

**The Block II Chemical Biological Mass Spectrometer –
Point Detection for Both Chemical and Biological Warfare Agents**

**Wayne H. Griest, Marcus B. Wise, Kevin J. Hart and Stephen A. Lammert
Oak Ridge National Laboratory
Oak Ridge, TN**

and

**Alexander P. Hryncewich and David W. Sickenberger
U.S. Army Soldier and Biological Chemical Command
Aberdeen Proving Ground, MD**

ABSTRACT

The Block II Chemical Biological Mass Spectrometer (CBMS) is a new instrument for point detection that integrates the detection and identification of both chemical warfare (CW) and biological warfare (BW) agents into a single compact unit. It is based upon a direct-sampling ion trap mass spectrometer interfaced to three sampling systems and is operated in full scan and tandem mass spectrometry (MS/MS) using ethanol chemical ionization (CI) or electron ionization (EI) modes.

INTRODUCTION

The Block II CBMS is a new vehicle-borne point detection (as well as reconnaissance) instrument that integrates chemical and biological warfare agent detection and identification into a single unit that is lighter, smaller, and less power-intensive than separate detectors. The instrument is soldier-friendly in operation and self-diagnosing in fault location, and resists nuclear radiation, temperature extremes, vibration and shocks from wheeled vehicle transport. New capabilities of the Block II instrument compared to the Block I instrument include the ability to rapidly switch between chemical and biological agent detection via the mode valve, *in situ* derivatization of biomarkers, a direct capillary inlet, inclusion of CW vapor detection, a vacuum system equipped with a turbomolecular pump, an ion trap mass spectrometer capable of using chemical ionization, automatic switching between full scan and MS/MS and between EI and CI by novel multiscan functions with broadband notches for multiple ion detection and confirmation.

INSTRUMENT

The Block II CBMS consists of four major modules – the Mass Spectrometer Module, the Sample Introduction Module (SIM), the Bioconcentrator Module and the Soldier Display Unit (SDU). Collectively, the four major modules occupy a volume of 5.8 ft³ and weigh ca. 170 lbs. Preliminary power figures indicate that the unit draws less than 500 Watts (average) of power under stabilized operating conditions. The following sections briefly describe the instrument. Greater detail is reported elsewhere¹.

Mass Spectrometer Module

The mass spectrometer module is divided into two halves that separately house the mass spectrometer assembly and the electronics card cage. The mass spectrometer assembly consists of an open-split capillary interface, an ion trap mass analyzer, turbomolecular pump and diaphragm rough pumps, and a manifold for the CI reagent and mass calibration gas. The electronics card cage is based on a 6U compact-PCI (CPCI) chassis to minimize the amount of external wiring harnesses. The 6U chassis houses a computer board that contains the CPU for low level instrument control (the Low Level Controller or LLC). Three additional 6U circuit card assemblies incorporate the broadband arbitrary waveform generator (including the high voltage rf control circuitry), the multifunction interface board (containing the temperature measurement subsystem, the RF-ramp generator, and much more of the general use analog and digital I/O circuitry), and the electrometer interface board (containing the control circuits for the ion-source lenses and filament, and related analog interface circuits).

Sample Introduction Module

The SIM module contains the hardware components for selecting one of the three sample introduction lines. These sample paths are controlled using a micro-electric multiposition valve actuator and 4-position valve (Valco Instruments Co., Inc, Houston, TX), referred to as the mode select valve, that allows one of the three sample inlets to be switched to the inlet of the open-split capillary vacuum interface. The three agent sample paths are described below. The SIM also houses the pyrolyzer.

Chem Ground Line

The Fox reconnaissance vehicle uses a pair of silicone wheels to alternately sample the ground surface for liquid CW agents and transport any adhering or adsorbed material to a heated desorption probe head mounted on the ground probe, which extends to the outside of the vehicle hull. The desorbed material is transported via a 2-meter heated capillary transfer line to the analysis instrument, which, in the case of the Fox vehicle, is currently a Bruker MM-1 Quadrupole Mass Spectrometer. The Block II design takes the effluent from the existing heated transfer line and directs the flow into the 4-port mode select valve and, when selected, to the CBMS Block II open-split vacuum interface.

Chem Air Line

A heated 1/16" Silicosteel™ (Restek Corp., Bellefonte, PA) line provides an interface with a port in reconnaissance vehicles to sample the outside air for CW agents at a rate up to ca. 100 mL/min. Results of a study comparing different materials for transfer lines indicates that the Silicosteel™ lines are particularly well suited for air sampling because these lines were found to be less adsorptive than Nylon™, polyethylene and Teflon™ lines and provide an opportunity to be resistively heated². As is the case for the other agent inlets, effluent from this line can be routed by the mode select valve to the open-split vacuum interface.

Bioconcentrator/Pyrolyzer

The Block II CBMS monitors for BW agents using an opposed jet virtual impactor particle concentrator and pyrolysis mass spectrometry. The bioconcentrator (MSP Corp., Minneapolis, MN) draws 330 L/min of air. A scalper with a 10 µm particle diameter cut point passes 300 L/min to the opposed jet virtual impactor stages and discards all particles with effective aerodynamic particle diameters greater than 10 µm to a venturi exhaust line coupled with the main pump exhaust. A two-stage opposed jet virtual impactor subsequently reduces the flow rate from 300 L/min to 1 L/min while retaining between 50% to 95% of all particle aerodynamic diameters between 2 µm and 10 µm. The improved efficiency for particle transmission is due in part to the opposed jet impactor design developed by MSP. The 1 L/min flow from the Biosampler is then transferred to a quartz

pyrolysis tube in the Sample Interface Module where the particles are deposited. After a sampling period of 2 minutes or longer, the flow from the Bioconcentrator is terminated to allow initiation of the pyrolysis cycle.

Toward the end of the biocollection, the pyrolyzer body heater is engaged to heat the pyrolyzer body to ca. 250 °C. This step requires approximately 1 minute to complete so it is important to begin this step prior to the completion of the collection of the sample to maintain a ca. 4 minute duty cycle for the bio monitoring mode. The temperature of the pyrolyzer body is then maintained at the elevated temperature until the end of the data acquisition (ca. 60 s). The temperature inside the pyrolyzer tube is only raised ca. 20-30 °C when the pyrolyzer body is heated to 250°C. The particles in the pyrolysis tube are then treated *in situ* with ca. 1-2 uL of 0.015 – 0.03 M tetramethylammonium hydroxide (TMAH) or tetrabutylammonium hydroxide (TBAH) using a newly designed automated injector. The pyrolysis tube is then ballistically heated to ca. 550 °C over 14-18 s where the polar components of the sample (e.g. the bacterial fatty acids) are derivatized using the reagent. The resulting derivatized pyrolysis products are then continuously transported via heated 1/16" Silicosteel™ lines through the Mode Select Valve to the inlet of the open-split capillary vacuum interface. A pump establishes a flow of ca. 1.3 mL/min from the pyrolyzer tube to the open-split interface when in the Bio Mode. A more typical flow rate of 10 mL/min has been used for the Chem Air and Chem Ground Modes. Data acquisition begins at the start of the pyrolysis cycle.

Software and Soldier Display Unit

In addition to the Low Level Controller, another CPU running Microsoft Windows NT Workstation 4.0 is dedicated to high level control of experimental design and data analysis software (High Level Controller – HLC). Communication between the two CPUs is accomplished using a TCP/IP ethernet connection. There are two options for the HLC. One option uses a ruggedized notebook computer running a full standard release of Windows NT and an expert level user interface. The second option uses an embedded version of Windows NT (Microsoft Windows NT Embedded) running on a Soldier Display Unit described below.

The expert level software is much more flexible for research and development purposes and includes a “research grade” scan function editor (SFE) interface. The SFE provides controls for instrument settings and allows the user to control the timing of signals to the ion trap electrodes (i.e. a scan function). The SFE also has the ability to display mass spectra, total ion profile and selected mass profile graphs during data acquisition. An additional tool has also been developed to provide offline analysis of data including the display and background subtraction of mass spectra, total ion profiles and selected mass profiles from data that are saved in either the same datafile or in different datafiles (separate runs). There are also options to automate instrument response calibration and quantitation. The work described in this paper was pursued using the expert user software.

The Soldier Display software is intended for end users with little or no knowledge of ion trap mass spectrometry and is being programmed using an instrument control language tailored to the Block II CBMS design. The Block II CBMS operator interface, referred to as the Soldier Display Unit (SDU), consists of the HLC single board computer and a gas plasma display in a box approximately 12" W X 7.5" H X 4.25" D. The SDU uses a gas plasma display because of the extreme, low temperature range specification. Mass storage is comprised of several solid state devices to promote this ruggedized design and includes a 40 MB IDE compatible flash drive for the operating system, a removable 40 MB PC Card for the application software and a removable 4 MB PC Card for a mission log. PC Cards were utilized to allow rapid software upgrades and to provide a removable mission log. The front panel of the SDU has a bank of LEDs that show instrument status and configuration information in the upper left quadrant. There are three user-input buttons on the right side of the plasma display that allow the user to interact with the CBMS in a manner similar to an automatic teller machine (ATM). The modes of operation are selected using these buttons. On the bottom of the SDU is a row of buttons that select the pages that are displayed to the user (e.g. the alarm page, the configuration page, etc.). The SDU software engages an audio and visual alarm, displays a message on the alarm page of the SDU that

consists of the inlet source (i.e. BIO mode), the agent (i.e. BG), the agent classification (i.e. bacteria) and a scaled bar graph that denotes relative intensity and sends a report message via a serial line.

PERFORMANCE

Bio/Air Mode

Ethanol chemical ionization (CI) in both full scan and tandem mass spectrometry (MS/MS) modes was selected to provide maximum selectivity and sensitivity to BW (as well as CW) agents in the presence of common battlefield interferents such as diesel engine exhaust and fuel. The ability of CI to minimize interferents in full scan MS is illustrated by comparison of the electron ionization (EI) and CI full scan spectra shown in Figure 1. Raw diesel engine exhaust from an old bus with a “dirty” 7 L diesel engine was sampled directly into the bioconcentrator of a Block II CBMS operated in EI or CI modes. A considerable reduction in the diesel exhaust signal is apparent in the CI mode versus the EI mode. For comparison with an agent spectrum, a full scan CI spectrum (TMAH derivatization) in Figure 2(A) shows *Francisella tularensis* SHU-4 sampled from an aerosol of about 31 agent-containing particles (3.2 μm aerodynamic diameter) per L of air. Biomarkers ranging from the 10:0 to 24:0 acids are readily visualized, and even lower aerosol concentrations could be detected. Figure 2(B) shows the spectrum of a 200 ng sample of crude castor bean extract introduced by direct liquid injection. Diketopiperazine derivatives produced from the thermolysis/methylation of proteins are marked (*).

Earlier biomarker work^{3,4} focused on fatty acid methyl esters produced by TMAH derivatization, but the very low intensities of unique fragment MS/MS product ions of fatty acid biomarkers severely limited CI MS/MS sensitivity to about 1% of the full scan CI. In addition, the methyl esters of unsaturated and cyclic fatty acids also fragment severely in full scan CI. Comparison of different esters identified the butyl derivatives as having good full scan MS and MS/MS properties. The unique MS/MS fragment ions are 10 to 70% as intense as their precursors, and the TBAH was selected as a drop-in replacement for the TMAH. The full scan CI spectrum of TBAH-derivatized *Francisella tularensis* SHU4 in Figure 3 (A) shows the expected 10:0 through 24:0. In Figure 3(B), the MS/MS spectrum of the protonated molecular ion of the 22:0 fatty acid (m/z 397) illustrates the two intense diagnostic product ions and the clean MS/MS spectrum. Higher line temperatures were required to transmit the heavier fatty acid butyl esters. Spectral libraries of the target BW agents are now being collected.

Chem/air Mode

The second operational mode of the Block II CBMS provides a means to monitor air for the presence of CW agent vapors via a heated Silicosteel® line. The line is typically heated to 180 °C. A solution of a simulant, dimethylmethyl phosphonate, was injected on the tip of the CW Air line to demonstrate a typical instrument response. The extracted mass profile for the protonated molecular ion of DMMP (m/z 125) is shown in Figure 4(A). Three blank injections of methanol were followed by 3 injections of 10 ng and 50 ng of DMMP in dissolved in methanol. Another test was performed using methyl salicylate (MS) diluted in a Tedlar bag at concentrations of 0.01 and 0.1 mg/m^3 . The mass profile obtained while the Tedlar bag was attached to the CW Air line is shown in Figure 4(B). The protonated molecular ion of MS at m/z 153 is clearly discernable at this level using ETOH-CI.

Chem/Ground Mode

Considerable work has been devoted to evaluating the detection of CW agent liquids sampled using the laboratory version of the ground probe. The data illustrate the selectivity which can be achieved for CW agent detection in the CI MS-MS mode of operation. Figure 5 shows mass profiles for tests using fog oil or DF2 with and without added agents GB or HD. Figure 5(A) shows the mass profile for m/z 99 (product ion from the protonated GB molecular ion of m/z 141) for 10 μg of fog oil (alone) and two runs of 120 ng of GB with 10 μg

of fog oil. Figure 5(B) shows the mass profile for m/z 123 (product ion from the protonated HD molecular ion of m/z 159) for 10 ug of DF2 (alone) and then two runs of 132 ng HD with 10 ug DF2. Both CW agents are easily detected in the presence of ca.100-fold mass excess of fog oil or DF2, and there is very little or no ion activity from the interferents. This indicates the ability of CI MS/MS to minimize false negatives and positives. Even greater excesses of interferent can be tolerated, but a clear-out period is necessary before agent can be detected. Ten ng of MS has been detected within 2 minutes of applying a road wheel dipped in DF2 to the lab probe head.

STATUS

Design and testing has been performed to ensure that the fielded instrument can survive temperature extremes and shock and vibrations from wheeled vehicle transport. The capability to survive radiation from a tactical nuclear event has been developed using specially selected components (where feasible) and custom-designed circumvention circuits. Tests have been performed at the White Sands Missile Range to confirm the radiation tolerance of critical components and subsystems⁵.

The Block II CBMS is undergoing tests with biological and chemical agents, and it participated in the JFT-6 trials at the Defense Research Establishment Suffield. It has been selected for trials on the JSLNBCRS HMMWV and LAV platforms and on the Block II FOX.

CONCLUSIONS

The development and preliminary testing of the Block II CBMS is being completed this year. Its sensitive and selective detection of BW and CW agents will provide improved protection to US and allied forces.

ACKNOWLEDGEMENTS

This research was sponsored by the U.S. Army Soldier and Biological Chemical Command, DOE No. 2182-K011-A1, U.S. Department of Energy under contract DE-AC05-00OR22725 with Oak Ridge National Laboratory, managed and operated by UT-Battelle LLC. The authors wish to acknowledge the CBMS Program Team in the development of the Block II: colleagues at ORNL for their design and testing of the instrument, MSP Corporation for their design of the biosampler, Colorado School of Mines for their work on identifying biomarkers and developing the reagent injector, and Orbital Sciences Corporation for their instrument design work and fabrication of preproduction units.

REFERENCES

1. K. J. Hart, M. B. Wise, W. H. Griest, and S. A. Lammert, "Design, Development and Performance of a Fieldable Chemical & Biological Agent Detector," *Field Analytical Chemistry and Technology*, 4 (22-3), 93-110 (2000).
2. C.V. Thompson, M.B. Wise and M.R. Guerin, "Effects of Transfer Line on MS Sampling and Analysis of VOC's in Air", Proceedings of the 43rd ASMS Conference on Mass Spectrometry and Allied Topics, Atlanta, GA, May 21-26, 1995 , p. 831.
3. S.A. Barshick, D.A. Wolf and A.A. Vass, "Differentiation of Microorganisms Based on Pyrolysis-Ion Trap Mass Spectrometry Using Chemical Ionization", *Anal. Chem.*, 71, 633-641 (1999).
4. F. Basile, M.B. Beverly, C. Abbas-Hawks, C.D. Mowry, T.L. Hadfield and K.J. Voorhees, "Direct Mass Spectrometric Analysis of in Situ Thermally Hydrolyzed and Methylated Lipids from Whole Bacterial Cells", *Anal. Chem.*, 70, 1555-1562 (1998).
5. R. M. Brady, "Initial Nuclear Radiation (INR) Detailed Test Report for the First Article Test (FAT) on the Block II Chemical/Biological Mass Spectrometer Electronics Test Stands (Block II CBMS)," TECOM

Project No. 8-CO-410-000-052, White Sands Missile Range, White Sands, NM (July, 1998), and following reports.

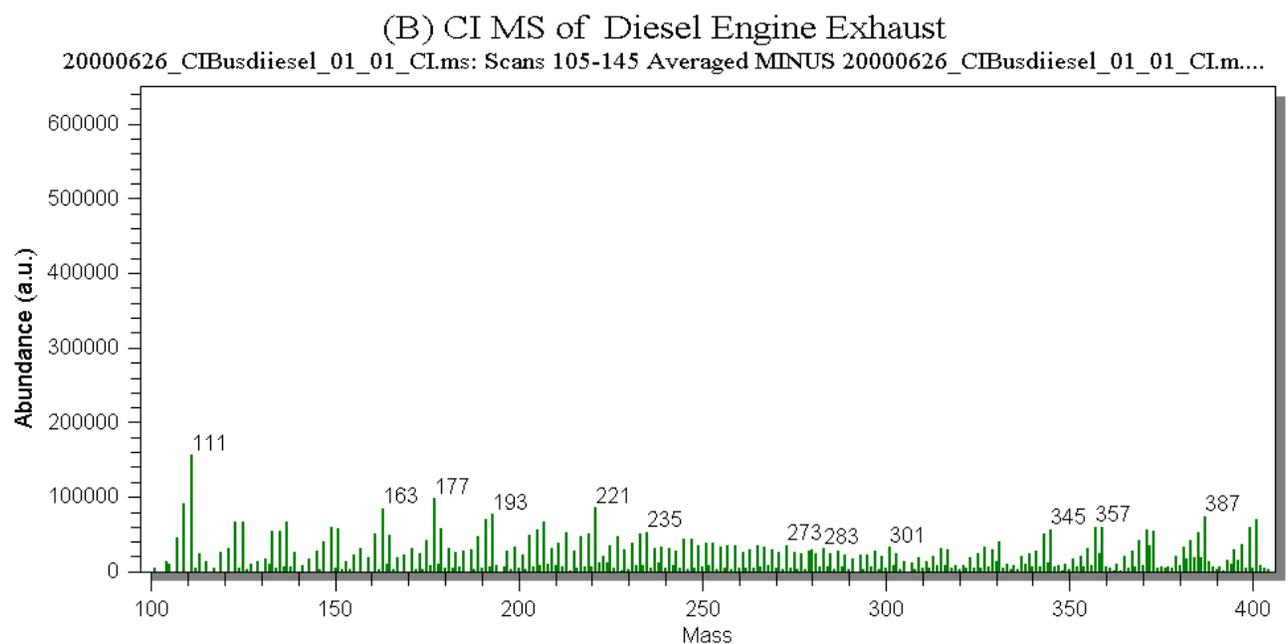
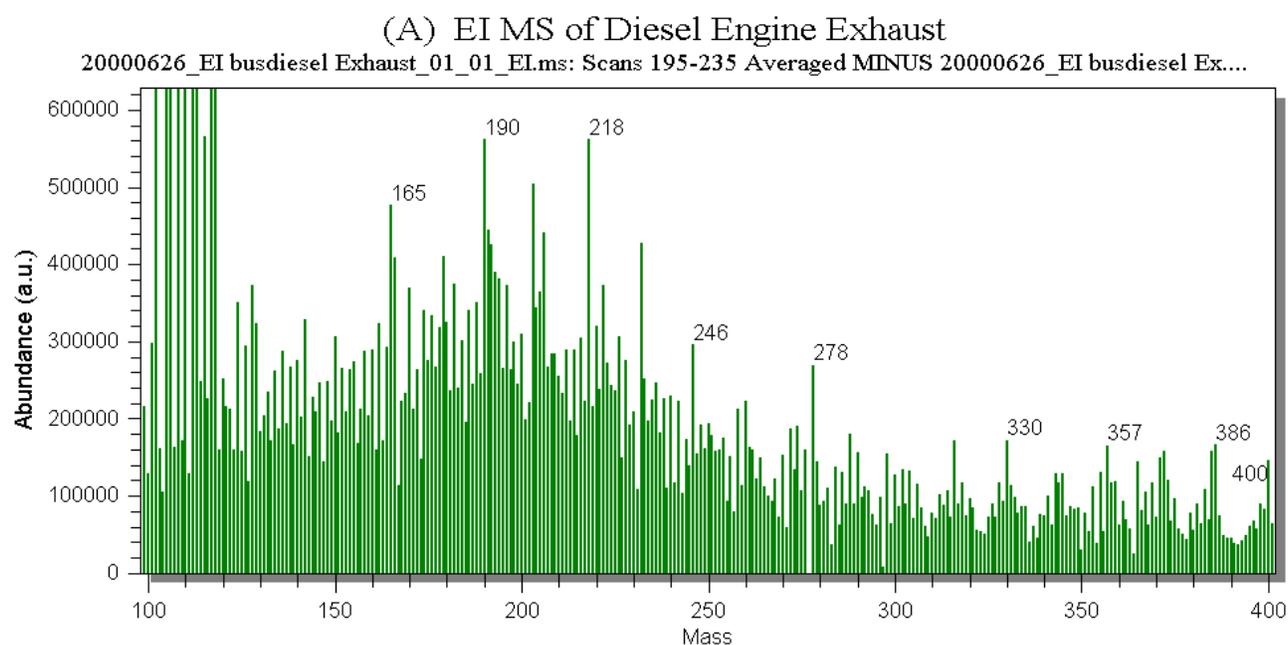
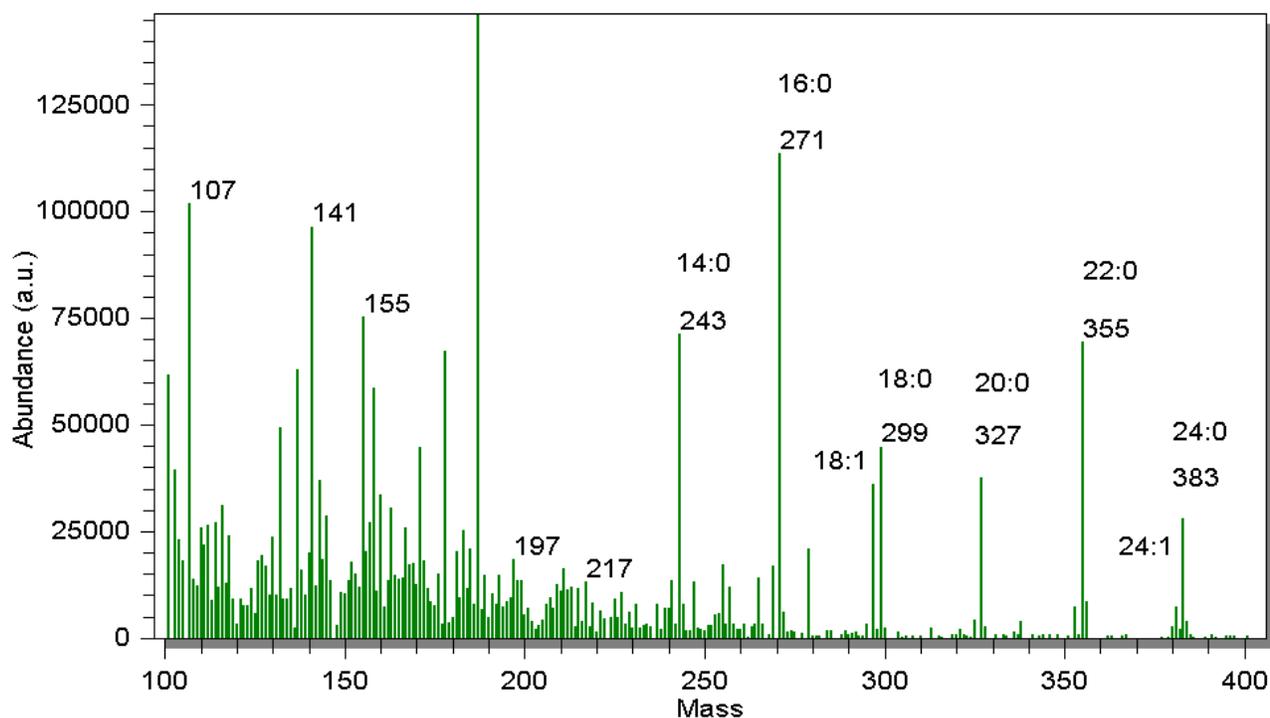


Figure 1. Comparison of EI and CI MS of diesel engine exhaust as TMAH derivatives.

(A) Francisella tularensis SHU-4, 31 ACPLA of 3.2 um particles

20000528_FT030_BC_03_01_CL.ms: Scans 12-52 Averaged MINUS 20000528_FT030_BC_03_01_CL.ms: Scans 5....



(B) Crude Precipitate from Castor Bean Extract, 200 ng Direct Injection

20000413_BTRA_01_01_CL.ms: Scans 615-655 Averaged MINUS 20000413_BTRA_01_01_CL.ms: Scans 254-294....

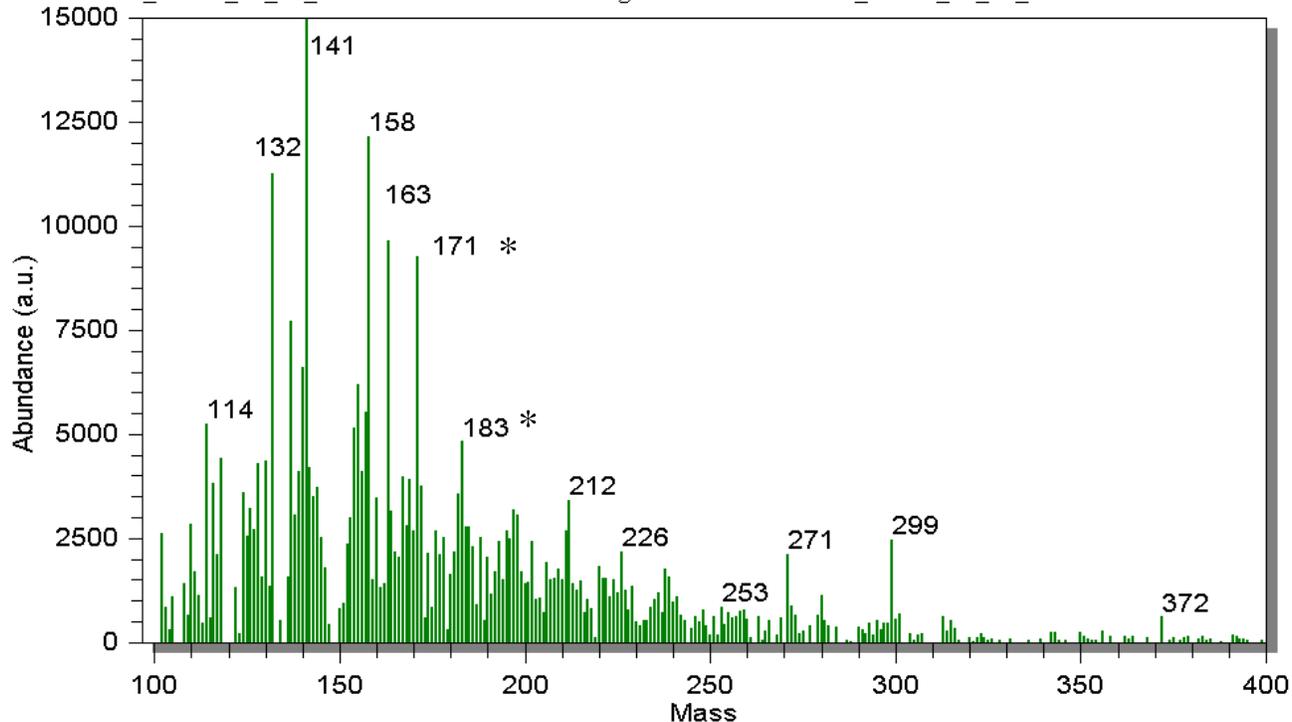
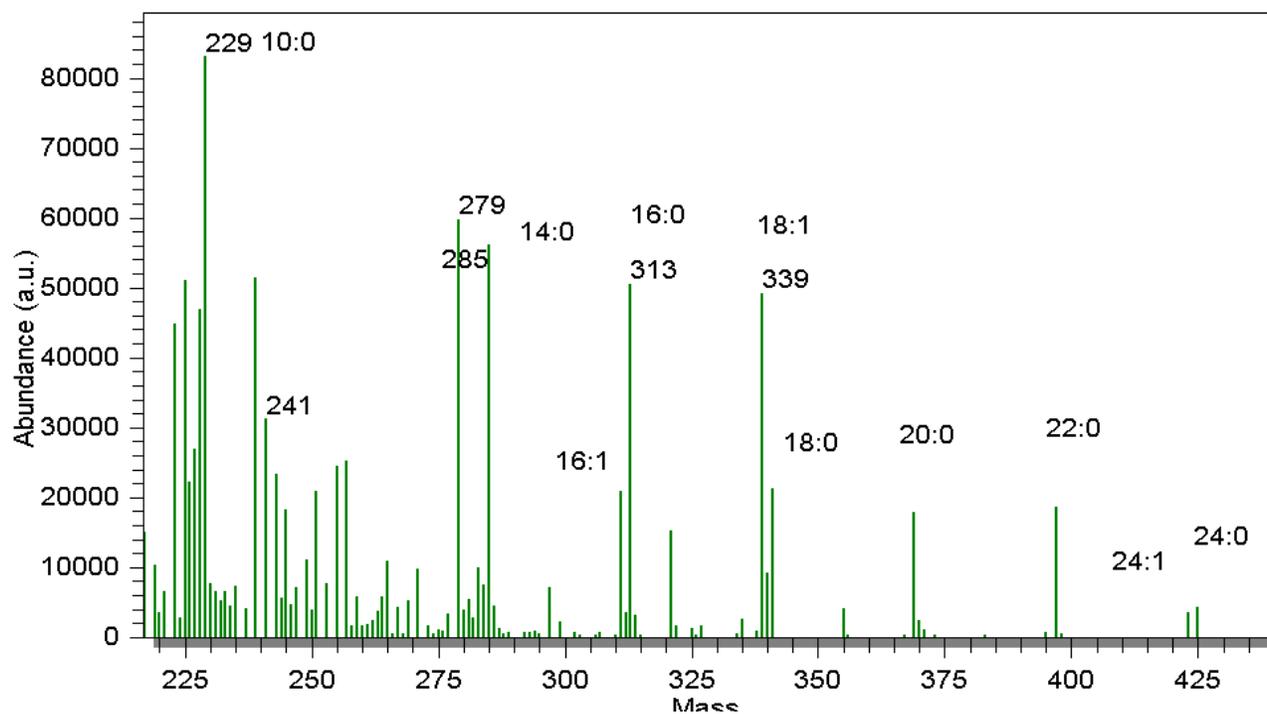


Figure 2. CI MS spectra of a bacterial aerosol and an injected crude toxin as TMAH Derivatives.

(A) *Francisella tularensis* SHU-4, 1.1 x 10E6 cells TBAH Derivatized

20000621_FT SHU4 TMAH_03_01_CIMS: Scans 280-340 Averaged MINUS 20000621_FT SHU4 TMAH_03_01_CIM...



(B) CI MS/MS of m/z 397, [MH]⁺ for 22:0 from *Francisella tularensis*

20000621_FT SHU4 TMAH msms397_01_01_CIMS: Scans 120-160 Averaged MINUS 20000621_FT SHU4 TMAH ms...

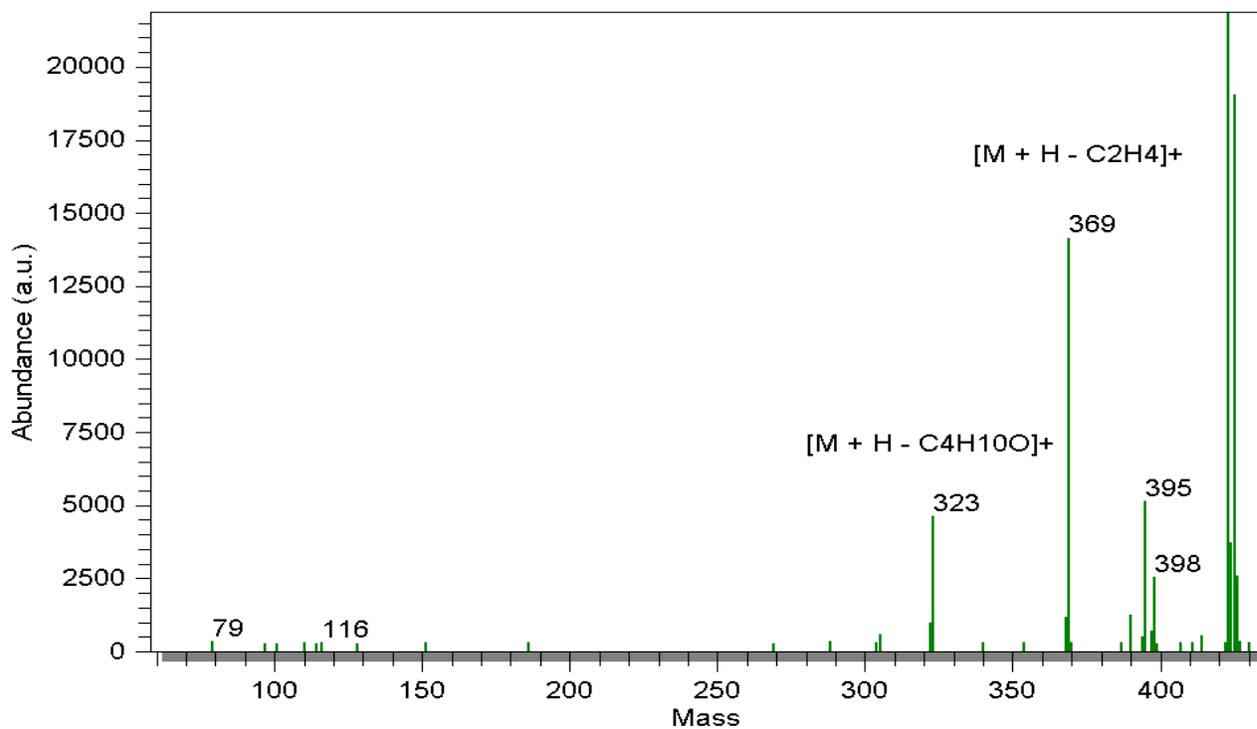
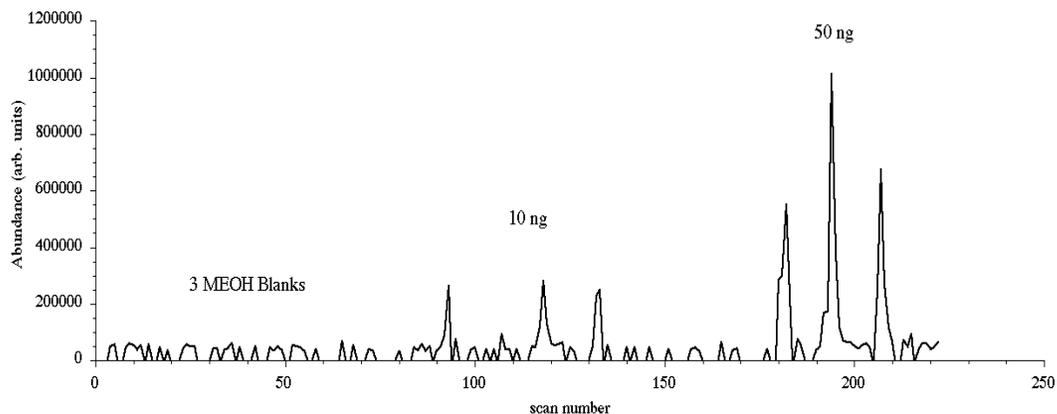


Figure 3. CI-MS of *Francisella tularensis* and CI MS/MS of 22:0 fatty acid as TBAH derivatives.

(A) Mass profile (m/z 125) for injection of methanol, 3 x 10 ng and 3 x 50 ng of DMMP



(B) Mass profile (m/z 153) for MES vapors at 0.01 and 0.1 mg/m³ from Tedlar bag

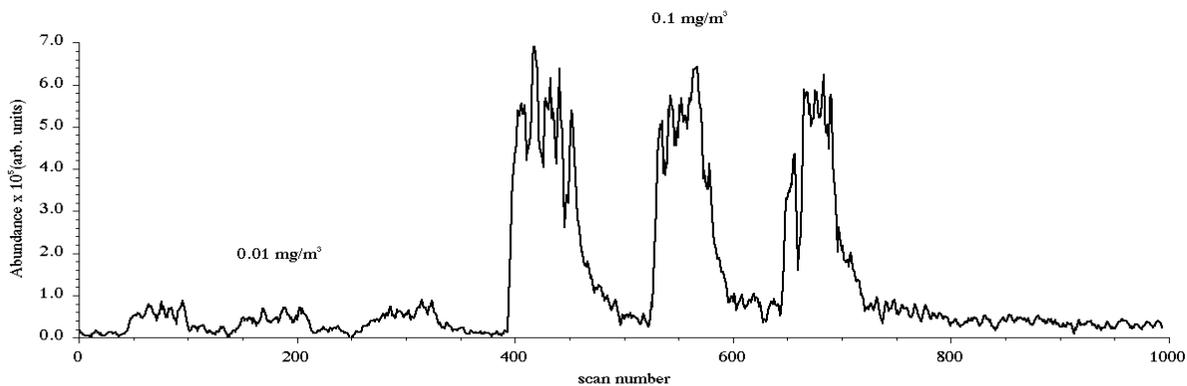
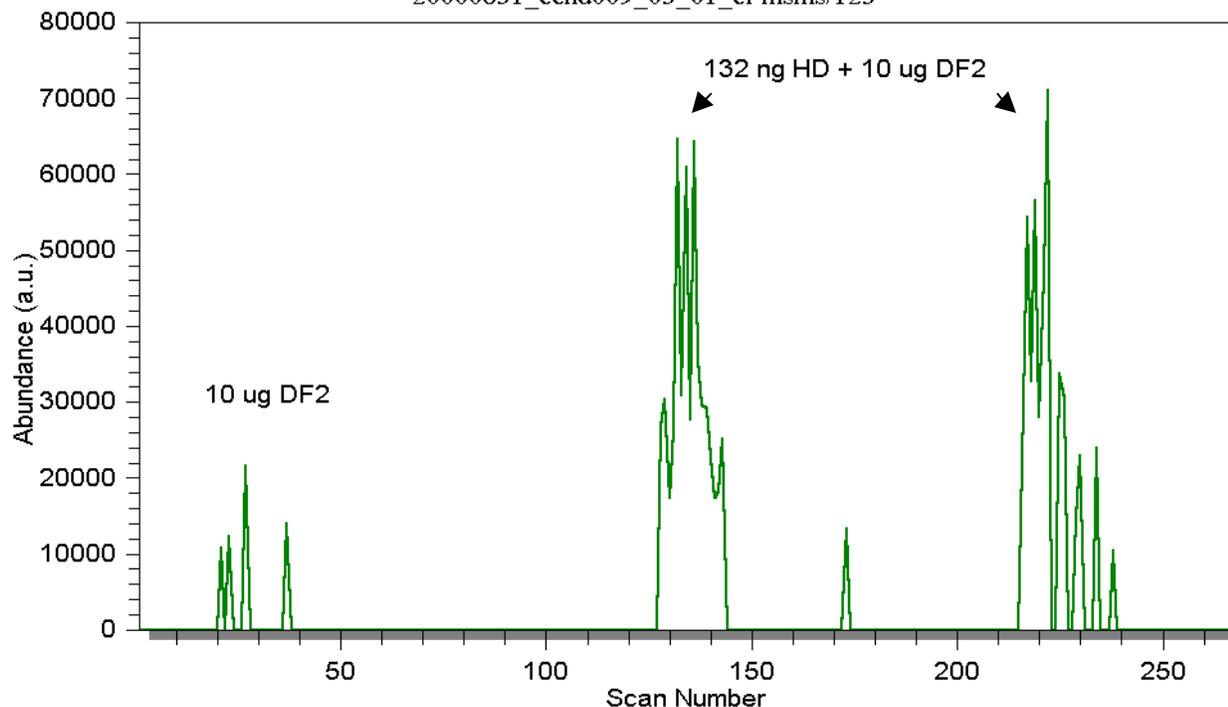


Figure 4. Simulant tests using Chem/Air sampling line and full scan CI MS mode.

(B) CI MS/MS (m/z 159 to 123) of DF2 and HD with DF2

20000831_cchd009_03_01_ci-msms/123



(A) CI MS/MS (m/z 141 to 99) of Fog Oil and GB with Fog Oil

20000811_ccgb007_06_01_ci-msms/99

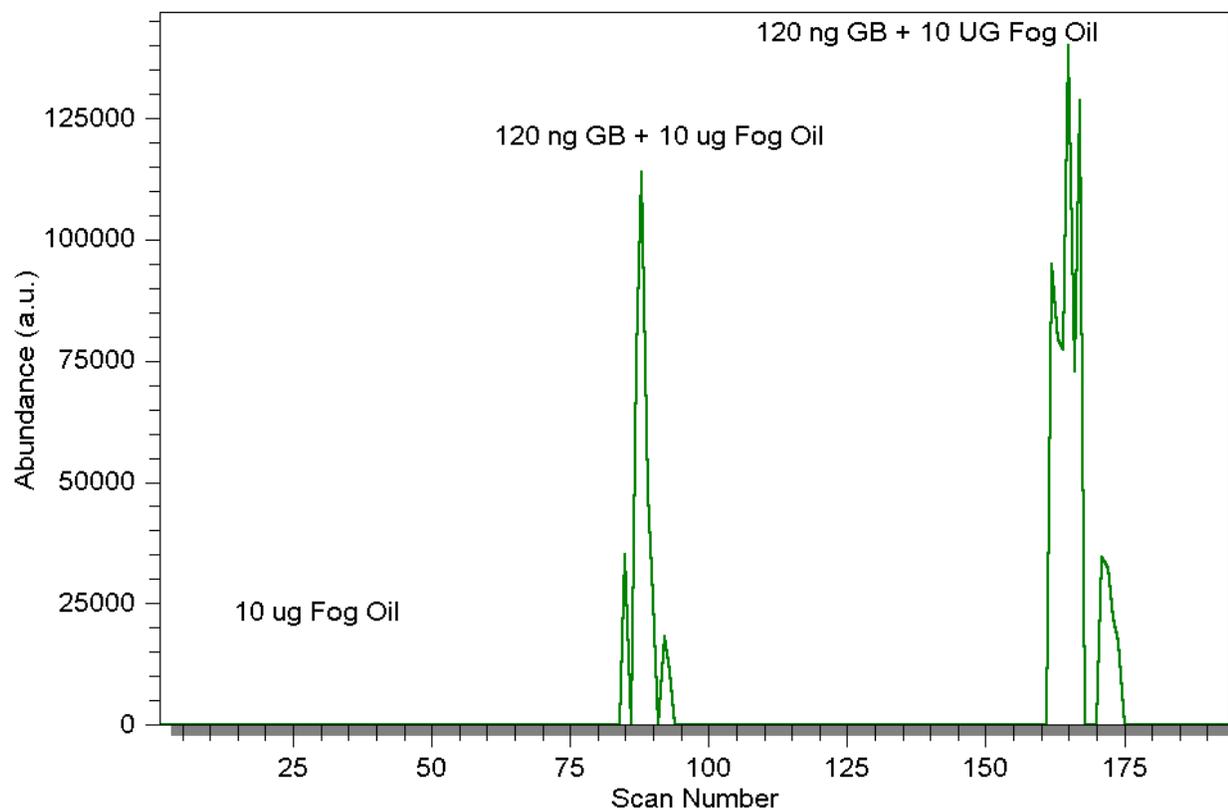


Figure 5. Mass profiles from CI MS/MS tests using GB, HD and battlefield interferents on lab probe.