93%
	3 d	52%
Diethyl ether	4 h	34%
	3 d	30%
No solvent	3 d	68%

The DMSP HCl was allowed to hydrate due to concerns of its purity decreasing over time while in storage. When a constant weight was achieved for the DMSP HCl, its moisture uptake was observed to stabilize at 9.8% mass fraction of water as determined by Karl Fischer titration. Static headspace GC-MS was used for the detection of occluded solvent, which indicated the presence of trace amounts of acetonitrile, estimated to be 0.003% mass fraction by the ^1H NMR spectroscopy. Thermo-gravimetric analysis showed no nonvolatile residue and elemental microanalysis confirmed the elemental composition and water content as a monohydrate: C = 32.1%; H = 6.9%; Cl = 24.8%; S = 17.1%; calculated: C = 31.8%; H = 6.9%; Cl = 18.8%; S = 17.0% for $C_5H_{11}ClO_2S.H_2O$.

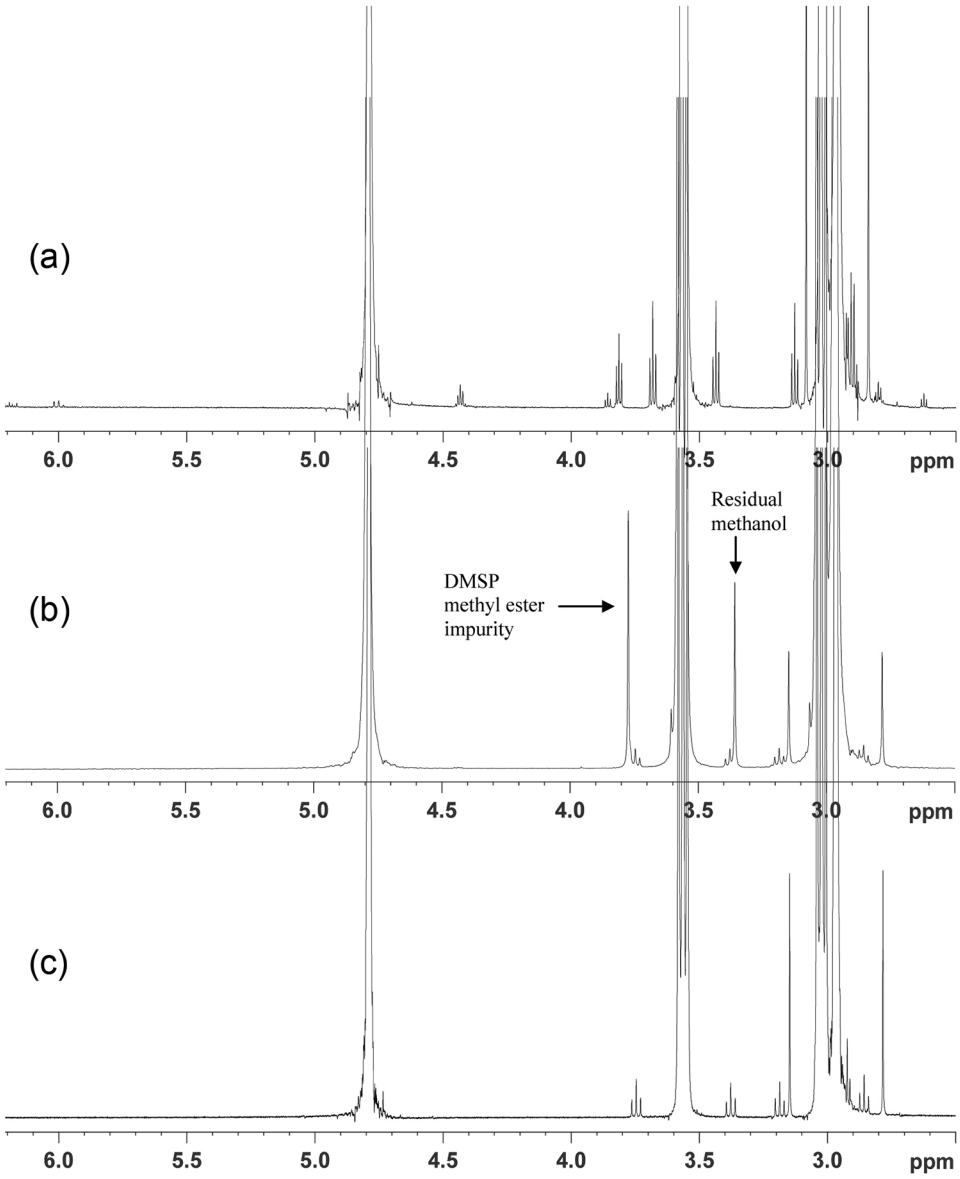

Fig. 1. ^{1}H NMR spectrum of DMSP.HCl in D_2O: (a) before recrystallization; (b) after recrystallization with methanol/diethyl ether; (c) after recrystallization with acetonitrile/water.

The DMSP HCl was certified to have a purity of 90.3 ± 1.8% mass fraction (95% coverage interval), which was obtained by quantitative NMR spectroscopy (qNMR) in deuterium oxide (D_2O) using 400 MHz field strength (Bruker Avance-III-400 NMR). A combination of the two proton triplet at 3.5 ppm and the combined two proton triplet and the six proton singlet at 2.9-3.0 ppm was used to determine its purity relative to a certified internal standard of maleic acid. Thereafter, periodic Karl Fischer titration and ^{1}H NMR analysis confirmed the stability of this material.

Hexa-deuterated DMSP hydrochloride (DMSP-d_6.HCl) was prepared by substituting DMS-d_6 for DMS as the starting reagent. It was prepared in 64% yield and was not subject to extended water uptake under ambient conditions. The same procedures were applied to its synthesis and to confirm its identity: ESI LC-MS (+ ve) *m/z* 141 ($M^+ + H$). ^{1}H NMR spectrum (D_2O) δ 3.02 (2H, t, J = 6.9 Hz, CH_2), 3.56 (2H, t, J = 6.9 Hz, CH_2). ^{13}C NMR spectrum (D_2O) δ 25.9 (quintet, J = 22 Hz, CD_3), 30.0 (CH_2), 39.9 (CH_2), 175.2 (C = O) ppm. Elemental microanalysis found: C = 34.2%; H = 2.9%; D = 7.0 %; Cl = 20.2 %; S = 18.3%; calculated: C = 34.0%; H = 2.9%; D = 6.8 %; Cl = 20.1 %; S = 18.2% for $C_5H_5D_6ClO_2S$. The purity of the DMSP-d_6.HCl was not certified because its intended use was as a stable isotope internal standard for isotope dilution analysis at the NMI. A purity estimate of 97% minimum was obtained for the DMSP-d_6.HCl from a single qNMR analysis using a

combination of the two proton triplet at 3.5 ppm and the two proton triplet at 2.9 ppm against a certified internal standard of sodium acetate.

Bell et al. (2012) observed variability of up to 30% in the slopes of calibration regression lines obtained from three different preparations of DMSP HCl. Thermo-gravimetric analysis indicated that these differences were mostly due to variable water content but it did not fully explain the observed variation. The authors identified the need for a CRM of DMSP HCl. As a component of the work conducted in this study, a commercially available DMSP HCl analytical reagent was purchased, and when analyzed by Karl Fischer titration was found on average (n = 3) to have a water content of 11.3% mass fraction. Additionally, analysis by ^{1}H NMR showed the presence of several unknown impurities that were estimated to constitute ~ 3% mass fraction. The purity of this particular DMSP HCl when analyzed by static HS-GC-IDMS against the NMI DMSP HCl CRM, found it to be 83.1 ± 1.9% (1σ, n = 7). It is common for commercially available DMSP HCl analytical reagent to be assumed to be 100% pure. The combination of water, organic impurities such as DMSP methyl ester (Fig. 1), and occluded solvents, can lead to discrepancies between standards and consequently contribute to variability in the quantitative results obtained from an ILC.

Validation of the static HS-GC-IDMS method for analysis of test samples

A primary method of analysis is the preferred technique by which to assign a reference value to the analyte in a test sample by the coordinating laboratory. Isotope dilution MS satisfies this requirement because it is a primary measurement method that has the potential for traceability to the International System of Units (De Bièvre and Peiser 1997). In this study, the stable isotope DMS-d_6 was applied as an internal standard surrogate of the DMS analyte to mimic its physical and chemical characteristics. The Henry's law coefficients for DMS-d_6 and DMS have been found to be equivalent within the experimental relative uncertainty of 3.0% relating to this measurement (Ridgeway Jr. et al. 1991; Warneck and Williams 2012). The presence of equilibrated DMS-d_6 in a sample can compensate for loss of the DMS analyte during the analysis procedure, thereby improving the precision and quantitative accuracy. For these reasons DMSP-d_6.HCl was synthesized and used to generate DMS-d_6 as an internal standard via the base-catalyzed reaction with NaOH.

DMS was quantified in the test samples from the sum of responses obtained from extracted ions m/z 62, 63, and 64 because collectively they have been reported to account for > 99% of naturally occurring DMS (Smith et al. 1999). This was initially assessed by calculating the relative mole fractions of thirteen DMS isotopomers that exist naturally in the environment (Ridgeway Jr. et al. 1991). By using the representative natural isotopic composition of the elements H, C, and S (De Laeter et al. 2003), the mole fractions of these DMS isotopomers were calculated. This analysis predicted normalized mole fractions for 62, 63, and 64 Dalton DMS of 93.95%, 1.77%, 4.22%, respectively, where mass 62 was derived from $^{12}C_2{}^{32}S^1H_6$, mass 63 from $^{12}C_2{}^{33}S^1H_6$, $^{12}C^{13}C^{32}S^1H_6$, $^{12}C_2{}^{32}S^1H_5{}^2H$, and 64 from $^{12}C_2{}^{34}S^1H_6$, $^{12}C^{13}C^{33}S^1H_6$, $^{13}C_2{}^{32}S^1H_6$. The sum of these DMS isotopomers for masses 62, 63, and 64 was calculated to account for 99.94% of naturally occurring DMS.

The predicted data were experimentally investigated using conventional 70 eV electron impact ionization MS which gave average isotopic proportions of 91.5%, 4.1%, and 4.4% for ions m/z 62, 63, 64, respectively, from seven replicate solutions of DMS. By comparison, Ridgeway Jr. et al. (1991) reported mole fractions for DMS in seawater to be 92.86%, 2.87%, and 4.16% for m/z 62, 63, and 64, respectively, using 30 eV electron impact ionization MS. For this study, DMS-d_6 was also analyzed and average isotopic proportions of 92.9%, 2.9%, and 4.1% were obtained for ions m/z 68, 69, 70, respectively (n = 7). These experimental results indicated that 99.9% of both DMS and DMS-d_6 could be accounted for by collectively monitoring these particular ions, therefore they were extracted from total ion chromatograms and the peak area responses ($PA_{62\text{-}64}$ and $PA_{68\text{-}70}$) were integrated and summed as part of the analytical procedure. The same deuterated internal standard solution was added to samples and calibration standards to produce two blends: an approach generally referred to as "double" IDMS. The $PA_{62\text{-}64}$ to $PA_{68\text{-}70}$ response ratios obtained from reagent blanks and calibration standards were plotted against the concentration of DMS in each standard to prepare calibration plots, from which line of best fit equations were determined and used to calculate the concentration of DMS in each test sample.

The stated 99% ^{2}H atom purity of the DMS-d_6 reagent used to synthesize the DMSP-d_6.HCl indicated that it should also contain a trace amount of natural DMS, and the mass spectrum for DMS-d_6 showed this as a minor response in the m/z 62-64 mass windows. This background response detected in reagent blanks containing only DMS-d_6 gave $PA_{62\text{-}64}$ to $PA_{68\text{-}70}$ response ratios of ~ 0.02, resulting in a limit of detection of 50 nmol L^{-1} for the static HS-GC-IDMS method. The background response ratio was applied to calibration plots but it was significantly less than the lowest level calibration standard, which gave a response ratio of ~ 0.2. The amount of DMSP-d_6.HCl added to the test samples gave $PA_{62\text{-}64}$ to $PA_{68\text{-}70}$ response ratios close to one to optimize the precision and accuracy of the isotope dilution approach. Accuracy and precision are greatest when the responses are matched because this can effectively cancel out systematic error in the derived response ratio (Henrion 1994).

Reference value, test sample homogeneity, and uncertainty

A DMS reference value of 2.51 ± 0.13 μmol L^{-1} for the test samples was obtained using the static HS-GC-IDMS method. This analytically derived value, which was determined on the day the samples were prepared, was very close to the calcu-

lated gravimetric and volumetric solution concentration for DMSP.HCl of 2.52 μmol L^{-1}. Static HS-GC-IDMS analysis also demonstrated that the test samples were sufficiently homogeneous. Random order duplicate analysis of six samples from each of the S1 and S2 sample sets gave means and 1σ standard deviations of 2.51 ± 0.05 μmol L^{-1} (n = 12) and 2.51 ± 0.04 μmol L^{-1} (n = 12), respectively. The uncertainty for the reference value was expanded, using a coverage factor of two, to provide a 95% confidence interval. It was estimated in accordance with the Eurachem/CITAC guide (Ellison and Williams 2012) by combining standard uncertainties for method precision (2.3%), the purity of the CRM (1.0%), and volumetric and gravimetric error (0.3%) in the preparation of standards and samples. Combining these standard uncertainties and applying a coverage factor of two gave a relative expanded measurement uncertainty of 5.1%.

The temporal and thermal stability of DMSP in artificial seawater

Examination of the stability of the test material is required as a component of an ILC, and this was carried out in accordance with prescribed guidelines (ISO/Guide-35 2006; Thompson et al. 2006). Sample S1/21 (stored at room temperature) and S2/16 (stored at 4°C) were analyzed in duplicate on the day they were prepared, in triplicate during the study period, and again in triplicate on the day the study results were due. The mean DMS concentrations and 1σ standard deviations obtained for S1/21 and S2/16 over this 105-d period were 2.43 ± 0.07 μmol L^{-1} and 2.44 ± 0.04 μmol L^{-1} (n = 8), respectively, demonstrating that the samples were sufficiently stable for the duration of the study to satisfy its intended purpose. The concentrations of DMS released from S1/21 and S2/16 over the 105-d period were not statistically different at the 99% confidence level ($T_{0.24} < t_{1\text{-}0.01/2,14}$ = 2.98, p = 0.82), which indicated that the results from participating laboratories for this ILC were unlikely to be biased by transport and storage of the test samples at room temperature.

The two samples S1/21 and S2/16 were again analyzed in triplicate 196 days after they were prepared and the average DMS concentrations had decreased by 0.5-0.7% from the average concentrations measured 91 days earlier. The mean DMS concentrations measured at day 0, 84, 105, and 196 were plotted against the elapsed time period, and it was apparent that very slow degradation of DMSP and subsequent loss of DMS from the samples had occurred. Linear regression slopes for S1/21 (ambient, r^2 = 0.8427) and S2/16 (4°C, r^2 = 0.9705) gave DMS losses of 0.81 and 0.34 nmol d^{-1}, which is equivalent to half-lives of 4.3 and 9.8 years for each sample, respectively. These findings are in agreement with the conclusion of Dacey and Blough (1987) that in sterile seawater at 10°C and pH of 8.2 the half-life of DMSP is ~ 8 years. The results of this stability study indicated that if low nano-molar DMSP HCl solutions were dispatched as test samples they would need to be frozen immediately after preparation and not thawed until immediately prior to analysis.

Interlaboratory calibration: Summary of techniques used and results reported

Most laboratories used GC with sulfur-specific detectors to measure DMS released from the test samples. Five laboratories used GC-Flame Photometric Detection (FPD), four used GC-Pulsed Flame Photometric Detection (PFPD), one used GC-Sulfur Chemiluminescence Detection (SCD) and one used Proton Transfer Reaction-Mass Spectrometry (PTR-MS). The DMS concentrations and expanded measurement uncertainties reported by the participating laboratories for the blind duplicate samples are given in Table 2 and shown graphically in Fig. 2. Table 2 also presents the differences between reported concentrations and the uncertainties for S1 and S2 on a relative basis. Summaries of analysis and calibration procedures reported by participating laboratories are transcribed into Tables 3 and 4. Ten laboratories that submitted results used the 'Purge-and-Trap' technique to recover DMS from the test samples after applying various dilution factors ranging from 4 to 2500, with the addition of alkali. Laboratory *13* used static headspace analysis and did not dilute the samples (Table 3). Following receipt of the interim report, two laboratories requested to amend their results. Laboratory *10* said that they reported the concentrations of DMSP in the samples instead of the DMS released. Laboratory *12* amended their results to include two decimal places instead of one. Unfortunately laboratories *1* and *9*, who indicated that they are presently using equilibrator equipment, were unable to report results by the due date.

Interlaboratory calibration: Within-laboratory variability

The within-laboratory variability between the blind duplicate test samples S1 and S2 was 5% or less for all participants except laboratories *2* and *8* (Table 2). The difference between the results for the duplicates reported from these two laboratories was 24% and 44%, respectively; however, this high variability was not significant for laboratory *8* when the reported

Table 2. Concentrations of DMS (μmol L^{-1}) and expanded uncertainties (95% confidence interval) for the blind duplicate test samples S1 and S2 analyzed by participating laboratories. ΔS1 & S2 is the relative percentage difference between the reported concentrations for the test samples.

Lab	Sample S1	Sample S2	ΔS1 & S2
2	2.52 ± 0.05	1.97 ± 0.02	24%
3	2.61 ± 0.19	2.67 ± 0.22	2%
4	2.56 ± 0.05	2.70 ± 0.05	5%
5	2.72 ± 0.14	2.65 ± 0.05	3%
6	2.34 ± 0.10	2.42 ± 0.05	3%
7	2.62 ± 0.23	2.75 ± 0.10	4%
8	2.49 ± 0.83	1.59 ± 0.53	44%
10	2.11 ± 0.22	2.18 ± 0.20	3%
11	2.21 ± 0.11	2.27 ± 0.13	3%
12	2.17 ± 0.10	2.14 ± 0.08	1%
13	2.42 ± 0.19	2.44 ± 0.18	1%

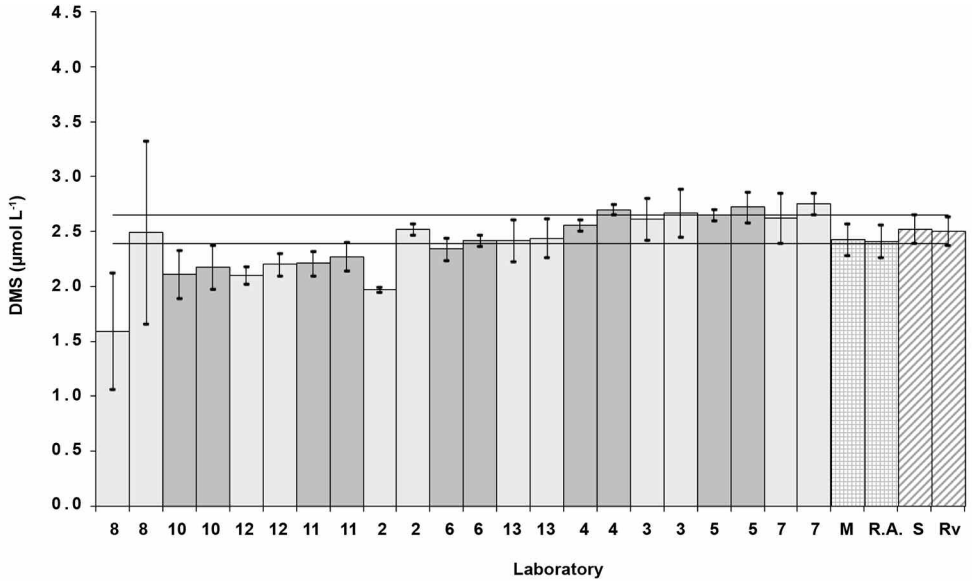

Fig. 2. Reported DMS concentrations and expanded measurement uncertainties (μmol L^{-1}) from participating laboratories for the blind duplicate test samples. The two horizontal lines represent the upper and lower limits of the expanded measurement uncertainty pertaining to the reference value. Legend: M is the mean value, R.A. is the robust average, S is the spiked concentration, Rv is the reference value.

Table 3. Sample dilution factors, analysis procedures, and instrumental techniques reported by participating laboratories.

Lab. code	Sample preparation	Dilution factor	Analysis technique	Equipment	GC column
2	Dilution	200	Purge and cryogenic trap	GC-PFPD	Zebron ZB-1, 30 m, 0.53 mm ID, 5.0 μm film thickness
3	Dilution	124	Purge and trap	GC-FPD	Varian CP-SIL 5B
4	Dilution	4	Purge and trap	GC-PFPD	SPB-1 SULFUR
5	Dilution in 1M NaOH	95	Sparge, trap, and purge	GC-SCD	Agilent DB-1
6	Dilution	10	Purge and trap	GC-FPD	
7	Dilution	2500	Purge and trap	GC-FPD	TCEP 25% ID3.2ϕ * 3.1 m
8	Dilution	199	Purge	PTR-MS	
10	Dilution	9	Purge and trap	GC-PFPD	BP1, 30m × 0.32mm × 4 μm
11	Dilution	50	Purge and trap	GC-PFPD	CP7529
12	Dilution	99	Purge and trap	GC-FPD	HP-1
13	No dilution. Samples equilibrated at 30°C	0	Headspace	GC-FPD	Varian CP-SIL 5CB

expanded measurement uncertainty was taken into account (Fig. 2). Laboratory *8* used PTR-MS for sample analysis and specified that they purged the samples to the detection system after applying a dilution factor of 199. It is not clear if this refers to a bubble-type equilibrator or whether a deviation from their normal sample introduction procedure was used, which may explain the large estimated measurement uncertainty. Therefore, it is not clear whether the high duplicate sample variability is a result of the technique or the sample introduction procedure used. Further ILC studies are required by PTR-MS laboratories to elucidate this issue. Laboratory *2* reported that they froze the test samples on arrival and when they were removed from the freezer both bottles were found to be cracked, presumably due to the minimal headspace volume. Consequently, the contents had to be decanted to alternative bottles, and it is possible that the 24% difference in the duplicate results reported by laboratory *2* was caused by this situation.

Interlaboratory calibration: Between-laboratory variability

The difference between the maximum and minimum concentrations reported from all laboratories for the S1 and S2 sample sets was 22.4% and 42.2%, respectively. The S2 sample set had the widest range of results which spanned from 1.59 μmol L^{-1} (lab *8*) to 2.75 μmol L^{-1} (lab *7*) (Fig. 2). If the results from laboratory *8* (which used PTR-MS) are removed, the difference between the maximum and minimum results for the

Table 4. Calibration standards reported by participating laboratories.

Lab. code	Calibration standard	Purity	Origin	Comment
2	Liquid standard (DMSP dissolved in MQ, followed by alkaline cold hydrolysis with 10M NaOH)	> 98%	CASS, University of Groningen	
3	DMSP	100%	CASS, University of Groningen	Purity is assumed based on the CASS post-synthesis NMR analysis
4	Permeation tube of DMS	100%	Kin-Tek Laboratories	Emission rate 547 ng/min
5	DMS permeation tube (ext. std) and MES permeation tube (int. std)		VICI	
6	Liquid	99%	Sigma	
7	Liquid standard made by DMSP chloride	>98.0%	Tokyo Kasei	
8	Liquid	>98%	Tokyo Chemical Industry	
10	Liquid	90.3% ± 1.8%	National Measurement Institute	
11	Permeation tube	100%	Kin-Tek Laboratories	Standard curve at $r^2 = 0.99$ emission rate 488 ng/min
12	Liquid	at least 95%	Chemical Synthesis at University of Essex	
13	DMSP-HCl	100%	CASS, University of Groningen	Purity is assumed based on the CASS post-synthesis NMR analysis

S1 and S2 sample sets is 22.4% and 28.4%, respectively. The average variability for both sets of samples is therefore 25.4% for the laboratories that used traditional GC techniques with sulfur-specific detectors to measure DMS in the test samples. This is in agreement with the estimate of Bell et al. (2012) who suggested the variability between existing measurements in the global surface seawater database is ≤ 25% according to the few intercomparison exercises that have previously been conducted. It should however be noted that this estimate of variability applies to measurement of ambient DMS concentrations in natural seawater samples. In contrast, the test samples in this study were in artificial seawater containing no algae and were analyzed by all but one laboratory using dilution factors that released DMS at super-ambient concentrations.

Interlaboratory calibration: Choice of calibration standard and comparison of analytical techniques

Laboratories *4*, *5*, and *11* reported that they used DMS permeation tubes for calibration. Laboratory *5* also noted that they also used a methylethylsulfide permeation tube as an internal standard. The remaining laboratories specified that they used liquid standards, which were clearly specified or at least indicated to be prepared from DMSP HCl. For laboratories *6* and *8*, it was not clear whether they used calibration solutions prepared from DMSP HCl or from DMS. There was no apparent bias or systematic difference between the results reported from calibration using liquid solutions versus permeation tubes. This was also found to be the case for a previous ILC between two laboratories of DMS measured in seawater (Turner et al. 1990).

Laboratories *5* and *7* reported similar DMS concentrations for the S1 and S2 duplicate pairs, these being relatively high amongst all results provided (Fig. 2). Laboratory *5* applied a dilution factor of 95 and uniquely used GC-SCD with calibration from permeated DMS, whereas laboratory *7* applied the largest dilution factor of 2500, used GC-FPD and a liquid calibration standard prepared from DMSP HCl. This comparison suggests that the relatively high results reported by laboratory *5* are not related to use of GC-SCD for analysis of the test samples nor DMS derived from a permeation tube.

The laboratory that was assigned codes *3* and *13* reported that they used GC-FPD and a Varian CP-SIL 5B GC column for two different sampling techniques, where code *3* was for 'Purge-and-Trap' and code *13* was for headspace sampling (Table 3). The 'Purge-and-Trap' technique produced higher results than the headspace technique, which were respectively above and below the reference value (Fig. 2). A DMSP HCl standard sourced from the same supplier, that was indicated to be 100% pure, was used for both techniques (Table 4).

Laboratory *8*, which uniquely used PTR-MS to analyze the test samples, reported a result for S1 that was very close to the reference value; however, the result for S2 was the furthest of all results relative to the reference value. These results indicate that PTR-MS can potentially provide accurate quantification of DMS in aqueous solution but may be subject to greater vari-

ability than the other established techniques. This is also suggested by the comparatively large measurement uncertainties reported by laboratory *8*. It is necessary for laboratories using PTR-MS to participate in future ILC studies to elucidate this preliminary observation.

Interlaboratory calibration: Estimation of measurement uncertainty

It is a requirement of the ISO Standard 17025 (ISO/IEC: 17025 2005) that laboratories have procedures to estimate the uncertainty of chemical measurements and to report this uncertainty when requested. All laboratories that participated in this ILC indicated that they are not accredited for this analysis; however, realistic estimation of measurement uncertainty is an important component of any analytical result. In this study, all participants reported expanded uncertainties, where the magnitude ranged from 1% to 33% relative to the result. Eleven out of the 22 expanded uncertainties provided for the S1 and S2 test samples were less than 5% relative to the result, which were considered to be unrealistically small according to the uncertainty estimated using static HS-GC-IDMS with undiluted samples. Laboratories *2* and *4* reported particularly small expanded uncertainties that did not account for the difference between duplicates. Laboratories *2*, *5*, *6*, and *7* reported very different measurement uncertainties for the S1 and S2 blind duplicates. The percentage differences between the relative uncertainties reported for S1 and S2 by these four laboratories ranged from 95% to 173%. Laboratory *8*, which used PTR-MS, reported comparatively large uncertainties; however, it was the only laboratory where the relative uncertainties were consistent for the blind duplicate samples (Table 2).

Discussion

Rationale for test sample preparation and conduct of the interlaboratory comparison

Natural seawater samples are often acidified to a pH of < 2 to preserve DMSP (Curran et al. 1998; Kiene and Slezak 2006) because the addition of strong acid to seawater can generally act as a biocide and enzyme inactivation agent to prevent the biological conversion of DMSP to DMS (Del Valle et al. 2011). The test samples used in this pilot study were prepared in artificial seawater rather than natural seawater to avoid the possible presence of DMSP-lyase. Samples were also passed through a 0.22 μm filter and acidified with HCl to pH 1 as a precautionary measure to maintain sterility.

The ILC test samples were prepared containing a concentration of DMSP.HCl that was orders of magnitude greater than ambient DMS seawater concentrations. There were a number of reasons for this decision, the main one being to encourage and enable those using equilibrator methods to participate. Equilibrators require a large sample volume, and the 100 mL test samples containing 2.5 μmol L^{-1} DMSP HCl facilitated a large dilution to be made that could provide at least 100 L of a near ambient nano-molar DMS concentration. A second reason for providing test samples containing 2.5 μmol L^{-1} of DMSP HCl was due to limited knowledge of the stability of DMSP HCl in aqueous solution over a three month period; the time schedule allocated from dispatch of samples to receipt of results from the participating laboratories. It was considered that the potential loss of DMS from ambient nano-molar test samples, regardless of the storage temperature during this period, could lead to greater error than that introduced from volumetric dilution of concentrated micro-molar test samples to ambient or near-ambient DMS concentrations immediately before analysis. To avoid potential invalidation of the ILC, it was decided for this pilot study to supply blind duplicate concentrated stock solutions at ambient temperature to assess within-sample variability due to the dilution step. A third reason for preparing the test samples at a super-ambient concentration was that it widened the choice of possible analytical approaches by allowing static headspace analysis to be used. This technique is unable to detect DMS in seawater at ambient nanomolar concentrations but nevertheless may be usefully applied where higher concentrations of DMS are generated such as in phytoplankton flask cultures. Static headspace analysis was used to assign the reference value to the test samples because it did not require the samples to be diluted and therefore eliminated this source of error in the estimation of measurement uncertainty relating to the reference value. Static headspace sampling in conjunction with GC-IDMS analysis was therefore used to provide a benchmark for the realistic assessment of measurement uncertainty associated with the results received by participating laboratories.

Information received from the advisory committee and intending participants for this pilot ILC suggested that a 90-d turnaround time was required because analytical equipment to measure DMS is regularly taken to sea and may not be immediately available. Additionally, the stability study conducted in conjunction with the ILC signaled the difficulty of providing stable test samples able to release near-ambient nanomolar concentrations of DMS over a 90-d period. Some of the participants in this pilot study indicated that they are well aware of this problem according to their comments and treatment of the samples after receipt. Laboratory *2*, for example, froze the test samples on receipt, presumably to prevent the thermal degradation of DMSP.HCl to DMS before analysis.

Synopsis of results for the interlaboratory comparison

The between-laboratory results obtained from this pilot study are considered to be reasonably good given that this is the first known ILC by multiple laboratories for the quantification of DMS in aqueous solution. The robust average for all laboratories was at the lower end of the expanded uncertainty of the reference value; however, it is consistent with the robust average by the overlap of the expanded uncertainties (Fig. 2). Whereas this is a good overall result, it is only an initial indication of the extent of random and systematic errors that exist between techniques and operators for the quantification of trace nanomolar concentrations of surface ocean seawater DMS. Only one laboratory diluted the test samples to an ambient DMS concentration for analysis, yet its results for the

duplicate samples were closer to the reference value than some other laboratories. Additionally, only one laboratory reported consistent measurement uncertainty for analysis of the duplicate samples.

Comments and recommendations

It is recommended that further ILC studies of this type are conducted because the results of this pilot study indicate that there is scope for the DMS measurement community to improve its proficiency to quantify DMS dissolved in aqueous solution. Specific recommendations for future ILC studies are (1) DMSP HCl reagent that is not purchased as a CRM with a specified purity should be desiccated. The reagent should then be stored at or below 20°C under dry nitrogen in an air-tight container. (2) Micro-molar DMSP.HCl test samples, which remain sufficiently stable for at least 105 days at room temperature, should be distributed. The door-to-door delivery of internationally consigned frozen test samples of DMSP HCl in the nanomolar range is not recommended because it cannot be guaranteed that they will remain frozen during transit. It would also increase participation costs and would exclude those using equilibrators that require large sample volumes. (3) A consensus reference material, such as the NMI DMSP.HCl CRM, should be used by all participants in a future ILC. (4) Future ILCs could incorporate instructions and references to assist laboratories quantify their measurement uncertainty.

An alternative intercomparison approach is to conduct a joint field study of ambient concentrations of DMS in seawater at or close to the sea. This is the only way to assess data intercomparability from natural samples. The need to gently filter phytoplankton from seawater is an essential component of such an analysis procedure yet can induce substantial variability in the results (Kiene and Slezak 2006). Due to the higher associated costs, it is recommended that improved overall proficiency in sample distribution ILC's precede joint field study intercomparisons.

There is a need to compare data variability that arises due to the range of analytical techniques and the different approaches for extracting DMS from solution (e.g., 'Purge-and-Trap' vs. equilibration). This ILC pilot study was unable to fully assess these differences because only one laboratory that used the equilibration technique participated. There is also a need to broaden participation to include those using new analytical approaches such as EI-PTR-MS and CI-MS, and directly compare them with established chromatographic techniques. By taking these actions, a better understanding of existing and future data comparability in the global surface seawater DMS database will be achieved.

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