EPA 600/R-14/399 | October 2014 | www.epa.gov/research

United States
Environmental Protection
Agency

Compatibility of Material and Electronic Equipment with Ethylene Oxide Fumigation

ASSESSMENT AND EVALUATION REPORT

Office of Research and Development
National Homeland Security Research Center

EPA 600-R-14-399
October 2014

Compatibility of Material and Electronic Equipment with Ethylene Oxide Fumigation

Assessment and Evaluation Report

National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Disclaimer

The United States Environmental Protection Agency, through its Office of Research and Development's National Homeland Security Research Center, funded and directed this investigation through EP-C-09-027 WAs 4-58 and 5-58 with ARCADIS U.S., Inc. This report has been peer and administratively reviewed and has been approved for publication as an Environmental Protection Agency document. It does not necessarily reflect the views of the Environmental Protection Agency. No official endorsement should be inferred. This report includes photographs of commercially available products. The photographs are included for purposes of illustration only and are not intended to imply that EPA approves or endorses the product or its manufacturer. Environmental Protection Agency does not endorse the purchase or sale of any commercial products or services.

Questions concerning this document or its application should be addressed to:

Shannon Serre
Decontamination and Consequence Management Division
National Homeland Security Research Center
U.S. Environmental Protection Agency (MD-E343-06)
Office of Research and Development
109. T.W. Alexander Drive
Research Triangle Park, NC 27711
Phone: 919-541-3817
Fax: 919-541-0496
E-mail: serre.shannon@epa.gov

Acknowledgments

This effort was directed by the principal investigator from the Office of Research and Development's (ORD's) National Homeland Research Center (NHSRC), utilizing the support from the US Environmental Protection Agency's (EPA's) Chemical, Biological, Radiological, and Nuclear (CBRN) Consequence Management Advisory Division (CMAD) within the Office of Emergency Management (OEM). The contributions of the entire team are acknowledged.

Project Team:

Shannon Serre (Principal Investigator)
NHSRC, ORD, US EPA
Research Triangle Park, NC 27711

R. Leroy Mickelsen
CBRN CMAD, OEM, Office of Solid Waste and Emergency Response, US EPA
Research Triangle Park, NC 27711

Jayson Griffin
CBRN CMAD, OEM, Office of Solid Waste and Emergency Response, US EPA
Research Triangle Park, NC 27711

This effort was completed under U.S. EPA contract #EP-C-09-027 with ARCADIS-US, Inc. The support and efforts provided by ARCADIS-US, Inc. are gratefully acknowledged.

Ramona Sherman and Eletha Brady-Roberts (Quality Assurance)
NHSRC, ORD, US EPA
Cincinnati, OH 45220

Joan Bursey (Quality Assurance and Editorial Assistance)
NHSRC, ORD, US EPA
Research Triangle Park, NC 27711

The peer reviewers of this report are also acknowledged for their input to this product:

Joseph Wood, NHSRC, Office of Research and Development, US EPA

Michael Nalipinski, CBRN CMAD, OEM, Office of Solid Waste and Emergency Response, US EPA

Lawrence Kaelin, CBRN CMAD, OEM, Office of Solid Waste and Emergency Response, US EPA

Table of Contents

List of Figures

List of Tables

List of Acronyms and Abbreviations

AC	Alternating current
ACPI	Advanced configuration and power interface
BI	Biological indicator
BIT	BurnInTest®
CBRN	Chemical biological radiological and nuclear
CD	Compact disc
CE	European Commission
CFC	Chlorofluorocarbon
CFU	Colony Forming Units(s)
CMAD	Consequence Management Advisory Division
CO	Carbon monoxide
DPG	Dugway Proving Ground
DQI	Data Quality Indicator
DQO	Data Quality Objective
DVD	Digital versatile disc
EPA	U. S. Environmental Protection Agency
ESD	Electrostatic discharge
EtO	Ethylene oxide
FM	Factory Mutual Research Corporation
HCFC	Hydrochlorofluorocarbon
HSPD	Homeland Security Presidential Directive
HSRP	Homeland Security Research Program
IS	Intrinsically safe
LCD	Liquid crystal display
LED	Light-emitting diode
MDI	Metered Dose Inhaler
MP	Megapixel
NHSRC	National Homeland Security Research Center
OEM	Office of Emergency Management
ORD	Office of Research and Development
OSHA	Occupational Safety and Health Administration
PBST	Phosphate Buffered Saline with Tween® 20
PC	Personal computer
PCMD	PC Doctor
PDA	Personal Digital Assistant
QA	Quality Assurance
QAPP	Quality Assurance Project Plan

QC	Quality Control
RH	Relative humidity
RTD	Resistance Temperature Detector
RTP	Research Triangle Park
SD	Secure Digital
SVGA	Super video graphic array
TSA	Tryptic Soy Agar
USB	Universal Serial Bus
WACOR	Work Assignment Contracting Officer Representative

Executive Summary

The objective of the research presented in this report was to determine the material compatibility of electronics and other materials to exposure with ethylene oxide gas (EtO) fumigation, a commercially available fumigation technology used widely in the medical industry for sterilization and considered for use to inactivate *Bacillus anthracis* spores. A secondary objective was to test the efficacy of EtO against a *Bacillus anthracis* surrogate. The current study was designed to provide direct information on the impact of EtO gas on sensitive electronic components and materials that can be viewed as surrogates for sensitive materials and high-end equipment (e.g., medical devices and airport scanners) that use similar types of components.

Bacillus atrophaeus, on commercial biological indicators and inoculated onto 18 mm rubber coupons, was used as a surrogate for the lethal biological agent, *Bacillus anthracis*. Manufacturer-suggested operational conditions were targeted for the study. The manufacturer-suggested fumigation method is an 11 g EtO cartridge activated within a specialized selectively permeable bag. The bag also contains Humidichips®, which contain water to humidify the environment inside the bag, as the ventilation cabinet containing the bag heats to the manufacturer-suggested temperature of 50 °C. As EtO is released from the cartridge (EtO is very volatile), the EtO slowly permeates through the bag wall into the ventilation cabinet over an 18-hour cycle. The cabinet removes the EtO through an abator, and the bag can be retrieved safely at the conclusion of the 18-hour cycle. The study also investigated the use of a higher EtO concentration with the use of an 18 g cartridge.

The study found that EtO was sporicidal with greater than 6 log reduction under all tested conditions.

Three different categories of materials were tested as surrogates for sensitive materials and high-end equipment. Category 1 materials (household building materials) which were previously tested with chlorine dioxide, hydrogen peroxide, and methyl bromide, were not included in this study. Because of size limitations, EtO would not be considered as an option for decontamination of Category 1 materials.

Category 2 materials included construction materials of low surface area but high functionality within a building. These construction materials included aluminum, copper, stainless steel, smoke detectors with and without carbon monoxide (CO) alarms, laser-printed paper, ink jet-colored paper, and color photographs.

Category 3 materials and equipment included pieces of small personal electronic equipment: Personal Digital Assistants (PDAs), cell phones, fax/phone/copier machines, compact discs (CDs), digital versatile discs (DVDs), Universal Serial Bus (USB) flash drives, and Secure Digital (SD) memory cards.

Category 4 materials included desktop computers and monitors.

The effects of EtO on all tested materials was minimal, with no recorded visual impacts on any of the materials. All fumigated electronic components maintained the same functionality as the control equipment.

1 Project Description and Objectives

This project supports the mission of the U.S. Environmental Protection Agency's (EPA's) Office of Research and Development (ORD), Homeland Security Research Program (HSRP) by providing information pertinent to the decontamination of contaminated equipment and materials where the contamination results from an act of terrorism. Under Homeland Security Presidential Directives (HSPDs)-5, 7, 8, and 10, the EPA, in a coordinated effort with other federal agencies, is responsible for "developing strategies, guidelines, and plans for decontamination of equipment, and facilities" to mitigate the risks of contamination following a biological agent contamination incident.

EPA's National Homeland Security Research Center (NHSRC) aims to help EPA address the mission of the HSRP by providing expertise and products that can be widely used to prevent, prepare for, and recover from public health and environmental emergencies arising from terrorist threats and incidents. One of the missions of NHSRC is to provide expertise and guidance on the selection and implementation of decontamination methods and to provide the scientific basis for a significant reduction in the time, cost, and complexity of decontamination events. Fumigation with ethylene oxide (EtO) for the decontamination of certain materials and equipment contaminated with anthrax spores has been suggested as a safe alternative to more harsh fumigants such as chlorine dioxide or hydrogen peroxide. Unlike hydrogen peroxide and chlorine dioxide, EtO is not an oxidizing agent and kills organisms through alkylation. Information on the compatibility of materials and equipment with typical EtO fumigation conditions effective for anthrax spores has not been determined in a systematic, reproducible way. Future guidance on selection and operation of decontamination technologies is dependent upon such information.

Data on the impact of fumigation with EtO on materials/equipment under sporicidal conditions relevant to facility decontamination are needed to define the guidance further with respect to the selection and use of fumigant technologies for small scale decontamination operations.

1.1 Purpose

The purpose of this study was to determine the impact of EtO fumigation on sensitive electronic components and materials that can be viewed as surrogates for sensitive materials and high-end equipment (e.g., medical devices and airport scanners) that use similar types of components. Decontamination conditions used were those achievable by commercial equipment. This effort investigated the impact on the physical appearance, properties, and functionality of the materials and equipment, as appropriate.

1.2 Process

Category 2, 3, and 4 materials and equipment were tested before exposure to EtO. The equipment was exposed to EtO according to the finalized test matrix. The diagnostic protocols (as outlined in Section 2.8) were repeated on all materials and equipment after fumigation and monthly for a period of at least nine months. The results of these testing protocols were used to evaluate the impact of fumigation on the materials tested. Fumigations were conducted in High Bay Room H-222 and compatibility testing was performed in Room E288 on EPA's Research Triangle Park, North Carolina, campus.

1.2.1 Overview of Compatibility Testing

Compatibility testing was performed to monitor the short- and long-term effects of EtO fumigation on materials and electronic equipment. Category 1 materials (household building materials) which were previously tested with chlorine dioxide, hydrogen peroxide, and methyl bromide, were not included in this study. EtO would be considered an option for decontamination of valuable items that might be damaged by other fumigants and would not be considered as an option for decontamination of Category 1 materials.

1.2.1.1 Category 2 and 3 Materials Testing

Category 2 includes construction materials of low surface area but high functionality within a building. These materials include aluminum, copper, stainless steel, smoke detectors with and without carbon monoxide (CO) alarms, color laser-printed paper, color ink jet-printed paper, and color photographs. The objective for this category of materials was to assess the visual and/or functionality (as appropriate) impact of the fumigation process on the materials. Building materials were tested to better compare to testing previously conducted with other fumigants [1, 2]. The impact was analyzed using visual inspections under each set of fumigation conditions and functionalities, where appropriate. The visual inspections were directed towards possible locations suspected of corrosion and possible material defects due to the fumigation process. Printed documents and pictures were inspected for possible alteration of their content. Inspection occurred at regular intervals over a ten-month period, with the material stored under ambient laboratory conditions throughout that time period. The visual inspections were documented in writing and by digital photography for each material before and after each fumigation.

Category 3 materials and equipment include small pieces of personal electronic equipment. These pieces of equipment included Personal Digital Assistants (PDAs), cell phones, fax/phone/copier machines, compact discs (CDs), digital versatile discs (DVDs), Universal Serial Bus (USB) flash drives, and Secure Digital (SD) memory cards. The objective for this category was to determine visual and functionality impacts on the equipment as a function of time post-fumigation. The assessment of the impact was visual inspection for aesthetic effects and evaluation of functionality pre-/post-fumigation. Inspection occurred at regular intervals over a ten-month period, with the equipment stored at ambient laboratory conditions throughout that time period. Visual inspections of the equipment were documented in writing and by digital photographs. Further, the functionality of each piece of equipment was assessed comparatively with similar equipment that was not subjected to the fumigant exposure.

1.2.1.2 Category 4 Materials Testing

Category 4 equipment included desktop computers and monitors. The objective for this category of equipment (and materials) was to assess the impact of the fumigation conditions using visual inspection, functionality testing, and a software personal computer (PC) diagnostic tool. The objective was to identify components and specific parts of components that may be susceptible to corrosion due to the fumigation process. This information can be used to make informed decisions about the compatibility of other equipment that may have similar components (at least similar in operation) and can at least reduce further testing or uncertainty in the field application. The equipment and materials included in this category are as follows:

- Dell OptiPlex 790 Desktop Computer (see Table 2-3 for specifications)

- Dell 19-inch flat panel monitor
- USB keyboard and mouse
- Computer and monitor power cords and connecting analog super video graphic array (SVGA) cable
- USB flash drives in all USB ports
- Network loopback adapter
- Serial loopback adapter.

1.3 Project Objectives

The primary objective of the work was to assess the impact of sporicidal fumigation with EtO on materials and electronic equipment. Specifically, the fumigation conditions of interest are those provided by the commercially used Andersen EOGas 333 system. Visual appearance of all items was documented before and after fumigation exposure. Some materials were not tested for functionality due to the multiplicity of potential uses. Since EtO is explosive under the target conditions, the state of operation of all electrical equipment was in the off state and de-energized for all test conditions involving EtO. Any electronic equipment with capacitors would need to have the capacitors discharged prior to fumigation with EtO.

An additional primary objective in this study was to obtain an indication of the potential impact that the local conditions inside the EtO bag may have on the effectiveness of the fumigation process to inactivate anthrax spores potentially located within equipment. For this purpose, inoculated rubber stubs were used. Under a previous study (Appendix A), rubber was determined to be the most resistant to EtO fumigation due to apparent adsorption and desorption of the fumigant.

2 Materials and Methods

2.1 Subject Materials and Equipment

Three categories of material and equipment were tested under the different fumigation conditions discussed in detail below; the categories can be separated based upon the conditions of testing and analysis performed to assess the impacts. Category 1 materials (building construction materials comprising high surface-area within a volume) were not included in this effort. The Category 2, 3, and 4 materials and equipment tested are listed in Tables 2-1, 2-2, and 2-3, respectively.

Table 2-1. Category 2 Materials

Material	Description	Supplier/ Manufacturer	Part Number	Coupon/ Sample Size
Type 3003 Aluminum	Textured 0.0625-inch thick sheet	McMaster Carr (Elmhurst, IL)	88685K12	2-inch by 2-inch, three pieces
Alloy 101 Copper	0.064-inch thick polished electrical grade, 99.99% pure	McMaster Carr (Elmhurst, IL)	3350K19	2-inch by 2-inch, three pieces
Type 316 Stainless Steel	0.0625-inch thick 2B finish	McMaster Carr (Elmhurst, IL)	9090K11	2-inch by 2-inch, three pieces
Type 304 Stainless Steel	0.0625-inch thick #3 finish	McMaster Carr (Elmhurst, IL)	9085K11	2-inch by 2-inch, three pieces
Smoke Detector	Battery-powered ionization sensor	First Alert (Aurora, IL)	SA304	one piece
Smoke Detector and CO alarm	Electrochemical CO sensor, Photoelectric sensing technology	First Alert (Aurora, IL)	SCO5CN	one piece
Laser-printed paper	Stack of 15 pages	RTO-E340-PS HP Color LaserJet (Palo Alto, CA)	NA	8-½-inch by 11-inch
Ink jet-colored paper	Stack of 15 color pages	HP DeskJet 932C Palo Alto (CA)	NA	8-½-inch by 11-inch
Color Photograph	4-inch by 6-inch Kodak processing	Walgreens (Springfield, IL)	NA	4-inch by 6-inch, three pieces

Table 2-2. Category 3 Materials and Equipment

Equipment	Description	Manufacturer	Model Number	Sample Size
PDA	Handheld	Palm (Sunnyvale, CA)	Pixi Plus	one piece
Cell Phone	Thin Flip Phone, 1.3 MP camera. 2.4" liquid crystal display (LCD)	Samsung (Ridgefield Park, NJ)	M400	one piece
Fax/Phone/Copier Machine	Plain-paper fax and copier with ten-page auto document feeder and up to 50-sheet paper capacity. 512KB memory stores up to 25 pages for out-of-paper fax reception	Brother (Bartlett, TN)	Fax 575	one piece
Data CD	Software CD	Snap! (Johns Creek, GA)	01-0170-026-000	one piece
Data DVD	Standard 21331 DVD Video	Warner Brothers (Los Angeles, CA)	Harry Potter and the Sorcerer's Stone DVD	one piece
USB Flash Drive	4 GB Flash Drive	Sandisk (Milpitas, CA)	SDCZ36-004G-A11	one piece
SD Memory Card	4 GB SD Card	Kingston (Fountain Valley, CA)	SD4/4GB/SKU# 9643151	one piece

Table 2-3. Category 4 (Personal Computer) Specifications

Base Unit:	OptiPlex 790 Minitower Base,90 PSU (225-0782)
Processor:	Opti 790,CORE i5 2500 Processor (3.3GHz, 6M) (317-6644)
Memory:	4GB,Non-ECC,1333MHz DDR3,2X2GB,Dell OptiPlex 990 (317-6987)
Keyboard:	Dell USB Entry Keyboard, No Hot Keys, English, OptiPlex (331-2024)
Monitor:	Dell 19 inch Flat Panel Display,E1911,Black,OptiPlex,Precision and Latitude (320-1762)
Video Card:	Integrated Video,HD Graphics 2000, Dell Optiplex 790 (320-2520)
Hard Drive:	250GB SATA 6.0Gb/s and 8MB Data Burst Cache,Dell OptiPlex (342-2453)
Operating System:	Windows 7 Professional,No Media, 64-bit, Optiplex, English (421-5606)
Operating System:	Windows 7 Label, Optiplex, Fixed Precision, Vostro Desktop (330-6228)
Mouse:	Dell MS111 USB Optical Mouse,OptiPlex and Fixed Precision (330-9458)
NIC:	Intel Standard Manageability, Dell OptiPlex 790 (331-2680)
CD-ROM or DVD-ROM Drive:	16X DVD+/-RW SATA,Data Only,Dell OptiPlex 790 Desktop or Minitower,Black (318-0623)
CD-ROM or DVD-ROM Drive:	Roxio Creator Starter,Media, Dell OptiPlex, Latitude and Precision Workstation (421-4540)
CD-ROM or DVD-ROM Drive:	Cyberlink Power DVD 9.5.1,Media, Dell OptiPlex, Latitude and Precision Workstation (421-5095)
Sound Card:	Heat Sink, Performance, Dell OptiPlex 790 Minitower (331-2023)
Speakers:	Internal Speaker, OPtiplex 990 (318-0319)
Cable:	Enable Low Power Mode for EUP Compliance,Dell OptiPlex (330-7422)
Cable:	Dell Data Protection Access,OptiPlex (421-5078)
Cable:	OptiPlex 790 Minitower Up to 90 Percent Efficient Power Supply (318-0875)
Cable:	Regulatory Label,Dell OptiPlex 790 Minitower (331-2689)
Documentation Diskette:	Power Cord,125V,2M,C13,Dell OptiPlex (330-1711)
Documentation Diskette:	Documentation,English,Dell OptiPlex (331-2030)
Factory Installed Software:	Energy Star 5.0 Category C (209kWh TEC), EPEAT Gold, Dell ESMART Settings, Dell OptiPlex 790 (331-2019)
Feature	No Resource DVD for Dell Optiplex, Latitude, Precision (313-3673)
Service:	Basic Hardware Service: Next Business Day Limited Onsite Service After Remote Diagnosis 2 Year Extended (938-7662)
Service:	Basic Hardware Service: Next Business Day Limited Onsite Service After Remote Diagnosis Initial Year (951-7510)

The Category 4 items, specifically the computers and monitors, were treated differently than the items included in the other categories. The computers and monitors were removed from their original packaging, labeled with a designated sample number, set up according to protocol, and tested for functionality. This equipment was transported to and from the EtO facility in anti-static and anti-corrosion bags (Corrosion Intercept Technology, http://www.staticintercept.com/CI_product.htm), specifically designed to protect equipment from exposure to potentially damaging electrostatic charge or corrosive gases. Computers and monitors remained energized and operated over the course of at least nine months to continually assess delayed effects due to the test conditions at which they were treated. All operations were done on an Electrostatic Discharge mat. Three control computers were used in this testing.

Computers were kept in operation simulating a five-day work week using BurnInTest® (BIT®), (Version 5.3 Pro, Passmark Software Pty. Ltd., Sydney, Australia). For each of five sequential days, BIT® was programmed to run as follows:

- 50 % load for eight hours (Figure 2-1)
- 16 hours in the ACPI standardized S3 state (standby mode)

- Computer is shut down and rebooted.

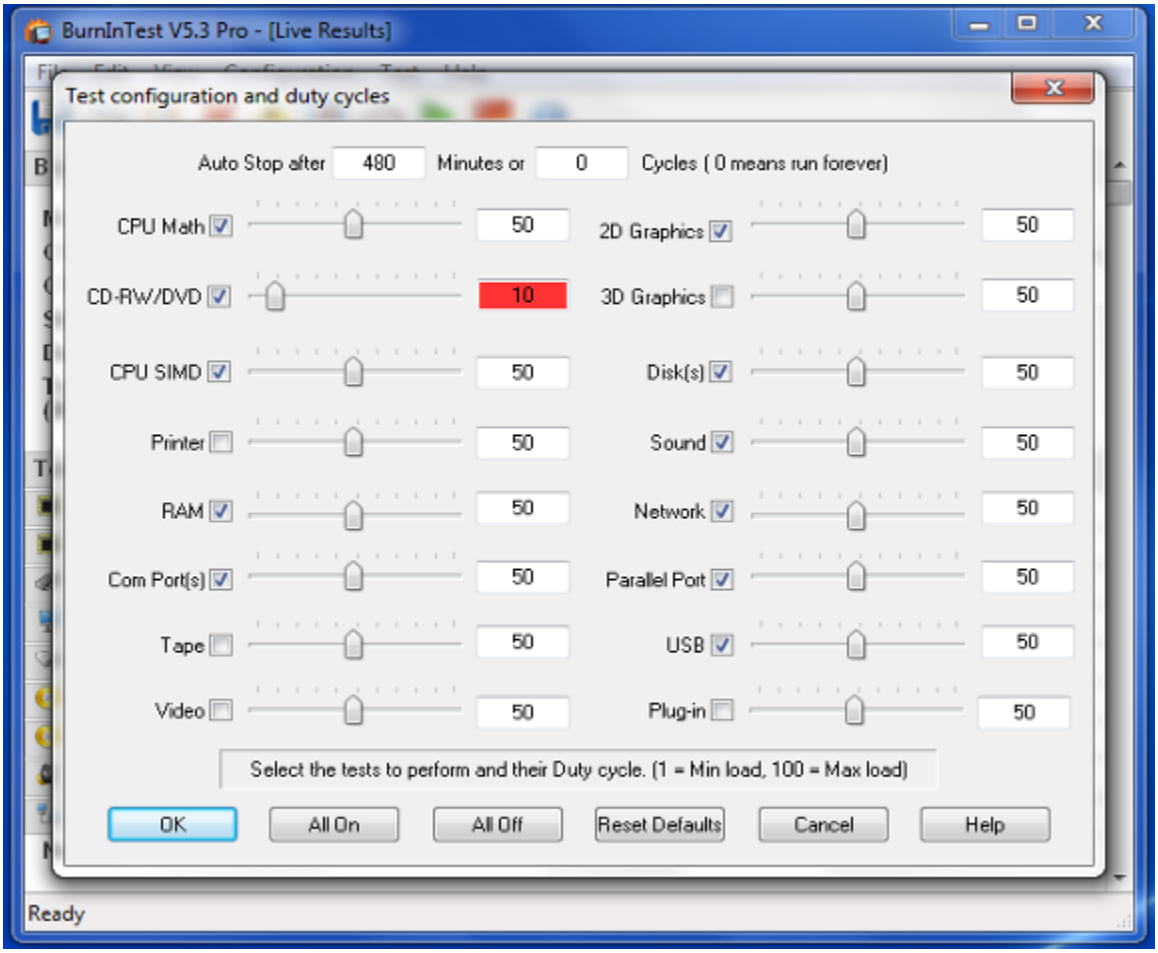

Figure 2-1. Test Configuration and Duty Cycles for BurnInTest® Software

After five days of this sequence, the computer was programmed to enter the advanced configuration and power interface (ACPI) standardized S3 state for 40 hours. After these 40 hours elapse, the test ends with the BIT® screen displayed. Probably due to an unidentified programming bug or operator error, some computers would only reboot 1-5 times instead of 6 during a testing cycle.

2.2 Laboratory Materials and Equipment

2.2.1 Ethylene Oxide Facility

The EtO fumigation facility, located in the EPA's Research Triangle Park (RTP) facility in High Bay Room H222, includes a heated and aerated cabinet (EOGas 333, Andersen Products, Inc., Haw River, NC, USA) which is used as an isolation chamber for the sterilization system (Figure 2-2).

Figure 2-2. EOGas 333 Cabinet

The EOGas 333 has the following features:

- 33 ft^3 capacity
- 100 % EtO, no chlorofluorocarbons (CFCs) or hydrochlorofluorocarbons (HCFCs)
- Sterilizes and aerates in the same cabinet
- Power outage backup protection
- Digital display with load tracking function
- EPA-registered; certifications to international standards
- Complies with current Occupational Safety and Health Administration (OSHA) regulations for personnel exposure
- Economical gas-disposal emissions-abatement equipment available
- All sterilizers available for either 110-V 60-Hz or 220-V 50-Hz.

The efficacy of EtO is dependent on both temperature and humidity during exposure. The cabinet was used to control the temperature at 50 °C during EtO exposure. Wetted sponges were included inside the permeable bags to moderate the relative humidity (RH) during exposure at or above the manufacturer-recommended humidity of 50-60 percent.

The permeable bags and wetted sponges were contained in premade kits (AN1006, Andersen Products, Inc., Haw River, NC, USA), which included 22-inch by 36-inch bags and 11 g EtO cartridges. Cartridges containing 18 g EtO were also used for this project.

Items for sterilization were de-energized (if applicable) and placed inside permeable bags with a cartridge of EtO (11 g and 18 g were used in this testing), a wetted sponge or humidichip, and a chemical indicator (AN-1087, Andersen Products, Inc., Haw River, NC, USA). The bag was then vacuum-sealed.

Once the cabinet reached the target conditions, bags were loaded into the cabinet. The cartridge was then activated, and the cabinet 18-hour cycle began. The temperature of the objects in the cabinet remained below the cabinet air temperature for a period of time due to the thermal mass. Typical exposure conditions are shown in Figure 2-3.

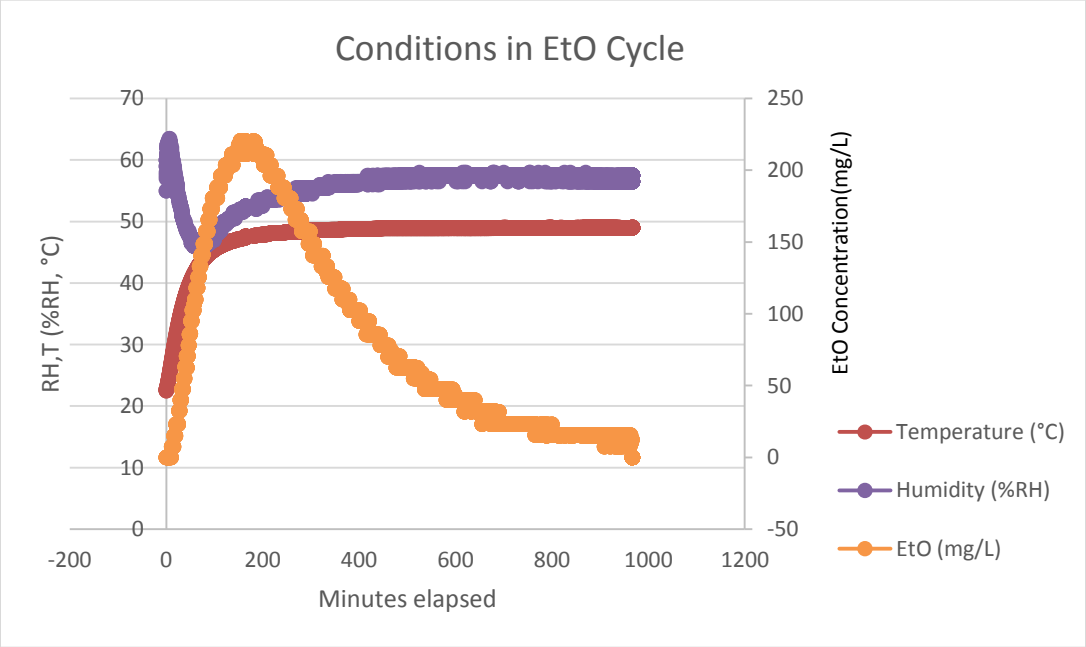

Figure 2-3. EtO Concentration over Time in a Standard Cycle

The chemical indicator (placed in each bag) changed color in proportion to the dose of sterilant and was a visual confirmation that the cycle operated as expected.

The EtO cabinet was used with an abator (ion exchange resin), which removed EtO from the exhaust of the cabinet before venting to the hood air handling system.

2.2.2 RH/Temperature Measurement

RH and temperature were monitored using a MadgeTech RHTemp Sensor (MadgeTech, Warner, NH) (Figure 2-4). These sensors were calibrated before use. At the conclusion of each fumigation, the sensors underwent post-test calibration. The data reduction procedure involved downloading the data from the sensor by using a USB cable and the Madgetech software (MadgeTech, Warner, NH).

Figure 2-4. MadgeTech RHTemp Sensor

The RHTemp1000IS is Factory Mutual Research Corporation (FM)-approved as intrinsically safe for hazardous environments. This certification makes the device well-suited for EtO sterilization, environmental studies, and numerous other hostile environment applications. The real-time clock ensures that all the data are time- and date-stamped. One RHTemp100IS sensor was placed inside the bag and another was placed inside the computer chassis for each Category 4 test. One MadgeTech device was placed inside the monitor bag. For each Category 2 and 3 test, one sensor was placed in each bag. Table 2-4 lists the specifications of the MadgeTech device.

Table 2-4. Madgetech (RH and Temperature) Sensor Specifications

Temperature sensor	Resistance temperature detector (RTD)
Temperature range	-20 °C to +80 °C (-4 °F to +176 °F)
Temperature resolution	0.01 °C
Temperature calibrated accuracy	±0.5 °C (0 °C to 55 °C)
Humidity sensor	Capacitive digital humidity sensor
Humidity range	0 to 100 % RH (non-condensing)
Humidity resolution	0.1 % RH
Humidity calibrated accuracy	±3 % RH maximum; ±2.0 % RH typical at 25 °C
Memory	16,350 readings per channel
Reading rate	1 second to 1 reading every 24 hours
battery life	two years typical at 25 °C, 15 minute reading intervals
Material	316 stainless steel
Dimensions	1.0" x 2.0" dia. (25.4 mm x 50.8 mm)
IP Rating	IP30
Operating environment	-20 °C to +80 °C, 0 to 95 % RH (non-condensing)
Required interface package	IFC400
Approvals	CE, Intrinsically Safe (IS) rated

2.2.3 Surface Roughness

A surface roughness tester (SRG-4000, Phase II Machine and Tool, Inc., Upper Saddle River, NJ, USA) uses a diamond stylus to measure average surface roughness to the nearest 0.001 micron. The stylus is pushed over the material surface, and the resistance to this action is measured. An average surface roughness is calculated from this measurement. Three surface roughness measurements were taken before and after exposure for each replicate Category 2 material coupon and recorded in the testing form.

2.2.4 Biological Indicators (BIs)

Commercial *Bacillus atrophaeus* biological indicators (BIs) (Catalog No. 1-6100, Mesa Laboratories, Inc., Lakewood, CO, USA) were used for all EtO fumigations. These are the standard BI used to measure EtO sterilization cycles. These BIs are paper strips inoculated with approximately 10^6 *Bacillus atrophaeus* spores, inside a Tyvek® pouch.

All BIs were maintained in their sterile Tyvek® envelopes until transferred to the NHSRC Biocontaminant Laboratory (Biolab) for analysis.

2.2.5 Spore Preparation

The test organism for this work was a powdered spore preparation of *Bacillus subtilis* and silicon dioxide particles. The preparation was obtained from the U.S. Army Dugway Proving Ground (DPG) Life Science Division. The preparation procedure is reported in Brown et al. [3]. Briefly, after 80 – 90 percent sporulation, the suspension was centrifuged to generate a preparation of approximately 20 percent solids. A preparation resulting in a powdered matrix containing approximately 10^{11} viable spores per gram was prepared by dry blending and jet milling the dried spores with fumed silica particles (Degussa, Frankfurt am Main, Germany). The powdered preparation was loaded into metered dose inhalers (MDIs) by DPG according to a proprietary protocol. Control checks for each MDI were included in the batches of coupons contaminated with a single MDI.

2.2.6 Inoculated Rubber Coupons

Rubber coupons were inoculated and included in each Category 4 fumigation bag to evaluate the efficacy of each individual cycle following the procedures listed below. Circles (18-mm diameter) were punched from 1/16-inch thick sheets of silicone rubber (Part #5787T11, McMaster-Carr, Atlanta, GA) and fastened to 18 mm aluminum stubs (P/N 16119, Ted Pella, Inc., Redding, CA) using double-sided, adhesive-backed tape (P/N 16084-8, Ted Pella, Inc., Redding, CA). The coupons were sterilized prior to use via an autoclave. Test coupons were inoculated with *Bacillus subtilis* at approximately 2×10^8 colony forming units (CFU) by placing the surface of the coupons a precise distance from the MDI during actuation by placing the coupons on custom-built stages (shown in Figure 2-5) contained in autoclaved glassware. The container consisted of a Petri dish on the bottom and a crystallization dish on top.

A procedural blank went through the same transfer process as the positives, but the inoculum was not applied. Three positive coupons on a separate stage were also inoculated but were not fumigated. Three negative rubber coupons remained in the dish in which they were sterilized and were not inoculated. The six-sample stage was placed inside the computer chassis with the BIs to help determine efficacy under the local conditions within the PC chassis.

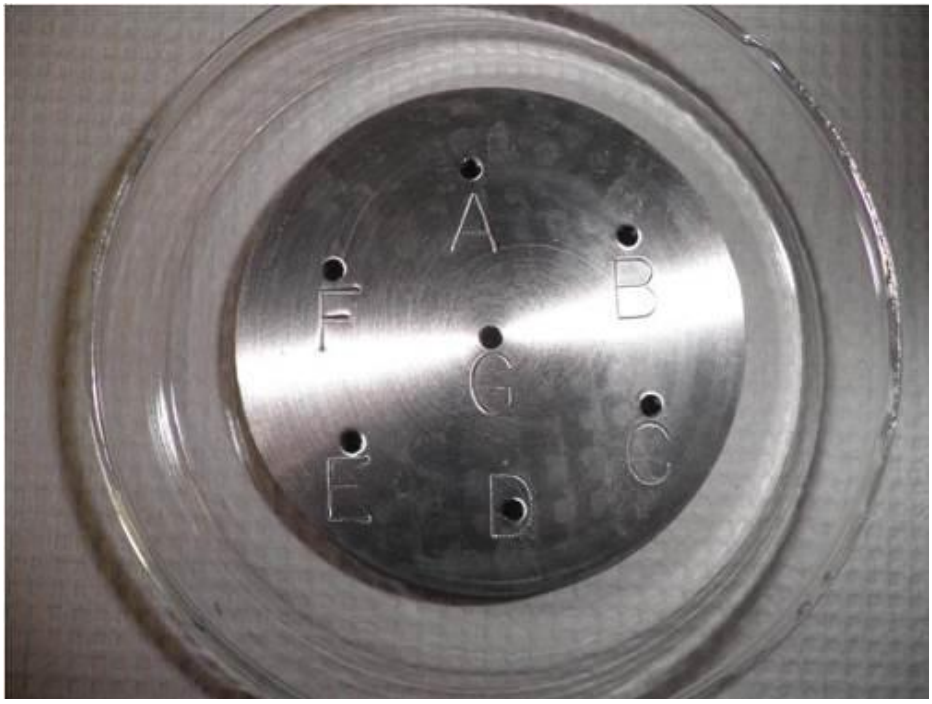

Figure 2-5. 18-mm Stub Stage in Container

Inoculated material coupons and BIs were placed inside the computer chassis to provide an indication of the effectiveness of the fumigation within each computer and are shown in Figures 2-6 and 2-7.

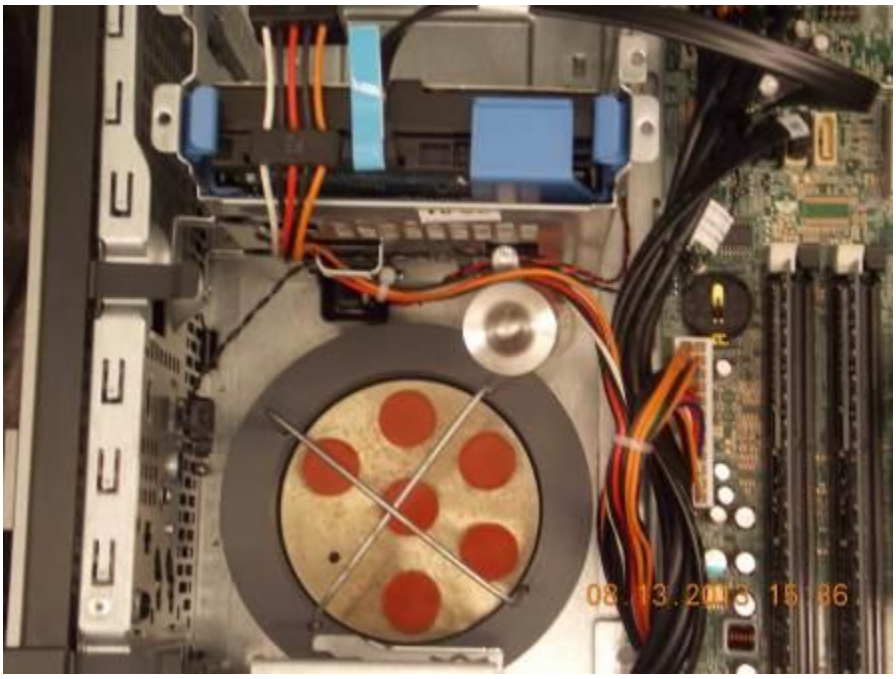

Figure 2-6. Location of MadgeTech device and rubber coupons within the computers (between the CD drives and hard drives)

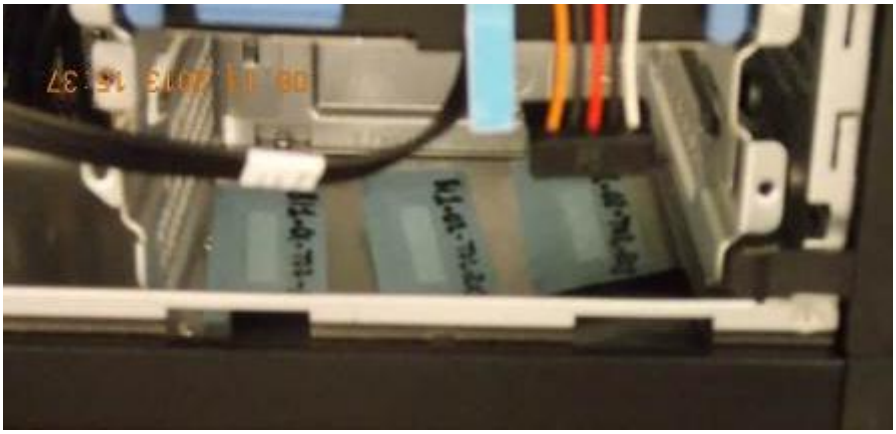

Figure 2-7. Location of BIs within the computers (inside the bottom panel)

2.3 Test Preparation

The testing chamber was conditioned prior to release of EtO inside the bag as described in Section 2.2. Individual bag loading was dependent on materials to be tested and the conditions to be tested, as specified by the test matrix. Table 2-5 lists the items included in each type of EtO fumigation cycle.

Table 2-5. Individual Bag Preparation

Cycle Type	Test Condition	EtO	Humidification	Biologicals	Monitoring Equipment	Materials/ Equipment	Chemical Indicator
Category 2	1	11 g cartridge	Two Humidichips®	BIs	Madgetech	Category 2 materials (as they fit)	Yes
Category 3	1	11 g cartridge	Two Humidichips®	BIs	Madgetech	Category 3 materials (as they fit)	Yes
Category 4	1	11 g cartridge	Two Humidichips®	BIs, rubber test coupons	Madgetech (one in each bag)	One PC in PC bag. Monitor, keyboard and mouse in separate bag.*	Yes
Category 4	2	11 g cartridge	Wetted sponge (w/18 g water)	BIs, rubber test coupons	Madgetech (one in each bag)	One PC in PC bag. Monitor, keyboard and mouse in separate bag.*	Yes
Category 4	3	18 g cartridge	Two Humidichips®	BIs, rubber test coupons	Madgetech (one in each bag)	One PC in PC bag. Monitor, keyboard and mouse in separate bag.*	Yes

Cycle Type	Test Condition	EtO	Humidification	Biologicals	Monitoring Equipment	Materials/ Equipment	Chemical Indicator
Category 4	4	No EtO	Two Humidichips®	BIs, rubber test coupons**	Madgetech (one in each bag)	One PC in PC bag. Monitor, keyboard and mouse in separate bag.*	Yes

* Both bags contain the EtO cartridge and humidification.

** Used for only one replicate.

Before fumigation of the Category 4 materials, it was necessary to open the computer chassis to insert the RH/temperature sensor (MadgeTech, Warner, NH), BIs, and inoculated rubber coupons into each desktop case. The inside of the desktop computers was digitally photographed. To maintain the integrity of the computer by avoiding static electricity, an electrostatic discharge (ESD) work station was set up to work on the computers in E288. All personnel were trained in operating these stations. In general, the ESD work station consists of an ESD work mat, an electrostatic monitor, and ESD wrist bands. All computers were inspected and operated (e.g., diagnostic testing, long-term operation of computers for analysis of residual effects) on the certified ESD work stations. During operation of the computers, all computers were energized using surge protectors.

2.4 Sampling Strategy

Local variations in temperature were expected, especially due to the thermal mass of the equipment or materials. This variation in temperature also affects RH. Because RH is a critical parameter in the effectiveness of the fumigant, the RH was checked by placing an RH/temperature sensor (MadgeTech, Warner, NH) in each fumigation bag. Each sensor was checked against a standard RH meter for calibration. For Category 2 and 3 testing, one sensor was placed in each bag. The sensors logged RH and temperature in real time, and the data were downloaded after the fumigation event was complete.

The testing strategy for the impact of the fumigation processes on Category 4 material and electronic equipment required monitoring the RH inside the bag (outside the computer chassis) and inside the computers. The sampling locations of the temperature and RH meters were identical to avoid any bias in the measurement. This positioning allowed direct comparisons between the bag and the localized RH after correcting for individual sensor bias.

2.4.1 Frequency of Sampling/Monitoring Events

Table 2-6 provides information on methods, test locations, and frequency for the measurement techniques used for this compatibility testing.

Table 2-6. Monitoring Methods for EtO fumigation

Monitoring Method	Test Location	Sampling Rate	Scope	Frequency and Duration
Visual Inspection	E288	NA	Effects of Fumigation	Monthly
RH/Temperature sensor	Inside bag Inside Cat. 4 computers	NA	0-100 % RH, -20-80 °C	Real-time six per minute
PC Doctor (PCMD) testing	E288	NA	Computer Functionality Hardware Compatibility	Monthly
BIT	E288	NA	Computer Functionality Hardware Compatibility	Weekly

2.4.2 Testing Approach

Two test matrices were used for the testing. Test Matrix T01 (Table 2-7) was used for Category 2 and 3 materials (combined) and Test Matrix T02 (Table 2-8) was used for Category 4 computers. The test matrices were built around the main objective of this project; to assess the damage, if any, to material and electronic equipment functionality after remediation of contaminated materials and equipment. Fumigated equipment was compared to control equipment not exposed to fumigation conditions.

Table 2-7. Test Matrix T01 (Category 2 and 3)

Test Condition	Treatment Conditions	Description
1	EtO (11 g), two Humidichips®, T=122 °F. All de-energized.	Standard Andersen Cycle
2	Controls, no exposure	Controls

Table 2-8. Test Matrix T02 (Category 4)

Test Condition	Treatment Conditions	Objective	Test Materials (Computer IDs)
1	EtO (11 g), two Humidichips®, T=122 °F. All de-energized.	Baseline - Standard Andersen Cycle	SS 04-08
2	EtO (11 g), wetted sponge (with 18 g water), T=122 °F. All de-energized.	Effect of higher RH on materials	SS 09-10
3	EtO (18 g), two Humidichips®, T=122 °F. All de-energized.	Effect of higher EtO Exposure	SS 14-16
4	No EtO, two Humidichips®, T=122 °F.	Effect of RH and temperature alone	SS 11-13
5	Controls, no exposure	Controls	SS 01-03

2.5 Measurements

Table 2-9 lists the critical and non-critical measurements associated with this project.

Table 2-9. Critical and Non-Critical Measurements

Sample Type	Sample Purpose	Critical Measurement	Non-Critical Measurements
Environmental conditions inside fumigation bags	Characterize T and RH	RH Temperature	
BIs	Indicative that sporicidal conditions were met during fumigation	Growth/No growth Incubation Temperature Incubation Time	
Inoculated coupons	Indicative that sporicidal conditions were met during fumigation	Incubation temperature CFU counts Extraction volume Plated volume	Incubation time
Category 2 Materials	Measures impact of fumigation	Visual Inspection Roughness Testing	
Category 3 Materials	Measures impact of fumigation	Visual Inspection Functionality Testing	
Category 4 Materials	Measures impact of fumigation	PC-Doctor Tests	BIT results

2.6 Sampling Procedures

Test samples, materials and equipment were stored under RH/temperature-controlled indoor ambient laboratory conditions until testing was performed. All samples, both test and control, were stored under the same conditions prior to and after the fumigation event. Sampling activities for this study include monitoring of environmental conditions (RH and temperature) and collecting microbiological samples for sporicidal efficacy. Tested materials and equipment were photographed before and after exposure and any visual changes were noted including color, legibility, and contrast.

2.6.1 Visual Inspection (Category 2-4)

Visual inspection focused on the possible effects of fumigation: a change in color or increase of corrosion. Color change may also affect legibility. Digital photographs of each test material were taken prior to fumigation. Digital photographs were taken after fumigation, with the fumigated sample next to a control sample when practical. Some electronic equipment was partially dismantled on an approved ESD work station to take digital photographs inside the casing. . The color of sections of digital photographs were compared to and described by a computer-based hexadecimal color chart. Any problems with legibility or contrast of materials before/after fumigation were recorded.

Photographs were taken of each material and device pre-exposure, immediately post-exposure, and then monthly thereafter for a period of ten months for Category 2 and 3 materials. Category 4 materials were only photographed periodically after Month 3, but no visual changes were noted. The purpose of this

physical documentation was so that comparisons could be made over time, looking for changes such as discoloration, corrosion, residue, and decrease in any device functionality.

2.6.2 Functionality Testing (Category 2-4)

All electronic equipment in Category 3 and 4 underwent functionality testing prior to and post-fumigation. Smoke Detectors and Smoke/CO detectors from Category 2 also underwent functionality testing.

Functionality testing was conducted by running a predefined routine specific to each of the Category 3 and 4 items. These routines were documented for each item and maintained in the item's log book. For the computer systems, PCMD Service Center 7.5 (PC Doctor, Reno, NV) was run to complete a hardware and software diagnostic investigation. The results were stored digitally and converted to PC scores throughout the testing period.

2.6.2.1 Smoke Detectors

Two functions of the smoke detectors were tested, the TEST/SILENT button and the smoke detection system. The TEST/SILENT Button was pressed and held. The expected result was a loud repeating three-beep horn pattern, the smoke light on, and light-emitting diode (LED) flashing once every second. To test the smoke detection system, smoke detector tester aerosol (SmokeCheck™, Home Safeguard Industries Fire and Safety Group LLC., Elk Grove Village, IL) was sprayed at a distance of 2 to 4 feet from the vent on top of the detector for 1-2 seconds. The expected result was a loud, repeating three-beep horn pattern, the smoke light on, and LED flashing once every second.

2.6.2.2 CO Detectors

The CO detector function on Smoke/CO alarms was tested using a CO detector test gas (CoCheck™, Home Safeguard Industries Fire and Safety Group LLC., Elk Grove Village, IL). The test gas was sprayed at the vent on top of the detector for 1-2 seconds from a distance of 2 to 4 feet. The expected result was a loud, repeating four-beep horn pattern, the CO light on, and LED flashing once every second.

2.6.2.3 Personal Digital Assistant (PDA) – Palm Pixi Plus

Two functions of the PDA were tested; transfer of files from the PDA to a personal computer, and transfer of files from the personal computer to the PDA. The transfer was considered successful if the file could be opened without noticeable corruption.

2.6.2.4 Fax/Phone /Copier Machine

The fax machine had to meet all of the following criteria to receive a PASS; otherwise, the unit failed:

- A fax was successfully transmitted. Successful transmission would be evident if a response was faxed back.
- A fax was successfully received.
- Outgoing and incoming telephone calls were successfully connected.

2.6.2.5 Cell Phone

Prepaid cell phones were refilled as necessary. An outgoing call was placed from the cell phone. The audibility of the ring tone was noted. The call (to a local phone) was answered and sound transmittal was

recorded. An incoming call was received on the cell phone. The audibility of the ring tone was noted. The call (from a local phone) was answered and sound transmittal was recorded. All buttons on the keypad were also tested.

2.6.2.6 Data CD

The first ten seconds of each track were evaluated for audible glitches or skips.

2.6.2.7 Data DVD

The first ten seconds of each track were evaluated for audible or visual glitches or skips.

2.6.2.8 USB Flash Drive

Two functions of the USB flash drives were tested: transfer of files from the USB flash drives to a personal computer and transfer of files from the personal computer to the USB flash drives. The transfer was considered successful if the file could be opened without noticeable corruption.

2.6.2.9 SD Memory Card

Two functions of the SD Memory Card were tested; transfer of files from the SD Memory Card to a personal computer and transfer of files from the personal computer to the SD Memory Card. The transfer was considered successful if the file could be opened without noticeable corruption.

2.6.2.10 PCMD Test Configuration

Functionality of Category 4 computers was tested using PCMD Service Center (Version 7.5, PC-Doctor, Rebo, NV, USA). The software identified and ran 96 distinct tests, including tests on the following subsystems:

- System Board
- Random Access Memory
- Central Processing Unit
- Complementary metal–oxide–semiconductor
- Graphics Card
- Hard Drive
- CD/DVD Drive
- Audio Visual Interleave
- Monitor
- Keyboard
- Mouse
- Network Connections and Protocols
- Peripheral Component Interconnect Buses
- Standby/Hibernate Functions
- Serial Communications Ports
- Universal Serial Bus (USB)Ports
- Audio Board.

PC Doctor testing was performed monthly to evaluate Category 4 equipment failures. All computers had individual cables and equipment to prevent corrosion material being transferred from one computer to another through reuse of cables or common equipment.

The PC-Doctor® Service Center™ 7.5 protocol was developed to have an industry-accepted standard method of determining pass versus failure of the computer subsystems. PC-Doctor® Service Center™ 7.5 functionality testing was conducted on each computer pre-fumigation, one day post-fumigation, then monthly.

For every monthly test, a standard protocol called for each test to be performed once. If any particular test failed the first time, the computer was tested a second time to correct for possible human error. A test that failed the second time was labeled "Fail". If the test failed the first time but passed the second time, it was labeled "Pass2". For tabulation, a score of 100 was assigned to each "Fail", while a "Pass2" received a score of 1. During each pre- and post-fumigation testing period, a total PCMD score was assigned to each computer based upon the number of tests that failed on the first or second attempt. A "Pass" results in a score of 0. Scores are only evaluated relative to controls and to the results of previous fumigation studies.

Statistical analysis was used to evaluate whether or not fumigated computers differed from control computers.

2.6.3 Microbiology Methods

2.6.3.1 Coupon Spore Enumeration

The day after EtO exposure, 18 mm rubber coupons (test, procedural blank, and positive control) were transferred aseptically into empty 50 mL sterile vials. The sample vials were then transported to the NHSRC Biolab, where 10 mL of sterile Phosphate Buffered Saline plus Tween® 20 (PBST) was aseptically added. The sample vials were then sonicated for 10 minutes using an 8510 Branson (Danbury, CT) ultrasonic cleaner at 44 kHz and 250 Watts. The sonication step was immediately followed by two continuous minutes of vortexing to further dislodge any viable spores. Each vial was briefly re-vortexed immediately before any solution was withdrawn for analysis. The solution was subjected up to a five-stage serial dilution. Each dilution (0.1 mL) was inoculated onto trypic soy agar (TSA) plates, spread with sterile beads, and incubated at 35 ± 2 °C for 18-24 hours. Plates with 30-300 CFU were counted manually. Any samples below countable criteria (30 CFU) on the primary dilution plates were filtered. The filters were incubated at 35 ± 2 °C for 18-24 hours prior to manual enumeration.

Extraction and initial plating of biological samples was performed within five days. Samples were stored under refrigeration until analyzed.

2.6.3.2 BI Analysis

BIs were processed as advised by manufacturer's instructions. Each BI was given a unique sampler identifier prior to the start of any experimentation. These BIS were analyzed qualitatively by aseptically transferring each individual sample into a sterile pre-labeled culture tube containing approximately 10 mL of growth media (tryptic soy broth). The culture tubes containing the growth media and the BI were agitated using a vortex mixer and then placed into an incubator at an appropriate temperature for the surrogate microbiological organism as found on the BI (as advised by manufacturer's instructions).

Following a seven- to ten-day incubation period, each of the culture tubes containing the growth media and biological indicators was analyzed for turbidity and tested for growth using a direct plating technique. Each of the samples were homogenized using a vortex mixer, and using a sterile pipette, 100 µL of liquid was removed from the culture tubes and plated onto tryptic soy agar and incubated at the temperature-time combination best suited for growth (as advised by manufacturer's instructions). The agar plates were then analyzed visually and notes were made concerning any observed growth.

3 Results

3.1 Category 2 Materials

3.1.1 Fumigation conditions

Not all Category 2 or 3 materials could fit into any one exposure bag, so three separate exposures were required. Conditions and contents of each bag are listed in Table 3-1. Fumigations were not sequential.

Table 3-1. Contents and Conditions of Category 2 and 3 EtO exposures

Fumigation	Treatment Conditions	Objective	Average Fumigation Conditions	Category 2 Materials	Category 3 Materials
1	EtO (11 g), two Humidichips®, T=122 °F. All de-energized.	Baseline: Standard Andersen Cycle	RH: 51.8 % T: 46.8 °C	Aluminum, type 304 and 316 stainless steel, smoke detector, laser-printed paper, ink jet-printed paper	Cellphones, CDs, DVDs, USB flashdrive, SD memory card
2	EtO (11g), two Humidichips®, T=122 °F. All de-energized.	Baseline: Standard Andersen Cycle	RH: 55.7 % T: 47.7 °C		Fax machine
8	EtO (11 g), two Humidichips®, T=122 °F. All de-energized.	Baseline: Standard Andersen Cycle	RH: 59.6 % T: 42.3 °C	Photographs, smoke/CO detector, laser-printed paper, ink jet-printed paper	PDA

Photographs were taken of each material pre-exposure, immediately post-exposure, and then monthly thereafter for a period of ten months.

The purpose of this physical documentation was so that comparisons could be made over time, looking for changes such as discoloration, loss of functionality, corrosion, residue, and decrease in the quality or readability of documents and photographs.

3.1.2 Visual Inspection

There was no recorded visual impact of the EtO fumigation on any Category 2 materials. No discoloration was found, even in high quality photographs and ink jet- and laser-printed documents. Certain individual colors in photographs were compared to and described by a computer-based hexadecimal color chart. No differences were noted over the course of testing. Figures 3-1 and 3-2 show the minimal visual impact on Category 2 materials (laser-printed paper and copper coupons) ten months post-fumigation.

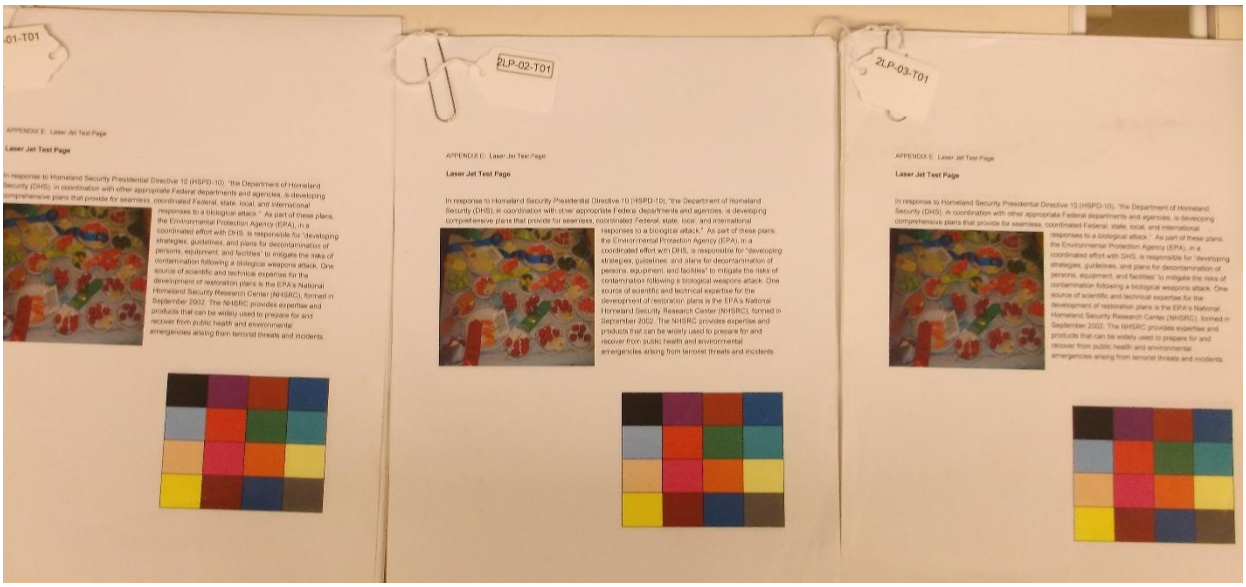

Figure 3-1. Laser Printer Paper exposed to EtO (left and center) and not exposed (right)

Figure 3-2. Copper exposed to EtO (2Cu-01 and 2Cu-02 on left) and not exposed (2Cu-03 on right)

The three peppers circled in Figure 3-3 were used to measure photograph fading by comparison to a hexadecimal color chart before and after testing. Some of the results are shown in Table 3-2. No change was apparent during testing.

Figure 3-3. Test Photograph

Table 3-2. Hexadecimal Color Comparison

Test Date	Red Pepper Code	Orange Pepper Code	Green Pepper Code
Pre-fumigation (9/4/13)	#E3170D	#FFB00F	#003300
Post-fumigation (11/29/13)	#E3170D	#FFB00F	#003300
End-testing (7/4/14)	#E3170D	#FFB00F	#003300

3.1.3 Functionality Testing

Functionality testing for Category 2 materials consisted of operation of the smoke and CO Detectors and material roughness testing, to determine if the surfaces of the metal coupons were affected by fumigation. Metal coupons were tested for surface roughness monthly throughout the testing campaign. Results were recorded for data analysis.

3.1.3.1 Smoke and Smoke/CO Detector Results

Table 3-3 shows the test results for the last month of testing. This table lists the tests required to pass and indicates that the detectors both passed. Both detectors passed similarly for every month they were tested.

Table 3-3. Smoke Detector Functionality Testing

Category 2	Material ID	PASS	FAIL
Smoke Detector 1. Test/silent button and escape light are functional 2. Smoke alarm with escape light is functional	2SD-01-T01	X	
	2SD-02-T01	X	
	2SD-03-T01	X	
Smoke Detector / CO Alarm 1. Test/silent button with smoke LED and CO LED 2. Smoke alarm with smoke led, 3. CO alarm with CO LED	2SC-01-T01	X	
	2SC-02-T01	X	
	2SC-03-T01	X	

3.1.3.2 Surface Roughness Testing Results

Measurements were taken incorrectly for pre-test sampling, post-test sampling and Month 1 sampling. The error was discovered before Month 2 sampling and the procedure was corrected. Therefore, the test coupon measurements are compared only to the controls that were not fumigated. As seen in Figure 3-4, there were a couple of clear outliers during testing. These measurements are clearly outliers because the readings returning to normal the following month and are most likely due to small scratches on the surface of the coupon. The coupon was not sampled in the exact same spot every time, and the instrument covers only a fraction of the surface. As is apparent in the graphs below, there was no trend of degradation in post-fumigation samples. Also, as seen in Table 3-4, there was no significant difference in roughness between test (n=3) and control samples (a P-value of <0.05 would indicate a significant difference). Figures 3-5 to 3-8 show the average surface roughness for each material compared to the controls.

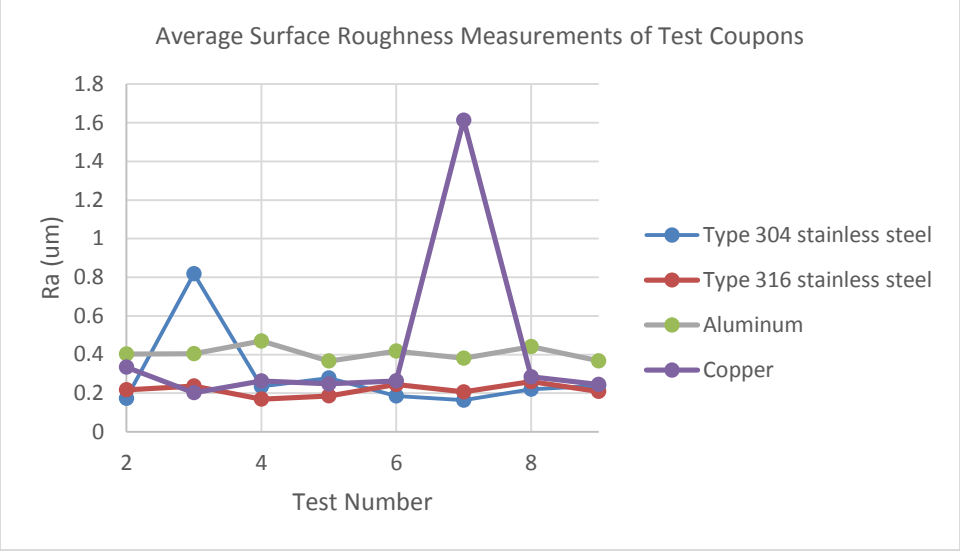

Figure 3-4. Average Surface Roughness Measurements of Test Coupons

Table 3-4. Average Roughness for Test Sets

Test Set	Average Test Coupon Roughness(µm)	Average Control Coupon Roughness (µm)	P-Value (control set compared to test set)
Type 304 stainless steel	0.29	0.22	0.06
Type 316 stainless steel	0.22	0.21	0.30
Aluminum	0.41	0.44	0.17
Copper	0.43	0.23	0.12

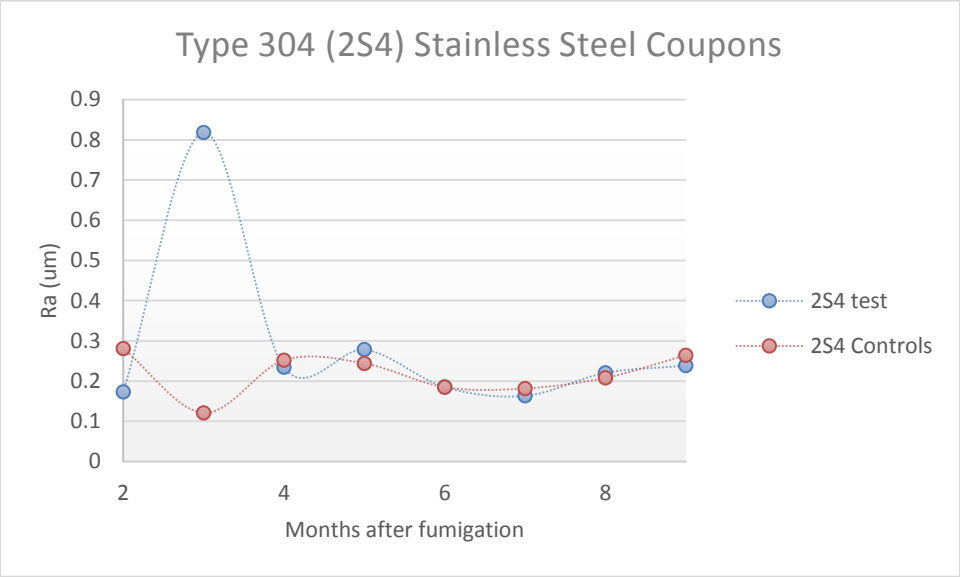

Figure 3-5. Type 304 Stainless Steel Roughness Measurements

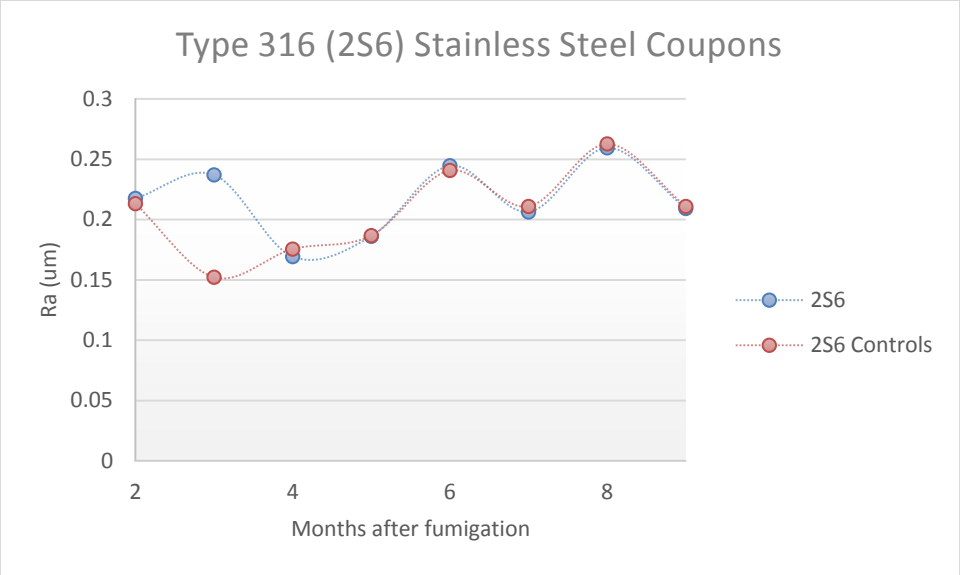

Figure 3-6. Type 316 Stainless Steel Roughness Measurements

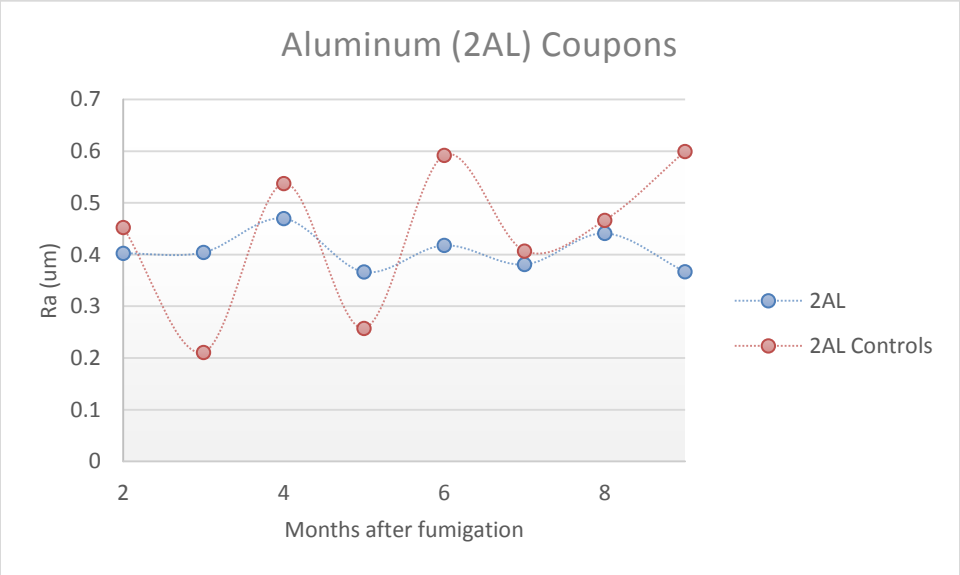

Figure 3-7. Aluminum Roughness Measurements

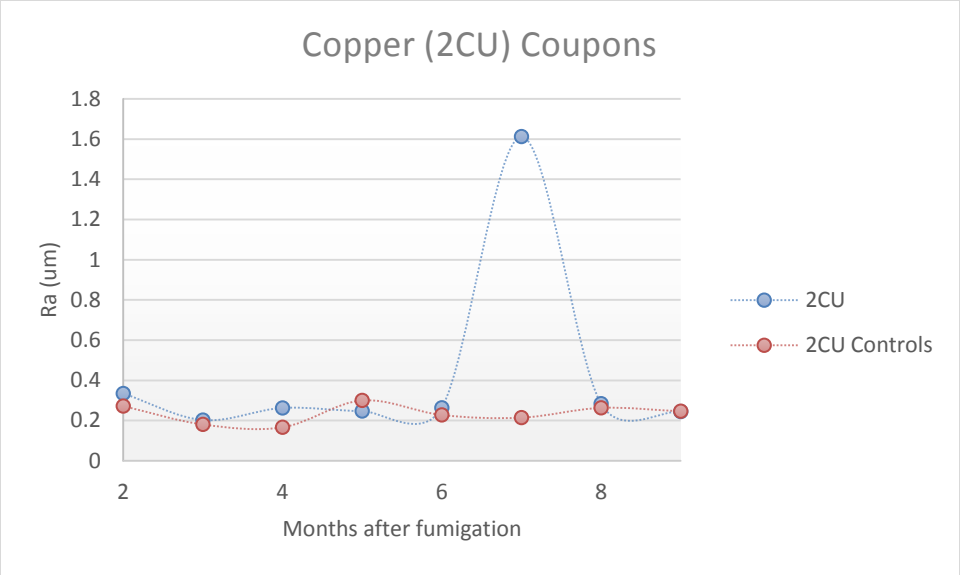

Figure 3-8. Copper Roughness Measurements

3.2 Category 3 Materials

Fumigation conditions are listed in Section 3.1.1.

3.2.1 Visual Inspection

There was no recorded visual impact of fumigation on any Category 3 materials (cell phone, DVD and CD). No discoloration was found in electronic displays. Figures 3-9 through 3-10 below show the minimal visual impact on digital photographs and CDs, respectively.

Figure 3-9. Photo of digital photographs taken over a year after exposure. There was no discernable difference between the control (2PH-03 on right) and test samples (2PH-01 and -02).

Figure 3-10. Exposed (left) and unexposed (right) CD 10 months later

3.2.2 Functionality Testing

Category 3 materials showed no functionality failures over the course of testing. The test results shown in Table 3-5 are from the last month of testing.

Table 3-5. Category 3 Functionality Testing Results

Category 3 Material	Material ID	PASS	FAIL
Cell Phone 1. A call made from the phone was connected. 2. A call made to the phone was connected and the ringtone was audible. 3. All buttons on the key pad are functional.	3PE-01-T01	X	
	3PE-02-T01	X	
	3PE-03-T01	X	
PDA 1. A data file can be transferred to the PDA and opened. 2. A data file can be transferred from the PDA and opened.	3PD-01-T01	X	
	3PD-02-T01	X	
	3PD-03-T01	X	
Fax/Phone/Copier 1. A fax was transmitted. 2. A fax was received.	3FA-01-T01	X	
	3FA-02-T01	X	
	3FA-03-T01	X	

Category 3 Material	Material ID	PASS	FAIL
3. Outgoing and incoming calls were connected.			
Data CD 1. The disk was readable. 2. The tracks were audible with no defects.	3CD-01-T01	X	
	3CD-02-T01	?*	
	3CD-03-T01	X	
Data DVD 1. The disk was readable. 2. The tracks were visible and audible with no defects.	3DV-01-T01	X	
	3DV-02-T01	X	
	3DV-03-T01	?*	
USB Flash Drive 1. A data file can be transferred to the USB and opened. 2. A data file can be transferred from the USB and opened.	4FD-01-T01	X	
	4FD-02-T01	X	
	4FD-03-T01	X	
Memory Card 1. A data file can be transferred to the memory card. 2. A data file can be transferred from the memory card and opened.	4SD-01-T01	X	
	4SD-02-T01	X	
	4SD-03-T01	X	

*3CD—02-T01 and 3DV-03-T01 went missing during testing

3.3 Category 4 Materials

3.3.1 Fumigation Conditions

Table 3-6 shows the average RH and temperature for each of the four controlled test conditions.

Table 3-6. Final Test Matrix

Test Condition	Treatment Conditions	Objective	Average Fumigation Conditions	Test Materials (Computer IDs)
1	EtO (11 g), two Humidichips®, T=122 °F. All de-energized.	Baseline - Standard Andersen Cycle	RH: 57.6 % T: 46.5 °C	SS 04-08
2	EtO (11 g), wetted sponge (with 18 g water), T=122 °F. All de-energized.	Effect of higher RH on materials*	RH: 57.9 % T: 45.2 °C	SS 09-10*
3	EtO (18 g), two Humidichips®, T=122 °F. All de-energized.	Effect of higher Ethylene Oxide Exposure	RH: 53.8 % T: 46.1 °C	SS 14-16
4	No EtO, two Humidichips®, T=122 °F.	Effect of RH and temperature alone	RH: 55.7 % T: 46.1 °C	SS 11-13
5	Controls, no exposure	Controls		SS 01-03

* This test was designed to show effects of higher RH, but the condition was not achieved

3.3.2 Testing Difficulties

The test matrix originally proposed had to be modified during the course of testing due to difficulties encountered during testing. Originally, the objective for the wetted sponge tests was to determine the effect of higher RH during fumigation on the computers. This test was based on scoping testing performed outside this effort. Unfortunately, this high RH condition could not be duplicated using EOGas permeable bags. Actual test conditions are listed in Table 3-6.

3.3.3 Visual Inspection

There was no recorded visual impact of fumigation on any Category 4 materials. There was no evidence of corrosion on any metal surfaces or edges. The lack of corrosion is especially important. Other fumigation technologies {USEPA, 2010 #3074} have shown significant corrosion on many components, especially unfinished metal edges. No discoloration was found in electronic displays. Figures 3-11 through 3-14 show the minimal visual impact on Category 4 materials. No visual impacts were seen after three months, and although visual inspection continued, digital photographs were no longer documented for the remainder of the testing period.

Figure 3-11. Inside SS04 (pre-fumigation)

Figure 3-12. Inside SS04 (two months post-fumigation)

Figure 3-13. SS04 back panel (pre-fumigation)

Figure 3-14. SS04 Back Panel (two months post-fumigation)

3.3.4 Functionality Testing

PCMD testing performed well, with one exception. Serial port tests (COM1) were sometimes excluded from testing for an unknown reason. When this occurred, the software would not recognize the COM1 hardware even though the Windows operating system reported it was operating normally. Multiple attempts were made to resolve the issue, but a resolution was not reached. While the problem persisted, any PCs that failed to run the test would be subject to an additional test. For these PCs, the Mouse Interactive Test was run with a serial mouse connected to the COM1 port to prove it was functional.

3.3.4.1 BIT

There were no BIT failures with the exception of those relating to failing DVD media (DVD media that had defects from the factory). In the case of BIT DVD errors, replacing the media always fixed the error.

3.3.4.2 PC-Doctor® Functionality Testing Results

Results from PC Doctor® testing are shown in Figures 3-15 through 3-19, below. To tabulate PCMD scores, a score of 100 was given to any failing test of the 96 conducted (subsystems are listed in Section 2.6.2.10). For any test that initially failed due to user error or bad media but that passed after correcting the issue (a “Pass 2” result), a score of 1 was given. The number of failures and “Pass 2” results can therefore be determined. For example, a score of 201 indicates two tests failed and one test was scored a “Pass 2”. Figure 3-19 shows the averages of each test set over the course of the period tested. Figures 3-15 to 3-18 show each subset of fumigated personal computer (PC) PCMD results as compared to the controls. Test results of “0” (all tests passed) will not show up on these figures. Averaged scores simply represent the average number of failures. Pass 2 results are barely accounted for mathematically since

they are commonly a result of human error and not indicative of a larger issue. An average score of 335 indicates an average of 3.35 total failures.

Since only a single fumigation could be performed for one PC at a time (overnight), and some test conditions were determined during the testing series, not all PCs were tested for the same amount of time. All PCs were tested for a minimum of nine months.

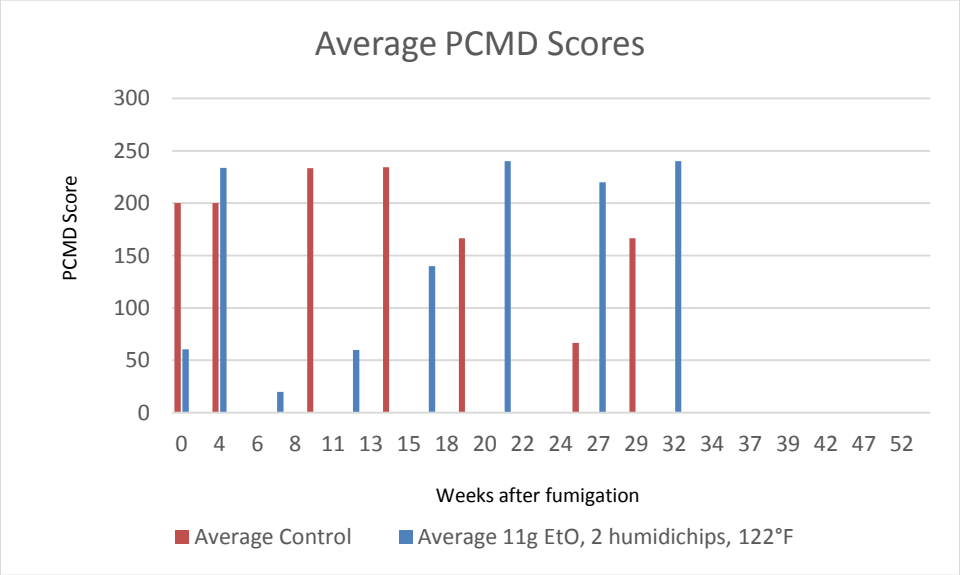

Figure 3-15. Test Condition 1 PCMD Scores Compared to Controls

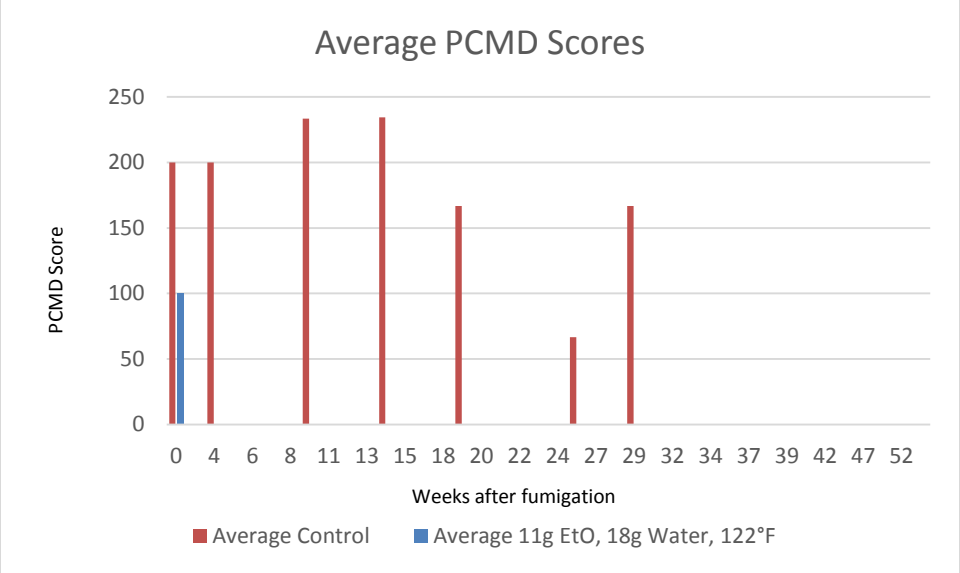

Figure 3-16. Test Condition 2 PCMD Scores Compared to Controls

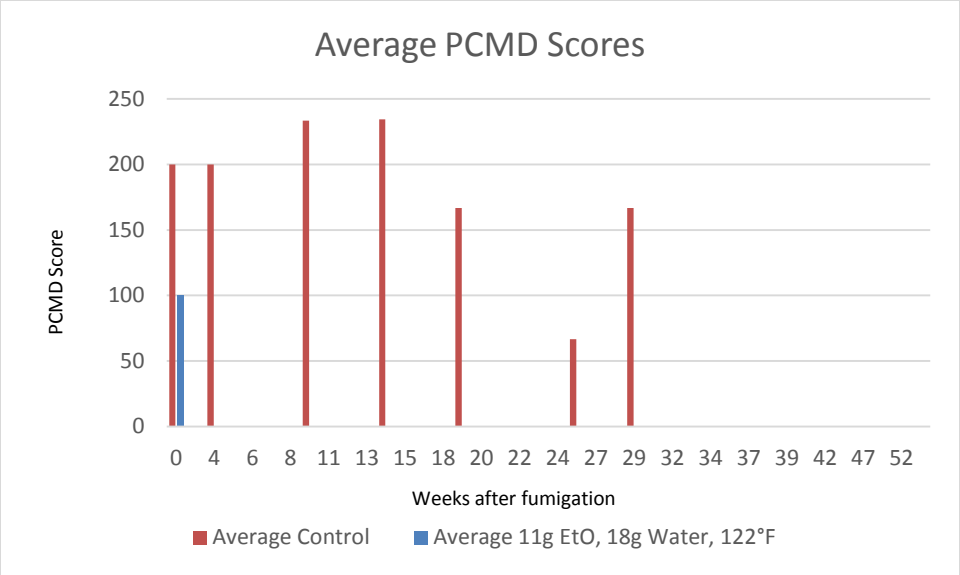

Figure 3-17. Test Condition 3 PCMD Scores Compared to Controls

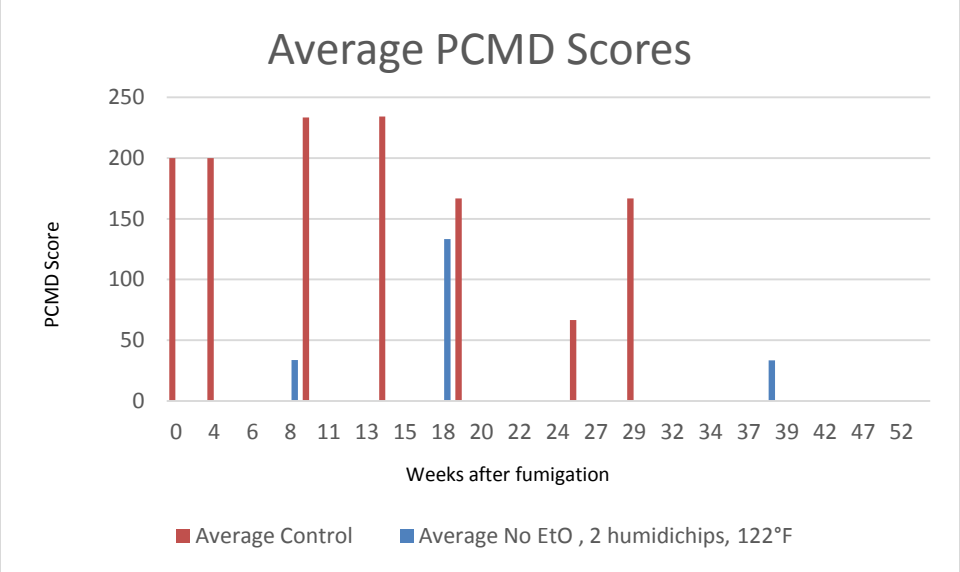

Figure 3-18. Test Condition 4 PCMD Scores Compared to Controls

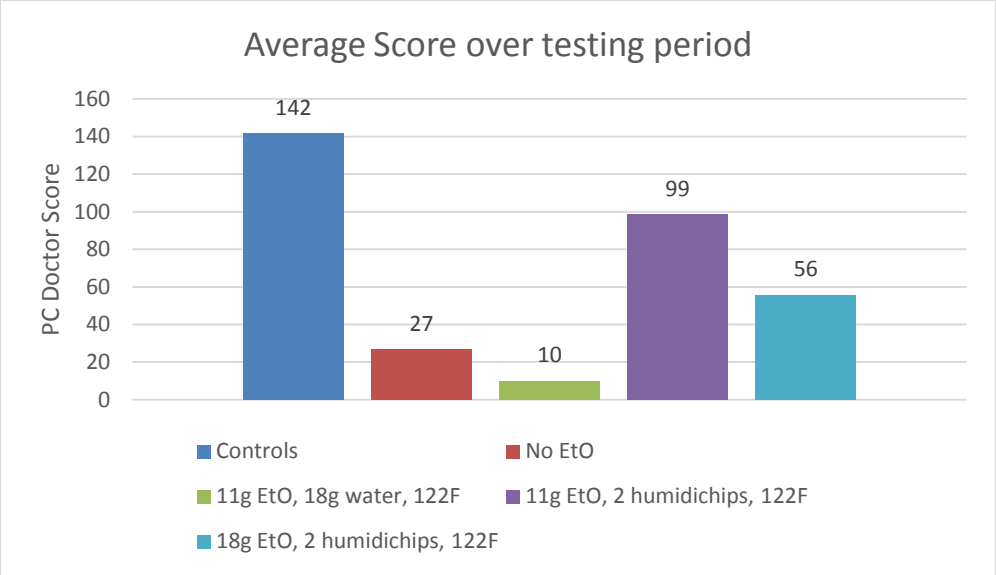

Figure 3-19. Average PC Doctor Scores over Testing Period

There were very few failures for Category 4 machines. The test failure rate across all machines was 0.4 %. Approximately 85 % of the failures were related to the CD/DVD drive or a result of an incorrect procedure with the audio loopback test. In some cases, new CD/DVD media would correct the error. Most of these failures were intermittent. A PC would fail one month's CD/DVD test and pass the next month. Some PCs experienced multiple persistent CD/DVD failures. Of the three PCs that experienced persistent failures, one was a control. This result indicates that the failure may not be a product of fumigation at all. The PCs used were susceptible to these failures even under normal usage conditions. Tests 83 and 84 required a very specific order of user interaction for the system to recognize the audio loopback cable. Tests where this failed were most likely due to this procedure not being carried out correctly. There is an identical test earlier in the procedure which never failed but uses different physical audio ports that are automatically recognized and activated by the software. Tests 83 and 84 failed approximately 20 % of the time across all PCs. The DVD tests (57, 58, and 59) failed approximately 10 % of the time across all PCs.

Since the control set had a higher average score, the failures witnessed were probably not a result of the fumigation. Tests conducted without EtO should show only the effects of increased temperature and RH. The results of tests without EtO are also consistent with fumigated test and control results. The p-values in Table 3-7 suggest that there are significant differences between only a few data sets. The PC subsets of Test Condition 2 (11 g EtO, 18 g water, 122 °F) Test Condition 4 (No EtO) resulted in the least failures over the testing period. These p-values are significantly different from Test Condition 5 (Controls). While the values are significantly different from the controls, it is likely not an effect of fumigation, since they actually performed better than the controls. The only other significant difference was between Test Condition 2 (11 g EtO, 18 g water, 122 °F) and Test Condition 1 (11 g EtO, two Humidichips®, 122 °F). As was shown in the Table 3-6 and described previously, there was no actual measured difference in fumigation conditions between these two subsets. The result is also unlikely to be any indication of an effect of the fumigation. The computers procured for this testing experienced intermittent failures, making it difficult to attribute these results to the fumigation conditions.

Table 3-7. p-Values between PCMD Scores of all PC Subsets*

Subset Being Compared	Compared to Subset	p-Value
Test Condition 5	Test Condition 1	0.447
Test Condition 5	Test Condition 2	0.018
Test Condition 5	Test Condition 4	0.015
Test Condition 5	Test Condition 3	0.244
Test Condition 1	Test Condition 2	0.048
Test Condition 1	Test Condition 4	0.057
Test Condition 1	Test Condition 3	0.549
Test Condition 4	Test Condition 2	0.291
Test Condition 4	Test Condition 3	0.092
Test Condition 3	Test Condition 4	0.155

* Values <0.05 (shown in red) indicate a significant difference.

3.4 Fumigation Effectiveness

For all test conditions, the EtO fumigations achieved greater than six log reduction, or non-detect. Test R14 was not filter-plated by the time of this report, and thus had a much higher detection limit. Though the test samples were non-detect for this test, this delay resulted in a lower (5.17) log reduction. These results are shown in Table 3-8, below. Non-detect results are shaded in yellow. Test R12 was the control condition without EtO.

Table 3-8. CFU Counts and Log Reduction for All Biological Tests

Test Number	Positive CFU	Test CFU	Log Reduction
R01	3.64×10^6	<1	6.76
R02	8.83×10^6	<1	7.01
R03	3.27×10^6	<2	6.30
R04	7.67×10^6	<1	7.09
R05	6.55×10^6	<1	7.01
R06	1.32×10^7	<1	7.33
R07	6.13×10^6*	<2	6.39
R08	7.19×10^6	<1	6.86
R09	9.60×10^6	<1	7.18
R10	1.18×10^7	<1	7.26
R12 (control)	1.71×10^7	9.35×10^6	0.26
R14	7.36×10^6	50	5.17
R15	2.11×10^7	<1	7.51
R16	2.28×10^7	<1	7.56

*Only four replicates for this test

All fumigated BIs exhibited no growth.

4 Quality Assurance

This project was performed under an approved Category III Quality Assurance Project Plan titled *The Impact of Decontamination Technologies (Ethylene Oxide) on Materials and Equipment (July 2013)*.

4.1 Sampling, Monitoring, and Analysis Equipment Calibration

Accuracy and precision of the RH measurements were determined during pre- and post-test calibrations. Temperature calibrations were performed on MadgeTech devices before use on this project. The accuracy and precision of volumetric measurements were assessed through calibration checks performed by the NHSRC Biolab. These calibration checks were performed before and after use of volume dispensing devices on the project, or once every four months.

There were operating procedures for the maintenance and calibration of all laboratory and NHSRC Biolab equipment. All equipment was verified as being certified calibrated or having the calibration validated by the EPA RTP on-site Metrology Laboratory at the time of use. Calibration of instruments was done at the frequency shown in Table 4-1. Any deficiencies were noted. The instrument was adjusted to meet calibration tolerances and recalibrated within 24 hours. If tolerances were not met after recalibration, additional corrective action was taken, possibly including recalibration or/and replacement of the equipment.

Table 4-1. Sampling and Monitoring Equipment Calibration Frequency

Equipment	Critical Measurement	Calibration/Certification	Expected Tolerance
MadgeTech Sensors	Relative humidity inside the bag	RH calibrated monthly to a salt cell calibrated Vaisala sensor.	$\pm$ 3 % RH
SRG-4000 Roughness Tester	Roughness measurements	Calibrated monthly before roughness testing to a standard surface.	$\pm$ 12 %
MadgeTech Sensors	Temperature inside the bag	Devices were factory calibrated. Calibration only required annually.	$\pm$ 0.5 °C

4.2 Data Quality

The Quality Assurance Project Plan (QAPP) for this project was followed with deviations noted as follows:

Test Condition 1 (18 g EtO, two Humidichips®, T=122 °F) was performed in quintuplet instead of triplicate. Also, all computers in Conditions 1 (SS 04-08) and 2 (SS 09-10) were fumigated with 11 g EtO instead of the 18 g EtO cartridge. While the original test matrix used an 18 g EtO cartridge for all tests, because of this error, it was decided to make the EtO exposure amount a variable as well. Category 4 materials were photographed only periodically and at the end of testing after Month 3 because no visual changes had been noted. Visual monitoring continued for the full ten months.

4.3 QA/QC Checks

Quantitative standards do not exist for biological agents. Quantitative determinations of organisms in this investigation did not involve the use of analytical measurement devices. Rather, CFU were enumerated manually and recorded. Critical QC checks are shown in Table 4-2. The acceptance criteria were set at the most stringent level that could be achieved routinely and are consistent with the data quality

objectives described in Section 4.4. Positive controls and procedural blanks were included along with the test samples in the experiments so that well-controlled quantitative values were obtained. Background checks were also included as part of the standard protocol. Replicate coupons were included for each set of test conditions. Qualified, trained, and experienced personnel ensured data collection consistency. When necessary, training sessions were conducted by knowledgeable parties, and in-house practice runs were used to gain expertise and proficiency prior to initiating the research.

The following Sample Acceptance Criteria were followed for this effort. Any deficiencies were noted and reported to the WACOR.

Table 4-2. Quality Assurance (QA)/Quality Control (QC) Sample Acceptance Criteria

QC Sample	Information Provided	Frequency	Acceptance Criteria	Corrective Action
Procedural Blank (coupon without biological agent)	Controls for sterility of materials and methods used in the sampling procedure.	one per test	No observed CFU	Identify and remove source of contamination. Consult WACOR*.
Inoculum Control (100 uL spike of inoculum into 10 mL PBST)	Initial contamination level on the coupons shows plate's ability to support growth.	three replicates per inoculation day	For high inoculation, target loading of 10^7 CFU per sample with a standard deviation of < 0.5 log. ($5x10^6$ – $5x10^7$ CFU/sample); Grubbs outlier test (or equivalent).	Outside target range: discuss potential impact on results with the WACOR; correct loading procedure for next test and repeat depending on decided impact. Outlier: evaluate stability of pipette.
Blank plating of microbiological supplies	Controls for sterility of supplies used in dilution plating	three of each supply per plating event	No observed growth following incubation	Sterilize or dispose of source of contamination. Re-plate samples.
Blank TSA Sterility Control (plate incubated, but not inoculated)	Controls for sterility of plates.	Each plate	No observed growth following incubation.	All plates are incubated prior to use, all contaminated plates will be discarded.
Field Blank Samples (Sample matrices handled in sampling area without contact with surfaces)	The level of contamination present during sampling	three per sampling event	No observed growth following incubation	Clean up environment. Sterilize sampling materials before use.
Biological samples	Number of CFU	Each replicate	Significant (reported) growth is between 30 and 300 colonies per plate. Replicate plates must agree within 100 %. Samples with fewer than 30 CFU on the undiluted plate may be filter-plated to reduce detection limit.	Replate.

*WACOR = Work Assignment Contracting Officer Representative

4.4 Acceptance Criteria for Critical Measurements

The Data Quality Objectives (DQOs) define the critical measurements needed to address the stated objectives and specify tolerable levels of potential errors associated with simulating the prescribed decontamination environments. The following measurements were deemed to be critical to accomplish part or all of the project objectives:

- Temperature
- RH
- Material inspection and electronic equipment functionality over time
- Growth/No Growth of the BIs CFU count.

The Data Quality Indicators (DQIs) listed in Table 4-3 are specific criteria used to quantify how well the collected data met the DQOs. Failure to provide a measurement method or device that meets these goals results in the rejection of results derived from the critical measurement. For instance, if the plated volume of a sample is not known, then that sample is invalid. In contrast, for the real-time RH measurements, some missing data would not invalidate a test. Visual inspection and operational testing of all categories of materials were performed to determine if the materials maintained their pre-exposure physical and functional characteristics throughout a ten-month observation period following an EtO decontamination event. PC-Doctor® Service Center™ 6, a commercially available software, was used to test the functionality of each computer pre-exposure, immediately post-exposure, and then up to monthly thereafter for a period of ten months looking to diagnose and detect computer component failures. If any particular test failed the first time, the computer was tested a second time to correct for possible human error. A test that failed the second time was labeled “Fail”. If the test failed the first time, but passed the second time, the test was labeled “Pass 2”.

Table 4-3. Data Quality Indicators

Critical Measurement	Measurement Device	Desired Accuracy	Achieved Accuracy	Desired Precision	Achieved Precision*	Detection Limits	Desired/ Achieved Completeness
RH	Vaisala Model 333 RH and temperature probe	± 3 % RH	± 3 % RH	± 15 % RH	± 12 % RH	0 to 100 % RH	95% /93 %***
Temperature		± 0.5 °C	± 0.5 °C**	± 5.0 °C	± 5.0 °C	-20 °C to 80 °C	95 %/93 %***
CFU counts		10 % of significant data will be recounted by a second person. Counts must agree within 10 %	Criteria met	10 % of significant data will be recounted by a second person. Counts must agree within 10 %	Criteria met	1 CFU	100 %/100%

* Precision for RH and temperature is defined as deviation from target conditions (50 °C, 60 % RH).

** This value was not checked with a post-testing calibration. Devices were calibrated by the manufacturer within a year of use, and manufacturer calibrations are required annually.

*** There were no intermittent failures for RH/T sensors. However, two tests lost entire device readings. One MadgeTech device failed for R03 inside the PC, and no data were collected. For test R05, only the monitor bag MadgeTech device data were downloaded. PC bag and PC measurements were lost.

EtO cartridges, Humidichips®, and sponges were weighed before and after use for fumigation to ensure exposures were consistent.

Visual inspections were performed before each test, at the end of each test, and at the start of each month thereafter for a period of ten months from the date of the fumigation event. Written documentation was augmented with high resolution digital photography. A comparison to the control materials/equipment was performed. For electronic equipment, the ESD work station was used to inspect the interior of the equipment.

Equipment functionality for electronic equipment was assessed before each test, at the end of each test, and at the start of each month thereafter for a period of ten months from the date of fumigation. The Category 4 equipment maintained in-house was set in operation mode on the ESD work station; temperature and RH of the area was typical of an office environment. The Category 3 equipment was maintained in the same area as the Category 4 equipment. The Category 3 equipment powered by alternating current (AC) was maintained in operational mode continuously; the battery powered equipment was turned on for functionality testing (e.g., maintained in the 'power off' mode).

For Category 4 items, the functionality was assessed using PCMD, a diagnostic software program used by PC manufacturers for pre-inspection of computer hardware and software. The results were stored in hard copy and electronically using a USB memory stick for each computer, along with the report of the visual inspection. The computers remained in the certified ESD work station area at a temperature and RH typical of an office environment for a period of ten months following the date of fumigation. The computers remained in the operational state. PCMD was run every month to assess changes in the performance of the computers.

Fumigation effectiveness: The sporicidal effectiveness was assessed for localized hot spots inside the computers, where the RH or temperature may be lower because of the thermal mass of the computers. A set of three BIs was placed inside each computer case, and another set was placed inside the bag to determine the effectiveness of the fumigation to inactivate the BIs as a function of location within the chamber (i.e., in the bag compared to inside computers). The BIs provided a qualitative result of growth or no growth after an incubation period of seven days. Inoculated rubber coupons were also enumerated for efficacy quantification.

Plated volume critical measurement goals were met. All pipettes are calibrated yearly by an outside contractor (Calibrate, Inc.).

Plates were analyzed quantitatively (CFU/plate) using a manual counting method. For each set of results (per test), a second count was performed on 25 percent of the plates with significant data (data found to be between 30-300 CFU). All second counts were found to be within 10 percent of the original count.

There are many QA/QC checks used to validate microbiological measurements. These checks include samples that demonstrate the ability of the NHSRC Biolab to culture the test organism, as well as to demonstrate that materials used in this effort do not themselves contain spores. The checks include:

- Negative control coupons: sterile coupons that remained in sterile packaging before biolab processing

- Procedural blank coupon: sterile coupons that go through the inoculation procedure without actually being inoculated and fumigated

- Positive control coupons: coupons inoculated but not fumigated.

4.5 Data Quality Audits

This project was assigned a QA Category III and did not require technical systems or performance evaluation audits.

4.6 QA/QC Reporting

QA/QC procedures were performed in accordance with the QAPP for this investigation.

5 Conclusions

EtO can be used safely on small electronic equipment and all materials tested in this study, with the use of adequate engineering controls. Little to no impact was recorded for any materials or equipment tested. Unfortunately, EtO is difficult to scale up due to the high temperature (122 ºF) and RH requirements. In addition, EtO is very toxic and flammable under the target conditions. EtO is not suitable for wide area fumigations such as a building or in any environment where a flame might be present or possible. While this work focused on using a commercial off the shelf system for fumigation, it is possible to use EtO in chamber fumigations when the safety issues are considered (flammability).

6 References

1. USEPA, *Compatibility of Material and Electronic Equipment with Methyl Bromide and Chlorine Dioxide Fumigation.* EPA/600/R-12/664. October, 2012.
2. USEPA, *Compatibility of Material and Electronic Equipment with Chlorine Dioxide Fumigation.* EPA/600/R-10/169. December, 2010.
3. Brown, G.S., Betty, R.G., Brockmann, J.E., Lucero, D.A., Souza, C.A., Walsh, K.S., Boucher, R.M., Tezak, M., Wilson, M.E., Rudolph, T., *Evaluation of a Wipe Surface Sample Method for Collection of Bacillus Spores from Nonporous Surfaces.* Appl. Environ. Microbiol., 2007. **73**(3): p. 706-710.

Appendix A: Andersen EOGas System Characterization

Task 1

The primary objective of this task was to characterize the exposure of materials during Andersen EOGas ethylene oxide (EtO) sterilization cycles, including the relative humidity (RH), temperature, and total exposure in terms of net EtO weight and exposure time. These tests were conducted using 5, 11, and 18 g EtO cartridges and two different bag sizes:

- The AN1005 (EOGas 5) is a 18" x 24" bag
- The AN1006 (EOGas 6) is a 22" x 36" bag

Electronic devices cannot be used during EtO exposure, so RH and temperature measurements were done during a cycle without EtO. The manufacturer (Anderson Products, Inc.) targets 750 mg/liter*hours at 50 °C and RH between 35 % and 80 %. Tests were performed with and without materials, using the manufacturer's recommendations of one water saturated sponge (Humidichip®) per bag. The measured RH during mock exposures using the EOGas 6 bag is shown in Figure 1.

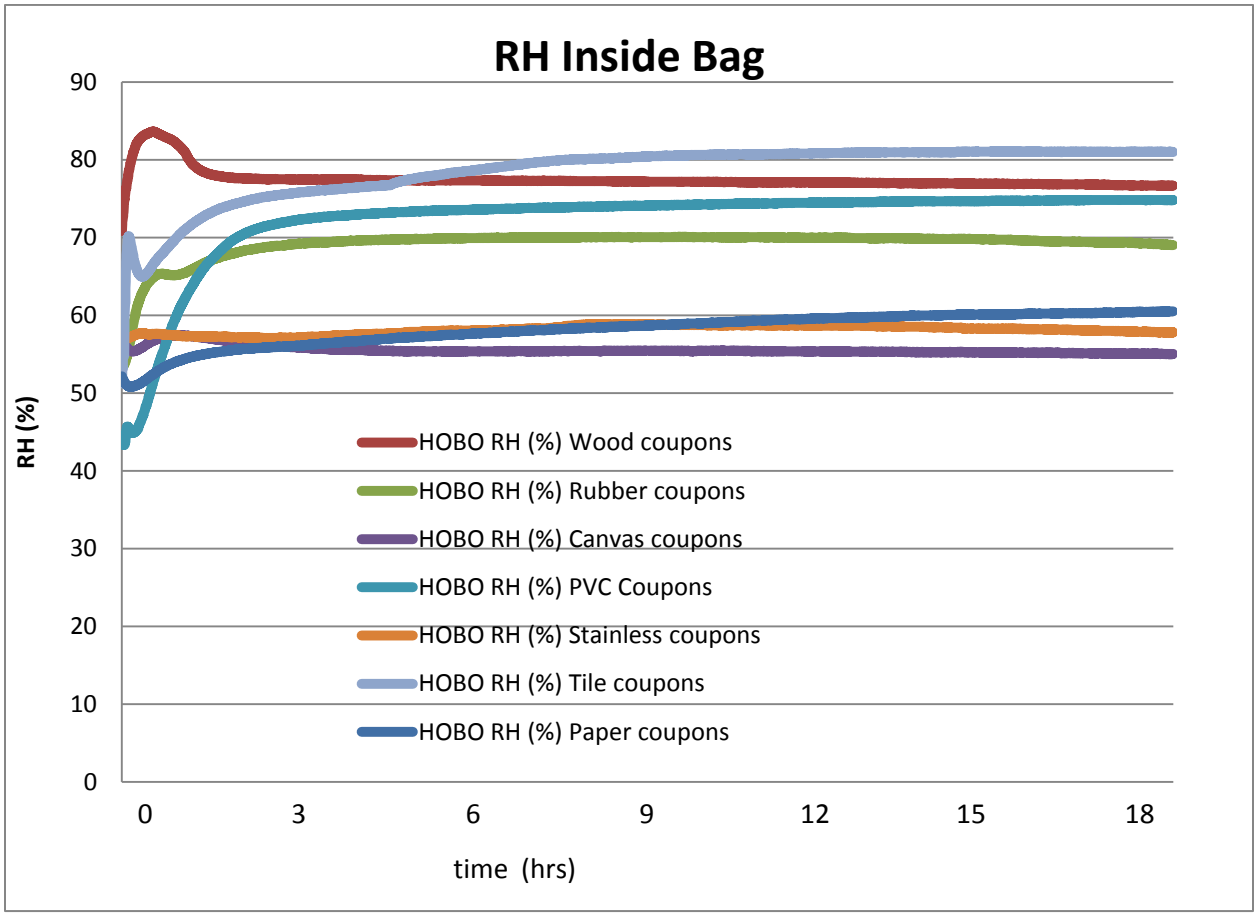

Figure 1. RH in EOGas 5 (small) and EOGas 6 (large) Bags with one Humidichip®

The temperature is very stable due to the control by the EOGas 333 sterilization cabinet. The average RH and temperature are shown in Table 1. Some minimum temperatures shown are during ramp-up and are not indicative of the exposure conditions.

Table 1. Measured Environmental Conditions inside Sterilizer Bags during Mock Exposures

Test	Bag Sizes	Materials in bag	RH (%)			Temperature (°C)		
			Min	Avg	Max	Min	Avg	Max
1A	18” x 24”	None	50.8	58.4	59.8	23.3	49.4	51.4
1B	22” x 36”	None	47.9	66.9	68.1	27.0	49.3	51.7
1C	22” x 36”	Wood coupons	76.7	77.5	83.6	49.4	50.7	52.2
1D	22” x 36”	Paper coupons	50.8	58.1	60.4	48.0	50.1	51.4
1E	22” x 36”	Ceramic (tile) coupons	65.0	74.4	78.3	48.2	50.0	51.3
1F	22” x 36”	Stainless coupons	49.1	58.1	58.9	46.4	49.6	51.1
1G	22” x 36”	PVC coupons	49.0	72.9	74.8	49.2	50.5	51.6
1H	22” x 36”	Canvas coupons	55.3	55.9	57.5	34.4	48.0	49.0
1I	22” x 36”	Rubber coupons	77.3	77.9	83.0	45.3	48.8	49.0

Figure 1 and Table 1 show that all environmental target conditions were met by the cabinet with one Humidichip® in the sterilizer bag.

The variation in RH seen in Figure 1 is not necessarily an effect of the materials present. Figure 2 shows the same type of data for bags that contained no materials.

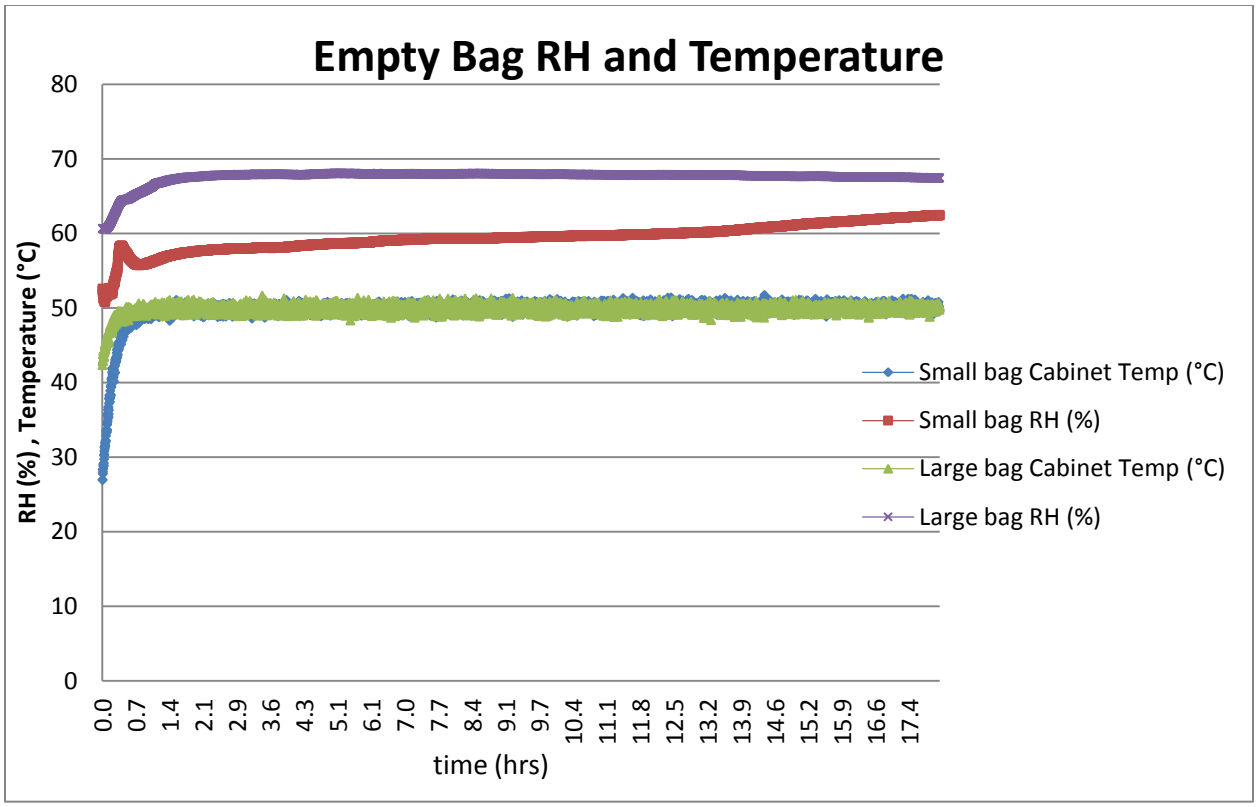

Figure 2. RH and Temperature in EOGas 5 (small) and EOGas 6 (large) bags with one Humidichip®

All things being equal, the RH in the smaller bag would be expected to be higher than the RH in the large bag due to the higher ratio of volume to Humidichip®. There were large variations in RH for all tests, suggesting that Humidichips® may have a large variation in water content.

Characterization of the EtO conditions inside the bag are difficult to determine directly. The EtO is explosive in the exposure range and cannot be measured using electronic devices such as electrochemical sensors. Use of extractive measurements would change the conditions by removing the target gas. Therefore, exposure conditions were measured indirectly by measuring the EtO permeating out of the sterilization bag. An FID was used to measure the exhaust EtO concentration. The FID was initially calibrated with propane calibration gases, but this procedure failed to close the mass balance. Other researchers had documented a significant response factor for EtO, and because EtO was the only hydrocarbon expected in these tests, an EtO calibration gas was used in subsequent calibrations. All results are reported in terms of ppm EtO, based on the EtO calibration gas. The response factor for EtO was measured as roughly 0.5, meaning that 100 ppm EtO would read 50 ppm ethane.

By measuring the concentration in and flow out of the cabinet exhaust, a total mass of EtO release can be calculated, and compared in turn to the total mass change of the EtO cartridge. An example graph of the exhaust concentration and calculated mass release is shown in Figure 3.

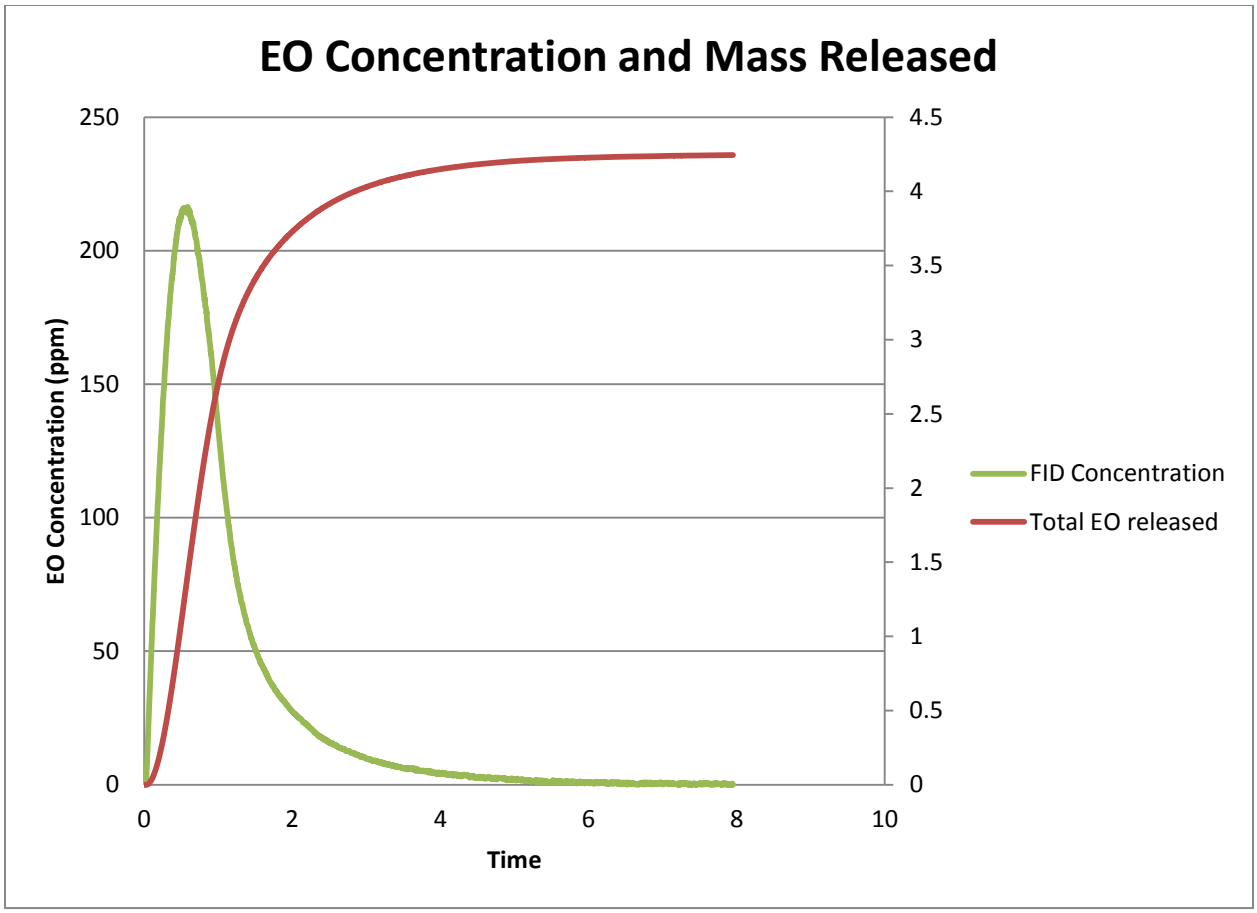

Figure 3. Exhaust Concentration and Total Mass Released

Table 2 shows the mass balance (recovery) on baseline (empty) sterilization bags. The EO released is based on the change in cartridge weight before and after exposure. The EO captured was measured in the exhaust.

Table 2. Mass Balance of EtO from Sterilization Bags without Materials

Test	Bag Size	EO Cartridge	EO Released (grams)	EO Captured (grams)	% Recovery
1J	18” x 24”	5 g	4.41	4.465	101.3
1K	22” x 36”	5 g	4.26	4.244	99.6
1L	18” x 24”	5 g	4.491	4.464	99.4
1M	22” x 36”	5 g	4.548	4.532	99.7
1N	18” x 24”	11 g	10.375	10.575	101.9
1O	22” x 36”	11 g	10.208	10.903	106.8
1P	18” x 24”	11 g	10.38	10.434	100.5

Test	Bag Size	EO Cartridge	EO Released (grams)	EO Captured (grams)	% Recovery
1Q	22” x 36”	11 g	10.59	10.303	97.3
1R	18” x 24”	18 g	17.606	18.225	103.5
1S	22” x 36”	18 g	17.41	18.649	107.1
1T	18” x 24”	18 g	17.638	17.9533	101.8
1U	22” x 36”	18 g	17.599	19.346	109.9

The percent recovery varied between 97 and 110 %. Integration of very low level concentrations over a long period of time during the aeration phase can lead to positive or negative bias due to instrument drift.

Exposure time can be estimated in a variety of ways. Exposure begins immediately upon rupture of the cartridge (and is detected as permeation in the exhaust within three minutes). At the conclusion of the 18-hour cycle, there is still measureable EtO inside the sterilization bag (approximately 40 ppm). It is unknown whether long durations at very low concentrations contribute materially to efficacy.

Determining the exposure concentrations is a more complicated affair. Sterilization bags are evacuated before sealing. The final pressure is not measured, but it can be assumed that the major components of the atmosphere during exposure are water vapor (from the RH provided by the Humidichip®) and EtO. The partial pressure of the water vapor is known, but, as discussed above, the EtO concentration cannot be directly determined. There are two processes determining the actual EtO concentration: release from the cartridge and permeation out of the sterilization bag. To help characterize both permeation and cartridge release rates, measurements were made using two different sized bags. The small bag (EOGas 5) is designed to reach target EtO concentration with a 5 gram cartridge, and the larger bag (EOGas 6) is designed for use with an 11 or 18 gram cartridge. The smaller bag has less area for permeation and should thus increase exposure within the bag as compared to the larger bag as illustrated in Figure 4 using the temporal EtO concentration traces during an 18-hour EtO cycle.

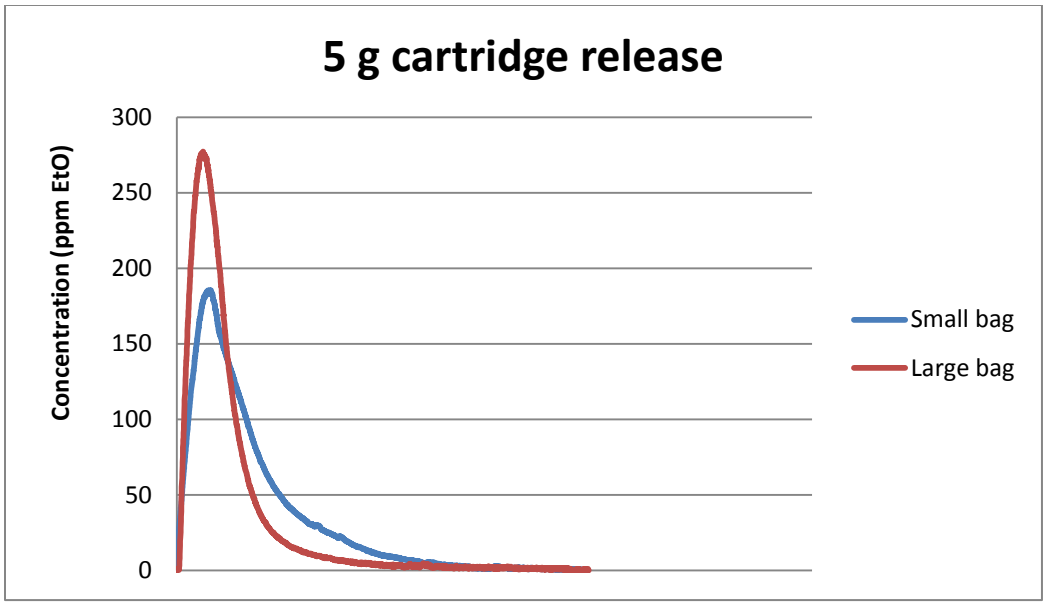

Figure 4. Small Bag vs. Large Bag with 5 g Releases

The cartridge release rate and amount for both of these tests is presumed to be identical (replicate 5 gram cartridges). The larger bag, with the larger surface area for permeation, released EtO more quickly, thus resulting in a higher concentration in the exhuast. The small bag released the EtO more slowly, increasing the contact time inside the bag.

Task 2

The two primary objectives of Task 2 were to determine any material demand commonly sterilized materials may have for EtO, and to determine the efficacy of EtO on deactivation of spores on these materials. Secondary objectives include determining any residue formed during sterilization and determining conditions required to obtain a 6 or greater log reduction (LR) in active spores following the EtO cycle.

Results show that rubber has the most significant material demand (see Figure 5 for different masses of rubber). All materials that have any significant material demand adsorb EtO, and then desorb EtO later in the cycle, resulting in complete recovery of EtO in all tests performed to date.

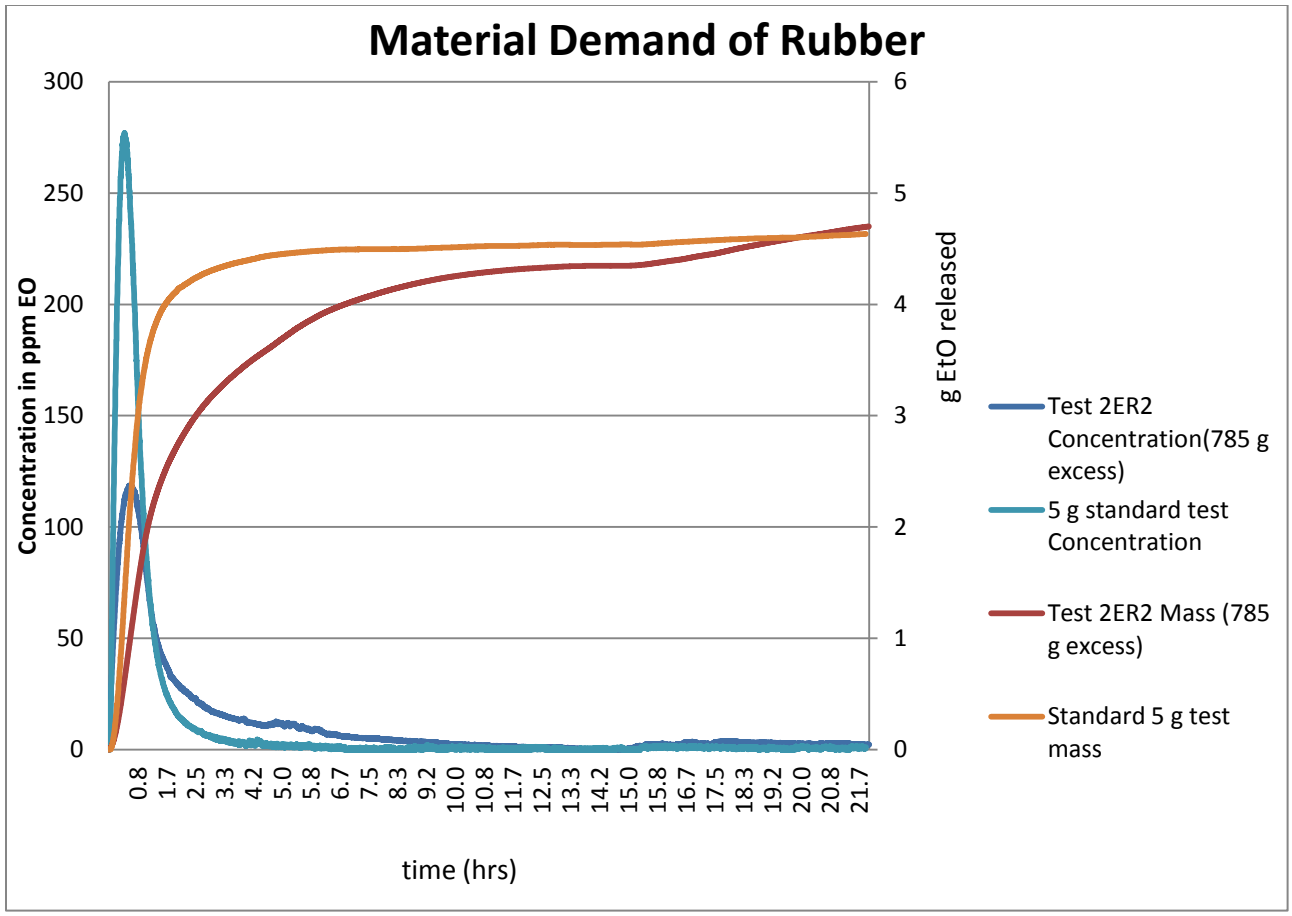

Figure 5. Exhaust Concentration of Rubber and Baseline Exposures

The upward trend seen in the exhaust concentration at the 15 hour mark in Figure 5 is unexpected, and none of the measured exposure parameters suggest a cause. A shift in the bag, due to settling, for instance, could cause a change in cartridge position or bag geometry, which could cause a spike in permeation.

Table 4 shows the results of efficacy tests with material demand effects. These tests were performed by inoculating coupons of the material with approximately 1x 10^6 *Bacillus subtilis* spores and placing them inside a sterilization bag with a large amount of material. The amount of material, though arbitrary, was determined more by volume than weight.

Table 4. Material Demand Efficacy Tests

Material	Excess Material (yes/no)	Cartridge Size	RH	Bag size	Sporicidal Efficacy
Paper	yes	5 g	No Humidichip® (<50 %)	Large (22” x 36”)	Not detected
PVC	yes	5 g	No Humidichip® (<20 %)	Large (22” x 36”)	Not detected
Wood	yes	5 g	No Humidichip® (<20 %)	Large (22” x 36”)	Not detected
Stainless	yes	5 g	No Humidichip® (<20 %)	Large (22” x 36”)	Not detected
Rubber	yes	5 g	No Humidichip® (<20 %)	Large (22” x 36”)	Approximately 20 CFU recovered
Ceramic	yes	5 g	No Humidichip® (<20 %)	Large (22” x 36”)	Not detected
Canvas	yes	5 g	No Humidichip® (<20 %)	Large (22” x 36”)	Not detected

In the case of rubber, there is some evidence to suggest that the longer exposure time at lower concentration (cause by material demand) is less sporicidal than the higher concentration of the exposure as designed (baseline fumigation with little demand). The EOGas system is designed by the manufacturer with a safety factor of 50 % more concentration*time exposure than is generally accepted as effective (750 mg/liter*hour vs. 500 mg/liter*hour). All tests wherein *Bacillus subtilis* spores were detected after exposure were performed at RH conditions below the manufacturer’s recommendations, and with half of the recommended EtO (5 gram cartridge in an 11 gram sterilization bag). These conditions had been chosen to better define cusp requirements for sterilization.

EPA
United States
Environmental Protection
Agency

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT NO. G-35

Office of Research and Development (8101R)
Washington, DC 20460

Official Business
Penalty for Private Use
$300