Final Study Report

Sponsor

American Spice Trade Association
2025 M Street NW
Suite 800
Washington, DC 20036

Study Title

Magnitude of the Residue of Ethylene Oxide and Ethylene Chlorohydrin in/on Spices

Data Requirements

OPPTS 860.1500 Magnitude of the Residue
40 CFR 160 (EPA FIFRA Good Laboratory Practice Standards)

Study Number

WA001-01

Study Director

Michael Wright
Wright Associates

Report Date

March 2005

Testing Facility

McKenzie/Wright Laboratories, LLC
3725 E. Atlanta Avenue, Suite One
Phoenix, Arizona 85040

Total Pages - 828

Statement of No Data Confidentiality Claims

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A),(B), or (C).

Sponsor: American Spice Trade Association

Sponsor Agent: Submitter

Cheryl Deem, Director
American Spice Trade Association

Date

This statement supersedes all other statements of confidentially that may occur elsewhere in this report.

Change of Address Statement

Following completion of all analytical work on the study, McKenzie/Wright Laboratories, LLC on October 31, 2001 relocated its primary facilities from 3725 East Atlanta Avenue, Phoenix, AZ 85040 to 1824 - 1830 Greenfield Plaza, Bryan, Texas, 77802. Because the analytical phase of the study was conducted at the Phoenix, AZ facility, the address for McKenzie/Wright Laboratories, LLC may appear as 3725 East Atlanta Avenue, Phoenix, AZ in various documents associated with the study reported herein. However, any inquiries for the analytical lab should now be addressed to:

McKenzie/Wright Laboratories, LLC
P. O. Box 10647
College Station, Texas 77845

Good Laboratory Practices Compliance Statement

We, the undersigned, hereby certify that the data contained in this report were generated in compliance with Good Laboratory Practice Standards (40 CFR Part 160) applicable to analytical testing facilities. There were no deviations from Good Laboratory Practice Standards during the analytical phase of this study except as noted below.

GLP Deviations to the Field Phase of the study

1. Independent GLP compliant analysis to verify the identity of the Test Substance identity and purity were not performed prior to initiation of the field phase of the study as required by 40CFR §160.105. The manufacturer of the active ingredient, ETO, supplied a Certificates of Analysis for the pure ETO. The formulator supplied gravimetric data characterizing the Test Substance mixture of active ingredient with the CO_2 inactive. These data were made part of the study record for the field phase of the study. A sample of the Test Substance has been retained in the event further characterization is required.

2. The SOP that was prepared to describe the sampling procedure at the commercial fumigation facility was not approved (signed) by ETO Sterilization management at the time the field study work was conducted as required by 40 CFR §160.81(a). Because the study sampling was a unique activity to the fumigation facility, all sampling was performed by the Study Director and recorded directly into the raw data of the study.

GLP Deviations to the Analytical Lab Phase of the study

1. Independent GLP compliant analyses to verify the identity of the ETO and ECH reference standards used in this study were not performed prior to the initiation of the study analyses as required by 40 CFR§160.105. The commercial supplier of the reference substances, Chem Services, Inc., provided certificates of analysis for determination of percent purity and retains chromatographic evidence of these determinations at their facility in West Chester, PA. During the course of the course of the conduct of this study, mass spectral data were developed at the testing facility for ECH, confirming the parent mass of this references substance. Archive samples of both reference substances have been retained in the event further characterization should be required by the Agency.

2. In deviation to requirements for instrument calibration set out in 40 CFR §160.63(a) the analysis of ground sage samples for residues of ECH was performed under circumstances where the analytical instrument could not be properly calibrated to establish an ECH limit of quantification (LOQ) that was lower than the treated sample residue. The validated ECH LOQ for ground sage was approximately two times higher than the residue found in the treated ground sage sample replicates. Therefore, with respect only to ECH residue data reported herein for treated ground sage, the values reported are only best estimates of the ECH residue resulting from the ETO sterilization treatment of ground sage. All other sample matrix analyses were performed with properly calibrated analytical instruments at a LOQ appropriate to treated sample residues.

Good Laboratory Practices Compliance Statement
(Continued)

Study Director:

Michael C. Wright
Wright Associates

Date

Sponsor Representative:

Susan Brown
McCormick & Co., Inc.

Date

Submitter:

Cheryl Deem, Director
The American Spice Trade Association

Date

Quality Assurance Statement

Study Title: **RESIDUES OF ETHYLENE OXIDE AND 2-CHLOROETHANOL IN SPICES.**

In accordance with the American Spice Trade Association policies and procedures for complying with the provisions of the EPA's FIFRA Good Laboratory Practice Standards (Final Rule, 40 CFR Part 160, August 17th 1989), the protocol, field phase and final report of this study has been inspected/audited by QUALITY ASSOCIATES, INC., 9017 Red Branch Road, Suite 102, Columbia, Maryland 21045. The methods described and results incorporated in this report accurately reflect the raw data produced during the study.

Inspection /Audit	Date	Report Date
Protocol	4/27/01	4/30/01
Facility (McKenzie/Wright Laboratories)	7/26/01	8/6/01
In –process	7/10/01	7/11/01
Final Field Report	5/9,14,15,21/03	5/21/03
Final Submission Report	??	??

Angela Psenicska
Quality Assurance Consultant
QUALITY ASSOCIATES, INC.

Date

Authentication

Study Number: **WA001-01**

Study Title: **Magnitude of the Residue of Ethylene Oxide and Ethylene Chlorohydrin in/on Spices**

We hereby declare that this summary report is an accurate and authentic representation of the condition and results of this study as reported in the appended study documents.

Study Director and Author: _____ Date: _____

Michael C. Wright
Wright Associates

Sponsor Approval: _____ Date: _____

Susan Brown
Sponsor Study Management
McCormick & Co., Inc.

Study Identification

Study Number:	WA001-01
Study Title:	Residues of Ethylene Oxide and 2-Chloroethanol in Spices
Testing Facility:	McKenzie/Wright Laboratories, LLC 3725 East Atlanta Ave. Phoenix, AZ 85040
Fumigation Facility:	ETO Sterilization 2500 Brunswick Avenue Building E Linden, NJ 07036
Sponsor:	Cheryl Deem American Spice Trade Association 2025 M Street, NW Suite 800 Washington, DC 20036
Sponsor Management Representative: **June 11, 2001 to December 6, 2002**	Susan Abbott McCormick & Co., Inc. 18 Loveton Circle Sparks Glencoe, MD 21152-9271
February 11, 2003 - Present	Susan Brown McCormick & Co., Inc. 10901 Gilroy Road Hunt Valley, MD 21031-1307
Study Director:	Michael C. Wright Wright Associates P. O. Box 10647 College Station, TX 77842-0647
Principal Field Investigator:	Michael C. Wright Wright Associates
Principal Analytical Investigator:	Melinda Lalko McKenzie/Wright Laboratories, LLC
Study Initiation Date:	11 June 2001
Field Study Start Date:	07 July 2001
Field Study Termination Date:	16 July 2001
Spice Field Sampling Interval:	09 July 2001 to 15 July 2001
Field Report Date:	7 July 2003
Analytical Start Date:	11 June 2001
Analytical Completion Date:	31 October 2001
Analytical Report Date:	22 December 2003
Final Report Date:	?? March 2005

Study Personnel

The following personnel were involved as the Report Author, and Study Management during the conduct of this portion of the study.

Title	Name
Study Director and Principle Field Investigator	Michael C. Wright, Wright Associates
Study Management	Susan Abbott, McCormick & Co., Inc.
Successor Study Management	Susan Brown, McCormick & Co., Inc.
Report Author	Michael C. Wright, Wright Associates

The following personnel of ETO Sterilization were involved at various stages of the field phase of this study.

Title:	Name
V. P., General Operations Manager	Karen Burns
V.P., Manufacturing Engineering	Marek Janasek
Chamber Operator – Forklift Operator	Andrzej Przysiadka
Sampler – Forklift Operator	Tomasz Bober

The following personnel of McKenzie/Wright Laboratories, LLC were involved in the analysis of samples at the Testing Facility.

Title:	Name
Principle Investigator:	Melinda Lalko
Laboratory Director:	Kathryn Koktavy
Laboratory Analyst:	Kathryn Nielson
President & Study Director	Michael Wright

Please refer to the Study Participants page in the raw data package for signatures and initials.

Archiving of Study Data and Retained Samples

All unused Test Substance and empty Test Substance containers used in conducting the field phase of this study were retained in accordance with the requirements of FIFRA GLP regulations, 40CFR Subpart F §160.195. A copy of the original raw data for the field phase of this study will be maintained in the archives of McKenzie/Wright Laboratories, 1824 - 1830 Greenfield Plaza, Bryan, TX 77802 and the original sent for permanent archiving at the Sponsor's direction.

A copy of all original raw data associated with the analytical phase of the study is archived at McKenzie/Wright Laboratories. The original data will be transferred for permanent archiving to a facility designated by the Study Sponsor, American Spice Trade Association. Following finalization of the report a representative sample of each test system, the test substance, and each reference substance used in this study will be transferred from McKenzie/Wright Laboratories, LLC to EPL Archive, Inc., 45610 Terminal Drive, Sterling, VA 20166 for permanent archiving or for as long as the quality of the material affords evaluation or for the duration of the product registration at the discretion of the Study Sponsor.

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STUDY EXECUTIVE SUMMARY

Introduction/Study Objectives

Under the Food Quality Protection Act (FQPA) of 1996, and proposed regulatory changes to 40 CFR Part 152, EPA reserved to itself the primary responsibility for the regulation of ethylene oxide uses in food by specific exception to § 152.5(d)(1). Under this authority, the Agency has reviewed the residue chemistry data submitted in response to an October 10, 1989 ETO DCI and finds that it requires additional data concerning 2-chloroethanol (ECH) residues in spices to satisfactorily complete the dietary risk assessment for ETO use as a spice sterilizing agent. The American Spice Trade Association (ASTA) has, in the mean time, identified a new ETO spice sterilization process, which it believes will result in a significant reduction of ETO and ECH residues in spices over previously available ETO sterilization processes.

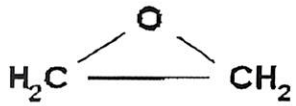
This study report presents data regarding residue levels of ethylene oxide (ETO) and its reaction product, ethylene chlorohydrin (2-chloroethanol or ECH) in/on 29 different whole and ground herb and spice matrices following their sterilization via the newly developed commercial scale ETO sterilization process. This new sterilization process is described in full detail within this report. The new ETO sterilization process was developed with the goal of reducing the residual ETO and ECH residues while maintaining the sterilization efficacy of ETO spice sterilization treatment.

The study was sponsored by the American Spice Trade Association and conducted by Wright Associates of College Station, Texas in July of 2001 using the commercial facilities of ETO Sterilization, Inc. (ESI) located in Linden, NJ. Following ETO sterilization at ESI, samples of the sterilized spices were taken at ESI, frozen and then shipped to the testing facility, McKenzie/Wright Laboratories, LLC in Phoenix, Arizona, where the analyses for ETO and ECH residues were performed.

Test Substance

The test substance was a commercial ETO sterilization gas sold under the trade name of Sterilizer Gas 5 by ARC Specialty Chemicals, Slate Hill, NY. ARC Specialty Products is a division of BALCHEM Corporation. Sterilizer Gas 5 is a commercial ETO sterilization gas mixture of 20% ETO in carbon dioxide, which is provided in 100 lb. compressed gas cylinders, usually loaded 9 cylinders to a steel pallet. This study utilized a single pallet of cylinders prepared by ACR Specialty Products as one lot of Sterilizer Gas 5. Additional descriptive details concerning the test substance may be found in Table I below and in Appendix A. Appendix A contains the manufacturer's gravimetric certification data. Independent certification of chemical composition will be provided at a later date under separate cover.

Table I
Test Substance Characteristics

Trade Name:	Sterilizer Gas 5
Physical State:	Liquefied compressed gas mixture
Active Ingredient Gas:	20% by weight Ethylene Oxide
Active Ingredient CAS #	75-21-8
Inactive Ingredient Gas:	80% by weight Carbon Dioxide
Inactive Ingredient CAS#	124-38-9
Sterilizer Gas 5 Lot Number:	00614A1
Source of Sterilizer Gas 5:	ARC Specialty Products, Slate Hill, NY
Receipt Date at ESI:	06 July 2001
Receipt Date at McKenzie/Wright Labs:	18 June 2001
Storage Conditions:	100 lb capacity compressed gas cylinder stored at ambient temperatures
Expiration Date:	None
Active Ingredient Name:	Ethylene Oxide (ETO)
Source of Active Ingredient	BASF Corporation
Active Ingredient Batch No.:	G1113EA10A (BASF)
Active Ingredient Purity:	99.99% (BASF)
Active Ingredient Molecular Weight:	44.06
Active Ingredient Molecular Structure:	

Reference Substances

Two reference substances were used at McKenzie/Wright Laboratories, LLC, Phoenix, AZ to perform the analysis of ETO and ECH in the herb and spice samples received from ESI, Linden, NJ. The ETO reference substance was obtained as an ampoule of ETO dissolved in toluene and the ECH reference standard was a neat liquid material. Both reference standards were obtained from Chem Service, Inc. of West Chester, PA and were used without further characterization as to purity or identity.

The characteristics of these two reference substances are presented in Tables II and III below.

Table II
ETO Reference Substance Characteristics

Trade Name:	Ethylene Oxide Solution
Physical State:	Ethylene Oxide dissolved in Toluene
CAS #: ETO	75-21-8
CAS #: Toluene	108-88-3
Source:	Chem Service, Inc. West Chester, PA
Lot No.:	252-148A
ETO Concentration:	1000 µg/mL ± 5% (Chem Service CoA)
Storage Conditions:	Refrigerator at ca. 4 °C
Receipt Date McKenzie/Wright Labs	15 May 2001
Expiration Date:	01 December 2003
Molecular Weight of ETO:	44.06

Molecular Structure:

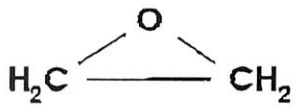
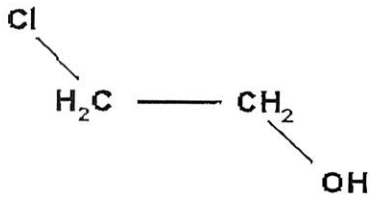


Table III
ECH Reference Substance Characteristics

Trade Name:	Ethylene Chlorohydrin, 2-Chloroethanol
Physical State:	Liquid
CAS #:	107-07-3
Source:	Chem Service, Inc. West Chester, PA
Lot No.:	241-125B
Percent Purity:	99.5% (Chem Service CoA)
Storage Conditions:	Refrigerator at ca. 4 °C
Receipt Date McKenzie/Wright Labs	15 May 2001
Expiration Date:	01 June 2004
Molecular Weight:	80.51

Molecular Structure:



ETO Sterilization of Herbs and Spices

I. Overview

The field phase of this study, conducted at ETO Sterilization, Inc., Linden, NJ (ESI) between June 28 and July 16, 2001, consisted of procuring the test system (spices and herbs) from commercial lines of trade, sampling each of the test systems prior to sterilization to obtain control samples of all herb and spice matrices, sterilization of the herb and spice test systems via ETO fumigation at elevated temperature and under variable pressure conditions, and then a post-treatment test system sampling scheme designed to provide three post treatment intervals of test samples for residue laboratory analysis. Test samples were collected from all test systems at three different post-treatment time intervals in order to provide data on residue declines during ambient temperature storage in the commercial warehouse environment of ESI. Sample intervals collected were zero-time, 24 hours and 72 hours post treatment for the residue decline portion of the study.

II. Identity of Test System

The test systems consisted of commercial sized pallets of 15 whole and 14 ground untreated spices obtained from the normal commercial line of trade. Once received at ESI, each pallet to be placed on test was labeled with a unique number and place in a specific holding area of ESI's warehouse. Commercial forklift equipment was used to move the pallets of spices to and from the fumigation chamber and about the warehouse. Additional details regarding the source, labeling and handling of the test systems may be found in the Field Phase Final Report in Appendix B.

III. ETO Fumigation Chamber Description

The sterilization treatment was performed using a new digitally controlled commercial scale sterilization process. The fumigation chamber was a commercial steel sterilization chamber of ca. 1591 cubic feet capacity capable of holding up to 11 pallets of spices at the same time, which were placed inside the chamber using a fork lift. Additional dimensional information and photographs of the chamber may be found in Appendix B.

IV. ETO Sterilization Process Description

After the pallets of spices were placed inside the chamber, temperature probes were placed into selected sacks or boxes of spices. These probes were connected to the digital process controller for the chamber. The chamber was then sealed airtight by closure and locking of a steel door. Initiating a sequence of temperature and pressure settings, which had been pre-programmed into a Honeywell digital recorder/controller module, started the sterilization cycle. In accordance with the Honeywell controller's temperature settings, the chamber was first heated to approximately 140 °C using hot water flowing through water jackets imbedded in all the chamber surfaces, including the door of the chamber. A vacuum was also drawn on the chamber to approximately 1.4 psia (pounds/square inch absolute) using a large commercial vacuum pump, with care being taken to scrub the pump

exhaust to eliminate any air pollution or potentially hazardous substances that might be exhausted. At this point in the cycle the Sterilizer Gas 5 was introduced into the chamber.

While the temperature and pressure cycles of the sterilization process were automatically controlled by the Honeywell controller for each sterilization run, introduction into the chamber of the Sterilizer Gas 5 had to be performed manually by the ESI facility staff. The ETO was injected by manually connecting a compressed gas cylinder of Sterilizer Gas 5 to a series of valves and pipes leading to the evacuated fumigation chamber. The treatment rate was determined gravimetrically using a commercial sized floor scale to weigh the amount of Sterilizer Gas 5 being injected into the chamber. For this study 1½ cylinder tanks of Sterilizer Gas 5 were used per treatment run. This corresponded to delivery of 150 lbs of 20% ETO in carbon dioxide to the fumigation chamber during each treatment, which is equal to a treatment rate of approximately 300 mg/L of ETO. The ETO exposure period was approximately 6 hours at one atmosphere and 140 °C after which most of the ETO and much of the ECH were removed from the chamber and spice samples by a cyclical steam distillation process.

For this study, three separate sterilization treatment runs were conducted, each run sterilizing 9 to 11 commercial sized pallets of spices and herbs. One treatment run per day was conducted starting on July 9 followed by treatment runs starting on July 10 and July 11, 2001. Each treatment run was conducted at a temperature range of 115 – 144 °F, with exposure to approximately 300 mg/Liter ETO for 6 hours at approximately one atmosphere of pressure during the first portion of the treatment run. The ETO exposure period was followed by a 9.5 hour cycle consisting of 21 shallow, steam assisted vacuum pulses between 1.5 and 4.7 psia. The purpose of the shallow vacuum pulse cycle was to remove all residual ETO from the chamber and spices and reduce ECH residues in the spices via a reduced pressure steam distillation mechanism. This period of 21 shallow vacuum cycles was followed by four pulsed deep vacuum cycles during which pressure was varied between one atmosphere (13.3 psia) down to 1.3 psia. The deep vacuum cycles were conducted with dry air to remove excess moisture introduced by the steam distillation process and return the spice product to normal moisture content prior to ending the fumigation process. Returning the chamber to atmospheric pressure and dropping the chamber temperature to less than 120 °F characterized the process termination.

The entire fumigation process, *per se*, required from 18.9 to 19.6 hours for completion. To accommodate typical work schedules in the commercial facility, the spice pallets were held sealed in the chamber at 113 to 120 °F and one atmosphere of pressure for an additional 2 to 5 hours at the end of the last vacuum cycle. Thus, the three treatment runs that constituted this study had a total residence time in the treatment chamber of 22.7, 21.6 and 23.6 hours respectively. Additional details regarding the sterilization process and data generated by the process controller may be found in the charts and tables in Appendix B.

ETO and ECH Sampling Of Sterilized Test Systems

Following the treatment run and removal of the spice pallets to a pre-selected warehouse location, each pallet of spice was hand sampled from two separate locations on the pallet. Typically a sample was taken from a bag, box or bale located on the top of the pallet and a separate sample was taken from a bag, box or bale located in the middle or bottom of the pallet. Samples were taken using latex gloves and stainless steel utensils. The utensils were thoroughly cleaned and gloves changed between each sampling.

Two separate samples for ECH analysis were taken for each herb and spice and placed directly into pre-labeled screw top polypropylene containers or plastic bags and then placed immediately on dry ice. After these duplicate samples had been taken from each pallet, the first sample of each pair of duplicates was further subsampled specifically for ETO analysis by weighing out six replicate 1 gram samples into 20 mL glass headspace vials. To each headspace vial, 100 µL of pesticide grade toluene was added using a calibrated micropipettor. Then the headspace vials were sealed with a septum and crimp-cap, and packaged up with the two bulk samples for that spice. The entire sample package was placed back on dry ice and maintained frozen until shipped by overnight express to the analytical laboratory.

After the initial sampling that immediately followed the completion of the treatment run, each spice was again sampled at 24 hours and again at 72 hours post fumigation. Each sampling was conducted in the same manner described above and all samples were sent to the analytical laboratory on dry ice by Federal Express overnight freight. Additional details for the study activities conducted at ESI may be found in Appendix B.

Sample Receipt and Processing at the Analytical Laboratory

The test sample of spices and herbs were received at the analytical laboratory between the dates of 10 July 2001 and 17 July 2001. All samples were received frozen and in good condition and placed after inventory into a walk-in freezer which was maintained at <15 °C. Additional control samples for completing the frozen storage stability portion of the study were received 24 and 25 July 2001. No further processing was required for ETO sample analyses. Certain samples sent for ECH analysis required grinding prior to extraction. For those samples that required grinding, the sample was removed from the walk-in freezer and grinding was performed on the frozen sample by hand using a hammer and pliers as crushing tools. Additional details regarding sample receipt and handling are tabulated in Appendix C.

Sample Analyses

I. Overview

The Analysis of spices for ETO and ECH residues was conducted using McCormick & Co., Inc. methods RA 10.3, "Determination of Ethylene Oxide Residues in Spices by Headspace Gas Chromatography" and RA 12.2,

"Determination of 2-chloroethanol Residues in Spices", respectively. The analytical instrument utilized in performance of Method RA10.3 was equipped with an Agilent heated headspace autosampler injector coupled to an Agilent 6890 gas chromatograph. Separations were performed using a 25 M x 0.52 mm Restek PLOT capillary column and detection of ETO was by a flame ionization detector. The analytical instrument utilized in performance of Method RA 12.2 was an Agilent 6890 gas chromatograph using a 30 M x 0.25 mm i.d. silica capillary column with 0.25 µm CP-WAX52CB film thickness and equipped with either a micro electron capture detector (µECD) or a mass selective detector (MSD).

The Limit of Detection (LOD) for the ETO method for all sample matrices was 0.25 ppm. The analytical method Limit of Quantification (LOQ) for ETO in all matrices was 1 ppm. The LOD and LOQ for the ECH method varied by sample matrix because of preexisting ECH residues in some control samples, co-extracted chromatographic interferences in some control samples and the presence of greater than 50 ppm of ECH in nearly all treated samples.

II. ETO Method Validation and Sample Analyses

The ETO method was validated for precision and accuracy concurrently with sample analysis. No residues of ETO were found in any matrix control sample. An ETO method precision performance analysis was conducted, consisting of six replicate analyses performed on seven treated spices sampled immediately following sterilization with ETO (0-time spice samples). Relative standard deviations for this precision performance evaluation ranged from 6.7% for ground cassia to a high of 37.3% for whole nutmeg. The degree of precision was most strongly correlated to the degree of homogeneity characteristic of the sample. In addition to the precision analysis, accuracy of the method was tested for each matrix concurrently with the sample analyses by concurrent analysis of laboratory fortified control matrix for each of the 29 herb and spice matrices tested. Average recoveries of ETO for control samples fortified at 1.0 ppm and 2.0 ppm were 92% and 88%, respectively. Table IV summarizes the results of ETO recovery from each sample matrix.

Because of the presumed volatility of the ETO residues, rapid analytical turn around time was a primary goal of the analytical procedure. All sterilization test samples were analyzed for ETO within 16 days of the time of sampling. Because of the need to maintain a rapid pace of analysis for ETO a standardized range of laboratory fortifications between 1 ppm and 5 ppm was used to evaluate ETO recovery from each sample matrix. Five of the 29 sample matrices contained time zero ETO residues that exceeded the 5 ppm level of ETO standard fortification to the corresponding control matrix. Time zero residues of ETO found in whole nutmeg, whole and ground cassia, ground black pepper and whole caraway seed exceeded 5 ppm. All of these over range residues were only slightly above the 5 ppm fortification, except whole nutmeg. Whole nutmeg time zero ETO residues averaged 20.5 ppm, just over 4 times the high ETO fortification of 5 ppm.

Despite the above standard fortification range responses for a few of the time zero analyses, all residues had declined to less than the highest fortification by the 24 hour sampling period. By the 72 hour sampling interval all spice and herb ETO residues had dissipated to <1.0 ppm, with the single exception of whole coriander whose 72 hour residue, nonetheless, fell well below the 5 ppm control sample fortification.

For all sample matrices except whole coriander, ETO residues showed a rapid dissipation to below the method limit of quantification within 72 hours. In the case of whole coriander, the time zero average ETO residue of 3.06 ppm did not dissipate significantly by the 72 hour post treatment sampling. The average ETO residue in treated whole coriander at 72 hours was still 2.45 ppm. However, this persistent ETO residue was found to be the probable result of treatment of the test sample with ETO prior to its selection for this study. This conclusion is supported by the recovery of very high ECH residues in the pre-study control sample, which could only have resulted from treatment of the whole coriander with ETO prior to its use in this study. Therefore, the ETO residues found in the whole coriander represent, for that spice, a worst case scenario of a double treatment of ETO. The rate or process by which the first ETO treatment may have been performed could not be determined because it was performed prior to importation into the United States.

Residues above the LOQ of 1.0 ppm ETO at time zero were found in only 10 of the 29 sample matrices. Ground black pepper, ground capsicum, whole cassia, ground cassia, whole caraway seed, whole coriander, whole cumin, ground nutmeg and whole sesame seed were found to have between 1.02 ppm to 6.93 ppm and whole nutmeg was found to contain the highest residue of 20.5 ppm.

Herb and spice matrices found to contain residues of ETO above 1 ppm for the day zero analysis, triggered analysis of the corresponding 24 hour sample interval. Zero-time matrices found to contain residues <1.0 ppm ETO or for which ETO residues were non-detectable (ND) were not further analyzed for ETO, even though samples had been taken at 24 hrs and 72 hrs and were available.

Of the 10 matrices tested for ETO residues from the 24 hour sample, 6 continued to show residues >1.0 ppm ETO. A total of 8 sample matrices were selected for analysis of the 72 hour samples, including 2 that had detectable residues at 24 hours of >0.25 ppm but <1.0 ppm. These later samples were analyzed as a precaution against the possibility the 24 hour results were only statistically <1.0 ppm with respect to the precision of the method. Seven of the eight 72 hour samples analyzed for ETO had declined to <1.0 ppm. The exception being whole coriander discussed above, whose average ETO residue at 72 hrs. was 2.45 ppm.

Table VI below summarizes the ETO residues found at each sampling interval for each spice matrix. A more detailed discussion of the ETO analyses may be found in Appendix C of this report. Residue results for ETO for all three sampling intervals are summarized by fumigation run in the analytical report Table VIII found in Appendix C. Appendix C, Table IX, presents detailed ETO residue data by spice matrix pair (ground versus whole).

III. ECH Method Validation and Sample Analyses

The accuracy and precision of the ECH method was evaluated from the results obtained for analysis of replicate fortifications of control samples from each herb and spice matrix. These fortified control samples were analyzed concurrently with treated samples. Fortification levels for the ECH analysis were adjusted for each sample matrix to correspond to ECH residue levels that were found in treated samples. Control sample fortification levels ranged from 10 ppm to 1950 ppm ECH. The analytical LOD and LOQ for each sample matrix was determined based on the lowest fortification level for which recoveries fell within the acceptable range of 60% to 130% for samples fortified at the LOQ and 70% to 120% for samples fortified at all other higher levels. Fortifications for which recoveries were <60% were use to establish the LOD for that particular matrix. A summary of these QC sample recoveries is presented in Table V.

Residues of ECH were found in control samples of ground basil (26, 30 and 24 ppm), ground oregano (11 and 10.2 ppm), ground sage (226, 252 and 296 ppm) and whole coriander (494, 450 and 494 ppm). The presence of ECH in control samples is indicative of treatment of the spice with ETO prior to its treatment in this study. Therefore, the treated sample residues found in this study for these four matrices might be considered indicative of the level of ECH residues likely to result from double ETO treatments of that spice and represent the worst case scenario for ECH residues in US spices.

Average residue levels of ECH in the other spices and herbs at time zero ranged from a low of <25 ppm in whole ginger to a high of 1663 ppm in whole basil and 1237 ppm in ground basil. Most residues fell within the narrower range of 25 ppm to 335 ppm.

Chromatographic interferences in the GC-ECD analysis of ground caraway seed required that this matrix be reanalyzed by GC-MS.

Residue declines for ECH over the 72 hour sampling period were not statistically significant for any of the spices.

Table VI below summarizes the residue results for ECH in treated herb and spice samples. A more detailed discussion of the ECH analyses is provided in Appendix C of this report. A more detailed summary of ECH residue data is provided for each sterilization run in Appendix C, Table X. Appendix C, Table VII, lists the LOD and LOQ determined for ECH in each herb and spice matrix. Appendix C, Table XI, provides detailed information concerning ECH residue data and recoveries by matrix pair (whole versus ground).

Table VI below presents a comparison of ETO and ECH residues for all spices at each of the three sampling intervals. Further detailed discussion of the analytical study can be found in Appendix C of this report.

Freezer Storage Stability

I. Overview

The frozen storage stability of ETO and ECH residues in 6 representative spice and herb samples was conducted over an interval of time equal to or greater than the longest freezer storage time for the sample between sampling date and the initial date of analysis. The six representative spices selected for assessment of freezer storage stability were: whole fennel, whole oregano, ground cassia, ground black pepper, ground celery seed and whole basil.

II. ETO Frozen Storage Stability

The ETO frozen storage stability testing interval covered 12 to 26 days of frozen storage. The ECH frozen storage stability testing interval covered 25 to 27 days. A reanalysis of ground caraway seed for ECH by GC-MSD did involve frozen storage of the ground caraway seed sample for a longer period of time than that which was tested in the storage stability study. Nevertheless, chromatography evidence developed during the course of the study demonstrated the stability of ECH in both sample extracts and spice samples held even under ambient storage conditions. As a result the GC-MSD data for ECH in ground caraway seed are considered valid and are the values reported herein.

The level of fortification for ETO freezer storage stability analysis was 2.0 ppm. All average recoveries for the triplicate method spike samples fell within the range of 98% to 106%. The duplicate storage stability samples were corrected for the average recovery of these method spikes and the average of these corrected values was reported as the frozen storage stability recovery for that particular matrix. Storage stability for ETO in whole oregano, ground cassia and whole fennel seed were conducted for a period of 25 days of frozen storage. ETO residue stability in ground black pepper and ground celery were tested for 26 days of frozen storage and whole basil storage stability data covers a frozen storage period of 12 days. Results ranged from lower stability of 64% in whole fennel seed to relatively stable frozen residue result of 96% and 91% for ground cassia and whole oregano, respectively. Ground black pepper and ground celery seed had stabilities of 77% and whole basil stability was 72%. Table VII below summarizes the frozen storage stability for ETO residues. See Appendix C for additional details regarding the storage stability of ETO on the six representative spice samples.

III. ECH Frozen Storage Stability

The same set of six spices was used to assess frozen storage stability of ECH residues. Fortification levels for ECH storage stability were 50 ppm because the LOQ for the ECH method was generally equal to or lower than this level of residue. Duplicate controls fortified on the day of extraction were extracted and analyzed for quality control purposes in addition to the duplicate pretreated frozen storage stability samples. The average recovery of the quality control samples was used to correct the recovery values for the storage stability samples. The average of these corrected storage stability recoveries was then reported as the percent stability for

the sample during the period of frozen storage tested. The frozen storage interval tested was 25 days for whole fennel and whole oregano; 26 days for ground cassia, ground black pepper and ground celery seed; and 27 days for whole basil. Average recovery of the duplicate fresh quality control spikes ranged between 84% for ground cassia to 112% for whole cassia. All samples except fennel seed showed good frozen storage stability of between 87% and 97%.

In the frozen storage stability study, ECH stability was found to be poor on fennel seed at only 43% of the original fortification following 25 days of frozen storage. On the other hand, residues of ECH on whole fennel seed as a result of the ETO sterilization treatment, which were significant at 275 ppm, 254 ppm and 343 ppm for the 0-time, 24 hour and 72 hour sampling intervals, appeared stable during the 1 to 11 days of frozen storage they underwent in the study.

The intervals of frozen storage between sampling and ECH analysis for treated whole fennel seed in this study were 1 day, 8 days and 11 days for the 0-time, 24 hour and 72 hour post-treatment samples, respectively. The longest sample frozen storage interval of 11 days is less than half the length of time tested in the frozen storage stability study. If it is assumed that the ECH residue decline in stability is an approximately linear function that would suggest that the appropriate storage stability adjustment percentage for each interval would be 97.7%, 81% and 74.9% for the 0-time residue, 24 hour residue and 72 hour treated sample residues, respectively. This is within the normally accepted range of stability adjustment percentages. Therefore, the poor results for the whole fennel ECH storage stability analysis after 25 days of frozen storage does not invalidate the treated whole fennel seed sample analytical results.

Storage Stability results for ECH in the six representative spices are presented below in Table VIII. See Appendix C for additional details regarding the storage stability testing.

Table IV
ETO Control Sample Fortification Recoveries

ETO Run #	Study Spice ID Code	Spice	Control Residue	0-Time % Recovery		24 Hr. % Recovery	72 Hr. % Recovery
				1.0 ppm	2.0 ppm	2.0 ppm	1.0 ppm
1	GOR	Ground Oregano	ND	89	81	NF	NF
1	GCE	Ground Celery Seed	ND	90	81	NF	NF
1	GCA	Ground Cassia	ND	88	83	85	91
1	WCA	Whole Cassia	ND	90	75	77	98
1	GBA	Ground Basil	ND	68	70	NF	NF
1	GCS	Ground Caraway Seed	ND	93	82	NF	NF
1	WNM	Whole Nutmeg	ND	99	87	97	95
1	GNM	Ground Nutmeg	ND	89	85	94	98
1	GGR	Ground Ginger	ND	90	96	NF	NF
1	WCU	Whole Cumin	ND	89	29*	NF	NF
1	WTU	Whole Turmeric	ND	95	80	NF	NF
2	WSA	Whole Sage	ND	NF	93	NF	NF
2	GSA	Ground Sage	ND	NF	97	NF	NF
2	WFN	Whole Fennel	ND	NF	90	NF	NF
2	WGR	Whole Ginger	ND	NF	105	NF	NF
2	GBP	Ground Black Pepper	ND	NF	90	96	98
2	WOR	Whole Oregano	ND	NF	96	NF	NF
2	GCO	Ground Coriander	ND	NF	92	86	97
2	WRP	Whole Capsicum	ND	NF	84	NF	NF
2	GRP	Ground Capsicum	ND	NF	84	97	NF
3	GCU	Ground Cumin	ND	NF	85	97	NF
3	WCS	Whole Caraway Seed	ND	NF	91	93	88
3	WCO	Whole Coriander	ND	NF	88	93	95
3	GFN	Ground Fennel	ND	NF	84	NF	NF
3	WBA	Whole Basil	ND	NF	76	NF	NF
3	WSE	Whole Sesame Seed	ND	NF	74	94	NF
3	WCE	Whole Celery Seed	ND	NF	81	NF	NF
3	GTU	Ground Turmeric	ND	NF	89	NF	NF
3	WBP	Whole Black Pepper	ND	NF	85	NF	NF

Average % Recovery at 1.0 ppm =	92	Std. Dev. = 6.9	n = 19
Average % Recovery at 2.0 ppm =	88	Std. Dev. = 7.7	n = 39

NF = No fortification performed
ND = No detectable residue found
* = loose/damaged headspace vial cap. Recovery excluded from statistics.

Table V
ECH Control Sample Fortification Recoveries

ETO Run #	Study Spice ID Code	Spice	Control Residue (ppm)	0-Time % Recovery				24 Hr. % Recovery						72 Hr. % Recovery								
				10 ppm	250 ppm	25 ppm	50 ppm	25 ppm	50 ppm	100 ppm	510 ppm	None	None	10 ppm	25 ppm	50 ppm	100 ppm	102 ppm	250 ppm	600 ppm	1500 ppm one	None
1	GOR	Ground Oregano	11/10	65	85	-	-	76	-	-	63	-	-	-	-	-	-	86	85	-	-	-
1	GCE	Ground Celery Seed	ND	38	86	-	-	60	-	-	67	-	-	-	-	-	-	-	93	97	-	-
1	GCA	Ground Cassia	ND	<10	86	-	-	59	-	-	72	-	-	-	45	76	-	-	-	-	-	-
1	WCA	Whole Cassia	ND	25	88	-	-	76	-	-	75	-	-	-	75	88	-	-	-	-	-	-
1	GBA	Ground Basil	26/30/24	88	71	-	-	59	-	-	82	-	-	-	-	-	-	-		106	109	-
1	GCS	Ground Caraway Seed	ND	-	-	96	104	-	72	112	-	-	-	-	88	-	122	-	-	-	-	-
1	WNM	Whole Nutmeg	ND	34	80	-	-	51	-	-	76	-	-	22	65	-	-	-	-	-	-	-
1	GNM	Ground Nutmeg	ND	25	84	-	-	63	-	-	79	-	-	-	-	81	-	104	-	-	-	-
1	GGR	Ground Ginger	ND	65	94	-	-	78	-	-	72	-	-	-	-	91	-	115	-	-	-	-
1	WCU	Whole Cumin	ND	38	94	-	-	55	-	-	78	-	-	-	-	-	-	98	93	-	-	-
1	WTU	Whole Turmeric	ND	41	98	-	-	73	-	-	80	-	-	-	87	-	-	110	-	-	-	-

ETO Run #	Study Spice ID Code	Spice	Control Residue (ppm)	0-Time % Recovery				24 Hr. % Recovery						72 Hr. % Recovery								
				10 ppm	250 ppm	500 ppm	None	25 ppm	None	None	510 ppm	None	None	10 ppm	25 ppm	50 ppm	102 ppm	250 ppm	600 ppm	None	None	None
2	WSA	Whole Sage	ND	ND	-	92	-	72	-	-	70	-	-	-	-	-	54	83	-	-	-	-
2	GSA	Ground Sage	226/252/296	463	-	75	-	179	-	-	86	-	-	-	-	-	-	55	102	-		-
2	WFN	Whole Fennel	ND	7	-	91	-	78	-	-	73	-	-	-	-	-	124	-	91	-	-	-
2	WGR	Whole Ginger	ND	67	-	86	-	87	-	-	71	-	-	<10	75	-	-	-	-	-	-	-
2	GBP	Ground Black Pepper	ND	43	-	82	-	96	-	-	88	-	-	-	-	-	97	88	-	-	-	-
2	WOR	Whole Oregano	<10	70	-	74	-	96	-	-	79	-	-	-	-	-	88	-	103	-	-	-
2	GCO	Ground Coriander	ND	16	-	71	-	86	-	-	72	-	-	-	72	-	118	-	-	-	-	-
2	WRP	Whole Capsicum	ND	ND	-	73	-	60	-	-	82	-	-	-	-	-	97	72	-	-	-	-
2	GRP	Ground Capsicum	ND	ND	-	73	-	55	-	-	63	-	-	-	-	-	82	76	-	-	-	-

ND = no detectable residues found

Table V
ECH Control Sample Fortification Recoveries
(Continued)

ETO Run #	Study Spice ID Code	Spice	Control Residue (ppm)	0 Time % Recovery				24 Hr. % Recovery						72 Hr. % Recovery								
				25 ppm	None	None	510 ppm	25 ppm	None	None	510 ppm	1500 ppm	1950 ppm	None	25 ppm	50 ppm	102 ppm	250 ppm	600 ppm	1050 ppm	1500 ppm	1950 ppm
3	GCU	Ground Cumin	ND	58	-	-	70	61	-	-	71	-	-	-	-	-	101	80	-	-	-	-
3	WCS	Whole Caraway Seed	ND	68	-	-	84	76	-	-	71	-	-	-	73	-	125	-	-	-	-	-
3	WCO	Whole Coriander	494/450/421	NA	-	-	82	224	-	-	91	-	-	-	-	-	-	-	127	104	-	-
3	GFN	Ground Fennel	ND	77	-	-	88	86	-	-	72	-	-	-	82	-	-	-	92	-	-	-
3	WBA	Whole Basil	<10	99	-	-	99	-	-	-		90	92	-	-	-	-	-	-	-	97	87
3	WSE	Whole Sesame Seed	ND	76	-	-	81	89	-	-	88	-	-	-	98	-	132	-	-	-	-	-
3	WCE	Whole Celery Seed	ND	99	-	-	78	109	-	-	91	-	-	-	-	-	104	-	105	-	-	-
3	GTU	Ground Turmeric	<10	67	-	-	70	99	-	-	75	-	-	-	-	-	105	84	-	-	-	-
3	WBP	Whole Black Pepper	ND	72	-	-	85	102	-	-	83	-	-	-	-	-	103	96	-	-	-	-

ND = no detectable residues found

Table VI
Magnitude and Decline of ETO and ECH Residues in Spices

ETO Run #	Spice ID Code	Spice Name	0 Time Samples		24 Hr. Samples		72 Hr. Samples	
			ETO[1] Ave. ppm	ECH[2] Ave. ppm	ETO[1] Ave. ppm	ECH[2] Ave. ppm	ETO[1] Ave. ppm	ECH[2] Ave. ppm
3	WBP	Whole Black Pepper	<1.0	154	-	176	-	171
2	GBP	Ground Black Pepper	6.10	250	0.84	280	<1.0	290
2	WRP	Whole Capsicum	<1.0	231	-	133	-	180
2	GRP	Ground Capsicum	1.10	179	ND	167	-	172
1	WCA	Whole Cassia	5.64*	25	1.77	29	<1.0	31
1	GCA	Ground Cassia	4.40*	<50	0.75	<50	<1.0	<50
2	WGR	Whole Ginger	ND	<25	-	ND	-	<25
1	GGR	Ground Ginger	<1.0*	99	-	79	-	86
1	WTU	Whole Turmeric	ND*	51	-	88	-	52
3	GTU	Ground Turmeric	<1.0	125	-	118	-	111
3	WBA	Whole Basil	<1.0	1663	-	1612	-	1428
1	GBA	Ground Basil	<1.0*	1088	-	1237	-	1173
2	WOR	Whole Oregano	<1.0	241	-	234	-	244
1	GOR	Ground Oregano	ND*	166	-	146	-	168
2	WSA	Whole Sage	<1.0	214	-	251	-	267
2	GSA	Ground Sage	ND	(203)	-	(270)	-	(288)
3	WCS	Whole Caraway Seed	5.44	77	1.24	56	<1.0	59
1	GCS	Ground Caraway Seed	<1.0*	78[4]	-	62[4]	-	62[4]
3	WCE	Whole Celery Seed	<1.0	299	-	273	-	243
1	GCE	Ground Celery Seed	<1.0*	335	-	306	-	290
3	WCO	Whole Coriander[3]	3.06	844	2.39	854	2.45	774
2	GCO	Ground Coriander	2.64	67	1.25	61	<1.0	59
1	WCU	Whole Cumin	ND*	128	-	139	-	129
3	GCU	Ground Cumin	1.68	181	<1.0	159	-	164
2	WFN	Whole Fennel	<1.0	275	-	254	-	343
3	GFN	Ground Fennel	ND	261	-	238	-	235
1	WNM	Whole Nutmeg	20.5*	<50	3.37	<50	<1.0	<50
1	GNM	Ground Nutmeg	1.77*	66	1.02	66	<1.0	54
3	WSE	Whole Sesame Seed	1.71	53	<1.0	30	-	32

[1] Averages are of 3 replicate samples taken from the ECH-1 container for each spice unless otherwise noted by an asterisk

[2] Average of the ECH residue found in two independently taken spice samples

[3] Unusually high ECH residues of 421 - 494 ppm in the control sample indicates sample was ETO treated prior to study treatment

[4] Samples Analyzed by GC-MSD due to chromatographic interferences in the GC-ECD analyses

* Indicates average is result of 6 replicate analyses instead of 3 replicates

ND Indicates that any residue which might be present is below the LOD of the method

\- Indicates sample for this interval was not extracted or analyzed.

< Indicates that residues are less than the reported LOQ but greater than the LOD

( ) Indicates a reported value is less than the validated LOQ for that matrix

Table VII
ETO Freezer Storage Stability Data Summary

Spice ID Code	Spice Name	Storage Interval (days)	Percent Recovery						
			Fresh Spike Rep 1	Fresh Spike Rep 2	Fresh Spike Rep 3	Fresh Spike Average	Storage Stability Rep 1	Storage Stability Rep 2	Average SS Recovery*
WFN	Whole Fennel	25	108	104	96	103	81	51	64
WOR	Whole Oregano	25	110	111	81	101	86	97	91
GCA	Ground Cassia	25	110	100	85	98	99	89	96
GBP	Ground Black Pepper	26	114	117	87	106	82	82	77
GCE	Ground Celery	26	116	115	85	105	85	77	77
WBA	Whole Basil	12	126	95	73	98	66	75	72

* Average of Storage Stability replicates corrected for average of Fresh Spike recoveries

Table VIII.

ECH Storage Stability Data Summary

Spice ID Code	Spice Name	Storage Interval (days)	Percent Recovery					
			Fresh Spike Rep 1	Fresh Spike Rep 2	Fresh Spike Average	Storage Stability Rep 1	Storage Stability Rep 2	Average SS Recovery*
WFN	Whole Fennel	25	102	96	99	46	40	43
WOR	Whole Oregano	25	89	97	93	77	85	87
GCA	Ground Cassia	25	82	85	84	72	74	87
GBP	Ground Black Pepper	25	111	100	106	103	101	96
GCE	Ground Celery	25	92	88	90	81	78	88
WBA	Whole Basil	27	111	113	112	109	102	94

* Average of Storage Stability replicates corrected for average of Fresh Spike recoveries

Conclusions

I. Sterilization Process

The fumigation process time shows a 45 minute variation between the three runs conducted which can not be controlled through control of equipment operational parameters. This variation in process time was expected because the rate that the chamber vacuum pump can pull down the chamber to the low set point pressure depends greatly on the type of packaging being used for the spice being treated and on how full the chamber is of spice. For instance, sealed plastic lined bags and boxes and multi-walled paper bags will resist evacuation of internal air for a longer period of time than burlap or synthetic polymer mesh bags. Variations in the resistance to air evacuation from sterilizer run to run is a source of variation in the time it takes for the chamber to reach the Honeywell controller's low-pressure set point during the pressure pulse phases of the sterilization cycle. Also, a nearly empty chamber has a greater volume of air to remove than a nearly full chamber and, therefore, will take longer to evacuate. As a consequence, the stages of the fumigation process that utilize a vacuum will vary in duration depending on what type of packaging is being treated and how much product volume is being treated relative to the chamber volume.

For this study, the chamber was loaded to at least 70% of capacity for each fumigation run. Each fumigation run was conducted on a chamber load of herbs and spices that are packaged in a variety of packaging types, including paper bags, polymer mesh bags and bales, cardboard boxes and burlap bags. An effort was made to obtain a similar mix of packaging types for each fumigation run. However, to the extent that the run-to-run uniformity of the packaging could not be exactly duplicated, variations in process run time are readily apparent from the data, even though each run was conducted using the same Honeywell Controller set-point values.

It would be expected that, under normal commercial operations, only a single type of spice and package would be treated in a run. We would expect run-to-run variability for this type of same-spice treatment to have considerably less process time variation than seen in the current study. However, process times between different spices and packaging might vary to a greater extent than observed in this study. The data from this study suggest a general overall process time of less than 24 hours with a 6-hour ETO gas exposure period. However, the data do not support establishment of any specific process times for the depuration stages of the process for the purposes of labeling the product use.

II. ETO and ECH Analytical Results

Residues of ETO in/on herbs and spices resulting from the tested sterilization process are very low and fully dissipate to below the 1.0 ppm analytical method limit of quantification within 72 hours of warehouse storage. Residues of ECH in/on herbs and spices resulting from the tested sterilization process are less than 343 ppm except for whole and ground basil, which contained residues between 1000 and 2000 ppm. Whole coriander ECH residues were also greater than 343 ppm, but contained pre-treatment ECH residues up to 494 ppm, indicating

probably treatment with ETO prior to being tested in this study. Following ETO treatment in this study whole coriander was found to contain residues of 854 ppm, which declined only slightly to 774 ppm during 72 hours of warehouse storage. As demonstrated by the dissipation results for whole coriander residues, ECH residues were generally persistent for all herbs and spices over the 72 hour warehouse holding time tested in this study.

III. ETO Product Use Label Support

Product use and process specifications that are supported by data from this study include effective ETO dose levels, ETO fumigation exposure time, temperature and pressure limit specifications during ETO exposure, and temperature and pressure limit specifications for an effective depuration process to be applied in the treatment chamber post-ETO exposure. The process description that these data support is as follows:

ETO treatment for 6 hours in an air tight steel chamber held at atmospheric pressure and 125 °F to 144 °F using a commercial grade 20% ETO in CO_2 sterilizer gas as the fumigant at an effective ETO dose level of approximately 300 mg/L, followed by 21 low vacuum steam assisted depuration vacuum cycles pulsed as quickly as possible between 1.5 psia and 5 psia at approximately 139 °F to 144 °F, followed by an additional four fresh air depuration cycles pulsed between 1.3 psia and 13 psia at 135 °F to 143 °F. The full ETO sterilization process cycle time for herbs and spices usually should be less than 24 hours.