EDGEWOOD CHEMICAL BIOLOGICAL CENTER
U.S. ARMY RESEARCH, DEVELOPMENT AND ENGINEERING COMMAND
Aberdeen Proving Ground, MD 21010-5424

ECBC-TR-1547

NMR METHOD TO DETERMINE NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT AGENT T

David J. McGarvey

RESEARCH AND TECHNOLOGY DIRECTORATE

William R. Creasy

LEIDOS, INC.
Abingdon, MD 21009-2161

October 2018

U.S. ARMY RDECOM

Disclaimer

The findings of this report are not to be construed as an official Department of the Army position unless so designated by other authorizing documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
XX-10-2018	Final	Jan 2017–Oct 2017

4. TITLE AND SUBTITLE
NMR Method to Determine NIST-Traceable Quantitative Weight Percentage Purity of Neat Agent T

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S
McGarvey, David J. (ECBC) and Creasy, William R. (Leidos)

5d. PROJECT NUMBER
W911SR-10-D-0004

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Director, ECBC, ATTN: RDCB-DRT-T, APG, MD 21010-5424
Leidos, Inc.; 3465 Box Hill Corporate Center, Abingdon, MD 21009-2161

8. PERFORMING ORGANIZATION REPORT NUMBER
ECBC-TR-1547

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Nuclear magnetic resonance (NMR) with ^{1}H or ^{13}C detection is described herein as a method to determine the weight percent purity of feedstock samples of agent T in a way that is National Institute of Standards and Technology (NIST)-traceable. A precision-and-accuracy test is also described.

15. SUBJECT TERMS
Purity determination
Nuclear magnetic resonance (NMR)
Sulfur mustard, bis-(2-chloroethyl) sulfide (HD)
National Institute of Standards and Technology (NIST)

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Renu B. Rastogi
U	U	U	UU	40	19b. TELEPHONE NUMBER (include area code) 410-436-7545

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Blank

PREFACE

The work described in this report was authorized under project no. W911SR-10-D-0004. This work was started in January 2017 and completed in October 2017.

The use of either trade or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

This report has been approved for public release.

Acknowledgments

The authors thank Suzanne Procell (U.S. Army Edgewood Chemical Biological Center; Aberdeen Proving Ground, MD).

Blank

CONTENTS

FIGURES

TABLES

Blank

NMR METHOD TO DETERMINE NIST-TRACEABLE QUANTITATIVE WEIGHT PERCENTAGE PURITY OF NEAT AGENT T

1. INTRODUCTION

This report provides information and details on the procedure to determine the purity of agent T {bis[2-(2-chloroethylthio)ethyl]ether} by nuclear magnetic resonance (NMR). This procedure is based on published technical report procedures for using NMR instruments to determine the purity of chemical warfare (CW) agent samples.[1–4] Previous National Institute of Standards and Technology (NIST; Gaithersburg, MD)-traceable methods were described for HN-3 [tris(2-chloroethyl)amine];[5] HN-1 (2,2′-dichlorotriethylamine);[6] and HD [bis-(2-chloroethyl) sulfide].[7] The procedure uses an internal standard with a known purity to establish an absolute weight percentage for the analyte of interest. Identifying the structures of other components in the mixture is not necessary. It is necessary to know the NMR chemical shifts of the major analyte, the internal standard, and the average molecular weights for the compounds of interest. The weight percent calculations are not negatively affected by the presence of unidentified compounds or undetectable components in the sample (e.g., inorganic salts, insoluble solids, etc.), as long as the sample is homogeneous or a thoroughly mixed suspension before it is portioned out from the storage container. In the previous reports for HD, HN-1, and HN-3, the procedure used a NIST standard material as an internal standard. An internal standard was purchased from Sigma-Aldrich Company (St. Louis, MO) that had a NIST-traceable purity. A balance that was calibrated with NIST-traceable weights was also used. All of these modifications made the method NIST-traceable.

For this method, a chemical from Sigma-Aldrich was used as an internal standard, but it was not NIST-certified by the vendor. A secondary step was used to determine the purity of the internal standard, and it was referenced to an NIST standard before the agent purity was calculated.

Technical issues with the NMR signal responses were the reason for the change from the previous HD method.[7] Because HD and agent T are chemically very similar, and because HD may be a contaminant in an agent T sample (along with other compounds), it is helpful to use carbon-13 (C-13 or ^{13}C) NMR to resolve the HD and agent T peaks. ^{13}C NMR has a higher resolution than proton NMR (H-1 or ^{1}H NMR); therefore, the peaks are spaced further apart. However, to use ^{13}C NMR for quantitative measurements, it is important to have an internal standard in which the carbon atoms have the same number of hydrogen atoms bonded to them as those of the analyte. (This is due to the transfer of excitation between carbon and hydrogen nuclei during proton decoupling, which improves signal strength and spectral resolution.) Both HD and agent T contain exclusively CH_2 groups; therefore, an appropriate internal standard must also contain a CH_2 group. There are not any commercially available NIST-traceable standards that meet this requirement; therefore, a secondary standard was used. By using the secondary standard, the purity could be determined by both ^{1}H and ^{13}C NMR using the same samples to minimize agent consumption and time for sample preparation. If the purity were determined by only ^{1}H NMR, the method described in the HD purity report[7] could be used for agent T.

To determine the purity of agent T, ^{1}H and ^{13}C NMR were both used for agent detection, and the results were compared to each other. Precision-and-accuracy (P&A) testing of the method for ^{1}H NMR data was performed.

2. PROCEDURE

2.1 Supplies

The following supplies can be used for the procedure. Equivalent supplies may be available from other vendors.

Agent T was obtained from the Chemical Agent Standard Analytical Reference Material (CASARM) program at the U.S. Army Edgewood Chemical Biological Center (ECBC; Aberdeen Proving Ground, MD) for this project.

The following supplies were purchased from Sigma-Aldrich: (1) A secondary internal standard of 1,1,1,2-tetrachloroethane (1,1,1,2-TCE; part number T7209-25G, Chemical Abstracts Service [CAS] no. 630-20-6, ReagentPlus 99% purity); (2) the NIST-traceable primary internal standard 1,2,4,5-tetramethylbenzene (1,2,4,5-TMB; part number 74658-5G, CAS no. 95-93-2), as a TraceCERT-certified reference material standard for quantitative NMR; and (3) chloroform, 99.9% D (part number 23,689-6).

The following laboratory supplies were purchased from Wilmad-Lab Glass (Vineland, NJ; http://www.wilmad-labglass.com/ordering/index.jsp): (1) 5 mm diameter, 8 in. long NMR tube (part number WG-1000-8-50); (2) Teflon inserts (part number 6005); and (3) Pasteur pipets, 9 in. (part number C-7095B-9).

A JEOL USA, Inc. (Peabody, MA) ECS-400 NMR spectrometer with a 400 MHz (9.8 T) superconducting magnet and 5 mm liquid analysis probe was used. A Sartorius Corporation (Göettingen, Germany) Cubis microbalance (model MSA6.6S-000-DM, precision of ±1 µg) was used for measuring weights because it allows small amounts of agent to be measured with good accuracy. The microbalance was installed in a fume hood and calibrated using NIST-traceable weights. For the P&A test of agent T, a Sartorius analytical balance was used, and it was also calibrated with NIST-traceable weights to ±20 µg. This balance was less accurate, but it is more commonly available than the microbalance. The microbalance is also more susceptible to static charges on the sample vials, which can introduce errors.

NMR systems and balances from other vendors should give comparable results, if the operators have the appropriate training.

Other common laboratory equipment was used, including a vortex mixer, spatulas, and volumetric pipets. This equipment is not critical to the accurate performance of the method.

2.2 Sample Preparation

This procedure was performed under proper engineering controls, in accordance with surety and safety regulations, equipment validations, and standing operating procedures that were approved by the ECBC Safety and Health Office. The balance was calibrated using NIST-traceable weights.

Follow these steps to prepare the samples:

a. Tare a screw-cap vial and its cap on the balance. Transfer 10–20 mg of neat internal standard into the vial. Replace the cap and determine the weight of the internal standard to an accuracy of 0.01 mg, and record the weight. Tare the balance after recording the weight.
b. Add 5–35 mg of feedstock agent sample to the vial. The liquid agent can be measured with a pipet (4 to 30 µL of liquid). (A P&A test of this method has been done over this range of agent amounts.) Record the weight to an accuracy of 0.01 mg in a laboratory notebook.
c. Add 0.4 mL of reagent-grade deuterated chloroform ($CDCl_3$).
d. Vortex or mix the sample for at least 15 s to dissolve both compounds in the solvent.
e. Transfer the solution into a polytetrafluoroethylene (PTFE) NMR tube insert. (Optional: A glass 4 mm insert tube may be used and flame-sealed, if desired.)
f. Place the tube insert into a 5 mm glass NMR tube, and push it to the bottom of the tube. Cap the insert with a PTFE stopper. Cap the NMR tube or flame-seal the outer tube without damaging the insert. This is done to doubly contain the agent sample so it can be removed from engineering controls.

2.3 Obtaining the NMR Spectrum

Operators of the NMR system must have sufficient training to understand the general operational principles and to use the instrument computer control to perform the required tasks. To validate that the NMR system is functioning correctly, a manufacturer sample, such as 0.01% ethylbenzene in deuterated acetone, can be analyzed to check the signal response. Detailed quality control specifications are not included in the following method.

a. Place the NMR tube into the spinner using a depth gauge to orient the tube at the correct depth relative to the detection coils. Lower the sample into the magnet bore. (Note: The doubly contained NMR tube that includes the agent will be outside of engineering controls.)
b. Lock the instrument on the deuterium signal from the $CDCl_3$.
c. Shim the magnet to maximize the lock signal.
d. Tune and match the probe.
e. Optional: Determine the T_1 (relaxation time) of the analytes in the sample solution by using an inversion recovery experiment and following the

instrument instructions. This procedure to determine the T_1 relaxation time should be done if there is an inconsistency in the purity determination, if a new instrument is being used, or if it is necessary to minimize the experiment acquisition time.

f. Load the instrument parameters to acquire a one-dimensional (1D) spectrum. For an ^{1}H NMR spectrum, if the T_1 relaxation time is not determined (i.e., step e is not performed), then set the relaxation time to 40 s. (This is typically 20 times longer than the longest T_1 in the solvent.) Do not use Nuclear Overhauser Enhancement, decoupling, or water peak suppression pulse sequences. For a ^{13}C NMR spectrum, set the relaxation time to 60 s or more and use ^{1}H decoupling.

g. Open a new data file on the NMR computer using a unique filename, the sample information, and a notebook reference. The following parameters are used. (Actual parameter names will vary depending on the make and model of the NMR and can be found in the NMR documentation.)

- Relaxation time: 40 s or as determined in step e or f.
- Excite pulse: 90° pulse. (Determining the time and amplitude for this pulse that corresponds to a 90° proton excitation should be found in the NMR instrument documentation.)
- Number of data points: 64K.
- Number of scans: 16 for ^{1}H NMR and 128 or more for ^{13}C NMR. (See discussion in Section 4.)
- Sweep width: 15 ppm for ^{1}H NMR and 300 ppm for ^{13}C NMR.
- Center frequency: 5 ppm for ^{1}H NMR and 100 ppm for ^{13}C NMR. (For the best results, the center frequency should be exactly equidistant between the internal standard peak and the analyte peak that will be integrated.)
- Automatic gain determination: on.

h. Acquire data.

i. Ensure that a total of seven or more replicate runs are acquired for statistical determination of the NMR variability, signal-to-noise (S/N) ratio, and integration errors. Several samples can be prepared by weight to determine the weighing statistical errors. For ^{13}C NMR spectra, perform as many sample repetitions as time permits.

2.4 Data Processing

Follow these steps to process the data acquired in Section 2.3:

a. Apply a window function (exponential multiplication). This may be done using a line-broadening parameter in the range of 0.5 to 2 Hz, which can be adjusted to enhance the S/N ratio. A larger line-broadening produces wider peaks, which can degrade the resolution between peaks. The same value for line-broadening must be used for all of the data files processed for the repeat runs.

b. Use fast Fourier transform to convert data from the time to frequency domain and to produce the NMR spectrum. A sample spectrum is shown in Figure 1 for an ^{1}H NMR spectrum and in Figure 2 for a ^{13}C NMR spectrum.

c. Phase all peaks in the spectrum, and correct the baseline if necessary.

d. If necessary for reporting, reference the chemical shift against the internal standard.

e. Integrate the relevant peaks in the spectrum to obtain the relative areas. A sample-integrated ^{1}H NMR spectrum is shown in Figure 3 with an expanded *y* scale. An integrated ^{13}C NMR spectrum is shown in Figure 4 with an expanded *y* scale. Some data systems will perform an automatic integration of the peaks. It is important for the operator to examine the integration to make sure that the correct parts of the peak are included in the integration. If the automatic integration is incorrect, the spectrum can be manually integrated. Figure 3 shows that each peak has satellite peaks on each side of the main peak. These peaks are produced by molecules that have a natural abundance of ^{13}C isotopes, and they each represent 0.55% of the center peak. The satellite peaks should be included in the integration of the central peak. (If the magnet is not well shimmed, the satellite peaks may not be resolved.)

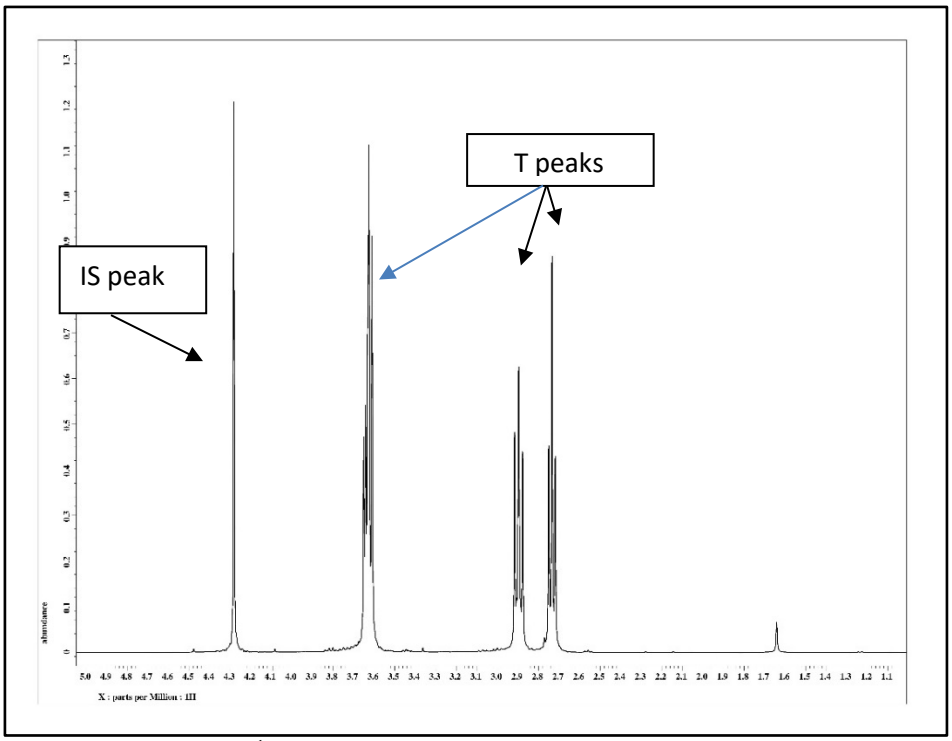

Figure 1. ^{1}H NMR spectrum of agent T (T peaks) and the internal standard 1,1,1,2-TCE (IS peak).

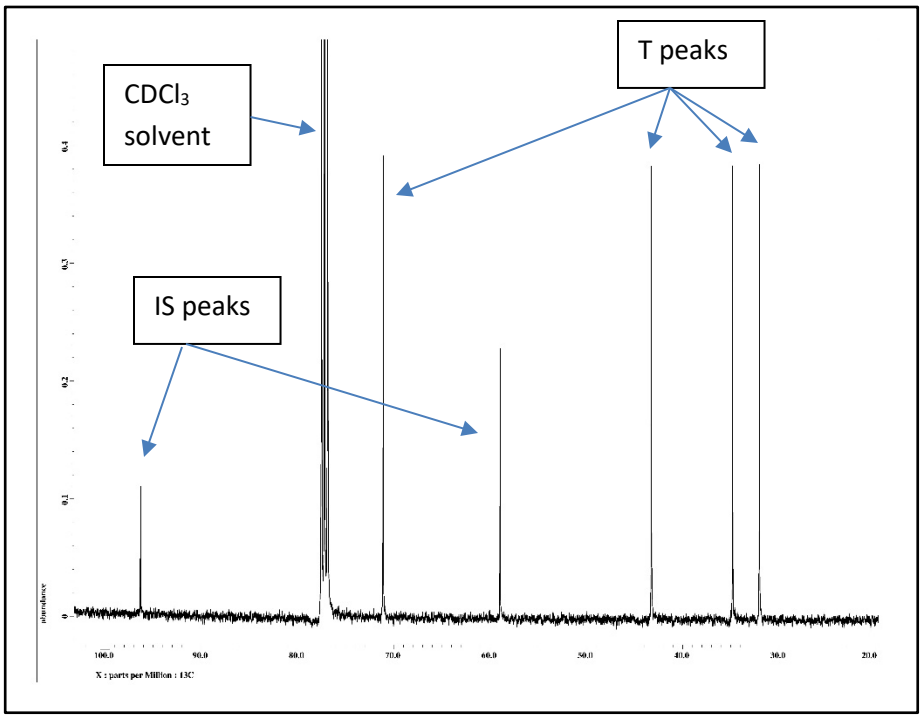

Figure 2. ^{13}C NMR spectrum of agent T (T peaks) and the internal standard 1,1,1,2-TCE (IS peaks) in $CDCl_3$ solvent.

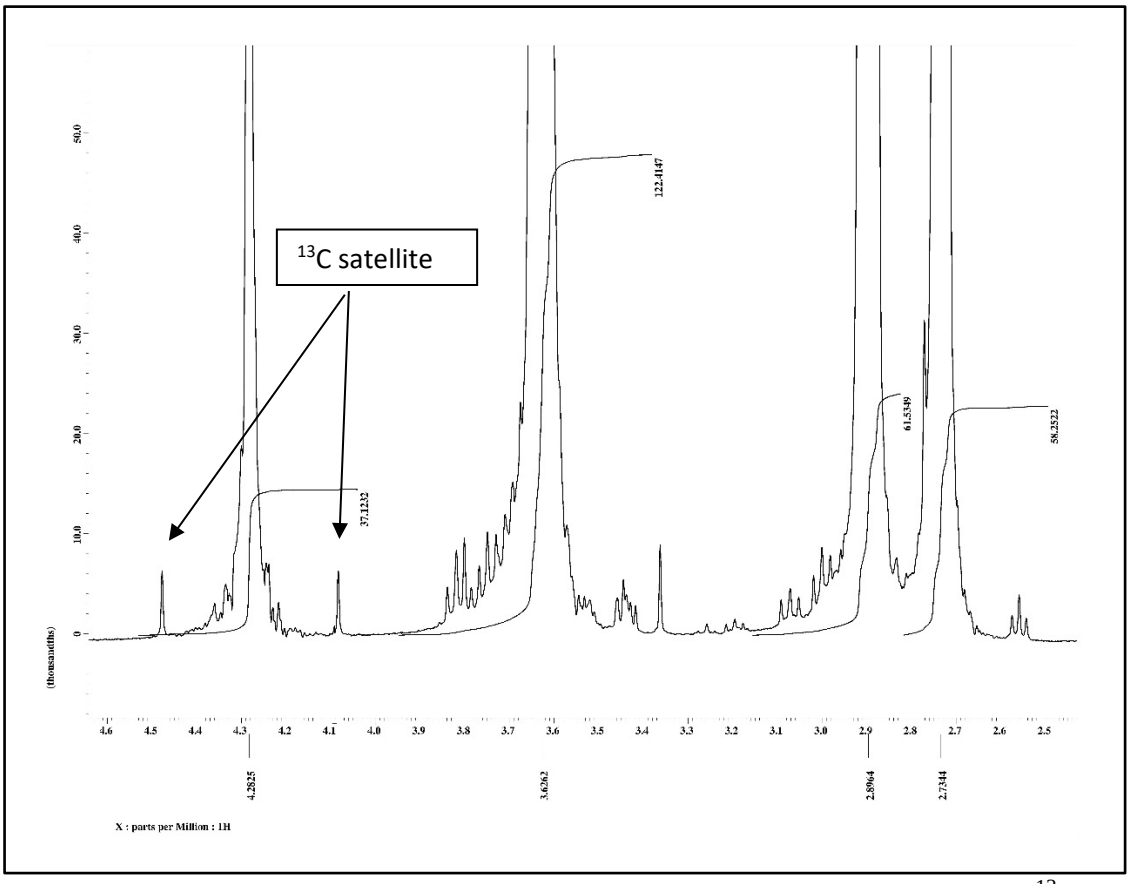

Figure 3. Plot showing the spectrum in Figure 1 with an expanded *y* scale. The small ^{13}C satellite peaks next to the central peaks and the integrals are shown. Note that the peaks at 2.7 and 2.9 ppm are not baseline-resolved, so there is some uncertainty in integrating them separately.

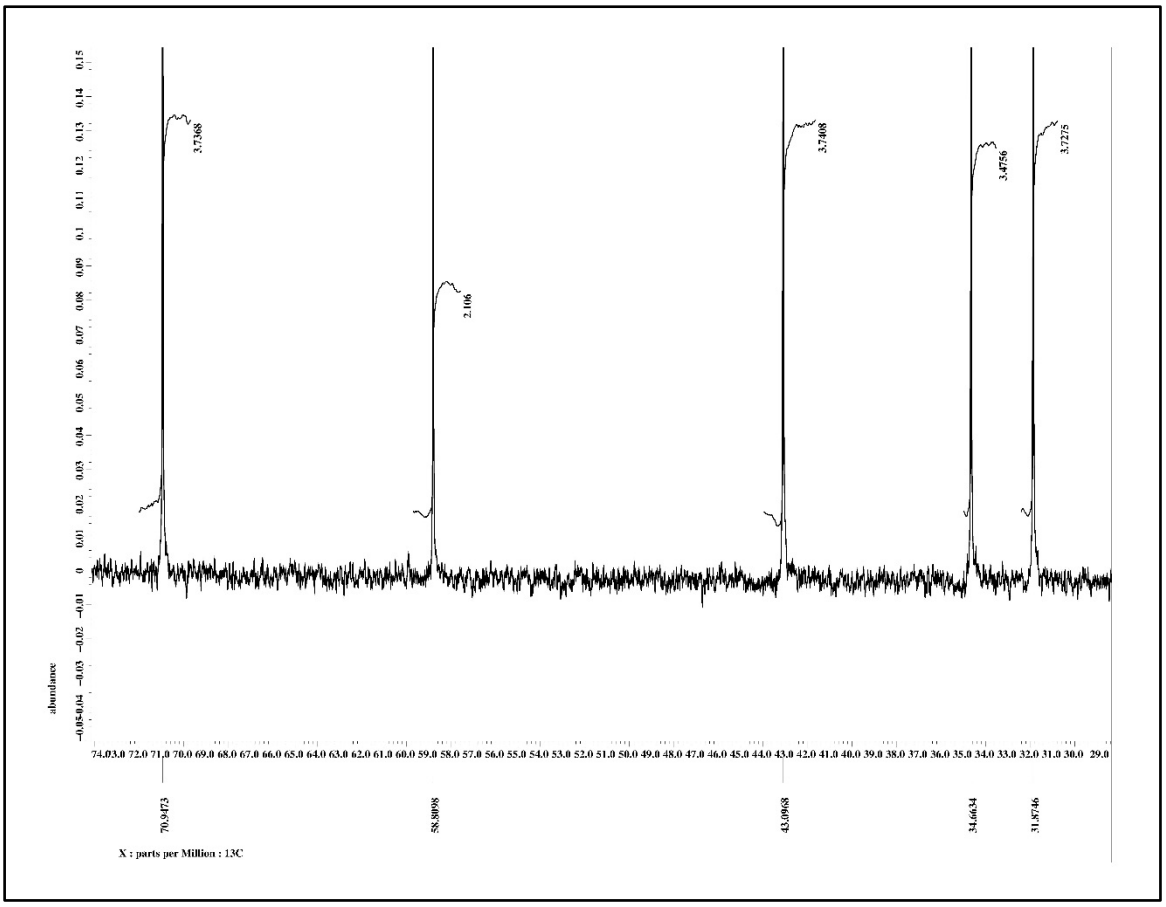

Figure 4. Plot showing the ^{13}C NMR spectrum in Figure 2 with an expanded *x* and *y* scale. Integrals are shown. Note that all of the peaks are baseline-resolved; therefore, there is no uncertainty in resolving them, although the spectrum has more noise due to the lower S/N ratio that is obtained with a ^{13}C NMR spectrum.

2.5 Purity Determination of Secondary Standard

Because the secondary standard 1,1,1,2-TCE is not a NIST-traceable standard, a second purity determination is needed to determine the accurate purity of the standard relative to a primary standard that is NIST-traceable. This determination is done using the same procedures as those found in Sections 2.2 to 2.4. The determination can be done either before or after the determination with the CW agent because no adjustment to the instrument is required. However, a calculation, based on the found purity result (Section 2.6), is required for this determination.

This step does not involve CW agent; therefore, some of the safety requirements can be relaxed. For example, the sample can be singly contained in a glass NMR tube rather than doubly contained. Several primary standards are commercially available and can also be used as alternatives to the standard 1,2,4,5-TMB that was used in the procedures described herein. The ^{1}H NMR spectra of the mixture of primary and secondary standards are shown in Figure 5, and the peaks are labeled.

Peaks are integrated as described in Section 2.4, step e. Even in the ^{1}H NMR spectrum, these peaks are very well resolved.

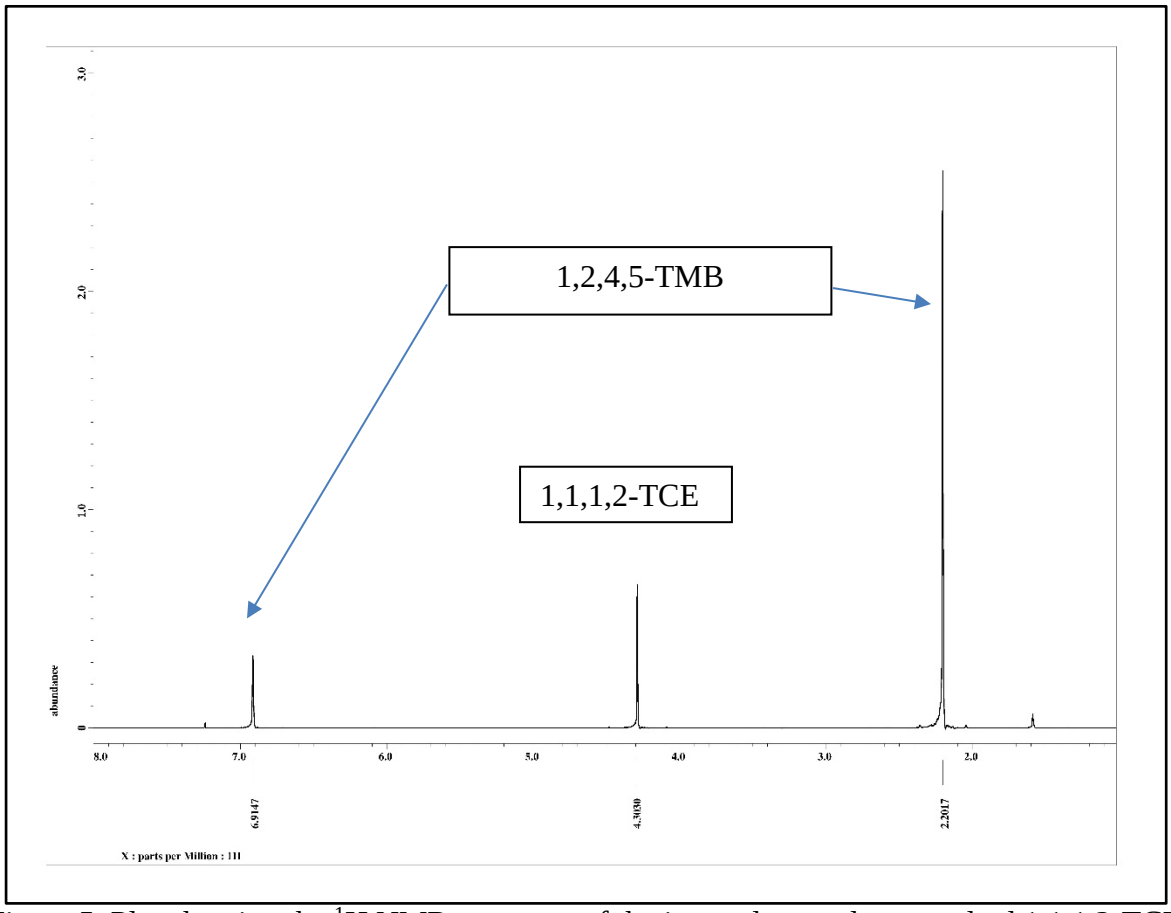

Figure 5. Plot showing the ^{1}H NMR spectrum of the internal secondary standard 1,1,1,2-TCE and the primary standard 1,2,4,5-TMB. Note that all of the peaks are baseline-resolved; therefore, there is no uncertainty when integrating them.

2.6 Purity Calculation

The weight percent of the analyte (wt % A) in the sample is calculated using the following formula, where analyte A is agent T, MW is molecular weight, and IS is the internal standard. The same formula is used for all spectra and internal standards, but the parameters will change based on the molecule that is being detected.

$$\text{wt \% A} = \frac{\text{area under A peak}}{\text{area under IS peak}} \times \frac{\text{MW of A}}{\text{MW of IS}} \times \frac{\text{weight IS}}{\text{weight A}} \times \frac{\text{no. identical } ^{1}\text{H (IS)}}{\text{no. } ^{1}\text{H (A)}} \times (\text{purity IS})\%$$

For the ^{1}H NMR spectrum of agent T, the parameters are as follows:

- area under A peak is the total sum of the area of the triplet peaks and the satellite peaks that are associated with them;
- area under IS peak is the total area of the singlet peak at 4.3 ppm and the satellite peaks;
- MW of A is the average molecular weight of agent T, which is 263.248 g/mol;
- MW of IS is the average molecular weight of the secondary internal standard, which is 167.849 g/mol. (Note that there is a difference between the average molecular weight and the monoisotopic molecular weight found in a mass spectrum.);
- weight IS is the balance-recorded weight of internal standard in the vial;
- weight A is the balance-recorded weight of feedstock agent T sample in the vial;
- no. identical ^{1}H (IS) is the number of identical protons in the internal standard, which is two;
- no. ^{1}H (A) is the number of protons in the integrated peaks of the analyte, which is four for each triplet; and
- purity IS is the purity of the secondary internal standard that is found from the primary purity determination.

For the ^{13}C NMR spectrum, the parameters are as follows:

- area under A peak is the total sum of the area of the peaks from the carbon atoms in agent T. Because the molecule has four different peaks, all four areas can be added for the best accuracy, although any one peak can also be used by itself;
- area under IS peak is the total area of the peak corresponding to the CH_2 carbon atom;
- MW of A is the average molecular weight of agent T, which is 263.248 g/mol;
- MW of IS is the average molecular weight of the internal standard, which is 167.849 g/mol;
- weight IS is the balance-recorded weight of internal standard in the vial;
- weight A is the balance-recorded weight of feedstock agent T sample in the vial;
- no. identical ^{1}H (IS) is the number of carbon atoms in the internal standard, which is one;
- no. ^{1}H (A) is the number of carbon atoms in the integrated peaks of the analyte, which is four if all four peaks are integrated and added; and
- purity IS is the purity of the internal standard that is found from the primary purity determination.

Several alternatives are possible for integrating the agent T peaks from the ^{1}H NMR spectrum. If the total area of both triplets at 2.7 and 2.9 ppm are used, then the total number of protons, no. ^{1}H (A), is eight. When only one triplet is used, which is usually at 2.9 ppm, then no. ^{1}H (A) is four. Only one triplet is used because the sample can contain a significant amount of the impurity dithiane. If it does, the NMR peaks for that compound may overlap with the triplet at 2.7 ppm and cause that triplet to have an area that is too large. Other impurities contain C–S bonds that may also have a chemical shift near the 2.7 ppm triplet. This can result in an inaccurate purity determination. However, the use of only one triplet requires estimating the minimum point between the two triplets and assuming that the overlap is symmetrical. This approximation is usually accurate, but in some cases of poor magnet shimming, there could be more tail from a peak in one direction than in another; therefore, the areas have some error associated with them.

A complex multiplet at 3.6 ppm also corresponds to eight hydrogen atoms. The multiplet can also be used for peak determination, but the complexity makes the peak broader, which indicates the possibility that other impurity peaks are hidden under it.

If the analytical statistical accuracy is reported, the calculated weight percentages for each replicate run can be averaged to find a mean (average) and standard deviation. For seven replicates, the mean ±2 × standard deviation provides the 95% confidence range.

3. P&A APPROACH

The agent T purity determination method was validated using a variation of the protocol used in a Class I P&A test.[5–7] This kind of test is typically used for validation of air-monitoring methods. The requirements are not entirely applicable to an NMR purity determination test; therefore, the P&A test was modified as needed.

A 4 day test was used. On each day of the test, 10 samples and 2 blanks were prepared. The 10 samples were prepared with the following amounts of agent T: 0.2Z, 0.5Z, 0.8Z, 1.0Z, and 1.5Z, where Z = 23 mg of agent T, and each sample was prepared in duplicate. As a result, the purity method was validated for a quantity of agent from 4.6 to 34.5 mg.

This testing was not performed in strict accordance with a normal P&A test. First, NMR is not a trace-detection method, and the purpose of the method is not to detect low amounts of agent for safety purposes, such as is required for air-monitoring applications. For a typical Class I P&A test, the amount of agent is measured in nanograms, which is usually dictated by the need to detect mandatory exposure limits. The NMR method is measured in milligrams, and the Z level is arbitrarily based on the detection limits of the current instrument.

The data from a P&A test is typically processed using the Certify software program (version 6.0, released November 2007 from U.S. Army Chemical Materials Agency [Aberdeen Proving Ground, MD]; this version runs on the Microsoft Access platform from Microsoft Corporation [Redmond, WA]). The Certify program contains statistical criteria for acceptance of the data or the test method within acceptable measurement limits. However, the

Certify program does not apply to the NMR purity determination very well. The levels are set in the program to be the same amount for all replicates from the 4 day test. For the NMR purity method, the actual amount of agent is determined by the weight of the agent taken from the NIST-traceable balance. The accurate amount is different for each measurement and is known for each sample. The amounts vary from day to day because the amounts cannot be portioned as accurately as the balance can measure, even if the nominal Target Z is the same. The accurate weight cannot be entered into the Certify program as an *x* coordinate; only the Target Z level can be entered.

The T_1 for the solutions (Section 2.3, step e) was not determined, and 40 s was used as the ^{1}H NMR relaxation delay time.

3.1. P&A Results for ^{1}H NMR

Tables 1 to 4 show the data sets collected on each day of the 4 day P&A test. Data were collected using ^{1}H NMR. The multiplet at 3.8 ppm was integrated, which corresponds to eight protons. The purity of the secondary standard was found using separate analysis runs of ^{1}H NMR, and the resulting data are shown in Table 5.

Figure 6 shows the data plotted together with the regression lines and correlation coefficients. Correlation coefficients for all of the days between Target Z (as a weight) and Found Z were >0.999.

Table 1. P&A Data from Day 1

Area of Analyte (Agent T) (arb. units)	Area of Standard (arb. units)	Weight of Standard (mg)	Sample Weight (mg)	Target Z (weight agent/ 23 mg) (unitless)	Found Z (unitless)
120.8880	35.5664	25.8000	34.1000	1.4826	1.4434
141.3212	42.1902	26.4000	34.4000	1.4957	1.4556
140.9128	57.102	25.3000	23.5000	1.0217	1.0277
123.7286	54.2501	26.0000	23.0000	1.0000	0.9761
133.0789	72.7285	27.2000	19.4000	0.8435	0.8192
100.1182	51.5948	25.1000	19.1000	0.8304	0.8017
93.8509	86.2544	25.5000	10.8000	0.4696	0.4567
95.7829	85.6903	25.5000	10.8000	0.4696	0.4692
62.2536	144.0111	25.6000	4.7000	0.2043	0.1822
63.7760	145.7041	25.5000	4.6000	0.2000	0.1837
0.4369	64.0789	12.0000	0.0000	0.0000	0.0013
0.6241	64.3318	12.0000	0.0000	0.0000	0.0019

Notes: arb. units, arbitrary units. Correlation coefficient of Target Z and Found Z is 0.99973334.

Table 2. P&A Data from Day 2

Area of Analyte (Agent T) (arb. units)	Area of Standard (arb. units)	Weight of Standard (mg)	Sample Weight (mg)	Target Z (weight agent/ 23 mg) (unitless)	Found Z (unitless)
136.8894	41.0458	26.2000	33.1000	1.4391	1.4383
115.4948	35.2313	26.5000	33.9000	1.4739	1.4299
99.7567	50.4632	29.0000	22.4000	0.9739	0.9436
116.2836	47.8805	25.0000	23.1000	1.0043	0.9994
132.0008	69.4565	26.3000	19.5000	0.8478	0.8227
97.3571	49.8433	25.3000	18.8000	0.8174	0.8134
93.5890	91.3556	27.0000	10.8000	0.4696	0.4553
93.3037	80.7958	24.6000	10.9000	0.4739	0.4676
59.5130	159.1149	28.3000	4.0000	0.1739	0.1742
62.6705	148.9727	26.8000	4.2000	0.1826	0.1856
0.0000	64.0789	12.0000	0.0000	0.0000	0.0000
0.0000	64.3318	12.0000	0.0000	0.0000	0.0000

Notes: arb. units, arbitrary units. Correlation coefficient of Target Z and Found Z is 0.99972849.

Table 3. P&A Data from Day 3

Area of Analyte (Agent T) (arb. units)	Area of Standard (arb. units)	Weight of Standard (mg)	Sample Weight (mg)	Target Z (weight agent/ 23 mg) (unitless)	Found Z (unitless)
120.2762	36.9684	26.5	34.2	1.4870	1.4192
119.2558	34.4716	25.2	33.8	1.4696	1.4350
109.6231	44.0030	25.1	23.0	1.0000	1.0293
122.7343	51.2005	24.6	21.9	0.9522	0.9707
130.8312	69.3341	26.3	18.9	0.8217	0.8169
87.3214	45.4066	24.7	18.5	0.8043	0.7819
105.0145	92.4882	24.1	10.7	0.4652	0.4504
94.4169	83.4824	24.5	10.8	0.4696	0.4561
65.0725	140.4324	24.9	4.5	0.1957	0.1899
75.0348	150.2150	25.1	4.9	0.2130	0.2064
0.0000	64.0789	12.0	0.0	0.0000	0.0000
0.0000	64.3318	12.0	0.0	0.0000	0.0000

Notes: arb. units, arbitrary units. Correlation coefficient of Target Z and Found Z is 0.99903926.

Table 4. P&A Data from Day 4

Area of Analyte (Agent T) (arb. units)	Area of Standard (arb. units)	Weight of Standard (mg)	Sample Weight (mg)	Target Z (weight agent/ 23 mg) (unitless)	Found Z (unitless)
146.0184	40.1840	25.0	34.8	1.5130	1.4953
142.5723	41.0257	25.3	34.1	1.4826	1.4472
127.2310	53.0061	25.2	23.6	1.0261	0.9956
96.2689	39.1159	24.7	23.5	1.0217	1.0006
123.1315	63.2326	25.0	19.6	0.8522	0.8013
162.0938	79.4183	24.8	19.6	0.8522	0.8332
99.0639	90.9612	25.6	10.8	0.4696	0.4589
109.1150	95.2440	24.2	10.9	0.4739	0.4564
74.7568	169.5480	25.2	4.1	0.1783	0.1829
58.8882	129.7230	24.3	4.3	0.1870	0.1816
0.0000	64.0789	12.0	0.0	0.0000	0.0000
0.0000	64.3318	12.0	0.0	0.0000	0.0000

Notes: arb. units, arbitrary units. Correlation coefficient of Target Z and Found Z is 0.99976497.

Table 5. Data for the Purity Determination of the Secondary Standard 1,1,1,2-TCE Relative to the NIST-Traceable Standard 1,2,4,5-TMB Using ^{1}H NMR

Parameter	Analysis Run Number						
	1	2	3	4	5	6	7
Area of 1,1,1,2-TCE	8.3534	8.4286	8.2551	8.2026	8.3024	8.0475	8.3032
Area of 1,2,4,5-TMB at 2.3 ppm	38.9361	39.5339	38.6233	38.4028	38.8389	37.6608	38.7925
MW of 1,1,1,2-TCE	167.849	167.849	167.849	167.849	167.849	167.849	167.849
MW of 1,2,4,5-TMB	134.22	134.22	134.22	134.22	134.22	134.22	134.22
Weight of 1,2,4,5-TMB (mg)	9.74	9.74	9.74	9.74	9.74	9.74	9.74
Weight of 1,1,1,2-TCE (mg)	16.02	16.02	16.02	16.02	16.02	16.02	16.02
Purity of 1,2,4,5-TMB (%)	99	99	99	99	99	99	99
No. of nuclei in 1,2,4,5-TMB	12	12	12	12	12	12	12
No. of nuclei in 1,1,1,2-TCE	2	2	2	2	2	2	2
Wt %	96.89	96.29	96.53	96.47	96.54	96.51	96.67
Average (7 repetitions)	96.56						
Standard deviation (n = 7)	0.19						
Confidence limits	0.37						

Notes: One sample was prepared and analyzed seven times. Areas provided in first two rows are in arbitrary units.

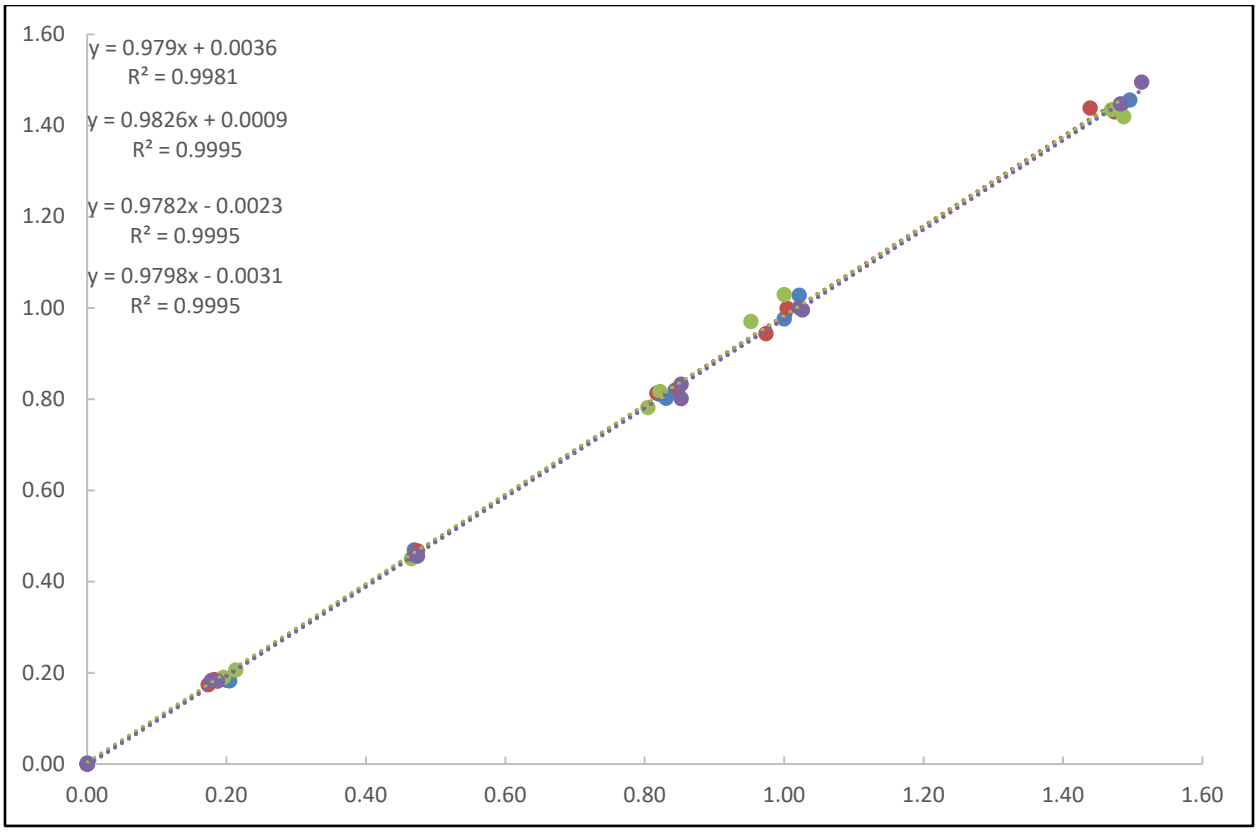

Figure 6. Plot of the data from 4 days, Found Z vs Target Z.

To calculate the Found Z, the formula from Section 2.5 was used, except it was normalized to 1Z = 23 mg, rather than use the actual Weight A. Using this method, the purity of the agent T sample can be determined from the slopes of the curves found in Figure 6. Using all four slopes gives an average purity of 98.0 wt %.

3.2 Purity Determination Using ^{1}H NMR

To determine the purity using this method, without performing an entire P&A study, the purity was calculated for each run using the formula in Section 2.6. Table 6 shows the calculations for Day 1 data, excluding the blank runs. The resulting average purity was 97.25 wt % with a standard deviation of 3.5%. The 95% confidence limit was 6.99%. The result was 98.5 wt % with a standard deviation of 1.7%, if the runs for 4.6 and 4.7 mg were excluded, which significantly reduced the error. Therefore, it may be more appropriate to exclude these runs.

Table 6. Data from Day 1 Used to Calculate Purity for Each Run

Area of Analyte (Agent T) (arb. units)	Area of Standard (arb. units)	Weight of Standard (mg)	Sample Weight (mg)	Wt %
120.8880	35.5664	25.8000	34.1000	97.363
141.3212	42.1902	26.4000	34.4000	97.325
140.9128	57.102	25.3000	23.5000	100.585
123.7286	54.2501	26.0000	23.0000	97.611
133.0789	72.7285	27.2000	19.4000	97.130
100.1182	51.5948	25.1000	19.1000	101.000
93.8509	86.2544	25.5000	10.8000	97.265
95.7829	85.6903	25.5000	10.8000	99.921
62.2536	144.0111	25.6000	4.7000	89.144
63.7760	145.7041	25.5000	4.6000	95.138
Average purity (wt %) (10 repetitions)	97.25			
Standard deviation (n = 10)	3.49			
Confidence limits	6.99			

arb. units, arbitrary units.

To minimize the amount of sample preparation, it is possible to prepare only one sample and rerun it multiple times. This approach minimizes the hazards from handling neat agent, the loss from consuming agent, and the waste from generating unneeded samples. However, the repetitions include only the errors that were generated by the NMR data acquisition and integration and do not include systematic or random errors from weighing and preparing samples. Table 7 shows data from repeated runs of a sample that was prepared as soon as the agent was received. The amount of agent that was used was about the same as that used for the 1.5Z samples. The triplet peak at 2.9 ppm was integrated for the determination, and the peak corresponds to four protons. The error shown by the standard deviation was smaller than those of the previous results that involved multiple sample preparations.

The found weight percentage was higher than the previous determinations, but it was within the standard deviation from the P&A data set. The weight percentage was also above 100%, which is not physically possible. The measurement method does not constrain the result to be 100% or less. Measurement errors can vary enough to produce a calculated result above 100%, if the error range or the measurement variation is large enough.

Table 7. Data from Repeated Runs of One Prepared Sample

Area of Analyte (Agent T) (arb. units)	Area of Standard (1,1,1,2-TCE) (arb. units)	Wt %
67.0285	66.7306	99.5550
67.1013	66.5285	99.9658
66.7882	66.2904	99.8568
67.0433	65.9967	100.6843
67.1748	65.8827	101.0563
66.7717	65.8701	100.4691
66.3783	65.9465	99.7615
Average purity (wt %) (7 repetitions)	100.19	
Standard deviation (n = 7)	0.212	
Confidence limits	0.425	

3.3 Certify Results

The results were analyzed with the program Certify 6.0, which was used for P&A data analysis. The screens that were generated by the program are shown in Figures 7 to 9. Parameters that were calculated by the program are shown on the screens.

The data that were obtained from this test were ±7.4%, which passed the Certify program pass/fail criteria of ±25%. This was a higher amount of error than was obtained with the accuracy of a purity determination. But because of the way the data are entered into the program, the Certify program is used to effectively test the correspondence of the Target Z with the weight. As shown in Figure 9, there was no scatter in the *x* coordinate in the Certify plot; however, there was scatter in the *x* coordinate for the data shown in Figure 6. The actual accuracy of the aforementioned data was better than the Certify program calculations suggested; therefore, using the Certify program to quantify the P&A results in this case did not accurately indicate the method performance. The results were best judged in terms of standard deviations and correlation coefficients of the data that included variation in the *x* coordinate.

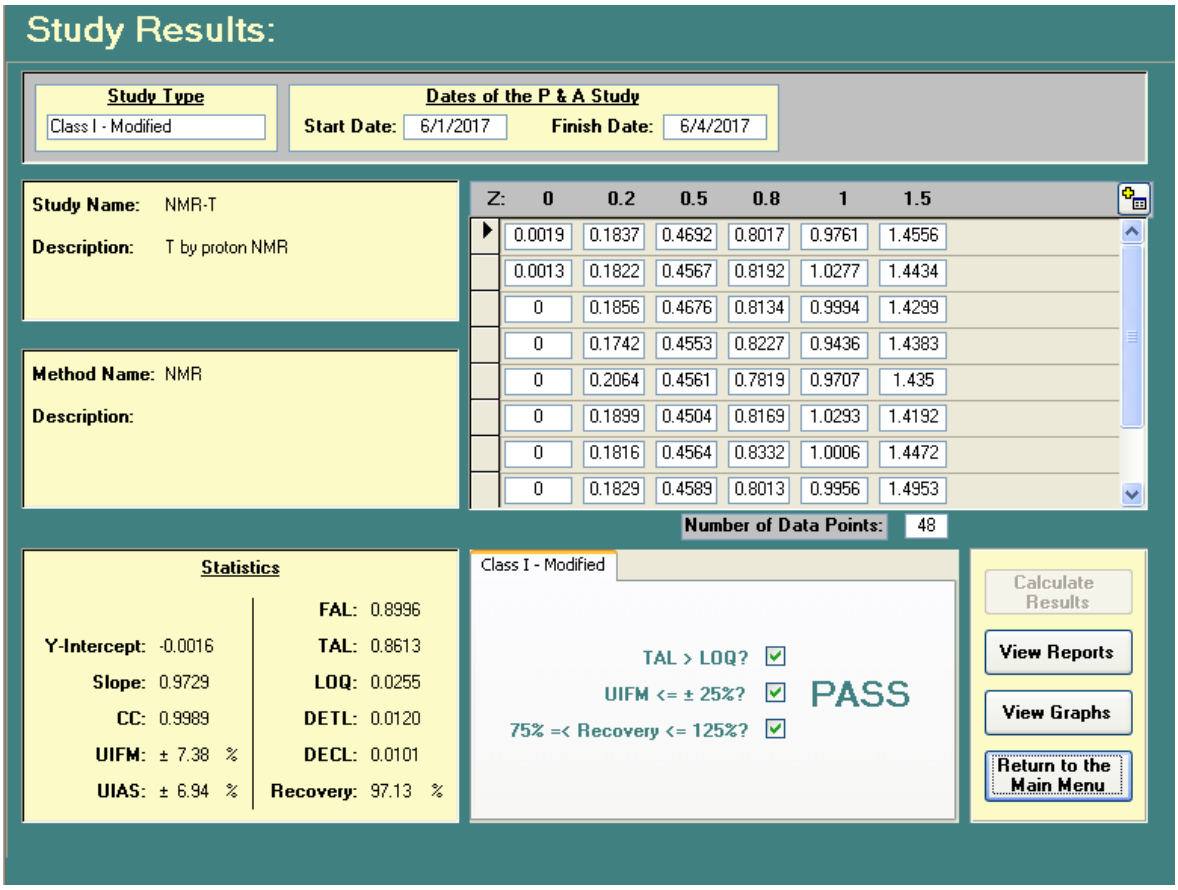

Figure 7. Certify program results page for the 4 day P&A study.

Report Summary

Class I - Modified

Study Name:	NMR-T	**Start Date:**	6/1/2017
Study Description:	T by proton NMR	**Finish Date:**	6/4/2017
Method:	NMR		
Laboratory:	Edgewood Chemical, Biological Center		
Agent:	GB		
Environment :	IDLH		
Sample Size:	48		

Target Levels

TC 1 =	0.0000Z
TC 2 =	0.2000Z
TC 3 =	0.5000Z
TC 4 =	0.8000Z
TC 5 =	1.0000Z
TC 6 =	1.5000Z

Target vs. Found Summary

Found Action Level:	0.8996	Z
Target Action Level:	0.8613	Z
Limit of Quantification:	0.0255	Z
Detection Limit:	0.0120	Z
Decision Limit:	0.0101	Z
Percent Recovery:	97.13	%
Uncertainty in Found Mass:	7.38	%
Uncertainty in Air Sample:	6.94	%

Statistical Parameters

Slope:	0.9729
Y-intercept:	-0.0016
Correlation Coefficient:	0.9989
Students-T Statistic:	2.01357

Outliers

Number of Outliers Detected:	0
Permissible Number of Outliers:	7
Percent of Permissible Outliers	0%

Pass/Fail Results

TAL greater than LOQ:	Passed
UIFM less than or equal to ±25%:	Passed
Recovery within 75% to 125%:	Passed

Figure 8. Certify program report summary.

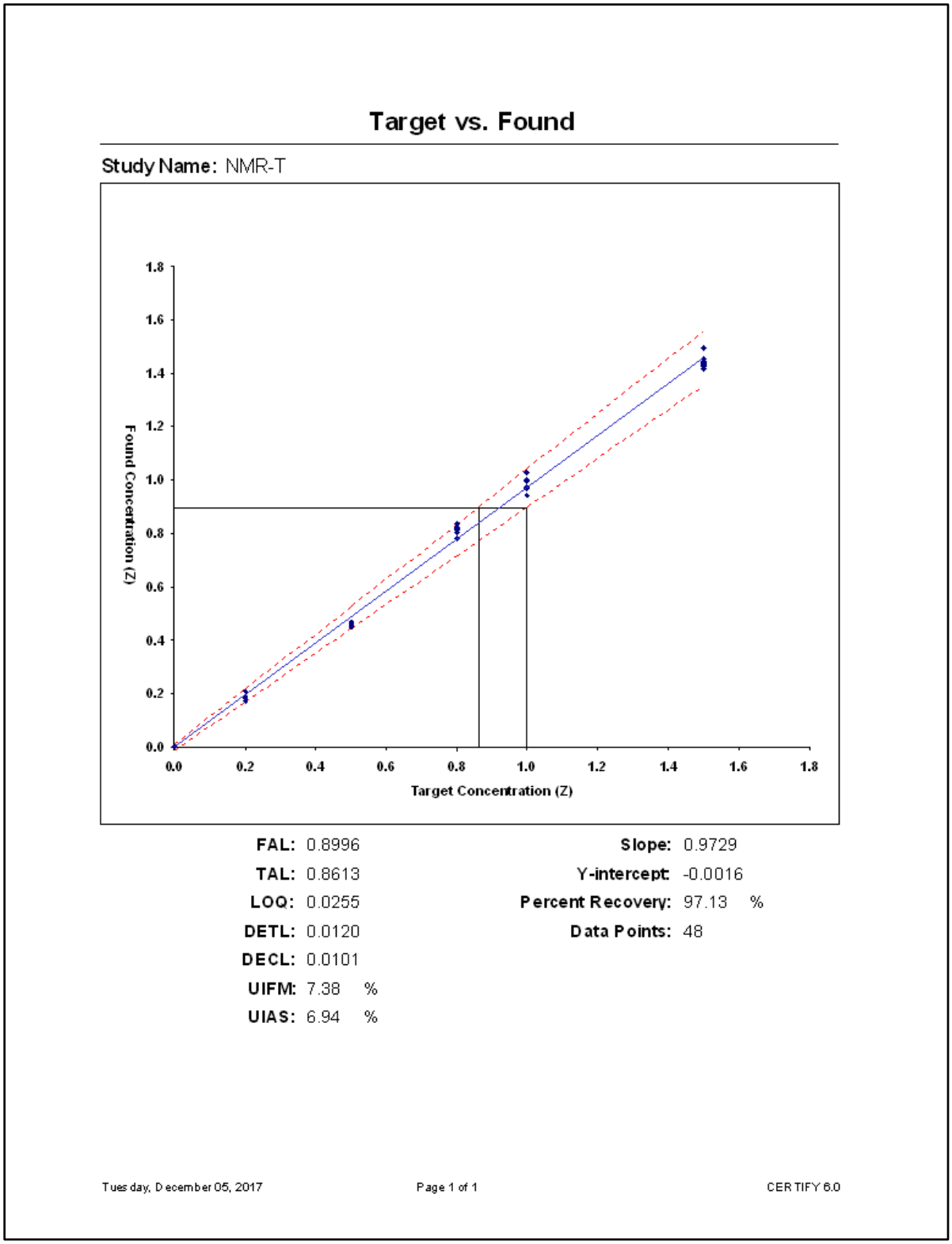

Figure 9. Certify program Target Z vs Found Z plot screen.

4. COMPARISON OF ^{1}H AND ^{13}C NMR PURITY DETERMINATIONS

The confirmation method for agent T purity determination using ^{13}C spectra was not validated using a full P&A test. The original intent was to perform both ^{1}H and ^{13}C NMR P&A tests on the same set of samples and use the same secondary internal standard for both tests. However, it was clear after obtaining the samples from Day 1 that the ^{13}C spectra for <1.0Z would not have an adequate quality due to the lower instrumental sensitivity to ^{13}C spectra. It was not possible to prepare a set of samples with a higher Z amount due to limited time and agent.

Accuracy of the results depends on having a sufficiently long relaxation delay time to prevent distortion of the peak areas. Measurement of the signal relaxation is used to determine how long the relaxation delay time should be. The recovery times (T_1) that were found for the two HD peaks in the previous study were 5.8 s (34 ppm) and 6.6 s (43 ppm).[7] We expect that agent T is similar. For the 1,1,1,2-TCE internal standard, the recovery time was longer at 8.7 s for the 59 ppm peak. Based on the longest recovery of 8.7 s, the delay time should be at least $10 \times T_1 = 87$ s.[7]

Several purity runs were done to determine the typical statistical uncertainty of the ^{13}C NMR test. The statistical information is dependent on the type of NMR system that was used (in this case, we used a JEOL ECS-400 NMR system), it is not a general property of the analytical method and must be determined for each particular instrument. The results for multiple runs are shown in Table 8.

The precision of the NMR measurement is associated with the S/N ratio of the spectrum, which depends on several factors, including the amount of analyte that is present and the number of scans that are averaged. However, the results in Table 8 indicate that there was not a direct relationship between S/N ratio and accuracy of the purity result. The averages of several runs are shown in the last column of Table 8. Within the standard deviations, the averages were similar for the runs, regardless of the S/N ratios.

In addition, there was not a strong relationship between accuracy and relaxation time for this data. The relaxation times varied from 90 s (the prescribed time) to 30 s. This relaxation time was 30% as much as the 90 s relaxation time that was indicated by the T_1 relaxation. The use of a relaxation time that is shorter than optimum causes some distortion of the signal responses, but it allows more signal-averaging in the same amount of time, which improves the S/N ratio. The peak with a longer recovery time was not quite relaxed to the baseline; therefore, the peak was slightly saturated and had a lower response. This affected the purity result, but as long as the distortion is less than the uncertainty produced by the noise level, the noise will be a larger limitation than will the distortion for the test.

Table 8. Relationship of S/N, Acquisition Time, Sample Amount, and Calculated Weight Percentage for ^{13}C NMR Spectra

Sample Name	No. Scans	Relax Time (s)	Total Run Time (min)	S/N for Agent T (71 ppm)	Weight of Standard (mg)	Sample Weight (mg)	Wt %	Average (no. reps)	Standard Deviation
P31B	32	90	55	21.1	45.58	34.82	99.64	100.77 (4)	5.5
P31B	32	90	55	18	45.58	34.82	93.50		
P31B	32	90	55	18.4	45.58	34.82	105.79		
P31B	32	90	55	20.6	45.58	34.82	104.14		
P35A	32	90	55.	40.7	11.02	35.54	92.54	–	–
P35B	32	90	55	10.3	12.68	36.55	107.21		
P35C	32	90	55	11.8	13.05	23.07	104.70		
P35D	32	90	55	7.7	11.75	24.65	102.56		
P35E	32	90	55	7.3	11.58	19.82	95.25		
P35F	32	90	55	9.3	11.76	18.58	101.16		
P35G	32	90	55	5.6	13.16	10.01	117.32		
P35H	32	90	55	4.2	11.85	11.32	96.08		
P35J	32	90	55	3.7	12.48	4.31	106.35		
P37A	256	60	269	135.8	11.67	33.80	97.20		
P37B	256	60	269	147.22	11.78	36.37	83.07		
P45A	512	60	534	33.87	26.20	33.10	91.57	97.21 (3)	13.70
P45B	512	60	534	55.66	26.50	33.90	102.86		
P45G	512	60	534	7.2	27.00	10.80	118.84		
P43A	512	30	276	60.09	25.80	34.10	110.75	100.52 (3)	8.88
P43B	512	30	276	62.4	26.40	34.40	95.98		
P43C	512	30	276	27.8	25.30	23.50	94.83		
P45A	512	30	276	33.9	26.20	33.10	91.57	104.42 (3)	13.70
P45B	512	30	276	55.7	26.50	33.90	102.86		
P45G	512	30	276	7.2	27.00	10.80	118.84		
P53A	300	60	314	119.4	25.00	34.80	97.81	102.38 (3)	8.40
P53A	300	60	314	119.1	25.00	34.80	97.26		
P53A	300	60	314	122.4	25.00	34.80	112.07		
							Overall average	101.06	

Notes: The average and standard deviation columns give the averages of the number of runs (no. reps) that were collected with the same acquisition conditions and provide their corresponding standard deviations.

Using a sample of 35 mg of agent T, 1 h was needed to acquire the spectrum shown in Figure 2, which involved 32 scans and a delay time of 90 s. This run gave a purity result of 100.4 wt % and a standard deviation of 5.2 % using seven repetitions on the same sample. The result was within the error limits of the purity determination for the ^{1}H NMR spectra. Four of these seven runs are in the first four lines of Table 8, and they show S/N ratios in the range of 18 to 21.

Regardless of the change in the number of scans from 32 to 512 and the range of S/N ratio from 18 to 150, the averages in Table 8 of three or four repetitions are in the range of 97 to 104 wt %, which is within the standard deviation of the purity result that was obtained from the ^{1}H NMR test.

An important quantity for accurate purity determination is the integration of spectral peaks. By integrating the peaks over a wide enough range, in a way that averages out the baseline noise, the average purity result (determined by the ratio of the internal standard peak area and the analyte peak area) may tend to cancel out the errors. More work is needed to improve the precision of the results because the standard deviations that are obtained are probably not acceptable for the purity determinations that are required for most applications.

^{13}C NMR spectra provide higher spectral resolution than do ^{1}H NMR spectra, and for this reason, the ^{13}C NMR spectra should be collected. For example, the compound dithiane is often present in HD samples at a concentration of about 1 wt %. However, the ^{1}H NMR spectrum does not have enough resolution to separate the 2.9 ppm triplet peak of HD from the dithiane peak. This example illustrates the need to trade off the resolution of the ^{13}C NMR spectrum for the sensitivity of the ^{1}H NMR spectrum. It is recommended that both types of spectra be collected for studies in which the purity and the potential impurities are both important requirements.

5. CONCLUSION

By using a NIST-traceable internal standard and a balance that was calibrated with NIST-traceable weights, the purity of the CW agent feedstock, agent T, was determined using a NIST-traceable method with ^{1}H NMR spectra. A method using ^{13}C NMR spectra involves a secondary standard that must be referenced to a NIST-traceable standard. This method has a lower precision, but it was capable of resolving and identifying some possible contaminants in agent T.

Blank

LITERATURE CITED

1. O'Connor, R.J.; Brickhouse, M.D.; McGarvey, D.; Durst, H.D.; Creasy, W.R.; Ruth, J.L. *NMR Method for the Quantitative Purity Analysis of Neat Feed Stock Samples*; ECBC-TR-253; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2002; UNCLASSIFIED Report (ADA406815).

2. O'Connor, R.J.; Brickhouse, M.D.; Rice, J.R.; Durst, H.D.; McGarvey, D.J.; Creasy, W.R.; Pence, J.; Montgomery J.L. *NMR Method for the Quantitative Analysis of Liquid Samples*; ECBC-TR-416; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2005; UNCLASSIFIED Report (ADA430358).

3. O'Connor, R.J.; Brickhouse, M.D.; Durst, H.D.; McGarvey, D.J.; Creasy, W.R. *NMR Method for the Quantitative Analysis of VX Hydrolysates*; ECBC-TR-417; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2005; UNCLASSIFIED Report (ADA430360).

4. Creasy, W.R.; McGarvey, D.J.; Rice, J.S.; O'Connor, R.J.; Durst, H.D. Study of Detection Limits and Quantitation Accuracy Using 300 MHz NMR. In *Proceedings of the 2002 Joint Service Scientific Conference on Chemical and Biological Defense Research*, 19–21 November 2002; Berg, D.A., Compiler; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2003; UNCLASSIFIED Report (ADA482893).

5. McGarvey, D.J.; Creasy, W.R. *NIST-Traceable NMR Method to Determine Quantitative Weight Percentage Purity of Nitrogen Mustard HN-3 Feedstock Samples*; ECBC-TR-1159; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2013; UNCLASSIFIED Report (ADA585492).

6. McGarvey, D.J.; Creasy, W.R. *NIST-Traceable NMR Method to Determine Quantitative Weight Percentage Purity of Nitrogen Mustard HN-1 Feedstock Samples*; ECBC-TR-1251; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2014; UNCLASSIFIED Report.

7. McGarvey, D.J.; Creasy, W.R.; Connell, T.R. *NIST-Traceable NMR Method to Determine Quantitative Weight Percentage Purity of Mustard (HD) Feedstock Samples*; ECBC-TR-1506; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2018; UNCLASSIFIED Report (AD1046144).

Blank

ACRONYMS AND ABBREVIATIONS

1D	one-dimensional
^{1}H NMR	proton or H-1 nuclear magnetic resonance
^{13}C NMR	carbon-13 or C-13 nuclear magnetic resonance
agent T	bis[2-(2-chloroethylthio)ethyl]ether; O-mustard
CAS	Chemical Abstracts Service
CASARM	Chemical Agent Standard Analytical Reference Material
CW	chemical warfare
ECBC	U.S. Army Edgewood Chemical Biological Center
HD	distilled mustard; bis-(2-chloroethyl) sulfide; EA 1033
HN-1	2,2′-dichlorotriethylamine; nitrogen mustard
HN-3	tris(2-chloroethyl)amine; nitrogen mustard
IS	internal standard
MW	molecular weight
NIST	National Institute of Standards and Technology
NMR	nuclear magnetic resonance
P&A	precision and accuracy
PTFE	polytetrafluoroethylene
S/N	signal to noise
T_1	relaxation time
TCE	tetrachloroethane
TMB	tetramethylbenzene
wt % A	weight percent of analyte

DISTRIBUTION LIST

The following individuals and organizations were provided with one Adobe portable document format (pdf) electronic version of this report:

U.S. Army Edgewood Chemical Biological Center (ECBC)
Analytical Toxicology Branch
RDCB-DRT-T
ATTN: McGarvey, D.
Creasy, W.
Weller, H.

Defense Threat Reduction Agency, R&D
J9-CBS
ATTN: Cronce, D.

Department of Homeland Security
RDCB-PI-CSAC
ATTN: Mearns, H.

Defense Technical Information Center
ATTN: DTIC OA

G-3 History Office
U.S. Army RDECOM
ATTN: Smart, J.

ECBC Technical Library
RDCB-DPB-BL
ATTN: Foppiano, S.
Stein, J.

Office of the Chief Counsel
AMSRD-CC
ATTN: Upchurch, V.

ECBC Rock Island
RDCB-DES
ATTN: Lee, K.
RDCB-DEM
ATTN: Grodecki, J.


EDGEWOOD CHEMICAL BIOLOGICAL CENTER
A U.S. Army RDECOM Laboratory