United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/petitioned-substances

Document Type:

☒ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☐ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

A.1 National List inclusion

Ethylene gas is listed on the National List in Section 205.605(b) "***Synthetics allowed.***" for use in postharvest ripening of tropical fruit and degreening of citrus. We hereby petition to add use of ethylene gas for potato and onion storage generated on-site by catalytic conversion of ethyl alcohol to this list. Given that ethylene is a naturally-occurring, plant produced growth hormone, NOP may wish to revise the "synthetic category, although the intended use in this case involves production of the ethylene via a catalytic conversion process from ethyl alcohol. It is our understanding that this use was not included at the the same time because it had not yet come into common use.

B.

1. Substance name:

Common name	ethylene
IUPAC name	ethene
CASRN	74-85-1
Structure	C_2H_4 

2. Petitioner:

Don O'Shaughnessy, Ph.D., DABT
President, D. O'Shaughnessy Consulting, Inc.
206 Traditions Blvd
Bowling Green, KY 42103
doctox@mac.com 270-537-5139

Manufacturer: Not applicable. Substance is generated on-site at crop storage facilities.

3. Current and Intended Use

The product is registered by US EPA for use as follows:
Ripening of avocados, bananas, citrus fruit, Kiwifruit, mangos, melons, papayas, pineapples, stone fruits (nectarines, peaches, plums), tomatoes, walnuts, and tobacco; and to prevent sprouting of potatoes and onions in storage. Ripening aid for greenhouse tomatoes and bell peppers. (*Appendix 1, sample EPA-registered label)*

It is the use in enclosed storage facilities for harvested **organic** onions and potatoes that is the subject of this petition.
Ethylene is a naturally occurring plant growth regulator with a significant history of human exposure via natural and anthropogenic sources, including use on food commodities and in anesthesia practices. The first product containing Ethylene was registered by the Agency in December, 1971. Currently, there are nine active Ethylene end-use product(s) (EPs) with active registrations with the Agency. These products are registered for use as plant growth regulators and herbicides. On May 18, 1990, the Agency classified Ethylene as a biochemical pesticide because it is a naturally occurring compound with a nontoxic mode of action to the target pest(s) / plant(s), and has a history of exposure to humans and the environment without any reported negative effects. (See EPA RED and Work Plan for ethylene, Appendix 2).

4. Activities and Application Rate

Ethylene is the primary component in most plastics. It is produced primarily by steam-cracking of hydrocarbons, but can alternatively be produced by the dehydration of ethanol, which can be produced from fermentation processes using renewable substrates such as glucose, starch and others.

Ethylene gas is highly flammable, and dangerous to store and transport. Accordingly, it is preferable to generate the ethylene on-site by catalytic dehydration of ethanol to ethylene and water.
In the activity intended in this petition, ethylene gas is generated by passing ethyl alcohol vapor over a heated γ-alumina surface. The amount of ethylene emitted and allowed to accumulate in the contained space is regulated by a sensor. The operator of the equipment simply connects a tube from a container of denatured* ethanol to the catalytic generator machine. The operation manuals for potato and onion storage uses are included in Appendix 3.

** Under US tax law, ethanol not intended for consumption as a beverage must be denatured in a manner that prevents its conversion by any practical means back to beverage grade ethanol. Ethanol for ethylene PGR production use is denatured with ethyl acetate (and referred to as "Special Denatured Alcohol, Formula #29), which is approved by US EPA for this use, and is similarly converted in the catalytic process to ethylene and water. The manufacturing process and formulation data are included in Appendix 4*

5. Manufacturing Process.
The process for fermentation and distillation of the alcohol, and addition of denaturant is included in Appendix 4. The synopsis is:

a) Grain (primarily corn) is ground, and fermented using yeast to a "beer" with a moderate ethanol content. This is distilled and re-distilled by heating and condensation of the vapors until the ethanol / water content reaches a minimum-boiling azeotrope of ~ 95% ethanol. Ethyl acetate is stirred in at 1% v/v equivalent to 0.99% w/w.

b) The process of catalytic dehydration is explained in Appendix 5, and the "user" process for production of ethylene from ethanol is included in the User Manuals (Appendix 3).

6. Ancillary Substances
The precursor substance to the ethylene is denatured alcohol (95% v/v with 1% v/v ethyl acetate, equal to 91.94 % ethanol by weight), and the remainder water. The substance for which inclusion in the National List is sought is the ethylene resulting from catalytic dehydration. A 5- batch analysis of the ethylene gas is included in Appendix 6. This shows that only ethylene and water are emitted.

7. Previous Reviews.
We are not in possession of previous reviews by USDA per se. However, we note that ethylene is listed in the National List under 205.605 (b) for postharvest ripening of tropical fruits and degreening of citrus. It is similarly listed in Canada, to include sprouting of potatoes, and in the EU to include inhibition of sprouting of potatoes and onions. Documentation is included in Appendix 7. A summary review document of the EC ad-hoc expert group on pesticides in organic food production is also included in Appendix 8.

8, 9 Regulatory Authority / CASRN / Labels
CASRN is indicated in Section B.1.
An example of one of the US EPA-registered products is included in Appendix 1. Again, it is noted that the EPA registered product is ethanol which is the product delivered to the storage facilities. However, it is the ethylene generated on-site that is at issue.

10. Physical / Chemical Properties
These are detailed in the OECD SIDS document Appendix 8. Ethylene is a gas with a BP of -103.71 ºC. It has marginal solubility in water.

11. Safety information
An SDS for ethylene is included (Appendix 9). However, it should be noted that this SDS is for compressed ethylene gas, and the principal hazard is flammability / explosion. The safety of ethylene in this use pattern is better represented in the EPA RED for ethylene (Appendix 2).

12 Research and Justification
The EPA RED (Appendix 2) details many advantages of using ethylene as a sprout inhibitor. It is effective at a very low rate of exposure, is of exceptionally low toxicity to non-target species and users, is naturally occurring in plants, and is a volatile gas that dissipates rapidly with no residue in crops or in soil, and

does not contaminate water. The use of ethanol-fueled on-site generators provides a much greater level of safety vs. transport of compressed flammable gas ethylene.
Appendix 10 includes several references to the utility of ethylene. In particular, it should be noted that sprout inhibition is an economic need for sustainable farming and food supply chains.
We are not aware of any presently nor historically existing *organic* alternatives to ethylene gas for these purposes.

13 **Justification Statement for Inclusion of a synthetic on the National List**

Ethylene gas, generated on-site from ethanol, is already listed in the National List for some uses in organic crops, and in other jurisdictions for storage of potatoes and onions. Inclusion in the National List should be expanded to include in-storage sprout inhibition of potatoes and onions. Although generated "synthetically" by catalytic dehydration of ethanol, it is also naturally produced and emitted by plants as a PGR. It leaves no residue in crops, soil, or water, and is of exceptionally low toxicity to non-target species. Use of ethylene displaces use of *chemical pesticides* such as CIPC, which may have crop residue and worker exposure issues.

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 1

EXEMPLAR EPA REGISTERED LABEL FOR AN ETHANOL PRECURSOR PRODUCT FOR GENERATION OF ETHYLENE ON-SITE IN CROP STORAGE

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Pesticide Programs
Biopesticides and Pollution Prevention Division (7511P)
1200 Pennsylvania Ave., N.W.
Washington, D.C. 20460

NOTICE OF PESTICIDE:
_X_ Registration
___ Reregistration
(under FIFRA, as amended)

EPA Reg. Number:	**Date of Issuance:**
92717-1	5/29/2020

Term of Issuance:
Unconditional

Name of Pesticide Product:
Restrain Generator Fuel

Name and Address of Registrant (include ZIP Code):

Restrain Company, Ltd.
Unit 7, The Forum, Minerva Business Park, Lynch Wood
PE2 6FT Peterborough, England

Note: Changes in labeling differing in substance from that accepted in connection with this registration must be submitted to and accepted by the Biopesticides and Pollution Prevention Division prior to use of the label in commerce. In any correspondence on this product, always refer to the above EPA Registration Number.

On the basis of information furnished by the registrant, the above named pesticide is hereby registered under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA or the Act).

Registration is in no way to be construed as an endorsement or recommendation of this product by the U.S. Environmental Protection Agency (EPA). In order to protect health and the environment, the Administrator, on his or her motion, may at any time suspend or cancel the registration of a pesticide in accordance with the Act. The acceptance of any name in connection with the registration of a product under the Act is not to be construed as giving the registrant a right to exclusive use of the name or to its use if it has been covered by others.

This product is unconditionally registered in accordance with FIFRA section 3(c)(5) provided that you:

1. Submit and/or cite all data required for registration or registration review of your product when the EPA requires all registrants of similar products to submit such data.

2. Submit storage stability and corrosion characteristics (Guidelines 830.6317 and 830.6320) data as these data requirements are not satisfied. A one-year study is required to satisfy these data requirements. You have 18 months from the date of this registration to provide these data to the EPA.

Signature of Approving Official:

Andrew Bryceland, Team Leader
Biochemical Pesticides Branch
Biopesticides and Pollution Prevention Division (7511P)
Office of Pesticide Programs

Date:

5/29/2020

EPA Form 8570-6

3. Make the following labeling change before you release this product for shipment:

    - Revise the EPA Registration Number to read, "EPA Reg. No. 92717-1."

4. Submit one (1) copy of the final printed labeling for the record before you release this product for shipment.

Should you wish to add/retain a reference to your company's website on your label, then please be aware that the website becomes labeling under FIFRA and is subject to review by the EPA. If the website is false or misleading, the product will be considered to be misbranded and sale or distribution of the product is unlawful under FIFRA section 12(a)(1)(E). 40 CFR § 156.10(a)(5) lists examples of statements the EPA may consider false or misleading. In addition, regardless of whether a website is referenced on your product's label, claims made on the website may not substantially differ from those claims approved through the registration process. Therefore, should the EPA find or if it is brought to our attention that a website contains false or misleading statements or claims substantially differing from the EPA-approved registration, the website will be referred to the EPA's Office of Enforcement and Compliance Assurance.

Your release for shipment of this product constitutes acceptance of these terms. If these terms are not complied with, this registration will be subject to cancellation in accordance with FIFRA section 6. A stamped copy of the labeling is enclosed for your records. Please also note that the record for this product currently contains the following acceptable Confidential Statements of Formula (CSFs):

- Basic Pre-formulation of Restrain Generator Fuel CSF dated 08/28/2019
- Basic Post-formulation of Restrain Generator Fuel CSF dated 03/03/2020

Any CSFs other than those listed above are superseded.

If you have any questions, please contact Menyon Adams of my team by phone at (703) 347-8496 or via email at adams.menyon@epa.gov.

Sincerely,

Andrew Bryceland, Team Leader
Biochemical Pesticides Branch
Biopesticides and Pollution
Prevention Division (7511P)
Office of Pesticide Programs

Enclosure

ACCEPTED
05/29/2020
Under the Federal Insecticide, Fungicide and Rodenticide Act as amended, for the pesticide registered under EPA Reg. No. 92717-1

RESTRAIN
Ethylene Generator Fuel

An ethylene treatment source for acceleration of ripening of avocados, bananas, citrus fruit, Kiwifruit, mangos, melons, papayas, pineapples, stone fruits (nectarines, peaches, plums), tomatoes, walnuts, and tobacco; and to prevent sprouting of potatoes and onions in storage. Ripening aid for greenhouse tomatoes and bell peppers. This product is only for use with the RESTRAIN ETHYLENE GENERATOR".

ACTIVE INGREDIENT:

Ethanol	91.94%
Other ingredients:	8.06%
TOTAL	100.0%

KEEP OUT OF REACH OF CHILDREN
CAUTION
NOT FOR HUMAN CONSUMPTION

READ THE ENTIRE LABEL INCLUDING ATTACHED BOOKLET BEFORE USING

FIRST AID	
If swallowed	• Call a poison control center or doctor immediately for treatment advice. • Have person sip a glass of water if able to swallow. • Do not induce vomiting unless told to do so by a poison control center or doctor. • Do not give anything by mouth to an unconscious person.
If inhaled	• Move person to fresh air. • If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth if possible. • Call a poison control center or doctor for further treatment advice.
If in eyes	• Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. • Call a poison control center or doctor for treatment advice.
If on skin or clothing	• Take off contaminated clothing. • Rinse skin immediately with plenty of water for l 5-20 minutes. • Call a poison control center or doctor for treatment advice.
Have the product container or label with you when calling a poison control center or doctor, or going for treatment. In case of spill or other emergency call CHEMTREC 1-800 - 424-9300	

Manufactured for:
Restrain Company Ltd.,
Unit 7 Minerva Business Park
LYNCHWOOD, PETERBOROUGH PE2 6FT, Great Britain
Phone # 270-781-8234

EPA Reg. No. 92717-X
NET CONTENTS 2.5 gallons

EPA Est. No. _____
Lot No. _____

PHYSICAL AND CHEMICAL HAZARDS

FLAMMABLE. Keep away from heat and open flames.

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS AND DOMESTIC ANIMALS

Caution. Prolonged or frequently repeated skin contact may cause allergic reaction in some individuals. Do not use this product if you are taking disulfiram (e.g. "Antabuse®").

PERSONAL PROTECTIVE EQUIPMENT (PPE)

Handlers and users must wear:

- Long-sleeved shirt and long pants
- Shoes and socks

DIRECTIONS FOR USE

It is a violation of federal law to use this product in a manner inconsistent with its labeling.

USE ONLY WITH THE RESTRAIN ETHYLENE GENERATOR.

CROPS

Avocados, Bananas, Citrus Fruit, Kiwifruit, Mangos, Melons, Papayas, Pears, Persimmons, Pineapples, Stone Fruits (Nectarine, Peach, and Plum), Tomatoes and Walnuts:
Use in a ripening room for the first 24 hours of ripening period. One (I) quart used as directed in 4,000 to 10,000 cubic feet ripening room produces ethylene concentrations varying from 4 to 1,200 P.P.M over a period 12- 48 hours. One (1) pint used as directed in 1,500 to 6,000 cubic feet ripening room produces ethylene concentrations varying from 5 to 500 P.P.M. over a period of 6-24 hours.

Tobacco:
Use immediately after filling curing system. Two (2) quarts used as directed in 1,500 to 2,500 cubic feet curing system produces ethylene concentrations varying from 15 to 300 P.P.M. over a period of 12 - 24 hours. Use additional two (2) quarts of RESTRAIN Ethylene Generator Fuel as required.

Storage of Potatoes:
The Restrain Generator should be located within the store that is being treated. The minimum cold store temperature is +37.5º F. This machine is not intended for use in temperatures below –34° F.

Fill the tank carefully using the funnel provided to prevent spillage. If spillage occurs it should be contained with the concave area on the top of the tank. Clear any spilled fuel before proceeding and dispose of any soiled paper and rags appropriately.

For the Restrain Generator B200 model, the room must be at least a volume of 7000 ft^3 (equivalent to approximately 60 one ton boxes) for normal cold stores.

The maximum size store depends on the standard construction, room air leakage, and the target level of “Restrain”. This equipment may be used in good quality cold stores of 4000 ton capacity.

The machine should be located at floor level of no greater than 3 feet above the floor in a position of good air circulation. It should be placed away from normal loading activity and protected by barriers from potential damage from fork lift trucks and similar hazards.

The machine must be within 5 degrees of level and be stable.

The machine must not be placed above or close to a source of heat or open flame.

Check the machine **DAILY** for correct operation.

The sensor to monitor the amount of ethylene being generated must be located remotely form the machine to maintain good control of the total room atmosphere. The location depends on the storage layout and design.

STORAGE POTATO APPLICATION			
Crop	**Rate**	**Interval**	**Remarks**
Storage of Potatoes (Sprout Inhibition)	0.0044 oz/ 35.3 ft^3 (10 ppm)	Apply continuously until potatoes are removed from storage.	Monitor storage temperature and commence application once eyes of potatoes begin to develop. • For use on seed potatoes remove the Restrain Generator five days prior to planting. • Check the Restrain Generator and the sensors daily during operation to ensure the proper rate of ethylene is being generated continuously. • Maintain an appropriate level of Restrain Fuel to ensure the proper delivery of the ethylene gas. • Monitor the potato seed storage temperature and ventilation to ensure the appropriate dispersion of the ethylene gas during application. • Ethylene gas levels must be checked prior to re-entry to ensure no exposure to users/workers.
Storage of Seed Potatoes	0.0066oz/ 35.3 ft^3 (15 ppm)	The ethylene generator will automatically gradually increase the ethylene concentration from 0.1 PPM to 15 PPM over a 21-day period. For seed potatoes, remove 5 days prior to planting	

Greenhouse Tomatoes and Bell Peppers:
Locate the Restrain Generator within the greenhouse that is being treated. The minimum use temperature is +63° F. This machine is not intended for use in temperatures below 30° F.

Fill the tank carefully using the funnel provided to prevent spillage. If spillage occurs it should be contained within the concave area on the top of the tank. Clear any spilled fuel before proceeding and dispose of any soiled paper and rags appropriately.

For the Restrain Generator B200 model the room should be at least an empty volume of 7000ft^3.

The maximum size greenhouse depends on the standard construction, air leakage, and the target level of “Restrain”. This equipment has been used satisfactorily in good quality of 5 acres capacity.

The machine should be located at floor level of no greater than 3 ft above the floor in a position of good air circulation. It should be placed away from normal loading activity and protected by barriers from potential damage from fork lift trucks and similar hazards.

The machine must be within 5 degrees of level and be stable.

The machine must not be placed above or close to a source of heat or open flame.

The sensor to monitor the amount of ethylene being generated must be located remotely form the machine to maintain good control of the total room atmosphere. The location depends on the storage layout and design.

GREENHOUSE TOMATO AND BELL PEPPERS APPLICATION			
Crop	**Rate**	**Interval**	**Remarks**
Greenhouse Tomatoes (Accelerated ripening)	0.00006 oz/ 35.3 ft^3 (1.4 ppm)	Apply at growth stage of berries in first - fructification stage: green to shriveled dark-seed for 4.5 days continuously.	• Monitor crops and commence application once the desired plant growth stage has been reached. • Check the Restrain Generator and the sensors daily during operation to ensure the proper rate of ethylene is being generated continuously. • Maintain an appropriate level of Restrain Fuel to ensure the proper delivery of the ethylene gas. • Monitor greenhouse temperature and ventilation to ensure the appropriate dispersion of the ethylene gas during application. • Ethylene gas levels must be checked prior to re-entry to ensure no exposure to users/workers.
Greenhouse Bell Peppers	0.00006 oz/ 35.3 ft^3 (1.4 ppm)	Apply at growth stage of berries in first - fructification stage: green to shriveled dark-seed for 4.5 days continuously	•

FOLLOWING APPLICATION, vent the greenhouse for 12 hours before re-entering.

Onions:
Load the store fully and follow normal procedures for drying and curing of the bulbs. This normally involves the use of high powered fan systems circulating ambient air through the boxes or through the bulk stack of bulbs. Once this phase is completed, the stores are then brought down to holding temperature, either by using ambient night time air or by the use of refrigeration or a combination of both.
The holding temperature will vary and will be between 33 - 38º F for cold stored onions and 48º F for ambient stored onions. Begin treatment in a store somewhere between early November and early December. Set the Restrain generator to operate the **onion control** program.
The minimum interval between removal of the crop from the store and release for consumption is 3 days.

STORAGE ONIONS APPLICATION			
Crop	**Rate**	**Interval**	**Remarks**
Storage of Onions	0.0066oz/ 35.3 ft^3 (15 ppm)	Apply continuously until onions are removed from storage.	• Monitor storage temperature and commence application once the stores have reached required temperature • Check the Restrain Generator and the sensors daily during operation to ensure the proper rate of ethylene is being generated continuously. • Maintain an appropriate level of Restrain Fuel to ensure the proper delivery of the ethylene gas. • Monitor the onion storage temperature and ventilation to ensure the appropriate dispersion of the ethylene gas during application. Daily fresh air flushing must not exceed 6 hr per day. • Ethylene gas levels must be checked prior to re-entry to ensure no exposure to users/workers.

USE PRECAUTIONS

Ensure that the ripening room or curing system is, to the extent reasonably possible, air-tight, with all vents and doors closed. Any cracks or holes in walls and doors must be repaired or covered over before use. Do not use in enclosures less than 1,500 cubic feet.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage or disposal.
PESTICIDE STORAGE: Store in dry locked storage area, at temperatures below 125°F.
PESTICIDE DISPOSAL: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility.
CONTAINER DISPOSAL: Non-refillable container. Do not reuse or refill this container. Offer for recycling, if available. Dispose of empty containers with normal non-incineration waste in compliance with any applicable State and Local regulations.

WARRANTY AND LIMITATION OF DAMAGES STATEMENT

Restrain Company, Ltd. warrants that this product conforms to the chemical description on the label thereof and is reasonably fit for the purposes stated on such label only when used in accordance with the directions of use under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Unintended effects may result because of such factors as storage conditions, presence of other materials or the manner of use of application, all of which are beyond the control of Restrain Company, Ltd.
To the extent permitted under applicable law, any damage arising from a breach of this warranty shall be limited to direct damages and shall not include consequential commercial damages, such as loss of profits or values or any other special or indirect damages.
Restrain Co. Ltd. makes no other express or implied warranty including any other express or implied warranty of FITNESS or MERCHANTABILITY.

If you do not agree with or do not accept any of directions for use, the warranty disclaimers, or limitation on liability, do not use the product, and return it unopened to the Seller, and the purchase price will be refunded.

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 2

US EPA RED AND WORK PLAN FOR ETHYLENE

United States
Environmental Protection
Agency

Office of Prevention, Pesticides
And Toxic Substances
(H-7508W)

EPA

Reregistration Eligibility Document (RED)

Ethylene

REREGISTRATION ELIGIBILITY DOCUMENT

ETHYLENE

LIST C

CASE 3071

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
SPECIAL REVIEW AND REREGISTRATION DIVISION
WASHINGTON, D.C.

ETHYLENE REREGISTRATION ELIGIBILITY TEAM

Office of Pesticide Programs:

Special Review and Reregistration Division

Ruby Whiters	Accelerated Reregistration Branch

Health Effects Division

Freshteh Toghrol	Chemistry & Reregistration Support Branch II
Roy Sjoblad	Science Analysis Branch
Winston Dang	Occupational and Residential Exposure Branch

Environmental Fate and Effects Division

Concepcion Rodriguez	Ecological Effects Branch
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Cynthia Giles-Parker	Fungicide and Herbicide Branch
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Mark Perry	Registration Support Branch

Biological and Economic Analysis Branch

James Saulmon	Biological Analysis Branch
Eric Maurer	Biological Analysis Branch

Policy and Special Projects Staff

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Office of General Counsel

Allen Corpien

GLOSSARY OF TERMS AND ABBREVIATIONS

CAS	Chemical Abstracts Service
EPA	U.S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
MRID	Master Record Identification (number) EPA's system of recording and tracking studies submitted.
RED	Reregistration Eligibility Document

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EXECUTIVE SUMMARY

This reregistration eligibility document (RED) is the United States Environmental Protection Agency ("EPA" or the "Agency") regulatory position on the continued registration of the pesticide ethylene and its uses. Products containing ethylene are currently registered as plant growth regulators and herbicides. Commercially, ethylene is used as a ripening agent for fruits and vegetables, a curing agent for tobacco, and to promote flower production in pineapples. It is also used to control witchweed in corn, cotton, peanuts and soybeans. The first registered product containing ethylene was registered in December, 1971.

The Agency has assessed the available scientific information about this compound in relation to all its registered uses to determine its eligibility for reregistration. The data base for ethylene is sufficient to allow the Agency to conduct a risk assessment for all uses. Therefore, the Agency has determined that the products containing ethylene for all uses are eligible for reregistration.

Before reregistering each product, the Agency is requiring confidential statements of formula and revised product labeling to be submitted within eight months from the issuance of this document. After reviewing these confidential statements of formula and revised labels, the Agency will determine whether or not the conditions of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) section 3(c)(5) have been met, that is, whether confidential statements of formula and labeling are acceptable and the product's uses will not cause unreasonable adverse effects to humans or the environment. If these conditions are met, the Agency will reregister the products. Those products which contain other active ingredients will be eligible for reregistration only when the other active ingredients are determined to be eligible for reregistration.

I. INTRODUCTION

In 1988, FIFRA was amended to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984. The amended Act provides a schedule for the reregistration process to be completed in nine years. There are five phases to the reregistration process. The first four phases of the process focus on identification of data requirements to support the reregistration of an active ingredient and the generation and submission of data to fulfill the requirements. The fifth phase is a review by the Agency of all data submitted to support reregistration.

Section 4(g)(2)(A) of FIFRA states that in Phase 5 "the Administrator shall determine whether pesticides containing such active ingredient are eligible for reregistration" before calling in data on products under section 4(g)(2)(B), and either reregistering products or taking "other appropriate regulatory action," under section 4(g)(2)(C) and (D). Thus, reregistration involves a thorough review of the scientific data base underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential hazards arising from the currently registered uses of the pesticide; to determine the need for additional data on health and environmental effects; and to determine whether the pesticide meets the "no unreasonable adverse effects" criterion of FIFRA section 3(c)(5).

This document presents the Agency's decision regarding the reregistration eligibility of ethylene. This document consists of five sections. Section I is this introduction. Section II describes ethylene, its uses and regulatory history. Section III discusses the human health and environmental assessment based on the data available to the Agency. Section IV discusses the decision on eligibility for reregistration for ethylene and Section V discusses product reregistration. Additional details concerning the Agency's review of available data are available on request.[1]

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[1] EPA's reviews of data on the set of registered uses considered for EPA's analysis may be obtained from the OPP Public Docket, Field Operations Division (H7506C), Office of Pesticide Programs, EPA, Washington, D.C. 20460.

II. CASE OVERVIEW

A. Chemical Overview

The following active ingredient is covered by this Reregistration Eligibility Document.

Chemical Name: ethylene

CAS Registry Number: 74-85-1

Office of Pesticide Programs Chemical Code: 41901

Empirical Formula: $C_2 H_4$

B. Use Profile

The following is information on the current registered uses and application methods. A detailed table of all uses of ethylene is in Appendix A.

Type of Pesticide: herbicide, plant growth regulator (to accelerate the ripening of harvested fruits and vegetables, curing agent for tobacco)

Target pest (herbicide): witchweed

Use Sites:
Terrestrial Food - fruits, vegetables
Indoor Food - fruits, vegetables
Indoor Nonfood - tobacco
Terrestrial food/feed - (herbicide) - corn, cotton peanuts, soybeans

Formulation Types

Technical Grade: 99.9%
Formulations: 6.2% - 99.5%

Method of Application:

Types of Treatment: ground soil injection (herbicide use), stored commodity fumigation, foliar spray

Equipment:	gas generator, soil injector, pressure sprayer
Timing:	postharvest (for stored commodities), May thru July (for soil injection - herbicide use)
Rates of Application:	See Appendix A

C. Regulatory History

As stated in the Executive Summary the first product containing ethylene was registered in December, 1971. The currently registered products (8) are used as plant growth regulators and herbicides in the sites identified in Section II.B above.

On May 18, 1990, the Agency designated ethylene as a biochemical pesticide based on the following scientific reasons: 1) it is a naturally occurring compound and 2) it has a nontoxic mode of action in target pests/plants.

III. SCIENCE ASSESSMENT OF ETHYLENE

A. Product Chemistry Assessment

Ethylene is a naturally occurring plant growth regulator with a molecular weight of 28.05. It is a colorless, flammable gas. Burns with a luminous flame. One volume of ethylene gas dissolves in about 4 volumes of water at 0°C, in about 9 volumes of water at 25°C, in 0.5 volumes of alcohol at 25°C, and in about 0.05 volume of ether at 15.5°C. It is soluble in acetone and benzene.[2]

[2] The Merck Index. Eleventh Edition, 1989. p. 597.

EPA has reviewed the scientific data base for ethylene primarily relying on information from the published literature. These sources are cited in Appendix B and C.

B. Human Health Assessment

1. Toxicology Data

The Agency believes there are sufficient data from the published literature to make a hazard assessment of the uses of ethylene. Therefore, the Agency is using published sources of information, cited below, rather than requiring new studies from registrants.

Ethylene is a gas and therefore, the only relevant route of exposure of toxicological concern is the pulmonary route. Widespread human exposure from the clinical use of ethylene as an anesthetic in the absence of any reports of significant toxicity are sufficient to allow the Agency to conclude that ethylene will be nontoxic to humans under the conditions of use as a plant regulator or in a witchweed control program.

Ethylene has been used as a clinical anesthetic since 1923. Anesthesia is complete within 20-30 minutes with 90% in oxygen. The percentage of ethylene may be reduced toward 80% in prolonged anesthesia. If the concentration is beyond 90% in animals, death results from respiratory failure. The lethal concentration for mice in air is 950,000 ppm ethylene.[3]

During established anesthesia, respiration is practically normal...and the pulse scarcely changed, excitability of the medullary centers is not lowered, the asphyxia is slight and does not proceed to cyanosis, sweating and salivation are slight or absent, temperature fall is relatively slight, renal efficiency... is not impaired, pulmonary irritation... appears to be absent, in obstetrical use, it does not materially reduce the uterine contractions, and permits prompt respiration to the delivered child, gastric movements are only slightly depressed, ... movements of the small and large intestines are stimulated.[4]

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[3] The Merck Index. Eighth edition, 1968.

[4] T. Sollmann, W.B. Sauders Company. Pharmacology and it's Applications to Therapeutics and Toxicology, 8th edition, 1964,

Ethylene is more advantageous than ether as an anesthetic because of safer induction and more rapid recovery. It is also more advantageous than nitrous oxide because of the practical absence of asphyxia.

The maximum exposure rate to ethylene under the current uses is 1000 ppm in the post-harvest treatment of stored commodities. By contrast, natural internal levels of ethylene in pineapples may reach as high as 1100 ppm and in apples as high as 2500 ppm.

No long-term problems have been attributed directly to the gas. The gas does not have local toxic effects.[5]

2. Dietary Exposure

Ethylene is exempt from the requirement of tolerance (40 CFR 180.1016) for residues when: a) used as a plant regulator on fruit and vegetable crops; or b) injected into the soil to cause premature germination of witchweed in fields of a number of crops as part of the U.S. Department of Agriculture witchweed control program. Therefore no residue data are required because of the lack of concern for mammalian toxicity.

3. Occupational and Residential Exposure

The Agency has waived these data requirements for the following reasons: a) low mammalian toxicity concerns and b) the high volatility of ethylene minimizes the post-application exposure to foliage, soil, dermal and inhalation. However, there is some hazard of dermal and ocular frost burns and of flammability posed by the compressed gas. Therefore, protective clothing, rubber gloves and goggles are required while handling cylinders or any application equipment under pressure.

4. Human Risk Assessment

With the exception of the physical/chemical hazards noted above, the potential risks to humans from occupational exposure are considered negligible due to: a) low toxicity concerns, b) ethylene's widespread use as an anesthetic and c) minimal dermal exposure.

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[5] J. Doull, C.D. Klaassen, M.O. Amdur. The Basic Science of Poisons, 2nd. Edition, 1980. Macmillan, New York.

C. Environmental Assessment

1. Ecological Effects Data

Ethylene is a naturally occurring gas that is produced in plants and acts on nontarget pest(s)/plants(s) through a nontoxic mode of action. Because it is naturally occurring and it has a nontoxic mode of action, ethylene has been classified as a biochemical. Ecotoxicity data are usually required for indoor use of biochemicals depending upon use pattern, production volume and other factors such as volatility. However upon these factors and its classification as a biochemical, no ecological effects studies are required for ethylene for indoor uses.

Data requirements for the outdoor uses have been waived because of its volatile nature, the method of application in the case of soil injection and its relatively low rate of application in the case of sprays to pineapples (2.5 lb/acre). The Agency believes that for the above reasons there will be minimal exposure to aquatic and terrestrial organisms for the outdoor uses of ethylene.

2. Environmental Fate Data

Environmental fate studies are not required for biochemical pesticides unless adverse effects on fish and wildlife observed as a result of acute testing (Tier I) for ecological effects. As stated above, the ecotoxicity studies have been waived for the outdoor uses of ethylene and therefore no environmental fate studies are required.

3. Environmental Risk Assessment

The Agency believes for the reasons stated above that the environmental risks for ethylene products are minimal.

IV. RISK MANAGEMENT AND REREGISTRATION DECISION FOR ETHYLENE

A. Determination of Eligibility

Section 4(g)(2)(A) of FIFRA requires the Agency to determine, after submission of relevant data concerning an active ingredient, whether products containing the active ingredient are eligible for reregistration. The Agency has waived all generic (i.e., active ingredient specific) data requirements except for technical chemistry data and additionally has relied on public literature for mammalian toxicology. The Agency has completed its review

of this information data and other factors and considerations, and has determined this information is sufficient to support reregistration of all products containing ethylene for all uses. Appendix B identifies the data that the Agency reviewed for the determination of reregistration eligibility for ethylene.

The Agency therefore finds that products containing only ethylene as an active ingredient are eligible for reregistration and may be reregistered once the confidential statements of formula and amended labeling are received and accepted by the Agency. Products that contain additional active ingredients will be reregistered once the Agency completes eligibility decisions on the other active ingredients and once product specific and amended labeling are received and accepted. The reregistration of particular products is addressed in Section V of this document ("Product Reregistration").

Although the Agency has found that all products containing ethylene are eligible for reregistration, it should be understood that the Agency may take appropriate regulatory action and/or require the submission of additional data to support reregistration of products containing ethylene, if significant new information of concern comes to the Agency's attention or if the data requirements for registration change.

B. Labeling Requirements for Manufacturing-Use Product(s) of ethylene

1. Under the heading "Directions for Use" add the following statement.

"Only For Formulation Into An_____, [fill blank with Insecticide, Herbicide, or the applicable term(s) which describe the type of pesticidal use(s)] For (1) The Following Use(s): _____ _____; or (2) Uses For Which US EPA Has Accepted The Required Data And/Or Citations of Data That The Formulator Has Submitted In Support of Registration; and (3) Uses For Experimental Purposes That Are In Compliance With US EPA Requirements."

2. The signal word is "DANGER".

3. The Precautionary Statements must read:

"Liquefied or pressurized gas can cause frost burns. Do not get in eyes or on skin. Wear long-sleeved shirt, long pants, boots, goggles and chemical-resistant gloves while handling cylinders or any application equipment under pressure. Harmful if inhaled. Avoid breathing vapors. Do not enter unventilated treatment areas unless wearing a respirator approved by NIOSH/ MSHA for this use."

4. The Statements of Practical Treatment (First Aid) must read:
"IF IN EYES: Flush with plenty of water. Call a physician."

"IF ON SKIN: Wash with plenty of soap and water. Get medical attention."

"IF INHALED: Remove victim to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention."

5. The Physical or Chemical Hazards statement must read:
[For the technical grade product]
"Contents under pressure. Do not store near heat or open flame. Do not puncture or incinerate container. Exposure to temperatures above 130 degrees Fahrenheit may cause bursting."

V. ACTIONS REQUIRED BY REGISTRANTS

A. Determination of Eligibility

Based on consideration of data and information submitted for the active ingredient, ethylene and the registered use patterns, the products containing this active ingredient are eligible for reregistration. Section 4(g)(2)(B) of FIFRA requires that the Agency obtain any needed product-specific data regarding the pesticide following a determination of eligibility. However, the Agency is not requiring any product specific data, it will review the confidential statements of formula and labels of these products to determine whether they may be reregistered.

B. Product Specific Data Requirements

The Agency is primarily relying on information from published literature to meet the data requirements for the technical material. Because the end-use products are similar in composition to the technical material, the Agency is not requiring any further product specific for the products containing ethylene as an active ingredient. Additionally, the labeling requirements prescribed in Section V.C. are sufficient to address the only product that does not have a similar percent amount of active ingredient.

C. Labeling Requirements for End-Use Products

1. The labels and labeling of all products must comply with EPA's current regulations and requirements. Follow the instructions in PR Notice 91-2 (Appendix D) and the Product Reregistration Handbook (Appendix E) with respect to labels and

labeling.

2. The labeling must include the following statement for the foliar spray (pineapple use).

"Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwater or rinsate."

3. The signal word is "DANGER".

4. The Precautionary Statements must read:

"Liquefied or pressurized gas can cause frost burns. Do not get in eyes or on skin. Wear long-sleeved shirt, long pants, boots, goggles and chemical-resistant gloves while handling cylinders or any application equipment under pressure. Harmful if inhaled. Avoid breathing vapors. Do not enter unventilated treatment areas unless wearing a respirator approved by NIOSH/MSHA for this use."

5. The Statements of Practical Treatment (First Aid) must read:

"IF IN EYES: Flush with plenty of water. Call a physician."

"IF ON SKIN: Wash with plenty of soap and water. Get medical attention."

"IF INHALED: Remove victim to fresh air. If not breathing, get artificial respiration, preferably mouth-to-mouth. Get medical attention."

6. The Physical or Chemical Hazards Statement must read:

"Extremely flammable. Contents under pressure. Keep away from fire, sparks and heated surfaces. Do not puncture or incinerate container. Exposure to temperatures above 130 degrees Fahrenheit may cause bursting."

APPENDIX A

ETHYLENE USE PATTERNS SUBJECT TO REREGISTRATION

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls.	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations: Allowed	Geographic Limitations: Disallowed	Use Limitations also see Abbreviations
USES ELIGIBLE FOR REREGISTRATION										
FOOD/FEED USES										
BANANA USE GROUP(S): Indoor Food										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm/1 cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	0.2664 cu.ft/hr/1K cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
				not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
CITRUS FRUITS USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
CORN (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
COTTON (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
GRAPEFRUIT USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls.	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations		Use Limitations also see Abbreviations
								Allowed	Disallowed	
LEMON USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
MELONS USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
MELONS, HONEYDEW USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
ORANGE USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.5	not spec.	not spec.	not spec.	not spec.
PAPAYAS USE GROUP(S): INDOOR FOOD										
PEANUTS (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
PEAR USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.5	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
PERSIMMON USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls.	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations: Allowed	Geographic Limitations: Disallowed	Use Limitations also see Abbreviations
PINEAPPLE USE GROUP(S): TERRESTRIAL FOOD + FEED CROP, INDOOR FOOD										
High volume spray (dilute), Foliar, pressure sprayer	PrGs	na	2.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
SOYBEANS (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
TANGERINES USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
TOMATO USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	150 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	3.33 cu.ft/hr/1K cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	200 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm/1 cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
WALNUT (ENGLISH/BLACK) USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	not spec.	1000 ppm	not spec.	not spec.	.5	not spec.	not spec.	not spec.	not spec.

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls.	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations		Use Limitations also see Abbreviations
								Allowed	Disallowed	
NON-FOOD/NON-FEED USES										
TOBACCO/CIGAR/CIGAR WRAPPING USE GROUP(S): INDOOR NON-FOOD (SEE ALSO ISSUE)										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	120 ppm/1K cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	300 ppm/2.5 K cu.ft.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	300 ppm	not spec.	not spec.	not spec.	not spec.	not spec	not spec.	not spec.

Abbreviations used

Header: not spec. = not specified;
Form: PrGs = Pressurized Gas;
Rate: na = not applicable; AI = Active Ingredient; A = Acre; ppm = parts per million;
Other: Minimum application rate is not in data base at this time.

APPENDIX B

Table of the Generic Data Requirements and Studies Used to Make the Reregistration Decision

GUIDE TO APPENDIX B

Appendix B contains listings of data requirements which support the reregistration for the active ingredient covered by this Reregistration Eligibility Document. This appendix contains generic data requirements that apply to the pesticide (active ingredient) in all products, including data requirements for which a "typical formulation" is the test substance.

The data tables are generally organized according to the following format:

1. Data Requirement (Column 1). The data requirements are listed in the order in which they appear in 40 CFR Part 158. The reference numbers accompanying each test refer to the test protocols set out in the Pesticide Assessment Guidelines, which are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

2. Bibliographic citation (Column 3). If the Agency has acceptable data in its files, this column lists the identifying number of each study. This normally is the Master Record Identification (MRID) number, but may be a GS number if no MRID number has been assigned. Refer to the Bibliography Appendices for a complete citation of the study.

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of ethylene

Guideline Citation	Title of study	Citation
§158.690 Product Chemistry		
151-10	Product Identity	(1)
151-11	Manufacturing Process	(1)
151-12	Discussion of Formation	(1)
151-13	Analysis of samples	41600901
151-15	Certification of limits	(1)
151-16	Analytical Method	(1)
151-17(a)	Color	(1)
151-17(b)	Physical State	(1)
151-17(c)	Odor	(1)
151-17(d)	Melting Point	(1)
151-17(e)	Boiling Point	(1)
151-17(f)	Density	(1)
151-17(g)	Solubility	(1)
151-17(h)	Vapor Pressure	waived
151-17(i)	pH	waived
151-17(j)	Stability	(1)
151-17(k)	Flammability	(1)
151-17(p)	Octanol/water partition	(1)

(1) for all requirements, except analysis of samples, information was obtained from public literature.

ECOLOGICAL EFFECTS

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III.

TOXICOLOGY

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III and relied on public literature.

ENVIRONMENTAL FATE

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III.

RESIDUE CHEMISTRY

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III.

The citations listed throughout this document and Appendix C were used to support these decisions.

APPENDIX C

ETHYLENE BIBLIOGRAPHY

Citations Considered to be Part of the Data Base Supporting the Reregistration of Ethylene

GUIDE TO APPENDIX C

1. CONTENT OF BIBLIOGRAPHY. This bibliography contains citations of all studies considered relevant by EPA in arriving at the positions and conclusions stated elsewhere in the Reregistration Eligibility Document. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions. Selections from other sources including the published literature, in those instances where they have been considered, will be included.

2. UNITS OF ENTRY. The unit of entry in this bibliography is called a "study". In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review, and can be described with a conventional bibliographic citation. The Agency has attempted also to unite basic documents and commentaries upon them, treating them as a single study.

3. IDENTIFICATION OF ENTRIES. The entries in this bibliography are sorted numerically by Master Record Identifier number, or "MRID". This number is unique to the citation, and should be used at any time specific reference is required. It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted studies; see paragraph 4(d)(4) below for further explanation. In a few cases, entries added to the bibliography late in the review may be preceded by a nine-character temporary identifier. These entries are listed after all MRID entries. This temporary identifier number also is to be used whenever specific reference is needed.

4. FORM OF ENTRY. In addition to the MRID, each entry consists of a citation containing standard elements followed, in the case of material submitted to EPA, by a description of the earliest known submission. Bibliographic conventions used reflect the standards of the American National Standards Institute (ANSI), expanded to provide for certain special needs.
   a. Author. Whenever the Agency could confidently identify one, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as author. As a last resort, the Agency has shown the first submitter as author.

   b. Document date. When the date appears as four digits with no question marks, the Agency took it directly from the

document. When a four-digit date is followed by a question mark, the bibliographer deduced the date from evidence in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c. Title. In some cases, it has been necessary for Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. Trailing parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission date. The date of the earliest known submission appears immediately following the word "received."

(2) Administrative number. The next element, immediately following the word "under," is the registration number, experimental use permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is the submitter, following the phrase "submitted by." When authorship is defaulted to the submitter, this element is omitted.

(4) Volume Identification (Accession Numbers). The final element in the trailing parentheses identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL," standing for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume. For example, within accession number 123456, the first study would be 123456-A; the second, 123456-B; the 26th, 123456-Z; and the 27th, 123456-AA.

APPENDIX C

ETHYLENE BIBLIOGRAPHY

MRID Citation

41600901 Weatherson, I. (1990) Ethylene: Product Chemistry: Product Identity and Composition: Lab Project Number: RR-2. Unpublished study prepared by Technology Services Group, Inc. 44 p.

41600902 Weatherson, I. (1990) Ethylene: Product Chemistry: Analysis and Certification of Product Ingredients: Lab Project Number RR-3. Unpublished study prepared by Technology Services Group, Inc.

41644201 Weatherson, I. (1988) Product Chemistry: Physical and Chemical Characteristics of Ethylene: Lab Project Number. Unpublished study prepared by Technology Services Group, Inc. 6 p.

41970001 Hawley, G. (1991) Ethylene--physical/chemical properties. Condensed Chemical Dictionary. 8 edition. 8 p.

41970002 Lewis, B., Von Elbe, G. (1991) Ethylene--flammability. Combustions, Flames and Explosions of Gases(3) 14 p.

41970003 Green, D.; Maloney, J. (1991) Ethylene--physical/chemical properties. Perry's Chemical Engineers Handbook (6): 19 p.

41970004 Weast, R. (1991) Ethylene--physical/chemical properties. CRC Handbook of Chemistry and Physics. 1 edition. 7 p.

42448501 Vilkas, A. (1992) Ethylene: Product Chemistry: Product Identity and Composition: study prepared by Union Carbide Industrial Gases, Inc. 9 p.

42448502 Vilkas, A. (1992) Ethylene; Product Chemistry: Analysis and Certification of Product Ingredients. Study prepared by Union Carbide Industrial Gases, Inc. 5 p.

APPENDIX D

PR Notice 91-2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 30 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

CERTIFIED MAIL

Dear Registrant:

I am pleased to announce that the Environmental Protection Agency (the "Agency") has completed its reregistration eligibility decision on the pesticide active ingredient ethylene.

Enclosed is a **Reregistration Eligibility Document (RED)** for the pesticide active ingredient ethylene. The RED is the Agency's evaluation of ethylene, its conclusions regarding human and environmental risks associated with the current product uses, and its decisions and conditions under which uses and products will be eligible for reregistration. Also enclosed is the **EPA RED facts** and the **Pesticide Reregistration Handbook** which provides instructions to registrants on how to respond to any labeling and data requirements specified in the RED and how to reregister products.

The RED identifies any specific labeling requirements such as restricted use classification, groundwater hazard statements, endangered species precautions, etc., necessary for reregistration based on a review of the generic data for the active ingredient. In addition, in order to be reregistered, all product labeling must be in compliance with format and content labeling as described in 40 CFR §156.10 and all labeling changes imposed by Pesticide Regulation (PR) Notices, and any label changes imposed by this RED.

The Pesticide Reregistration Handbook contains detailed instructions for compliance with the RED and must be followed carefully. There are several key points to remember in preparing your response to the RED:

Within 8 Months of the Date of this Letter

1. For **each product**, you must submit a completed **Application for Reregistration** (EPA Form 8570-1), **five copies of the label and labeling** revised as specified by the RED and in accordance with current requirements, **two completed copies of the Confidential Statement of Formula** (CSF) (EPA Form 8570-4).

2. The labeling and CSF which you submit for **each** product must **comply with P.R. Notice 91-2** (Appendix D). That Notice requires that the amount of active ingredient declared in the ingredient statement must be stated as the **nominal concentration** rather than the lower certified limit. You have two options for submitting a CSF: (1) accept the standard certified limits (see 40 CFR §158.175) or (2) provide certified limits that are supported by the analysis of five batches. If you choose the second option, you must submit or cite the data for the five batches along with a certification statement as described in 40 CFR §158.175(e).

3. Send your Application for Registration to the **Registration Division Product Manager 22 (PM 22) who is assigned to the case, Cynthia Giles-Parker.** Use the correct address shown on page 6 of the enclosed Product Reregistration Handbook (Appendix E). Note that the mailing distribution code for your response is RED-RD-PM22.

Questions on **confidential statement of formula and labeling** (for both End-use and Manufacturing-use products) should be directed to the **Registration Division Product Manager** for ethylene, **Cynthia Giles-Parker at (703) 305 -5540.** Questions on the **generic data requirements** should be directed to **Ruby Whiters, the Chemical Review Manager** in the **Special Review and Reregistration Division at (703) 308-8079.**

The Agency is prepared to meet with any registrants who have questions about responding to the ethylene RED. **If you wish to meet with the Agency, you must contact Mrs. Cynthia Giles-Parker within two weeks of your receipt of the RED.** The Agency intends to have one combined meeting with interested registrants. If there are any requests for such a meeting, the Agency will notify all registrants who requested a meeting of the date, location and time. Requests for a meeting will not extend the 90-day or 8-month response deadlines.

Sincerely yours,

Peter Caulkins for

Daniel M. Barolo, Director
Special Review and
Reregistration Division

Enclosures

registered uses of ethylene do not pose an unreasonable risk to the environment.

Additional Data Required

EPA has waived all generic (that is, active ingredient- specific) data requirements for ethylene except for technical chemistry studies, which have been received and reviewed.

Product Labeling Changes Required

The labels of all registered ethylene products must comply with EPA's current pesticide labeling requirements. A summary of the label additions/changes required for ethylene technical or manufacturing use products appears in the RED.

The following additions/changes are required in the labeling of ethylene end-use products:

- The signal word is "DANGER".
- The Precautionary Statement must read, "Liquefied or pressurized gas can cause frost burns. Do not get in eyes or on skin. Wear long-sleeved shirt, long pants, boots, goggles and chemical-resistant gloves while handling cylinders or any application equipment under pressure. Harmful if inhaled. Avoid breathing vapors. Do not enter unventilated treatment areas unless wearing a respirator approved by NIOSH/MSHA for this use."
- The First Aid Statement of Practical Treatment must read, "IF IN EYES: Flush with plenty of water. Call a physician."

  "IF ON SKIN: Wash with plenty of soap and water. Get medical attention."

  "IF INHALED: Remove victim to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention."
- The Physical or Chemical Hazards Statement must read, "Extremely flammable. Contents under pressure. Keep away from fire, sparks and heated surfaces. Do not puncture or incinerate container. Exposure to temperatures above 130 degrees Fahrenheit may cause bursting."

Regulatory Conclusion

- All registered pesticide products containing the active ingredient ethylene are not likely to cause unreasonable adverse effects in people or the environment, and are eligible for reregistration. These products will be reregistered once the required confidential statement of formula and revised labeling are received and accepted by EPA.

For More Information

EPA is requesting public comments on the Reregistration Eligibility Document (RED) for ethylene during a 60-day time period, as announced

in a Notice of Availability published in the Federal Register. To obtain a copy of the RED or to submit written comments, please contact the Public Response and Program Resources Branch, Field Operations Division (H-7506C), Office of Pesticide Programs (OPP), US EPA, Washington, DC 20460, telephone 703-305-5805.

In the future, the ethylene RED will be available from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, telephone 703-487-4650.

For more information about ethylene or about EPA's pesticide reregistration program, please contact the Special Review and Reregistration Division (H-7508W), OPP, US EPA, Washington, DC 20460, telephone 703-308-8000. For information about reregistration of individual ethylene products, please contact PM Team 22, Registration Division (H-7505C), OPP, US EPA, Washington, DC 20460, telephone 703-305-5540.

For information about the health effects of pesticides, or for assistance in recognizing and managing pesticide poisoning symptoms, please contact the National Pesticides Telecommunications Network (NPTN). Call toll-free 1-800-858-7378, 24 hours a day, seven days a week, or fax your inquiry to 806-743-3094.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PR NOTICE 91-2

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

NOTICE TO MANUFACTURERS, PRODUCERS, FORMULATORS, AND REGISTRANTS OF PESTICIDES

ATTENTION: Persons Responsible for Federal Registration of Pesticide Products.

SUBJECT: Accuracy of Stated Percentages for Ingredients Statement

I. PURPOSE:

The purpose of this notice is to clarify the Office of Pesticide Program's policy with respect to the statement of percentages in a pesticide's label's ingredient statement. Specifically, the amount (percent by weight) of ingredient(s) specified in the ingredient statement on the label must be stated as the nominal concentration of such ingredient(s), as that term is defined in 40 CFR 158.153(i). Accordingly, the Agency has established the nominal concentration as the only acceptable label claim for the amount of active ingredient in the product.

II. BACKGROUND

For some time the Agency has accepted two different methods of identifying on the label what percentage is claimed for the ingredient(s) contained in a pesticide. Some applicants claimed a percentage which represented a level between the upper and the lower certified limits. This was referred to as the nominal concentration. Other applicants claimed the lower limit as the percentage of the ingredient(s) that would be expected to be present in their product at the end of the product's shelf-life. Unfortunately, this led to a great deal of confusion among the regulated industry, the regulators, and the consumers as to exactly how much of a given ingredient was in a given product. The Agency has established the nominal concentration as the only acceptable label claim for the amount of active ingredient in the product.

Current regulations require that the percentage listed in the active ingredient statement be as precise as possible reflecting good manufacturing practices 40 CFR 156.10(g)(5). The certified limits required for each active ingredient are intended to encompass any such "good manufacturing practice" variations 40 CFR 158.175(c)(3).

The upper and lower certified limits, which must be proposed in connection with a product's registration, represent the amounts of an ingredient that may legally be present 40 CFR 158.175. The lower certified limit is used as the enforceable lower limit for the product composition according to FIFRA section 12(a)(1)(C), while the nominal concentration appearing on the label would be the routinely achieved concentration used for calculation of dosages and dilutions.

The nominal concentration would in fact state the greatest degree of accuracy that is warranted with respect to actual product composition because the nominal concentration would be the amount of active ingredient typically found in the product.

It is important for registrants to note that certified limits for active ingredients are not considered to be trade secret information, under FIFRA section 10(b). In this respect the certified limits will be routinely provided by EPA to States for enforcement purposes, since the nominal concentration appearing on the label may not represent the enforceable composition for purposes of section 12(a)(1)(C).

III. REQUIREMENTS

As described below under Unit V. " COMPLIANCE SCHEDULE," all currently registered products as well as all applications for new registration must comply with this Notice by specifying the nominal concentration expressed as a percentage by weight as the label claim in the ingredient(s) statement and equivalence statements if applicable (e.g., elemental arsenic, metallic zinc, salt of an acid). In addition, the requirement for performing sample analyses of five or more representative samples must be fulfilled. Copies of the raw analytical data must be submitted with the nominal ingredient label claim. Further information about the analysis requirement may be found in the 40 CFR 158.170. All products are required to provide certified limits for each active, inert ingredient, impurities of toxicological significance(i.e., upper limit(s) only) and on a case by case basis as specified by EPA. These limits are to be set based on representative sampling and chemical analysis(i.e., quality control) of the product.

The format of the ingredient statement must conform to 40 CFR 156-Labeling Requirements For Pesticides and Devices.

After July 1, 1997, all pesticide ingredient statements must be changed to nominal concentration.

IV. PRODUCTS THAT REQUIRE EFFICACY DATA

All pesticides are required to be efficacious. Therefore, the certified lower limits may not be lower then the minimum level to achieve efficacy. This is extremely important for products which are intended to control pests which threaten the public health, e.g., certain antimicrobial and rodenticide products. Refer to 40 CFR 158.640.

In those cases where efficacy limits have been established, the Agency will not accept certified lower limits which are below that level for the shelf life of the product.

V. COMPLIANCE SCHEDULE

As described earlier, the purpose of this Notice is to make the registration process more uniform and more manageable for both the agency and the regulated community. It is the Agency's intention to implement the requirements of this notice as smoothly as possible so as not to disrupt or delay the Agency's high priority programs, i.e., reregistration, new chemical, or fast track (FIFRA section 3(c)(3)(B). Therefore, applicants/registrants are expected to comply with the requirements of this Notice as follows:

(1) Beginning July 1, 1991, all new product registrations submitted to the Agency are to comply with the requirements of this Notice.

(2) Registrants having products subject to reregistration under FIFRA section 4(a) are to comply with the requirements of this Notice when specific products are called in by the Agency under Phase V of the Reregistration Program.

(3) All other products/applications that are not subject to (1) and (2) above will have until July 1, 1997, to comply with this Notice. Such applications should note "Conversion to Nominal Concentration" on the application form. These types of amendments will not be handled as "Fast Track" applications but will be handled as routine requests.

VI. FOR FURTHER INFORMATION

Contact Tyrone Aiken for information or questions concerning this notice on (703) 557-5024.

Anne E. Lindsay

Anne E. Lindsay, Director
Registration Division (H-7505

APPENDIX E

Pesticide Reregistration Handbook

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 30 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

CERTIFIED MAIL

Dear Registrant:

I am pleased to announce that the Environmental Protection Agency (the "Agency") has completed its reregistration eligibility decision on the pesticide active ingredient ethylene.

Enclosed is a **Reregistration Eligibility Document (RED)** for the pesticide active ingredient ethylene. The RED is the Agency's evaluation of ethylene, its conclusions regarding human and environmental risks associated with the current product uses, and its decisions and conditions under which uses and products will be eligible for reregistration. Also enclosed is the **EPA RED facts** and the **Pesticide Reregistration Handbook** which provides instructions to registrants on how to respond to any labeling and data requirements specified in the RED and how to reregister products.

The RED identifies any specific labeling requirements such as restricted use classification, groundwater hazard statements, endangered species precautions, etc., necessary for reregistration based on a review of the generic data for the active ingredient. In addition, in order to be reregistered, all product labeling must be in compliance with format and content labeling as described in 40 CFR §156.10 and all labeling changes imposed by Pesticide Regulation (PR) Notices, and any label changes imposed by this RED.

The Pesticide Reregistration Handbook contains detailed instructions for compliance with the RED and must be followed carefully. There are several key points to remember in preparing your response to the RED:

Within 8 Months of the Date of this Letter

1. For **each product**, you must submit a completed **Application for Reregistration** (EPA Form 8570-1), **five copies of the label and labeling** revised as specified by the RED and in accordance with current requirements, **two completed copies of the Confidential Statement of Formula** (CSF) (EPA Form 8570-4).

2. The labeling and CSF which you submit for **each** product must **comply with P.R. Notice 91-2** (Appendix D). That Notice requires that the amount of active ingredient declared in the ingredient statement must be stated as the **nominal concentration** rather than the lower certified limit. You have two options for submitting a CSF: (1) accept the standard certified limits (see 40 CFR §158.175) or (2) provide certified limits that are supported by the analysis of five batches. If you choose the second option, you must submit or cite the data for the five batches along with a certification statement as described in 40 CFR §158.175(e).

3. Send your Application for Registration to the **Registration Division Product Manager 22 (PM 22) who is assigned to the case, Cynthia Giles-Parker.** Use the correct address shown on page 6 of the enclosed Product Reregistration Handbook (Appendix E). Note that the mailing distribution code for your response is RED-RD-PM22.

Questions on **confidential statement of formula and labeling** (for both End-use and Manufacturing-use products) should be directed to the **Registration Division Product Manager** for ethylene, **Cynthia Giles-Parker at (703) 305 -5540.** Questions on the **generic data requirements** should be directed to **Ruby Whiters, the Chemical Review Manager** in the **Special Review and Reregistration Division at (703) 308-8079.**

The Agency is prepared to meet with any registrants who have questions about responding to the ethylene RED. **If you wish to meet with the Agency, you must contact Mrs. Cynthia Giles-Parker within two weeks of your receipt of the RED.** The Agency intends to have one combined meeting with interested registrants. If there are any requests for such a meeting, the Agency will notify all registrants who requested a meeting of the date, location and time. Requests for a meeting will not extend the 90-day or 8-month response deadlines.

Sincerely yours,

Peter Caulkins for

Daniel M. Barolo, Director
Special Review and
Reregistration Division

Enclosures

4. The Statements of Practical Treatment (First Aid) must read:
"IF IN EYES: Flush with plenty of water. Call a physician."

"IF ON SKIN: Wash with plenty of soap and water. Get medical attention."

"IF INHALED: Remove victim to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. Get medical attention."

5. The Physical or Chemical Hazards statement must read:
[For the technical grade product]
"Contents under pressure. Do not store near heat or open flame. Do not puncture or incinerate container. Exposure to temperatures above 130 degrees Fahrenheit may cause bursting."

V. ACTIONS REQUIRED BY REGISTRANTS

A. Determination of Eligibility

Based on consideration of data and information submitted for the active ingredient, ethylene and the registered use patterns, the products containing this active ingredient are eligible for reregistration. Section 4(g)(2)(B) of FIFRA requires that the Agency obtain any needed product-specific data regarding the pesticide following a determination of eligibility. However, the Agency is not requiring any product specific data, it will review the confidential statements of formula and labels of these products to determine whether they may be reregistered.

B. Product Specific Data Requirements

The Agency is primarily relying on information from published literature to meet the data requirements for the technical material. Because the end-use products are similar in composition to the technical material, the Agency is not requiring any further product specific for the products containing ethylene as an active ingredient. Additionally, the labeling requirements prescribed in Section V.C. are sufficient to address the only product that does not have a similar percent amount of active ingredient.

C. Labeling Requirements for End-Use Products

1. The labels and labeling of all products must comply with EPA's current regulations and requirements. Follow the instructions in PR Notice 91-2 (Appendix D) and the Product Reregistration Handbook (Appendix E) with respect to labels and

APPENDIX D

PR Notice 91-2

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PR NOTICE 91-2

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

NOTICE TO MANUFACTURERS, PRODUCERS, FORMULATORS, AND REGISTRANTS OF PESTICIDES

ATTENTION: Persons Responsible for Federal Registration of Pesticide Products.

SUBJECT: Accuracy of Stated Percentages for Ingredients Statement

I. PURPOSE:

The purpose of this notice is to clarify the Office of Pesticide Program's policy with respect to the statement of percentages in a pesticide's label's ingredient statement. Specifically, the amount (percent by weight) of ingredient(s) specified in the ingredient statement on the label must be stated as the nominal concentration of such ingredient(s), as that term is defined in 40 CFR 158.153(i). Accordingly, the Agency has established the nominal concentration as the only acceptable label claim for the amount of active ingredient in the product.

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It is important for registrants to note that certified limits for active ingredients are not considered to be trade secret information, under FIFRA section 10(b). In this respect the certified limits will be routinely provided by EPA to States for enforcement purposes, since the nominal concentration appearing on the label may not represent the enforceable composition for purposes of section 12(a)(1)(C).

III. REQUIREMENTS

As described below under Unit V. " COMPLIANCE SCHEDULE," all currently registered products as well as all applications for new registration must comply with this Notice by specifying the nominal concentration expressed as a percentage by weight as the label claim in the ingredient(s) statement and equivalence statements if applicable (e.g., elemental arsenic, metallic zinc, salt of an acid). In addition, the requirement for performing sample analyses of five or more representative samples must be fulfilled. Copies of the raw analytical data must be submitted with the nominal ingredient label claim. Further information about the analysis requirement may be found in the 40 CFR 158.170. All products are required to provide certified limits for each active, inert ingredient, impurities of toxicological significance(i.e., upper limit(s) only) and on a case by case basis as specified by EPA. These limits are to be set based on representative sampling and chemical analysis(i.e., quality control) of the product.

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VI. FOR FURTHER INFORMATION

Contact Tyrone Aiken for information or questions concerning this notice on (703) 557-5024.

Anne E. Lindsay

Anne E. Lindsay, Director
Registration Division (H-7505

APPENDIX E

Pesticide Reregistration Handbook

labeling.

2. The labeling must include the following statement for the foliar spray (pineapple use).

"Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of equipment washwater or rinsate."

3. The signal word is "DANGER".

4. The Precautionary Statements must read:

"Liquefied or pressurized gas can cause frost burns. Do not get in eyes or on skin. Wear long-sleeved shirt, long pants, boots, goggles and chemical-resistant gloves while handling cylinders or any application equipment under pressure. Harmful if inhaled. Avoid breathing vapors. Do not enter unventilated treatment areas unless wearing a respirator approved by NIOSH/MSHA for this use."

5. The Statements of Practical Treatment (First Aid) must read:

"IF IN EYES: Flush with plenty of water. Call a physician."

"IF ON SKIN: Wash with plenty of soap and water. Get medical attention."

"IF INHALED: Remove victim to fresh air. If not breathing, get artificial respiration, preferably mouth-to-mouth. Get medical attention."

6. The Physical or Chemical Hazards Statement must read:

"Extremely flammable. Contents under pressure. Keep away from fire, sparks and heated surfaces. Do not puncture or incinerate container. Exposure to temperatures above 130 degrees Fahrenheit may cause bursting."

APPENDIX A

ETHYLENE USE PATTERNS SUBJECT TO REREGISTRATION

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Apps	Max. # Apps. @ Max. Rate	Min. Interval Between Apps. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations: Allowed	Geographic Limitations: Disallowed	Use Limitations also see Abbreviations
USES ELIGIBLE FOR REREGISTRATION										
FOOD/FEED USES										
BANANA USE GROUP(S): Indoor Food										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm/1 cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	0.2664 cu.ft/hr/1K cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
				not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
CITRUS FRUITS USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
CORN (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
COTTON (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
GRAPEFRUIT USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls.	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations		Use Limitations also see Abbreviations
								Allowed	Disallowed	
LEMON USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
MELONS USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
MELONS, HONEYDEW USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
ORANGE USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.5	not spec.	not spec.	not spec.	not spec.
PAPAYAS USE GROUP(S): INDOOR FOOD										
PEANUTS (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
PEAR USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.5	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
PERSIMMON USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls.	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations		Use Limitations also see Abbreviations
								Allowed	Disallowed	
PINEAPPLE USE GROUP(S): TERRESTRIAL FOOD + FEED CROP, INDOOR FOOD										
High volume spray (dilute), Foliar, pressure sprayer	PrGs	na	2.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
SOYBEANS (UNSPECIFIED) USE GROUP(S): TERRESTRIAL FOOD + FEED CROP										
Soil injection treatment, May, June, July, soil injection equipment	PrGs	na	1.5 lb AI/A	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
TANGERINES USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	5 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
TOMATO USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	150 ppm	not spec.	not spec.	.25	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	3.33 cu.ft/hr/1K cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	200 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm/1 cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	1000 ppm	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
WALNUT (ENGLISH/BLACK) USE GROUP(S): INDOOR FOOD										
Stored commodity fumigation, Postharvest, cylinder	PrGs	not spec.	1000 ppm	not spec.	not spec.	.5	not spec.	not spec.	not spec.	not spec.

APPENDIX A - Case 3071, [Ethylene] Chemical 041901 [Ethylene]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max. # Appls	Max. # Appls. @ Max. Rate	Min. Interval Between Appls. @ Max. Rate (Days)	Restricted Entry Interval (Days)	Geographic Limitations		Use Limitations also see Abbreviations
								Allowed	Disallowed	
NON-FOOD/NON-FEED USES										
TOBACCO/CIGAR/CIGAR WRAPPING USE GROUP(S): INDOOR NON-FOOD (SEE ALSO ISSUE)										
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	120 ppm/1K cu.ft	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	300 ppm/2.5 K cu.ft.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.	not spec.
Stored commodity fumigation, Postharvest, cylinder	PrGs	na	300 ppm	not spec.	not spec.	not spec.	not spec.	not spec	not spec.	not spec.

Abbreviations used

Header: not spec. = not specified;
Form: PrGs = Pressurized Gas;
Rate: na = not applicable; AI = Active Ingredient; A = Acre; ppm = parts per million;
Other: Minimum application rate is not in data base at this time.

APPENDIX B

Table of the Generic Data Requirements and Studies Used to Make the Reregistration Decision

GUIDE TO APPENDIX B

Appendix B contains listings of data requirements which support the reregistration for the active ingredient covered by this Reregistration Eligibility Document. This appendix contains generic data requirements that apply to the pesticide (active ingredient) in all products, including data requirements for which a "typical formulation" is the test substance.

The data tables are generally organized according to the following format:

1. Data Requirement (Column 1). The data requirements are listed in the order in which they appear in 40 CFR Part 158. The reference numbers accompanying each test refer to the test protocols set out in the Pesticide Assessment Guidelines, which are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

2. Bibliographic citation (Column 3). If the Agency has acceptable data in its files, this column lists the identifying number of each study. This normally is the Master Record Identification (MRID) number, but may be a GS number if no MRID number has been assigned. Refer to the Bibliography Appendices for a complete citation of the study.

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of ethylene

Guideline Citation	Title of study	Citation
§158.690 Product Chemistry		
151-10	Product Identity	(1)
151-11	Manufacturing Process	(1)
151-12	Discussion of Formation	(1)
151-13	Analysis of samples	41600901
151-15	Certification of limits	(1)
151-16	Analytical Method	(1)
151-17(a)	Color	(1)
151-17(b)	Physical State	(1)
151-17(c)	Odor	(1)
151-17(d)	Melting Point	(1)
151-17(e)	Boiling Point	(1)
151-17(f)	Density	(1)
151-17(g)	Solubility	(1)
151-17(h)	Vapor Pressure	waived
151-17(i)	pH	waived
151-17(j)	Stability	(1)
151-17(k)	Flammability	(1)
151-17(p)	Octanol/water partition	(1)

(1) for all requirements, except analysis of samples, information was obtained from public literature.

ECOLOGICAL EFFECTS

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III.

TOXICOLOGY

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III and relied on public literature.

ENVIRONMENTAL FATE

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III.

RESIDUE CHEMISTRY

EPA waived 40 CFR Part 158 requirements for reasons discussed in section III.

The citations listed throughout this document and Appendix C were used to support these decisions.

APPENDIX C

ETHYLENE BIBLIOGRAPHY

Citations Considered to be Part of the Data Base Supporting the Reregistration of Ethylene

GUIDE TO APPENDIX C

1. CONTENT OF BIBLIOGRAPHY. This bibliography contains citations of all studies considered relevant by EPA in arriving at the positions and conclusions stated elsewhere in the Reregistration Eligibility Document. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions. Selections from other sources including the published literature, in those instances where they have been considered, will be included.

2. UNITS OF ENTRY. The unit of entry in this bibliography is called a "study". In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review, and can be described with a conventional bibliographic citation. The Agency has attempted also to unite basic documents and commentaries upon them, treating them as a single study.

3. IDENTIFICATION OF ENTRIES. The entries in this bibliography are sorted numerically by Master Record Identifier number, or "MRID". This number is unique to the citation, and should be used at any time specific reference is required. It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted studies; see paragraph 4(d)(4) below for further explanation. In a few cases, entries added to the bibliography late in the review may be preceded by a nine-character temporary identifier. These entries are listed after all MRID entries. This temporary identifier number also is to be used whenever specific reference is needed.

4. FORM OF ENTRY. In addition to the MRID, each entry consists of a citation containing standard elements followed, in the case of material submitted to EPA, by a description of the earliest known submission. Bibliographic conventions used reflect the standards of the American National Standards Institute (ANSI), expanded to provide for certain special needs.
   a. Author. Whenever the Agency could confidently identify one, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as author. As a last resort, the Agency has shown the first submitter as author.

   b. Document date. When the date appears as four digits with no question marks, the Agency took it directly from the

document. When a four-digit date is followed by a question mark, the bibliographer deduced the date from evidence in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c. Title. In some cases, it has been necessary for Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d. Trailing parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1) Submission date. The date of the earliest known submission appears immediately following the word "received."

(2) Administrative number. The next element, immediately following the word "under," is the registration number, experimental use permit number, petition number, or other administrative number associated with the earliest known submission.

(3) Submitter. The third element is the submitter, following the phrase "submitted by." When authorship is defaulted to the submitter, this element is omitted.

(4) Volume Identification (Accession Numbers). The final element in the trailing parentheses identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL," standing for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume. For example, within accession number 123456, the first study would be 123456-A; the second, 123456-B; the 26th, 123456-Z; and the 27th, 123456-AA.

APPENDIX C

ETHYLENE BIBLIOGRAPHY

MRID Citation

41600901 Weatherson, I. (1990) Ethylene: Product Chemistry: Product Identity and Composition: Lab Project Number: RR-2. Unpublished study prepared by Technology Services Group, Inc. 44 p.

41600902 Weatherson, I. (1990) Ethylene: Product Chemistry: Analysis and Certification of Product Ingredients: Lab Project Number RR-3. Unpublished study prepared by Technology Services Group, Inc.

41644201 Weatherson, I. (1988) Product Chemistry: Physical and Chemical Characteristics of Ethylene: Lab Project Number. Unpublished study prepared by Technology Services Group, Inc. 6 p.

41970001 Hawley, G. (1991) Ethylene--physical/chemical properties. Condensed Chemical Dictionary. 8 edition. 8 p.

41970002 Lewis, B., Von Elbe, G. (1991) Ethylene--flammability. Combustions, Flames and Explosions of Gases(3) 14 p.

41970003 Green, D.; Maloney, J. (1991) Ethylene--physical/chemical properties. Perry's Chemical Engineers Handbook (6): 19 p.

41970004 Weast, R. (1991) Ethylene--physical/chemical properties. CRC Handbook of Chemistry and Physics. 1 edition. 7 p.

42448501 Vilkas, A. (1992) Ethylene: Product Chemistry: Product Identity and Composition: study prepared by Union Carbide Industrial Gases, Inc. 9 p.

42448502 Vilkas, A. (1992) Ethylene; Product Chemistry: Analysis and Certification of Product Ingredients. Study prepared by Union Carbide Industrial Gases, Inc. 5 p.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 30 1992

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

CERTIFIED MAIL

Dear Registrant:

I am pleased to announce that the Environmental Protection Agency (the "Agency") has completed its reregistration eligibility decision on the pesticide active ingredient ethylene.

Enclosed is a **Reregistration Eligibility Document (RED)** for the pesticide active ingredient ethylene. The RED is the Agency's evaluation of ethylene, its conclusions regarding human and environmental risks associated with the current product uses, and its decisions and conditions under which uses and products will be eligible for reregistration. Also enclosed is the **EPA RED facts** and the **Pesticide Reregistration Handbook** which provides instructions to registrants on how to respond to any labeling and data requirements specified in the RED and how to reregister products.

The RED identifies any specific labeling requirements such as restricted use classification, groundwater hazard statements, endangered species precautions, etc., necessary for reregistration based on a review of the generic data for the active ingredient. In addition, in order to be reregistered, all product labeling must be in compliance with format and content labeling as described in 40 CFR §156.10 and all labeling changes imposed by Pesticide Regulation (PR) Notices, and any label changes imposed by this RED.

The Pesticide Reregistration Handbook contains detailed instructions for compliance with the RED and must be followed carefully. There are several key points to remember in preparing your response to the RED:

Within 8 Months of the Date of this Letter

1. For **each product**, you must submit a completed **Application for Reregistration** (EPA Form 8570-1), **five copies of the label and labeling** revised as specified by the RED and in accordance with current requirements, **two completed copies of the Confidential Statement of Formula** (CSF) (EPA Form 8570-4).

2. The labeling and CSF which you submit for **each** product must **comply with P.R. Notice 91-2** (Appendix D). That Notice requires that the amount of active ingredient declared in the ingredient statement must be stated as the **nominal concentration** rather than the lower certified limit. You have two options for submitting a CSF: (1) accept the standard certified limits (see 40 CFR §158.175) or (2) provide certified limits that are supported by the analysis of five batches. If you choose the second option, you must submit or cite the data for the five batches along with a certification statement as described in 40 CFR §158.175(e).

3. Send your Application for Registration to the **Registration Division Product Manager 22 (PM 22) who is assigned to the case, Cynthia Giles-Parker.** Use the correct address shown on page 6 of the enclosed Product Reregistration Handbook (Appendix E). Note that the mailing distribution code for your response is RED-RD-PM22.

Questions on **confidential statement of formula and labeling** (for both End-use and Manufacturing-use products) should be directed to the **Registration Division Product Manager** for ethylene, **Cynthia Giles-Parker at (703) 305 -5540.** Questions on the **generic data requirements** should be directed to **Ruby Whiters, the Chemical Review Manager** in the **Special Review and Reregistration Division at (703) 308-8079.**

The Agency is prepared to meet with any registrants who have questions about responding to the ethylene RED. **If you wish to meet with the Agency, you must contact Mrs. Cynthia Giles-Parker within two weeks of your receipt of the RED.** The Agency intends to have one combined meeting with interested registrants. If there are any requests for such a meeting, the Agency will notify all registrants who requested a meeting of the date, location and time. Requests for a meeting will not extend the 90-day or 8-month response deadlines.

Sincerely yours,

Peter Caulkins for

Daniel M. Barolo, Director
Special Review and
Reregistration Division

Enclosures

United States Environmental Protection Agency | Office of Prevention, Pesticides And Toxic Substances (H-7508W) | EPA-738-F-92-012 September 1992

EPA R.E.D. FACTS

Ethylene

Pesticide Reregistration

All pesticides sold or used in the United States must be registered by EPA, based on scientific studies showing that they can be used without posing unreasonable risks to people or the environment. Because of advances in scientific knowledge, the law requires that pesticides which were first registered years ago be reregistered to ensure that they meet today's more stringent standards.

In evaluating pesticides for reregistration, EPA obtains and reviews a complete set of studies from pesticide producers, describing the human health and environmental effects of each pesticide. The Agency imposes any regulatory controls that are needed to effectively manage each pesticide's risks. EPA then reregisters pesticides that can be used without posing undue hazards to human health or the environment.

When a pesticide is eligible for reregistration, EPA announces this and explains why in a Reregistration Eligibility Document, or RED. This fact sheet summarizes the information in the RED for ethylene.

Use Profile

The pesticide ethylene is registered for use as a plant growth regulator and a herbicide. Ethylene is used commercially as a ripening agent for fruits and vegetables, a curing agent for tobacco, and a flower-producing agent in pineapples. It also is used to control witchweed in corn, cotton, peanuts and soybeans.

Regulatory History

The first pesticide product containing ethylene as an active ingredient was registered in December 1971. In May 1990, EPA designated ethylene as a biorational pesticide because it is naturally occurring and has a nontoxic mode of action in controlling target pests. Currently, eight pesticide products containing ethylene are registered with EPA.

Ethylene is exempt from the requirement of a tolerance (or maximum residue level) when used as a plant growth regulator on fruit and vegetable crops, or when injected into the soil to cause premature germination of witchweed, as part of the U.S. Department of Agriculture (USDA) witchweed control program. (Please see 40 CFR 180.1016.)

Human Health Assessment

Toxicity

EPA used information from the published literature rather than requiring new studies from registrants to assess the toxicity of ethylene.

Ethylene is a gas; therefore, the only exposure of toxicological concern is exposure to the lungs. Ethylene is naturally occurring and has been used widely as an anesthetic since 1923 without reports of significant toxicity. Therefore, EPA concludes that ethylene will be nontoxic to humans under its approved conditions of use as a plant growth regulator and in witchweed control programs.

Dietary Exposure

Ethylene is exempt from tolerance requirements, as mentioned earlier. EPA is requiring no residue data for reregistration because ethylene poses no dietary risk concerns.

Occupational and Residential Exposure

EPA has waived requirements for applicator and residential exposure studies because ethylene poses no mammalian toxicity concerns. In addition, due to its high volatility, people are not likely to be exposed to ethylene once it has been applied to fruit, vegetables or soil.

Human Risk Assessment

The potential risks to people from the pesticide uses of ethylene are considered negligible because ethylene is of low toxicity, high volatility (so exposure to treated foliage and foods as well as skin and lungs is minimal), and has had years of safe use as an anesthetic.

Environmental Assessment

Environmental Fate

Since ethylene is a biorational pesticide, environmental fate studies would not be required unless adverse effects on fish and wildlife were noted in ecological effects studies. As explained below, all ecotoxicity studies have been waived. Therefore, environmental fate studies are not required.

Ecological Effects

EPA has waived the ecological effects data requirements for both the indoor and outdoor uses of ethylene. Because it is a volatile gas, ethylene used indoors is not likely to result in exposure to nontarget species. The outdoor uses, soil injection and pineapple sprays, will result in only negligible exposure to aquatic and terrestrial organisms. Ethylene is naturally occurring and of low toxicity. Therefore, no data are required for reregistration of the outdoor uses.

Environmental and Ecological Risk Assessment

Ethylene is a naturally occurring, volatile gas, regarded as a biorational pesticide due to its low toxicity. Therefore, EPA finds that the

Ethylene
Final Work Plan

Registration Review Case 3071
Docket Number EPA-HQ-OPP-2009-0877

Ethylene
Final Work Plan
Registration Review Case Number: 3071

Approved By:

W. Michael McDavit

W. Michael McDavit, Acting Director
Biopesticides and Pollution Prevention Division

Date: 6/18/10

I. INTRODUCTION

This document is EPA's Final Work Plan for Ethylene. The work plan includes the expected registration review timelines. The final work plan is intended to address any public comments received concerning the Preliminary Work Plan in the Summary Document, which was posted in Ethylene's registration review docket (EPA-HQ-OPP-2009-0877) concerning the initial docket postings. The Summary Document provided information on what EPA knows about the pesticide and what additional risk analyses and data or information the Agency believes are needed to make a registration review decision.

The Agency is implementing the Registration Review program and plans to review each registered pesticide every 15 years to determine whether it continues to meet the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) standard for registration. Changes in science, public policy, and pesticide use practices will occur over time. The registration review program is intended to make sure that, as the ability to assess risk evolves and as policies and practices change, all registered pesticides continue to meet that statutory standard. The public phase of registration review begins when the initial docket is opened for each case. Information on this program is provided at: http://www.epa.gov/oppsrrd1/registration_review/.

Ethylene is a naturally occurring plant growth regulator with a significant history of human exposure via natural and anthropogenic sources, including use on food commodities and in anesthesia practices. The first product containing Ethylene was registered by the Agency in December, 1971. Currently, there are nine active Ethylene end-use product(s) (EPs) with active registrations with the Agency. These products are registered for use as plant growth regulators and herbicides. On May 18, 1990, the Agency classified Ethylene as a biochemical pesticide because it is a naturally occurring compound with a nontoxic mode of action to the target pest(s) / plant(s), and has a history of exposure to humans and the environment without any reported negative effects.

II. RESPONSES TO COMMENTS

During the 60 day comment period that closed on February 26, 2010, the Agency received one comment from the Olefins Panel (producers of Ethylene as a commodity chemical) of the American Chemistry Council in response to the Preliminary Work Plan for Ethylene associated with Registration Review Docket# EPA-HQ-OPP-2009-0877.

The Olefins Panel informed the Agency that they are currently sponsoring a guideline 90-day inhalation study on Ethylene in rats, as well as several metabolism studies preformed *in vivo* (rats and mice) and *in vitro* (rat, mouse, and human tissue). The Panel indicated that they will provide the Agency with the final report upon completion of the study.

In response, the Agency thanked the Panel for this comment and looks forward to reviewing the final report should the Panel decide to submit it. The Agency regards this information as supplemental. Based on the current risk assessment for Ethylene, the Agency does not foresee the need for any additional toxicity information.

III. RISK ASSESSMENT AND DATA NEEDS

Product Chemistry

All product chemistry data requirements for Ethylene have been satisfied. Assessment of these data shows that they continue to meet the standard for registration under FIFRA, as amended by the FQPA. No further product chemistry data are required. Please refer to the Summary Document posted in the initial docket (EPA-HQ-OPP-2009-0877) for a more detailed discussion.

Human Health Risk Assessment

Based on the available information, the Agency does not foresee the need for new data or a new human health risk assessment. At this time, the currently registered EPs contain the signal word and label precautionary statements consistent with Toxicity Category I. Label PPE requirements of long-sleeved shirt, long pants, boots, goggles, and chemical-resistant gloves during handing of gas cylinders and product application are consistent with acute dermal Toxicity Category III. Label language also requires the use of a NIOSH/MSHA approved respirator when entering unventilated treatment areas. In addition, based on Ethylene's physical and chemical properties, it is considered to be non-persistent in the environment. It degrades rapidly in the environment and, therefore, human exposure to pesticidal residues of Ethylene is expected to be minimal. The Agency has also investigated the potential for Ethylene to be classified as a carcinogen. These concerns were raised by an OECD report in 1996. Specifically, the Agency has reviewed a previously submitted two-year chronic inhalation study for Ethylene submitted by Permviro Systems, Inc. (MRID 41600904). Based on these reviews, the Agency has concluded with reasonable certainty that no harm to the general population will result from the use of products containing Ethylene as their active ingredient when used according to label instructions.

All data requirements per 40 CFR 158.2050 have been fulfilled for Ethylene. Please refer to the Summary Document posted in the initial docket (EPA-HQ-OPP-2009-0877) for a more detailed discussion.

Environmental Fate and Ecological Risk Assessment

1. Effects on Nontarget Organisms

All nontarget toxicity data requirements for Ethylene have been fulfilled and meet the standard for registration required under FIFRA, as amended by FQPA. Based on the available information for Ethylene, the Agency does not foresee the need for additional

ecotoxicity data or a new risk assessment. Please refer the Summary Document posted in the initial docket (EPA-HQ-OPP-2009-0877) for a more detailed discussion.

2. Endangered Species Assessment

Non-target studies and information reviewed by the Agency indicate that Ethylene is not harmful to terrestrial or aquatic plants and wildlife at the current label use rates. Physical properties show that ethylene is volatile and will rapidly dissipate in the atmosphere.

Pesticide use of Ethylene in the field is environmentally insignificant compared to other emissions that occur naturally in the environment. The Agency evaluated this scenario by using the Terrestrial Residue Exposure Model (T-REX) Version 1.4.1 in order to perform a terrestrial risk assessment that included numerous calculations of dietary exposure for multiple weight class animals. The expected ethylene residues were calculated for avian and mammalian species along with dissipation rate of the chemical applied to foliar surfaces (single or multiple applications) in order to estimate acute and chronic risk quotients (RQs) relative to weight class for various sized birds and mammals. The model generated output included median bound Kenega dietary values that suggest this ethylene exposure has a low potential for toxic impact to birds (RQ = 0.01 – 0.06) and mammals (RQ= 0.01- 0.06) that may be feeding in the area of application. These low RQ values combined with information that ethylene is highly volatile in the environment, show that ethylene exposure should not exceed the Agency's level of concern (LOC = 0.2-0.5) for avian and mammalian non target organisms and endangered/threatened species (LOC = 0.1). As a result of these analyses, the Agency has determined that the registered uses of Ethylene will have "No Effect" on endangered and threatened terrestrial or aquatic species, or any designated critical habitat, as listed by the United States Fish and Wildlife Service and the National Marine Fisheries Service. Ethylene's Endangered Species Assessment' and the results of the Individual Effects Model can be found in the Registration Review docket EPA-HQ-2009-0877.

The Agency is also in the process of reviewing the conventional ethephon, an organophosphonate that releases ethylene as a degradate, and may be asking for additional data due to uncertainty related to potential non-target plant exposure from this compound as it degrades in the environment. However, as detailed in this document, the Agency believes that the use of pesticides currently formulated with active ingredient ethylene do not appear to raise the same concern for reasons outlined above.

IV. ESTIMATED TIME

EPA has created the following estimated timeline for completion of Ethylene's registration review case.

Activities	Estimated Month/Year
Phase 1: Opening the docket	
Open Public Comment Period for Ethylene	December 2009
Close Public Comment Period	February 2010
Phase 2: Case Development	

Develop Final Work Plan (FWP)	June 2010
Phase 3: Registration Review Decision	
Open Public Comment Period for Proposed Reg. Review Decision	November 2010
Close Public Comment Period	January 2011
Final Decision and Begin Post-Decision Follow-up	February 2011
*Estimated Total (years)	14 months

* This schedule is subject to revision should unforeseen issues arise during the registration review process.

V. NEXT STEPS

EPA will issue a Proposed Final Decision for public comment in November, 2010

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

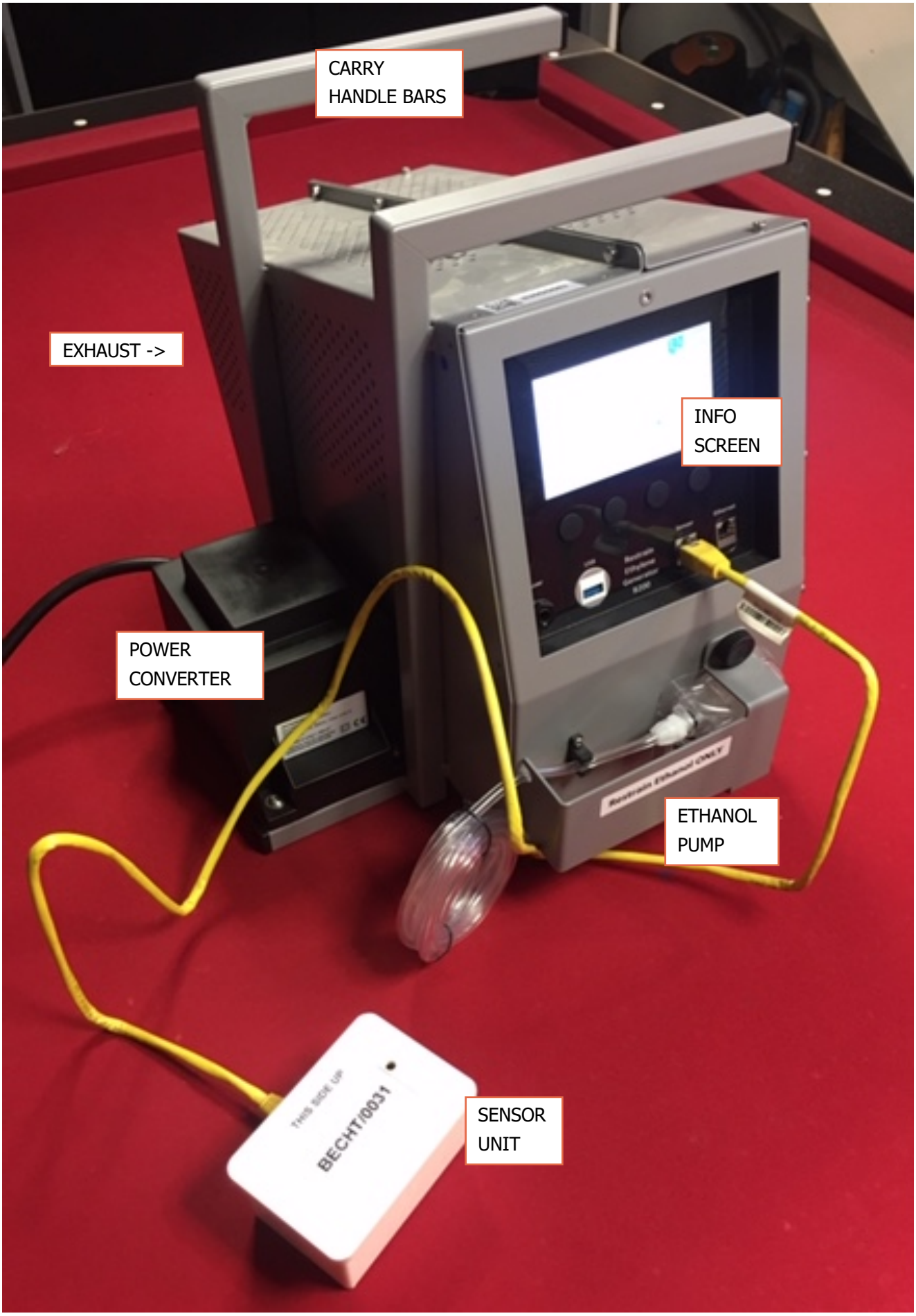
APPENDIX 3

OPERATOR MANUALS FOR ETHYLENE GENERATOR - ONION AND POTATO STORAGE

MODEL B200

Manual POTATO

Version Dec 2020


CARRY
HANDLE BARS
EXHAUST ->
INFO
SCREEN
POWER
CONVERTER
ETHANOL
PUMP
SENSOR
UNIT
BECHT/0031

PURPOSE

The B200 Restrain Generator is used for the production and maintenance of a stable atmosphere of ethylene within a cold store. The protocols of use for potatoes are detailed in **Appendix 1** of this manual. The application and use of this product is the user's responsibility and should be in accordance with relevant legislation.

LOCATION

The Restrain Generator should be located within the store that is being treated.

This machine is not intended for use in temperatures below 30F (–1.0 °C).

The room should be at least empty volume 200m3/7000 Cubic Feet (equivalent to approximately 60 one-ton boxes) for normal cold stores, or 500m3/17500 Cubic Feet if it has been totally sealed to controlled atmosphere standards.

The maximum size store depends on the standard of construction, room air leakage, and the target level of %Restrain(= calculation in PPM ethylene concentration).

This equipment has been used satisfactorily in good quality cold stores of 4,000 tonne capacity. The machine should be located at floor level or no greater than 1m above the floor in a position of good air circulation. It should be placed away from normal loading activity and protected by barriers from potential damage from fork lift trucks and similar hazards.

The Restrain generator produces water in the form of vapour or steam depending on the surrounding environment. Some water will condense forming drips, which will fall on to the floor underneath the machine, and will evaporate. A small amount of adsorbent material under the exhaust should be used to soak up this condensate.

POWER

The machine is fitted with a 120 V 15A 3-prong plug (NEMA 5-15), and should be connected to a GFCI receptacle. Power specifications: 120V AC, 60 Hz and 300 VA. The power converter is mounted outside the generator.

If the supply cord is damaged it must be replaced with the same type by the manufacturer Restrain or its service agent.

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SAFETY

THE GENERATOR SHOULD BE PLACED WITHIN 5 DEGREES OF LEVEL AND NOT ABOVE OR CLOSE TO A SOURCE OF IGNITION.

DO NOT PLACE THE GENERATOR AGAINST A WALL OR BINS. KEEP AT ALL TIME 0.6 METER/2 Ft. SPACE FREE AROUND THE MACHINE. THE EXHAUST BECOMES VERY HOT. AVOID THE CABLES AND WIRES TO CONTACT THE EXHAUST AT ALL TIMES!

THE GENERATOR SHOULD AT ALL TIMES BE KEPT DRY AND FREE OF MOISTURE. AVOID WATER LEAKAGE ON/IN THE GENERATOR.

THE ROOM MUST BE AT LEAST EMPTY VOLUME 200 M3/7,000 CUBIC Ft.

IN CASE OF DAMAGED OR BROKEN PARTS TO ELECTRICAL POWER CABLE OR TRANSFORMER, IMMEDIATELY DISCONNECT THE GENERATOR FROM THE POWER SUPPLY. DO NOT REMOVE COVERS. CONTACT RESTRAIN - DO NOT ATTEMP TO DO REPAIRS YOURSELF.

THE CATALYST HOUSING CONTAINS A HEATER WHICH IS VERY HOT. THE FURNACE IS WELL INSULATED AND TAKES AT LEAST 6 HOURS TO FALL TO A TEMPERATURE THAT IS SAFE TO HANDLE THE GENERATOR.

ETHANOL IS EXTREMELY FLAMABLE AND THERFORE WHEN HANDLING THE FUEL DO NOT SMOKE OR USE NEAR SPARKS OR OPEN FLAME, RADIO AND MOBILE PHONES.

MAINTENANCE

At least once per month the following items should be checked:
· The connection cable has not been damaged.
· All covers are properly secured.
The machine should be checked DAILY for correct operation.

ETHANOL

Only authorised Restrain Ethanol should be used. This is exclusively supplied by the Restrain Company Ltd. The safe running & life expectancy of the Restrain Generator is dependant on the correct formulation of Restrain ethanol being used.

Refer to the Restrain Fuel Safety sheet in **Appendix 2** of this manual.

PLACE THE SENSOR FIRST

- The output of the B200 RESTRAIN Generator responds to the demand needed to maintain the %Restrain level in the storage room. This is measured with the B-ECHT Sensor, and connected with the yellow UTP cable, which plugs into the UTP socket on the front of the machine, marked with SENSOR.
- The B-ECHT Sensor monitors and records the store temperature, CO_2 and humdity at the same time as the %Restrain reading. Each season the sensor needs to be calibrated before starting up the new storage season.
- **Before** using Restrain Ethanol make sure to place the sensor (WITH THIS SIDE UP) at least 15 meters/50 ft. away from the generator and in an area of good air circulation.

Avoid contamination of the sensor. The sensor can also be affected by motorized equipment exhaust fumes. Incorrect readings will be obtained when these are operating in the vicinity, but are not of high enough concentrations under normal operating conditions to cause permanent damage to the sensor.

- 

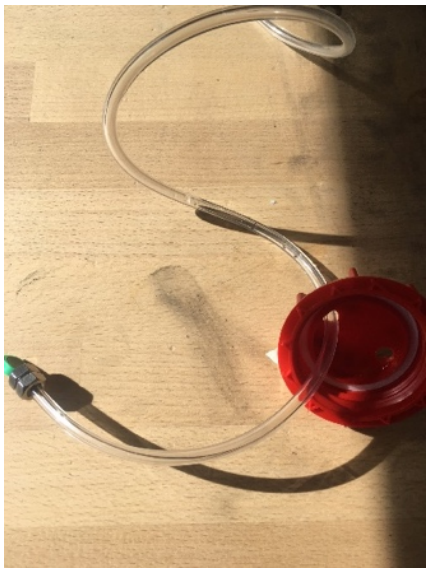
Warning: The Restrain Sensor for measuring the ethylene levels in store must be kept away from Restrain ethanol at all times. (Even when switched off or in transport). Fumes from the fuel will permanently damage the sensor. For protection against contamination the sensors are transported and stored in a sealed plastic bag/container. They should be removed from the container and placed in position only when machine is about to be started up.

PREPARE THE GENERATOR

- Put the power plug into a 110 - 120 V AC outlet. First the software will start up. This takes about 2 minutes

PREPARE THE JERRYCAN

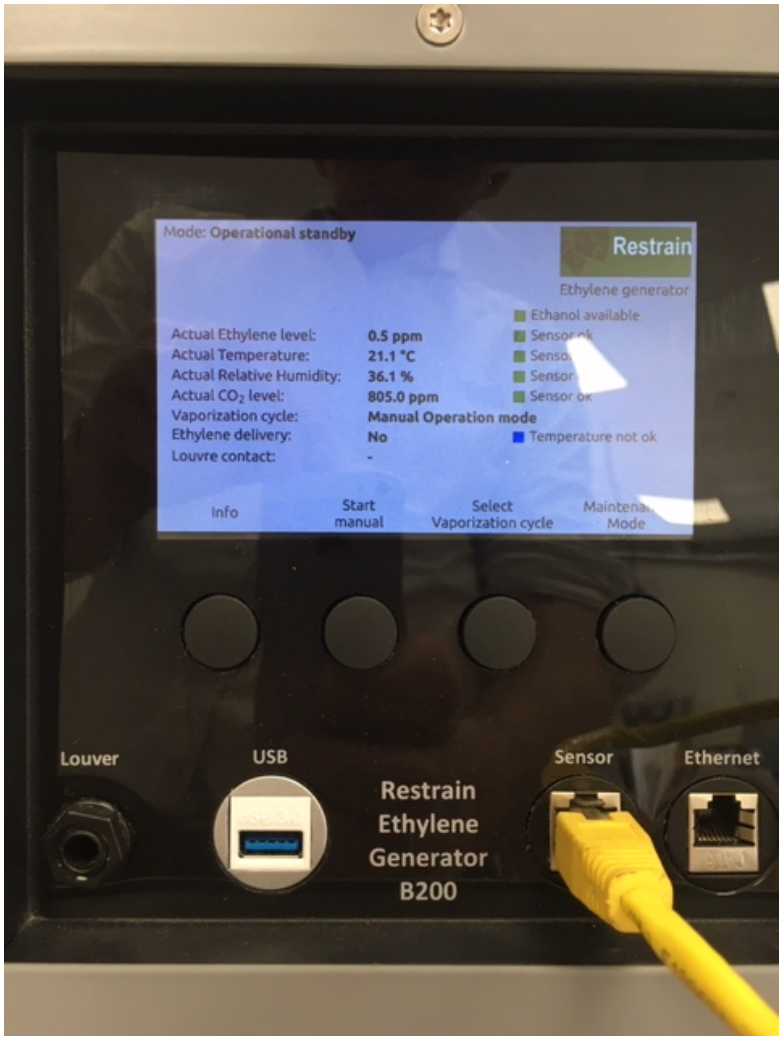
- Use Restrain fuel jerrycan and unscrew the cap. Drill 2 holes of 8mm / 3/8 inch diameter each in the cap.
- In 1 hole guide the suction tube through the cap.
- Add the two weights and fix the filter on the suction end.



- Screw the cap on the jerrycan and make sure the filter touches the bottom of the jerrycan.

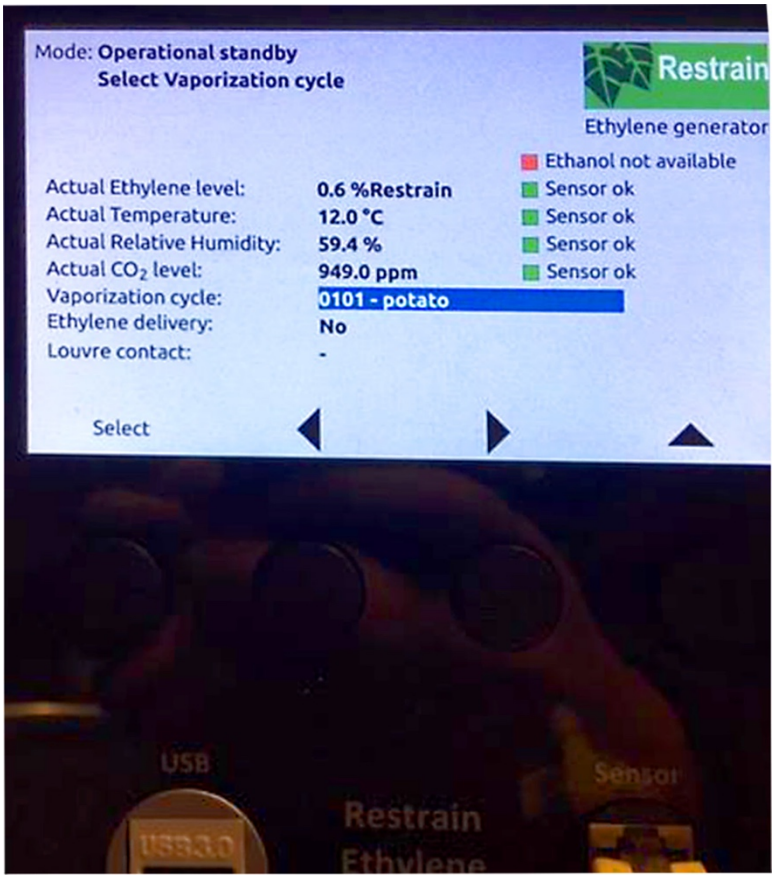
PREPARE THE GENERATOR

- Initial screen: Operational Standby and there is no Vaporization cycle (programme) selected.

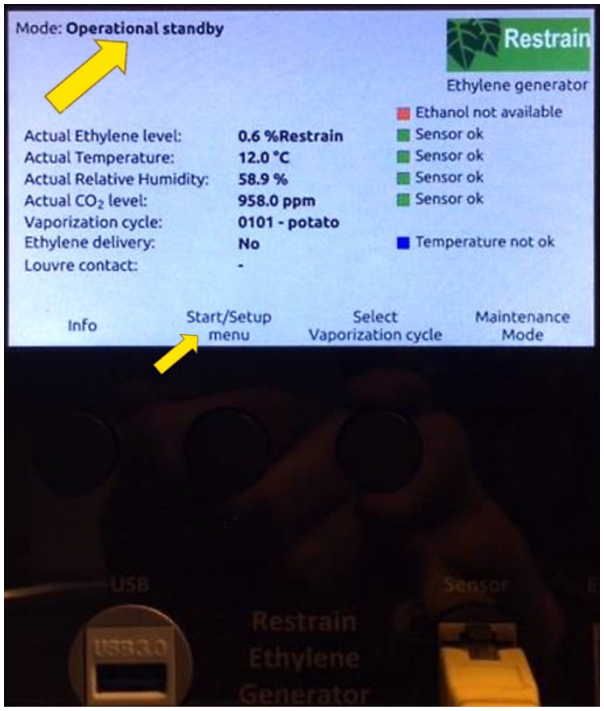


- Now select the vaporization cycle 0101 POTATO with the 2nd button on the right below the screen.

  And confirm your potato programme with 1st button on the left with Select.

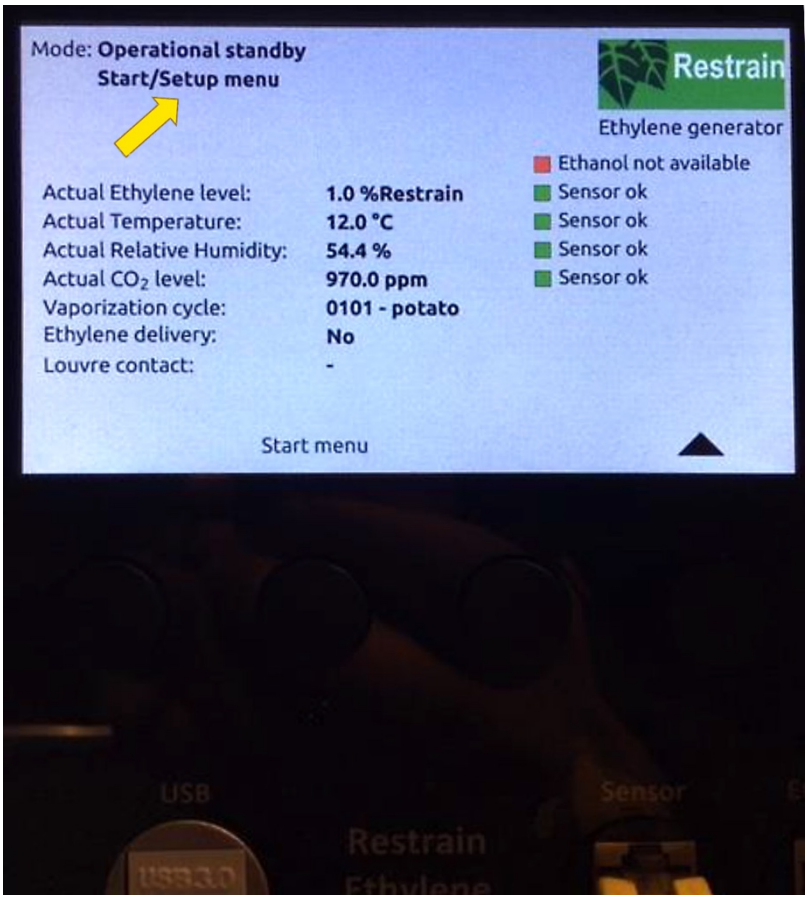


- You have now selected and confirmed the program 0101 POTATO

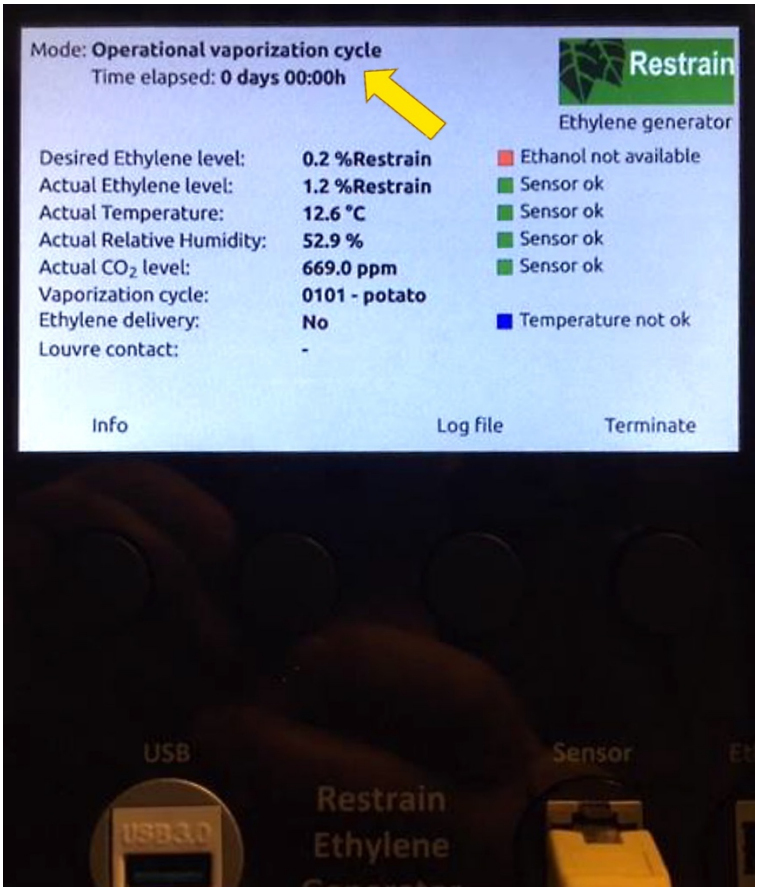


- On the screen you will see that you are in **Operational Standby** mode and you selected **0101 Potato** Vaporization cycle. Now you need to start the programme. Start/Setup 2nd button on the left.

- You are now in the start/setup menu. Now go to Start menu by 2nd button left.



- You are now in start menu. For potatoes it is advised to start the Slow Start* programme as standard procedure. Press 2nd button left Start and the Restrain Generator automatically and gradually increases the level of ethylene in the store from a starting level of 0.2% Restrain (0.1 ppm ethylene) to 20% Restrain (10 ppm ethylene) over a 21-day period.
- Once the Slow Start programme has run for 21 days, the level of ethylene being produced and maintained in the store will have reached 20% Restrain and will subsequently maintain that level throughout the storage season.



- Now you have started the standard slow start programme from day 0.

  The ethanol is not yet pumped in the tube near the generator, so you see a red indicator. The temperature of the catalyst need to warm up and takes about 40 minutes from the start. The indicator is blue. And when warmed up to operational temperature it will switch to green.

  The ethanol pump will only start when the catalyst temperature is reached (green indicator) and when the desired ethylene level 0,2% Restrain is higher than the actual ethylene level.

- The indicators for Ethylene level, Temperature, Relative Humidity and CO_2 level should all be green.
- When the self priming ethanol pump starts to pump and the ethanol reaches the calalyst, the "Ethanol not available" in red will switch to Ethanol available in green.
- If the current is cut off, the programmed machine will remember the settings and when power is switched back on, the catalyst will be heated up and continue with the programme automatically.

Appendix 1
Prepack Ware Potato Storage Protocol

Minimising the respiration rate increase in the stored crop during the introduction of ethylene is important. Following the simple steps below will enable you to use Restrain system successfully.

The RESTRAIN B-ECHT sensor should have been serviced and calibrated by an authorised engineer before the storage season is started.

1) The potato crop must be of good quality and settled in store and reached the holding temperature.

2) Only the pre-programmed **Slow Start Up** Restrain program should be used on prepack potatoes.
3) Restrain can only be introduced when the crop shows the first signs of eye appearance.

4) When ethylene is introduced, air must be circulated continuously within the store for the first 3 weeks to avoid the localised build up of CO_2 (carbon dioxide). Elevated CO_2 above 2500 PPM (0.25%) increases respiration rates.

5) Flush the store daily with fresh air if carbon dioxide levels are likely to exceed 2500 PPM 0.25%.

Use of Ambient Air

To minimise ethanol usage during ambient air use an additional louver cable can be connected from the B200 Generator to the storage room controller. When louvers are openend to flush with fresh air, the B200 Generator will be turned off. No ethanol will be pumped while louver(s) is (are) openend.

It takes 3 days to uptake ethylene in the potatoes and 3 days to release the ethylene from the potatoes. Daily flushing fresh air for maximum 6 hours is possible without losing potato dormancy.

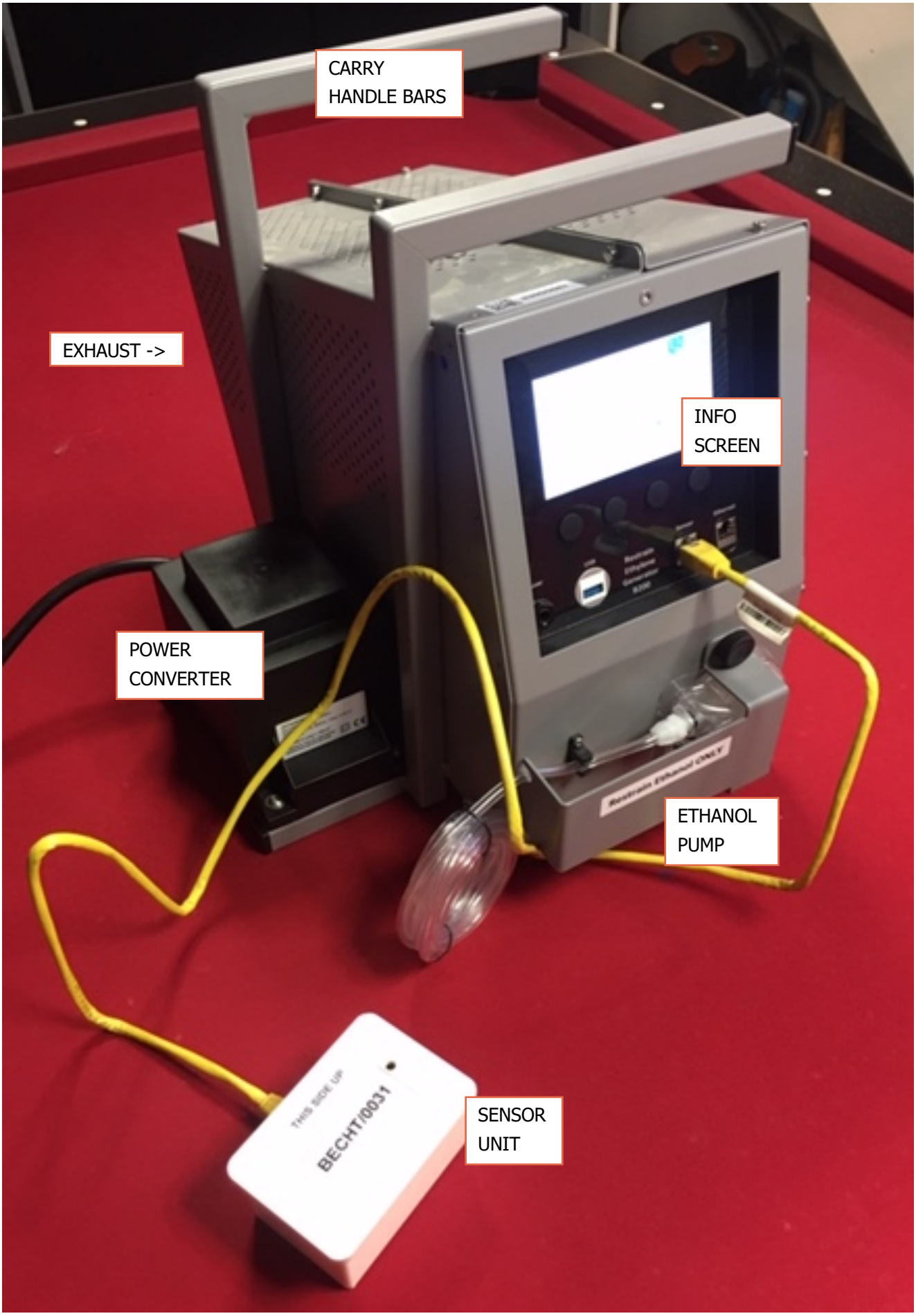
1.1 Store Unloading

- The Restrain Generator should be left in operation until all of the potatoes have been removed from store & released for shipping.
- Direct processing or consumption is advised, as after 3 days the potato sprouts will begin to grow.

MODEL B200

Manual ONION

Version December 2020


CARRY
HANDLE BARS
EXHAUST ->
INFO
SCREEN
POWER
CONVERTER
ETHANOL
PUMP
SENSOR
UNIT
BECHT/0031

PURPOSE

The B200 Restrain Generator is used for the production and maintenance of a stable atmosphere of ethylene within a cold store. The protocols of use for onions are detailed in **Appendix 1** of this manual. The application and use of this product is the user's responsibility and should be in accordance with relevant legislation.

LOCATION

The Restrain Generator should be located within the store that is being treated.

This machine is not intended for use in temperatures below 30F (–1.0 °C).

The room should be at least empty volume 200m3/7000 Cubic Feet (equivalent to approximately 60 one-ton boxes) for normal cold stores, or 500m3/17500 Cubic Feet if it has been totally sealed to controlled atmosphere standards.

The maximum size store depends on the standard of construction, room air leakage, and the target level of %Restrain(= calculation in PPM ethylene concentration).

This equipment has been used satisfactorily in good quality cold stores of 4,000 tonne capacity. The machine should be located at floor level or no greater than 1m above the floor in a position of good air circulation. It should be placed away from normal loading activity and protected by barriers from potential damage from fork lift trucks and similar hazards.

The Restrain generator produces water in the form of vapour or steam depending on the surrounding environment. Some water will condense forming drips, which will fall on to the floor underneath the machine, and will evaporate. A small amount of adsorbent material under the exhaust should be used to soak up this condensate.

POWER

The machine is fitted with a 120 V 15A 3-prong plug (NEMA 5-15), and should be connected to a GFCI receptacle. Power specifications: 120V AC, 60 Hz and 300 VA. The power converter is mounted outside the generator.

If the supply cord is damaged it must be replaced with the same type by the manufacturer Restrain or its service agent.

SAFETY

THE GENERATOR SHOULD BE PLACED WITHIN 5 DEGREES OF LEVEL AND NOT ABOVE OR CLOSE TO A SOURCE OF IGNITION.

DO NOT PLACE THE GENERATOR AGAINST A WALL OR BINS. KEEP AT ALL TIME 0.6 METER/2 Ft. SPACE FREE AROUND THE MACHINE. THE EXHAUST BECOMES VERY HOT. AVOID THE CABLES AND WIRES TO CONTACT THE EXHAUST AT ALL TIMES!

THE GENERATOR SHOULD AT ALL TIMES BE KEPT DRY AND FREE OF MOISTURE. AVOID WATER LEAKAGE ON/IN THE GENERATOR.

THE ROOM MUST BE AT LEAST EMPTY VOLUME 200 M3/7,000 CUBIC Ft.

IN CASE OF DAMAGED OR BROKEN PARTS TO ELECTRICAL POWER CABLE OR TRANSFORMER, IMMEDIATELY DISCONNECT THE GENERATOR FROM THE POWER SUPPLY. DO NOT REMOVE COVERS. CONTACT RESTRAIN - DO NOT ATTEMP TO DO REPAIRS YOURSELF.

THE CATALYST HOUSING CONTAINS A HEATER WICH IS VERY HOT. THE FURNACE IS WELL INSULATED AND TAKES AT LEAST 6 HOURS TO FALL TO A TEMPERATURE THAT IS SAFE TO HANDLE THE GENERATOR.

ETHANOL IS EXTREMELY FLAMABLE AND THERFORE WHEN HANDLING THE FUEL DO NOT SMOKE OR USE NEAR SPARKS OR OPEN FLAME, RADIO AND MOBILE PHONES.

MAINTENANCE

At least once per month the following items should be checked:
· The connection cable has not been damaged.
· All covers are properly secured.
The machine should be checked DAILY for correct operation.

ETHANOL

Only authorised Restrain Ethanol should be used. This is exclusively supplied by the Restrain Company Ltd. The safe running & life expectancy of the Restrain Generator is dependant on the correct formulation of Restrain ethanol being used.

Refer to the Restrain Fuel Safety sheet in **Appendix 2** of this manual.

PLACE THE SENSOR FIRST

- The output of the B200 RESTRAIN Generator responds to the demand needed to maintain the %Restrain level in the storage room. This is measured with the B-

ECHT Sensor, and connected with the yellow UTP cable, which plugs into the UTP socket on the front of the machine, marked with SENSOR.

- The B-ECHT Sensor monitors and records the store temperature, CO_2 and humdity at the same time as the %Restrain reading. Each season the sensor needs to be calibrated before starting up the new storage season.
- **Before** using Restrain Ethanol make sure to place the sensor (WITH THIS SIDE UP) at least 15 meters/50 ft. away from the generator and in an area of good air circulation.

Avoid contamination of the sensor. The sensor can also be affected by motorized equipment exhaust fumes. Incorrect readings will be obtained when these are operating in the vicinity, but are not of high enough concentrations under normal operating conditions to cause permanent damage to the sensor.

Warning: The Restrain Sensor for measuring the ethylene levels in store must be kept away from Restrain ethanol at all times. (Even when switched off or in transport). Fumes from the fuel will permanently damage the sensor. For protection against contamination the sensors are transported and stored in a sealed plastic bag/container. They should be removed from the container and placed in position only when machine is about to be started up.

PREPARE THE GENERATOR

- Put the power plug into a 110 - 120 V AC outlet. First the software will start up. This takes about 2 minutes

PREPARE THE JERRYCAN

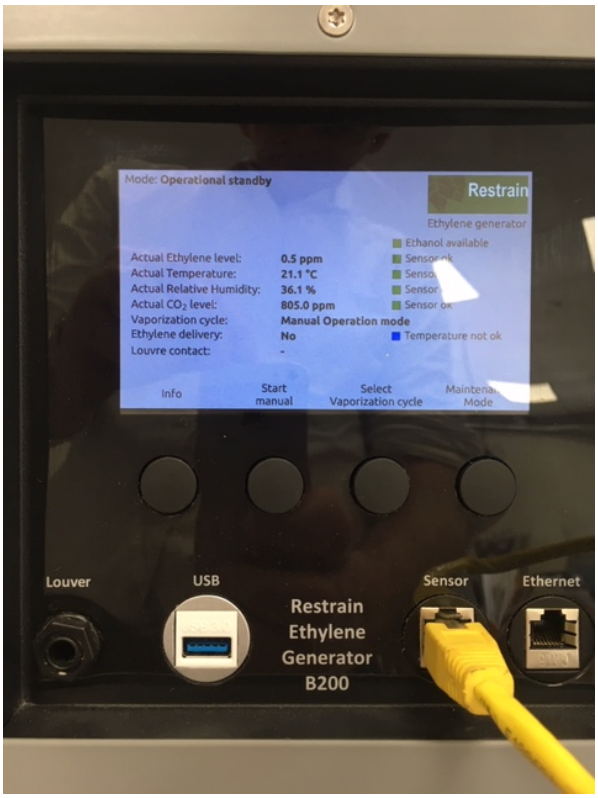
- Use Restrain fuel jerrycan and unscrew the cap. Drill 2 holes of 8mm / 5/16 inch diameter each in the cap.

- Guide the suction tube through one hole in the cap.
- Add the two weights and fix the filter on the suction end.

- Screw the cap on the jerrycan and make sure the filter touches the bottom of the jerrycan.

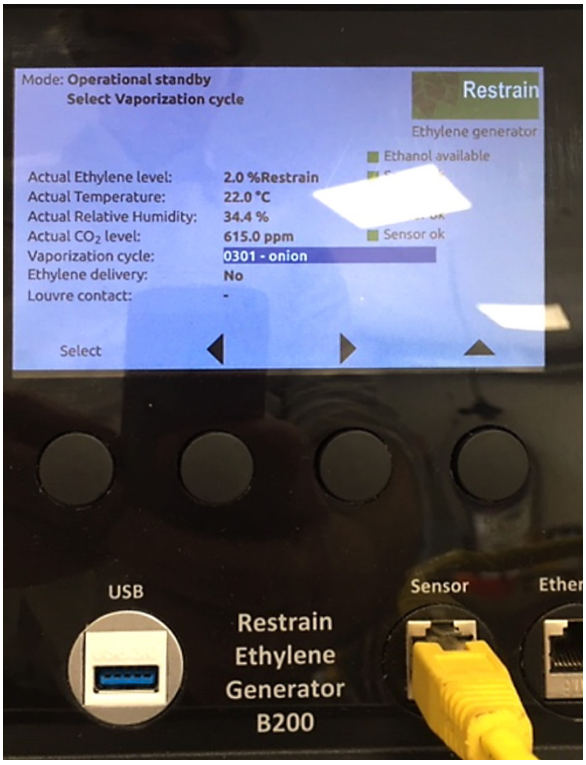
PREPARE THE GENERATOR

- Initial screen: Operational Standby and there is no Vaporization cycle (programme) selected.

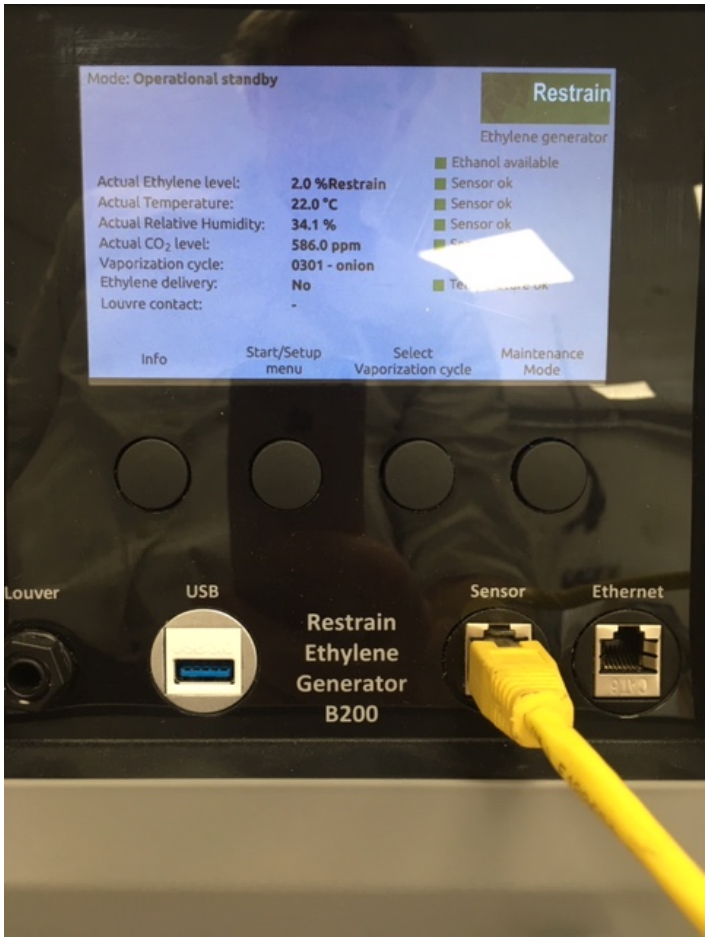


- Now select the vaporization cycle 0301 ONION with the 2nd button on the right below the screen.

  And confirm your ONION programme with 1st button on the left with Select.

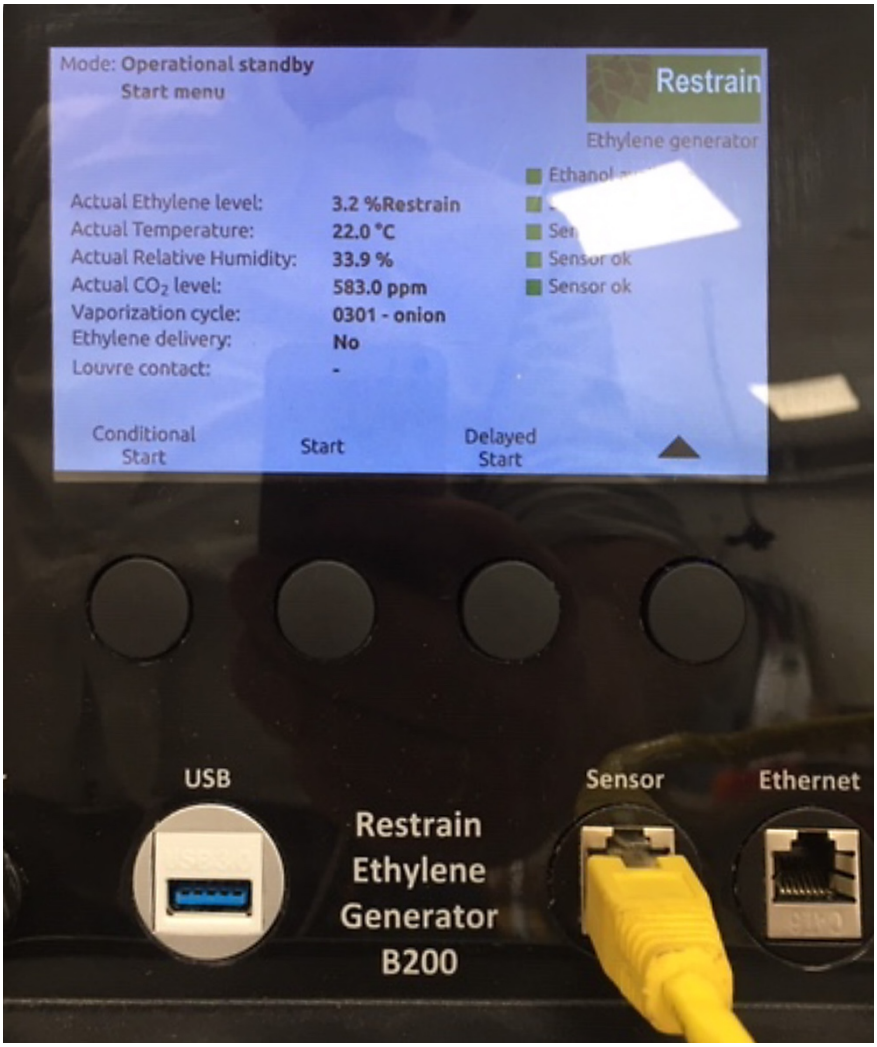


- You have now selected and confirmed the program 0301 ONION

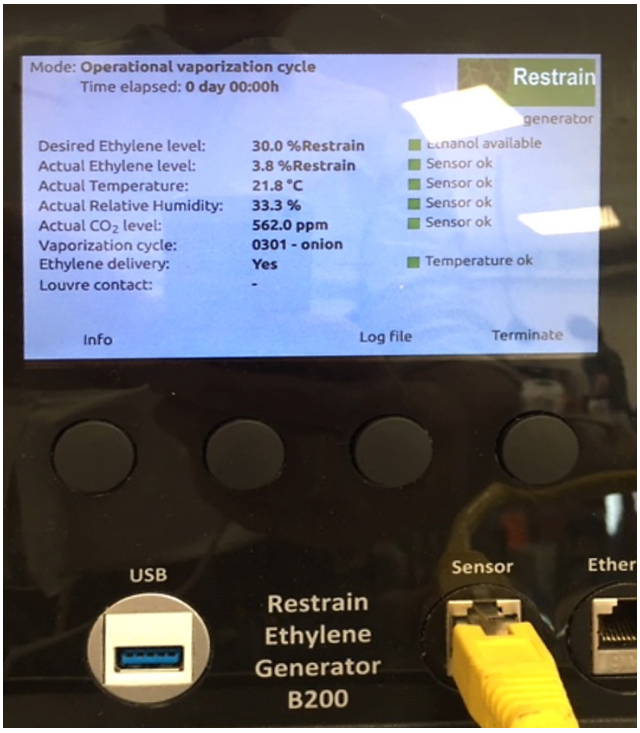


- On the screen you will see that you are in **Operational Standby** mode and you selected **0301 ONION** Vaporization cycle. Now you need to start the programme. Start/Setup 2nd button on the left.

- You are now in the start/setup menu. Now go to Start menu by 2nd button left.



- You are now in start menu. For onions to start the programme press 2nd button left Start and the Restrain Generator and automatically the concentration of ethylene in the store will be mainained to 30% Restrain (15 ppm ethylene) throughout the storage season.

- 

- Now you have started the standard slow start programme from day 0.

  The ethanol is not yet pumped in the tube near the generator, so you see a red indicator. The temperature of the catalyst needs to warm up and this takes about 40 minutes from the start. The indicator is blue. And when warmed up to operational temperature it will switch to green.

  The ethanol pump will only start when the catalyst temperature is reached (green indicator) and when the desired ethylene level 30% Restrain (15 ppm) is higher than the actual ethylene level.

- The indicators for Ethylene level, Temperature, Relative Humidity and CO_2 level should all be green.
- When the self priming ethanol pump starts to pump and the ethanol reaches the calalyst, the "Ethanol not available" in red will switch to "Ethanol available" in green.
- If the current gets cut off, the programmed machine will remember the settings and when power is switched back on, the catalyst will be heated up and continue with the programme automatically.

Appendix 1
Prepack Onion Storage Protocol

Minimising the respiration rate increase in the stored crop during the introduction of ethylene is important. Following the simple steps below will enable you to use Restrain system successfully.

The RESTRAIN B-ECHT sensor should have been serviced and calibrated by an authorised engineer before the storage season is started.

1) The onion crop must be of good quality and settled in store and have reached the holding temperature.

Use of Ambient Air

To minimise ethanol usage during ambient air use an additional louver cable can be connected from the B200 Generator to the storage room controller. When louvers are opened to flush with fresh air, the B200 Generator will be turned off. No ethanol will be pumped while louver(s) is (are) openend.

It takes 3 days to uptake ethylene in the onions and 3 days to release the ethylene from the onions. Daily flushing fresh air for maximum 6 hours is possible without losing onion dormancy.

1.1 Store Unloading

- The Restrain generator should be left in operation until all of the onions have been removed from store.

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 4

MANUFACTURING PROCESS FOR ETHANOL INTENDED FOR USE IN GENERATING ETHYLENE GAS BY CATLYTIC DEHYDRATION

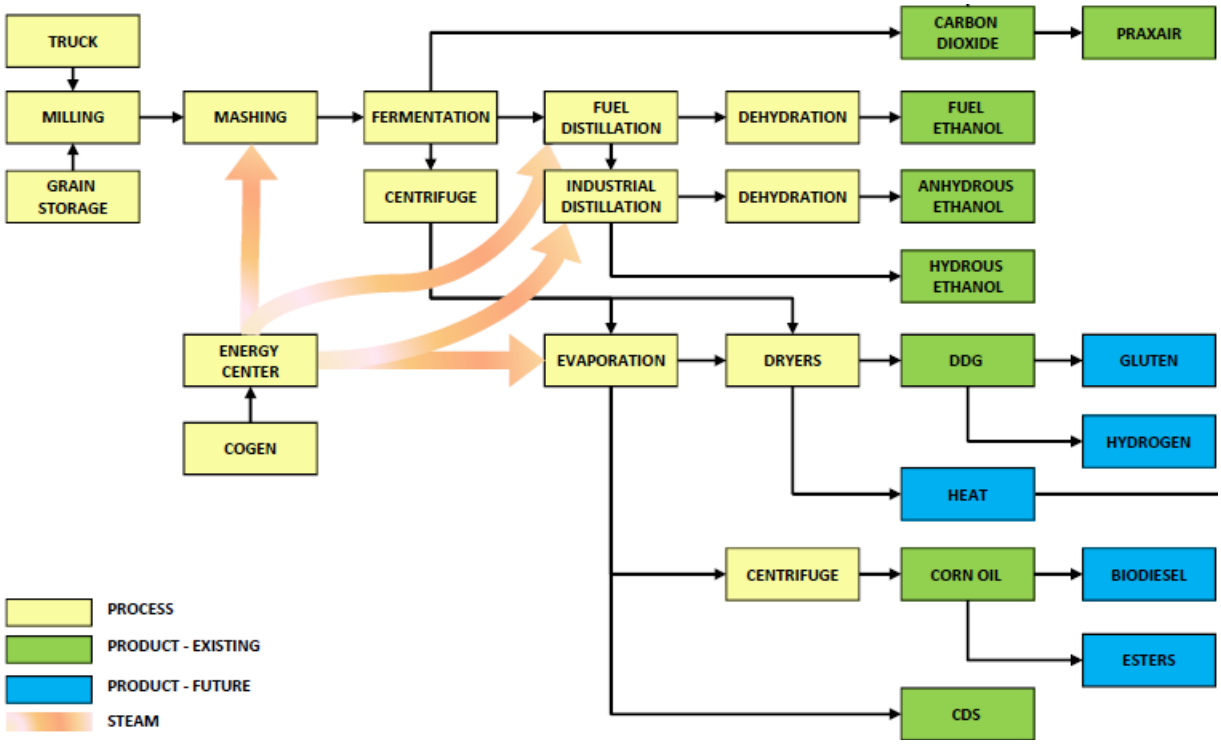
Manufacturing Process for Restrain Generator Fuel

Step 1: Manufacture of non-denatured ethanol minimum boiling azeotrope "190 Proof"

1. **Chemical Reaction:**
   Glucose is fermented with the aid of yeast producing ethyl alcohol and carbon dioxide as a by-product.

   $C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$

2. **Manufacturing Flow Chart:**



3. **Primary Manufacturing Steps and Manufacture Method**
   a. *Milling*
   Dried corn kernels are used as raw material.

b. *Cooking*
   Milled corn is mixed with water.
   The pH is adjusted to approximately 6 using ammonium hydroxide solution.
   Enzymes are added to the corn starch slurry.
   Slurry is heated to approximately 110C for 10 minutes and then cooled to 85C to initiate enzyme conversion of the corn starch into dextrose.
   Small amount of phosphoric acid is added to provide a source of phosphate mineral nutrient to the downstream yeast.
c. *Sacharification*
   Starch slurry is cooled to approximately 60C for approximately 8 hours.
   Sulfuric acid is added to adjust pH to near 4.
   Enzymes are added to further break down starch / dextrose molecules to sugars
d. *Fermentation*
   The sacharified slurry is further cooled to 30C and routed in continuous mode through a series of fermenters.
   Yeast consumes sugars which have been produced from the corn and convert then to ethyl alcohol and carbon dioxide.
   Fresh yeast and yeast nutrients are added.
   Ammonium hydroxide is added continuously to provide inorganic nitrogen to the yeast.
   Sulfuric acid is added to maintain optimal pH.
e. *Distillation*
   Fermented broth containing 12% ethanol, 12% solids with the remainder of primarily water is routed through a series of distillation columns in order to remove water and solids and purify ethanol:
   - Separation column separates the corn solids and most of the water from the ethanol present in the fermented mash
   - The ethanol / water product leaving from near the top of the separation column enters the rectification column.
   - The rectification column serves to remove most of the remainder of the water from the ethanol fed to it.
   - Ethanol then purified on Hydroselection column, downstream rectification column, and methanol columns. Overall it is distilled 5 times.
   - To obtain anhydrous ethanol, material is further passed through aluminosilicate molecular sieves. Microscopic pores in sieves bind water molecules but allow the larger ethanol molecule to pass through.

   The above series of columns and purification steps serve to purify the ethanol product so that it may be sold into high purity pharmaceutical, fragrance, flavor and other markets requiring high purity product.

The maximum theoretical yield is 4.55 liters of anhydrous Ethyl alcohol per 10 kg of dry corn.

Ethanol is produced by a continuous process. Material is tested per approved specification before it is routed to the holding tank. Samples of the product from the holding tank are routinely tested. Aliquots of finished product are loaded into railcars and tanker trucks, tested, and delivered to a packaging plant.

Formulation Process Part 2
To every 100 kg of ethanol 190 Proof add: 1.01 kg ethyl acetate. Stir under dehumidified air. Package in air-tight containers.

Discussion of Formation of Impurities

Refer to the 5-batch analysis of the 190 Proof Ethanol. The only impurities identified were water (6.9 to 7.3%) and ash (~0.2%) otherwise not identifiable.
Water is expected as an impurity due primarily to the distillation process, in which a minimum boiling azeotrope of ethanol / water of approximately 95% is the maximum ethanol purity attainable. Water above 5% is due to the fact that concentrated ethanol is highly hygroscopic, and the sampling and analysis process itself allows for absorption of moisture from air. A small variation in water content will have no effect on the purity of the ethylene emitted by the catalytic generator.

Following the distillation of the ethanol itself, the denaturation process involves only stirring in of ethyl acetate. No new impurities are formed beyond any present in the input materials.

MANUFACTURING METHOD AND FORMATION OF IMPURITIES RESTRAIN GENERATOR FUEL

SDSs of Input Materials

SAFETY DATA SHEET

Creation Date 13-Oct-2009 **Revision Date** 17-Jan-2018 **Revision Number** 5

1. Identification

Product Name **Ethyl acetate**

Cat No. : **E145-1; E145-4; E145-4LC; E145-20; E145-200; E1452PR; E145-500; E145FB-19; E145FB-50; E145FB-115; E145FB-200; E145POP-50; E145POPB-50; E145RB-19; E145RB-50; E145RB-115; E145RB-200; E145RS-28; E145RS-50; E145RS-115; E145RS-200; E145S-4; E145SK-4; E145SK-4LC; E145SS-28; E145SS-50; E145SS-115; E145SS-200; E145SS-1350; NC1489568**

CAS-No 141-78-6
Synonyms Acetic acid ethyl ester

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Flammable liquids	Category 2
Serious Eye Damage/Eye Irritation	Category 2
Specific target organ toxicity (single exposure)	Category 3
Target Organs - Central nervous system (CNS).	

Label Elements

Signal Word
Danger

Hazard Statements
Highly flammable liquid and vapor
Causes serious eye irritation
May cause drowsiness or dizziness

Precautionary Statements
Prevention
Wash face, hands and any exposed skin thoroughly after handling
Do not breathe dust/fume/gas/mist/vapors/spray
Use only outdoors or in a well-ventilated area
Keep away from heat/sparks/open flames/hot surfaces. - No smoking
Keep container tightly closed
Ground/bond container and receiving equipment
Use explosion-proof electrical/ventilating/lighting/equipment
Use only non-sparking tools
Take precautionary measures against static discharge
Wear protective gloves/protective clothing/eye protection/face protection
Keep cool
Response
Get medical attention/advice if you feel unwell
Inhalation
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Call a POISON CENTER or doctor/physician if you feel unwell
Skin
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
Fire
In case of fire: Use CO2, dry chemical, or foam for extinction
Storage
Store in a well-ventilated place. Keep container tightly closed
Store locked up
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
Repeated exposure may cause skin dryness or cracking

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Ethyl acetate	141-78-6	>95

4. First-aid measures

General Advice If symptoms persist, call a physician.

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. If skin irritation persists, call a physician.

Inhalation Move to fresh air. If not breathing, give artificial respiration. Get medical attention if

symptoms occur.

Ingestion Clean mouth with water and drink afterwards plenty of water.

Most important symptoms and effects Breathing difficulties. May cause central nervous system depression: Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Unsuitable Extinguishing Media Water may be ineffective, Do not use a solid water stream as it may scatter and spread fire

Flash Point -4 °C / 24.8 °F

Method - Closed cup

Autoignition Temperature 427 °C / 800.6 °F

Explosion Limits
Upper 11.5 vol %
Lower 2.0 vol %

Oxidizing Properties Not oxidising

Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
Flammable. Risk of ignition. Vapors may form explosive mixtures with air. Vapors may travel to source of ignition and flash back. Containers may explode when heated.

Hazardous Combustion Products
Carbon monoxide (CO) Carbon dioxide (CO_2)
Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA

Health	Flammability	Instability	Physical hazards
2	3	0	N/A

6. Accidental release measures

Personal Precautions Use personal protective equipment. Ensure adequate ventilation.
Environmental Precautions Should not be released into the environment. See Section 12 for additional ecological information.

Methods for Containment and Clean Up Soak up with inert absorbent material. Keep in suitable, closed containers for disposal.

7. Handling and storage

Handling Ensure adequate ventilation. Wear personal protective equipment. Do not get in eyes, on skin, or on clothing. Avoid ingestion and inhalation.

Storage Flammables area. Keep away from heat and sources of ignition. Keep container tightly closed in a dry and well-ventilated place.

8. Exposure controls / personal protection

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH	Mexico OEL (TWA)
Ethyl acetate	TWA: 400 ppm	(Vacated) TWA: 400 ppm (Vacated) TWA: 1400 mg/m³ TWA: 400 ppm TWA: 1400 mg/m³	IDLH: 2000 ppm TWA: 400 ppm TWA: 1400 mg/m³	TWA: 400 ppm TWA: 1400 mg/m³

Legend

ACGIH - American Conference of Governmental Industrial Hygienists
OSHA - Occupational Safety and Health Administration
NIOSH IDLH: The National Institute for Occupational Safety and Health Immediately Dangerous to Life or Health

Engineering Measures Ensure adequate ventilation, especially in confined areas. Use explosion-proof electrical/ventilating/lighting/equipment. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Long sleeved clothing.

Respiratory Protection No protective equipment is needed under normal use conditions.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Liquid
Appearance	Colorless
Odor	sweet
Odor Threshold	50 ppm
pH	No information available
Melting Point/Range	-83.5 °C / -118.3 °F
Boiling Point/Range	75 - 78 °C / 167 - 172.4 °F
Flash Point	-4 °C / 24.8 °F
Method -	Closed cup
Evaporation Rate	6.2
Flammability (solid,gas)	Not applicable
Flammability or explosive limits	
Upper	11.5 vol %
Lower	2.0 vol %
Vapor Pressure	103 mbar @ 20°C
Vapor Density	3.04
Specific Gravity	0.902
Solubility	Slightly soluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	427 °C / 800.6 °F
Decomposition Temperature	No information available
Viscosity	0.45 cP @ 20 °C
Molecular Formula	C4 H8 O2
Molecular Weight	88.11
Surface tension	24 mN/m @ 20°C

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Stable under normal conditions.

Conditions to Avoid Incompatible products. Keep away from open flames, hot surfaces and sources of ignition.

Incompatible Materials Strong oxidizing agents, Strong acids, Amines, Peroxides

Hazardous Decomposition Products Carbon monoxide (CO), Carbon dioxide (CO_2)

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Ethyl acetate	10,200 mg/kg (Rat)	> 20 mL/kg (Rabbit) > 18000 mg/kg (Rabbit)	58 mg/l (rat; 8 h)

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation Irritating to eyes

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Ethyl acetate	141-78-6	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects No information available

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure Central nervous system (CNS)

STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed May cause central nervous system depression: Inhalation of high vapor concentrations may cause symptoms like headache, dizziness, tiredness, nausea and vomiting

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

Do not empty into drains.

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Ethyl acetate	EC50 = 3300 mg/L/48h	Fathead minnow: LC50: 230 mg/l/ 96h Gold orfe: LC50: 270 mg/L/48h	EC50 = 1180 mg/L 5 min EC50 = 1500 mg/L 15 min EC50 = 5870 mg/L 15 min EC50 = 7400 mg/L 2 h	EC50 = 717 mg/L/48h

Persistence and Degradability Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility Will likely be mobile in the environment due to its volatility.

Component	log Pow
Ethyl acetate	0.6

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

Component	RCRA - U Series Wastes	RCRA - P Series Wastes
Ethyl acetate - 141-78-6	U112	-

14. Transport information

DOT

UN-No UN1173
Proper Shipping Name ETHYL ACETATE
Hazard Class 3
Packing Group II

TDG

UN-No UN1173
Proper Shipping Name ETHYL ACETATE
Hazard Class 3
Packing Group II

IATA

UN-No UN1173
Proper Shipping Name ETHYL ACETATE
Hazard Class 3
Packing Group II

IMDG/IMO

UN-No UN1173
Proper Shipping Name ETHYL ACETATE
Hazard Class 3
Packing Group II

15. Regulatory information

All of the components in the product are on the following Inventory lists: X = listed

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Ethyl acetate	X	X	-	205-500-4	-		X	X	X	X	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule

T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA This material, as supplied, contains one or more substances regulated as a hazardous substance under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302)

Component	Hazardous Substances RQs	CERCLA EHS RQs
Ethyl acetate	5000 lb	-

California Proposition 65 This product does not contain any Proposition 65 chemicals

U.S. State Right-to-Know Regulations

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Ethyl acetate	X	X	X	-	X

U.S. Department of Transportation

Reportable Quantity (RQ): Y
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade Serious risk, Grade 3

16. Other information

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 13-Oct-2009
Revision Date 17-Jan-2018
Print Date 17-Jan-2018
Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 5

MANUFACTURING DESCRIPTION OF THE CATALYTIC DEHYDRATION PROCESS TO PRODUCE ETHYLENE FROM ETHANOL

Materials **2013**, *6*, 101-115; doi:10.3390/ma6010101

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Review

Ethylene Formation by Catalytic Dehydration of Ethanol with Industrial Considerations

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Abstract: Ethylene is the primary component in most plastics, making it economically valuable. It is produced primarily by steam-cracking of hydrocarbons, but can alternatively be produced by the dehydration of ethanol, which can be produced from fermentation processes using renewable substrates such as glucose, starch and others. Due to rising oil prices, researchers now look at alternative reactions to produce green ethylene, but the process is far from being as economically competitive as using fossil fuels. Many studies have investigated catalysts and new reaction engineering technologies to increase ethylene yield and to lower reaction temperature, in an effort to make the reaction applicable in industry and most cost-efficient. This paper presents various lab synthesized catalysts, reaction conditions, and reactor technologies that achieved high ethylene yield at reasonable reaction temperatures, and evaluates their practicality in industrial application in comparison with steam-cracking plants. The most promising were found to be a nanoscale catalyst HZSM-5 with 99.7% ethylene selectivity at 240 °C and 630 h lifespan, using a microreactor technology with mechanical vapor recompression, and algae-produced ethanol to make ethylene.

Keywords: ethanol; dehydration; ethylene; catalyst selectivity; industry; catalyst stability

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1. Introduction

Ethylene is the most widely produced organic compound in the chemical industry. The large global demand for the compound stems from its various uses as precursors to polymers such as polyethylene, found in most plastics, or surfactant chemicals such as ethylene oxide or ethylene glycol (Figure 1), according to Chemical and Engineering News (2006) [1,2]. The Organization of Economic Cooperation and Development reported that production of ethylene is well over 100 million tons annually, and this market size attracts both industrial companies and scientific researchers alike, as shown by the study history represented graphically in Figure 2 [3]. Figure 2 shows patents and article papers on published year from 1998 to 2011, which is illustrated in the result of the patent, emerged as a rising trend year by year from 1998 to 2005, and dropped to show a rising trend after 2006 and 2008. Of particular interest are alternative methods for synthesizing ethylene, especially with fossil fuel reserves diminishing and, consequently, gas prices and production costs steadily rising. Traditionally, ethylene is produced by steam cracking hydrocarbons, Kniel *et al.* (1980) claims, and this method continues to dominate the industry today [4]. True (2012) reported the top ethylene producing complexes listed in Table 1 (ranked by capacity in tons per year), which are all steam cracking plants, and Conti (2012) reported the capacities of the Braskem and Solvay Indupa ethanol to ethylene plants, while Voegle (2012) reported the capacity of the Dow Chemical plant currently under construction [5–7]. However, attention has recently shifted towards green alternatives for manufacturing ethylene, to reduce greenhouse gas emissions and dependency on limited fossil fuels. Leading this green trend is the production of ethylene by catalytic bioethanol dehydration.

Figure 1. Main uses of ethylene in industry (left to right): polyethylene, ethylene dichloride (precursor to vinyl chloride, below), ethylene oxide (precursor to ethylene glycol, below), and ethylbenzene (precursor to styrene, below).

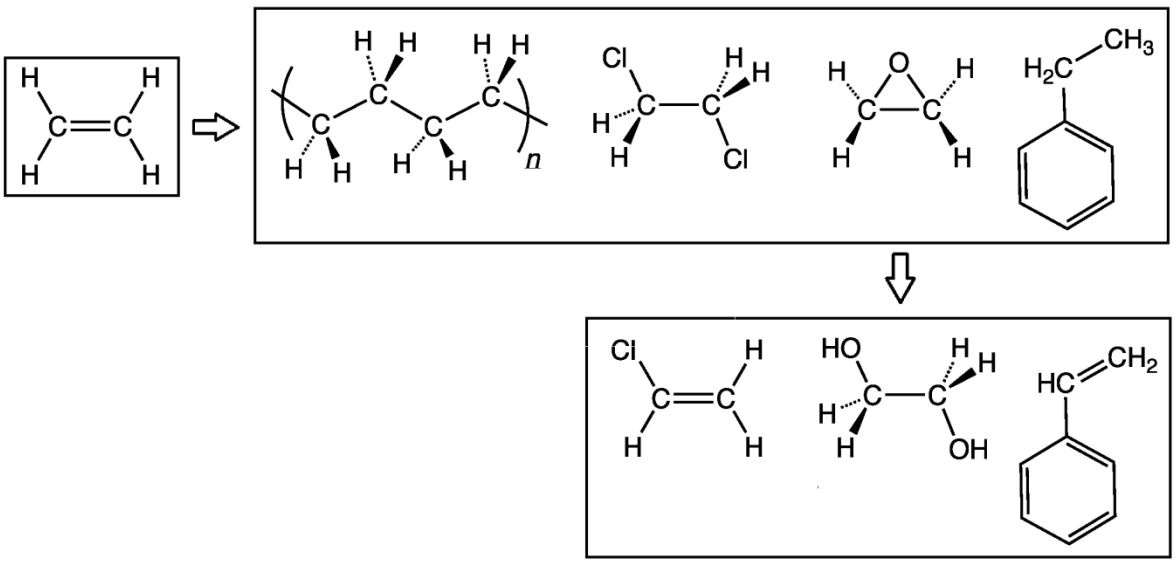


Figure 2. Trend in number of patents (dash-dot line) and publications (solid line) since 1998. Research done on Scopus with key words: ethanol dehydration and ethylene, ethanol dehydration and ethene, ethanol and ethylene production. Total results per type per year for the three searches were summed together.

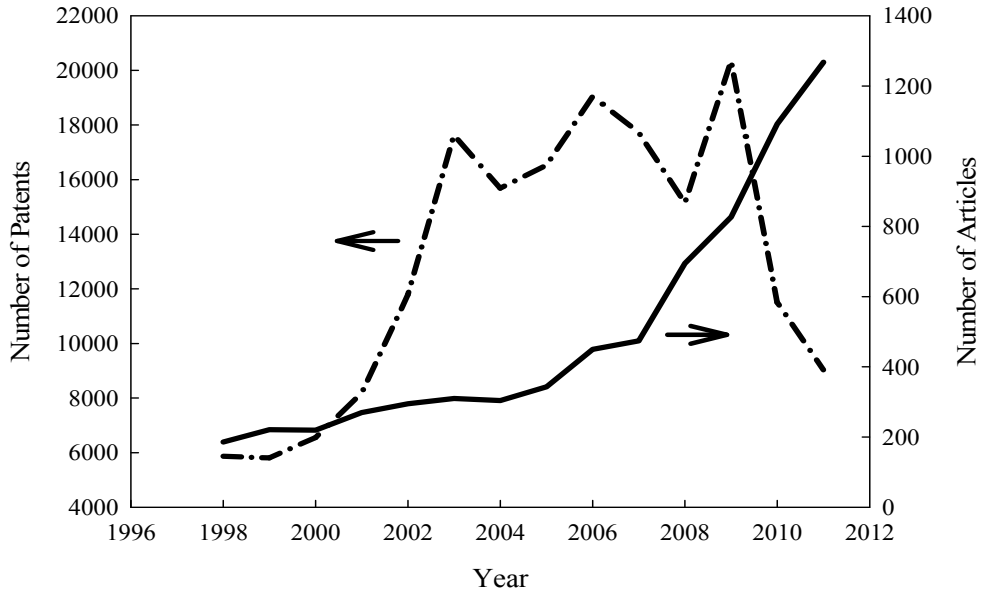


Table 1. Top industrial ethylene complexes and their locations ranked by capacity (tons of ethylene produced per year).

Company	Location	Ton/year
Steam-cracking plants	–	–
Formosa Petrochemical Corporation	Mailiao, Taiwan,	2,935,000
Nova Chemicals Corporation	Joffre, Alberta, Canada	2,811,792
Arabian Petrochemical Company	Jubail, Saudi Arabia	2,250,000
ExxonMobil Chemical Company	Baytown, TX, USA	2,197,000
ChevronPhillips Chemical Company	Sweeny, TX, USA	1,865,000
Dow Chemical Company	Terneuzen, Netherlands	1,800,000
Ineos Olefins & Polymers	Chocolate Bayou, TX, USA	1,752,000
Equistar Chemicals LP	Channelview, TX, USA	1,750,000
Yanbu Petrochemical Company	Yanbu, Saudi Arabia	1,705,000
Equate Petrochemical Company	Shuaiba, Kuwait	1,650,000
Ethanol to ethylene plants	–	–
Braskem	Triunfo, Brazil	200,000
Dow Chemical Company	Santa Vitoria, Brazil (under construction)	190,000
Solvay Indupa	Santo Andre, Brazil	60,000

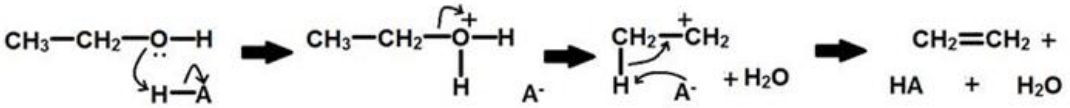
In the catalytic dehydration of ethanol to form ethylene, an acid catalyst first protonates the hydroxyl group, which leaves as a water molecule. The conjugate base of the catalyst then deprotonates the methyl group, and the hydrocarbon rearranges into ethylene. This mechanism is depicted in Figure 3. The reaction is endothermic, and because of this, the optimal reaction temperature is fairly high, ranging from 180 °C to 500 °C. Maintaining the reaction temperature constitutes much of the energy cost in industrial

application of the reaction, since competing reactions into diethyl ether or acetaldehyde are favored outside of the temperature range and so decrease ethylene yield.

To make ethanol dehydration more industry-friendly, many researchers have investigated different catalysts to increase ethylene yield and lower reaction temperature. Researched catalysts began with alumina and transition metal oxides, but have now expanded to include many modified versions of old catalysts, silicoaluminophosphates (SAPO), HZSM-5 zeolite catalyst, and heteropolyacid catalysts. While current catalysts have achieved much better results than the original ones in terms of yield and reaction temperature, most are still not ready for commercialization. Of the SAPO catalysts studied by Zhang *et al.* (2008), Chen *et al.* (2010), and Wu *et al.* (2011), SAPO-11-4 had the best results with 98.0% ethylene selectivity at 250 °C, but with many catalysts such as modified HZSM-5 and MCM-41 achieving over 99.0% selectivity, SAPO catalysts are not competitive enough [8–10]. Likewise, H-mordenites studied by Takahara *et al.* (2005) achieved results like 99.9% ethylene selectivity at 180 °C, but due to the inaccessibility of the catalysts, they are not ideal for commercialization [11]. Bokade *et al.* (2011) studied montmorillonite catalysts, and Zaki (2005) studied manganese oxide and iron oxide modified alumina and silica, but high reaction temperatures of 500 °C for Zaki and low ethylene selectivity for Bokade eliminated those catalysts as possible industrial catalysts [12,13].

The Braskem (Brazil) ethanol to ethylene plant began operation in 2010 and is currently the only plant of its kind at the commercial scale [14]. Although it is considered commercial scale, it produces 200,000 tons of ethylene per year, which pales in comparison with the millions of tons of ethylene capacity that the top steam cracking plants have. In order to make green ethylene plants more competitive, further advancements must be made from Braskem's first step towards environmentally friendly ethylene production. This review article will discuss the catalysts recently studied that have the potential to be applied in an industrial scale ethanol to ethylene plant competitive with steam cracking plants. In addition, other innovative technologies that should be considered for such a plant are presented. Because other research papers on the dehydration of ethanol to ethylene do not report findings in the context of industry, catalysts and technology in relation to production costs and feasibility are also evaluated.

Figure 3. Mechanism for the dehydration of ethanol to ethylene.



2. Catalysis

2.1. Modification of γ-Al_2O_3

From the very beginnings of the studies of ethanol dehydration to ethylene, γ-alumina has been used as a catalyst for the reaction. However, due to the high reaction temperature of 450 °C required and the relatively low ethylene yield of 80%, researchers now look to modify the catalyst to reduce the reaction temperature and increase ethylene yield to make it more economically efficient. Phillips Oil Company (USA) utilizes γ-alumina treated with KOH and ZnO/Al_2O_3 in its production of ethylene and

Halcon SD (USA) has applied MgO-Al_2O_3/SiO_2 based SynDol catalyst in their facilities [15]. Research on more modifications continues, with Doheim *et al.* (2002) achieving 97% ethanol conversion at 300 °C with Na_2O-doped Mn_2O_3/Al_2O_3 [16]. Chen *et al.* (2007) investigated the catalytic ability of TiO_2 modified γ-Al_2O_3 [15]. The TiO_2/γ-Al_2O_3 catalyst was prepared by mixing commercial γ-Al_2O_3 powder with Na_2CO_3 solution, then adding $Ti(SO_4)_2$ and Na_2CO_3 solution. SO_4^{2-} was eliminated by washing and centrifugation; then, the material was dried and calcined to produce TiO_2/γ-Al_2O_3. Tests on the catalytic ability resulted in up to 99.4% ethylene selectivity at 500 °C, significantly higher than the maximum 90.1% ethylene selectivity at 475 °C of γ-Al_2O_3 reported by Zhang *et al.* (2008) [10]. Ethanol conversion and ethylene selectivity were found to increase from 75% conversion and 40% selectivity to 100% conversion and 99.4% selectivity with temperature increases from 360 °C to 500 °C. They also decreased from 100% conversion and 97% selectivity to 96% conversion and 96% selectivity with a space velocity increased from 52 h^{-1} to 230 h^{-1}. Ethanol conversion increased from 64% to 88% at 380 °C with an increase in ethanol concentration of 10 wt % to 90 wt %, while ethylene selectivity decreased from 94% to 86% at 380 °C with the same increase in ethanol concentration. The catalyst deactivated quickly after 400 h of stability testing with temperatures be between 410 and 430 °C. Although the modification of γ-Al_2O_3 has produced much more favorable results than the original catalyst, the reaction temperature is still high, considering the modern industrial standards. On the other hand, the stability of the modified catalyst is relatively high as compared to other catalysts currently being developed, lasting 400 before deactivating.

2.2. Modification of HZSM-5

The ability of HZSM-5 to catalyze the dehydration of ethanol to ethylene at lower temperatures (200 to 300 °C) has made it commercially valuable and promising for further improvements in efficiency. The acidity and porosity of zeolites play an important role in the ethanol transformation into hydrocarbons [17]. At 300 °C, HZSM-5 can reach an ethanol conversion level of 98% and 95% ethylene selectivity. The main disadvantage of HZSM-5 is its acidity, which reduces its stability and coking resistance. As a solution, Zhang *et al.* (2008) and Ramesh *et al.* (2009, 2010, 2012) found that modification with phosphorous to reduce acidity maintained high ethylene selectivity (99.8%) while also increasing anti-coking ability [18–21]. These results were a trade-off with reaction temperature, as temperatures at or above 300 °C were necessary. Lanthanum modification was reported by Mao *et al.* (1989) and Ouyang *et al.* (2009) to improve catalytic ability at low temperatures [22,23]. Thus, Zhan *et al.* (2010) investigated the properties of lanthanum-phosphorous modified HZSM-5 [13]. Commercial HZSM-5 zeolite was impregnated with H_3PO_4 and $La(NO_3)_3$, stirred and evaporated, then dried and calcined to yield La-P-HZSM-5 of different compositions. The most favorable of the compositions was 0.5% La-2% P-HZSM-5, which reached 99.9% ethylene selectivity with 100% ethanol conversion at 240 °C. Moreover, the catalyst was able to maintain ethylene selectivity above 97% after 72 h, although no information was reported on its stability for longer periods of time. The catalyst most likely has a long life span, since La-P-HZSM-5 had a life span of 830 h before losing reactivity in the study done by Ouyang *et al.* (2009), but the inclusion of phosphorus could reduce the life span [23]. TG/DTG/DTA analysis showed restricted coke formation, as expected with the addition of lanthanum.

By modifying commercial catalyst HZSM-5, Zhan *et al.* (2010) managed to produce ideal data for the catalysis of ethanol dehydration to ethylene [13]. With almost 100% ethylene selectivity and ethanol conversion and low temperatures of 240 °C, 0.5% La-2% P-HZSM-5 is currently one of the most promising catalysts for industrial use. On top of achieving such high catalytic performance, the modifications made were inexpensive, another advantage to using 0.5% La-2% P-HZSM-5. More information on long term stability of the catalyst and effects of different reaction conditions is necessary before it can be employed in large scale plants.

Another modification developed by Guo *et al.* is nanoscale HZSM-5 zeolite catalysts [24–27]. The nanoscale HZSM-5 zeolite catalyst labeled as nano-CAT was obtained. The microscale HZSM-5 zeolite powder (crystal size: 1–3 μm) was commercially available [25]. By modifying commercial catalyst HZSM-5, Sendesi *et al.* (2012) has found that modification with Si/Al ratio. Based on TPR results, increasing Si/Al ratio from 25 to 250 was improved the redox properties, which will favor the speed of electron transfer [28]. Compared to conventional-sized HZSM-5 zeolites, the nanocatalysts reached 99.7% ethylene selectivity and 100% ethanol conversion at 240 °C, and maintains both ethanol conversion and ethylene selectivity above 98% for 630 h of reaction, exhibiting high stability. On top of that, the catalyst demonstrated good coking resistance. The nanoscale HZSM-5 appears to be the most ideal catalyst out of the current ones being studied, but before applying it in industry, the feasibility of scaling nanoscale catalysts to large commercial scale production plants while retaining laboratory results should be considered.

2.3. Heteropolyacids

Heteropolyacid salts have attracted attention as potential catalysts due to the multifunctional properties of the salt formation. Particularly, $Ag_3PW_{12}O_{40}$ has demonstrated high catalytic ability, making it a promising catalyst for the dehydration of ethanol to ethylene, but its high acidity reduces its stability. Gurgul *et al.* (2011) investigated the influence of surface composition of $Ag_3PW_{12}O_{40}$ on its catalytic ability and stability, especially observing the effects of air humidity [29]. The catalyst was prepared by reacting silver nitrate with tungstophosphoric acid, and the resulting salt was dried. From the catalytic tests, $Ag_3PW_{12}O_{40}$ was found to achieve 99.8% ethylene selectivity at 2% humidity and 220 °C, but only about 70% ethanol conversion. At 9% humidity, ethylene selectivity was lowered to 99.2% at 220 °C, but ethanol conversion increased to almost 100%. Thus, Gurgul *et al.* (2011) concluded that the presence of water stabilized the surface composition of AgPW salt (e.g., $Ag_3PW_{12}O_{40}{\cdot}3H_2O$) [29]. Long term stability of the catalyst was not detailed in the study, so whether the application of AgPW salt in industry is economically favorable or not is still left to be determined. However, the high ethylene selectivity (99.2%) at a low reaction temperature of 220 °C suggests that areas of the world with an average relative humidity of 9% should investigate the catalyst further, since the reaction temperature is lower than most of the current catalysts being studied. The lattice parameter for $Ag_3PW_{12}O_{40}{\cdot}3H_2O$ structure at 473 K was estimated to 11.93 Å [29]. It was also observed when the structure of $H_3PW_{12}O_{40}{\cdot}6H_2O$ with 12.15 Å [30].

Ciftci *et al.* (2012) also studied the modification of tungstophosphoric acid (TPA) by impregnating it into MCM-41 [31]. The resulting TPA-MCM-41 catalyst achieved 99.9% ethylene selectivity at 300 °C, with about 98% ethanol conversion. This far outperforms the results of pure TPA reported by

Varisli *et al.* (2007) with only 77% ethylene selectivity at 250 °C [32]. Activities of silica supported tungstophosphoric acid (TPA), and salts of TPA were tested for the dehydration of ethanol [31]. TPA incorporated silicate structured new mesoporous catalysts were synthesized following one-pot hydrothermal and impregnation procedures. Surface area of TPA-MCM-41, which was prepared by impregnating TPA into MCM-41, was two orders of magnitude higher than the surface area of pure TPA and this catalyst showed very high activity in dehydration reactions of both ethanol and methanol [32]. Very stable ethanol conversion data near 100% was also reported, but no quantitative data on the long term stability was included. The use of TPA-MCM-41 may be more advantageous for areas without enough humidity for AgPW salt in terms of heteropolyacids, but the reaction temperature of 300 °C makes TPA-MCM-41 less favorable than catalysts such as 0.5% La-2% P-HZSM-5.

Varisli *et al.* (2008) studied the impregnation of silicotungstic acid in MCM-41, and found that the catalyst had 99.9% ethylene selectivity with about 99% ethanol conversion at 250 °C [33]. Due to higher surface area, it showed higher activity than TPA impregnated MCM-41. However, no data on the life span or long term stability of the catalyst was reported, so the catalyst should be further studied before it is considered for industrial application.

A W-Silicate-based heteropolyacid catalyst (TRC-92) developed by Varisli *et al.* (2010) showed 99% ethylene selectivity at 280 °C, but only about 70% ethanol conversion [34]. The W-Silicate-basedcatalyst (TRC-92), modified version of the hydrothermal synthesis procedures for the synthesis of vanadium, palladium, nickel and tungsten incorporated silicate structured mesoporous catalysts [34]. Despite the relatively low ethanol conversion though, the catalyst is capable of liquid phase reactions, because of the high stability of the solid catalyst and its having both meso and macropores. Although the low ethanol conversion may appear disadvantageous, the elimination of the need to vaporize ethanol before feeding it to the reactor may prove even more cost-efficient than 100% ethanol conversion.

A summary of the catalysts is listed in Table 2, along with a currently used industrial catalyst, SynDol, for a basis of comparison. SynDol catalyst based on $MgO–Al_2O_3/SiO_2$ developed by Halcon SD has been applied commercially [35].

Table 2. Summary of catalysts for the dehydration of ethanol to ethylene and their catalytic ability.

Catalyst	Max ethylene selectivity	Ethanol conversion	Reaction Temperature	LHSV [a]/ WHSV [b]/ GHSV [c]	Lifespan, Stability	Comments	Reference
$TiO_2/\gamma\text{-}Al_2O_3$	99.4%	100%	360–500°C	26–234 h^{-1} [a]	400 h, stable	Lab modified	[15]
0.5% La-2% P-HZSM-5	99.9%	100%	240–280°C	2 h^{-1} [b]	Very stable	Lab modified	[13]
Nano-CAT	99.7%	100%	240°C	1 h^{-1} [b]	630 h, very stable	Lab modified	[25]
$Ag_3PW_{12}O_{40}$	99.2%	100%	220°C	6000 h^{-1} [c]	Stable in 9% humidity	Lab synthesized	[29]
TPA-MCM-41	99.9%	98%	300°C	2.9 h^{-1} [b]	Very stable	Lab modified	[31]

Table 2. *Cont.*

Catalyst	Max ethylene selectivity	Ethanol conversion	Reaction Temperature	LHSV [a]/ WHSV [b]/ GHSV [c]	Lifespan, Stability	Comments	Reference
STA-MCM-41	99.9%	99%	250°C	2.9 h^{-1} [b]	Stable	Lab modified	[33]
TRC-92	99.0%	70%	280°C	2.9 h^{-1} [b]	Very stable	Lab synthesized	[34]
SynDol (Halcon) (SD, USA)	96.8%	99%	450 °C	26–234 h^{-1} [a]	Very stable	Commercial catalyst	[15]

[a] liquid hourly space velocity (LHSV); [b] weight hourly space velocity (WHSV); [c] gas hourly space velocity (GHSV).

3. Reaction Conditions

Many studies included data on the effects of changing certain reaction conditions on catalytic ability (summarized in Tables 3 and 4), but it cannot be assumed that the effects apply to any catalyst of the ethanol dehydration reaction. For example, higher space velocity appears to lower ethanol conversion and ethylene selectivity, according to studies done on TiO_2/γ-Al_2O_3 and SAPO catalysts by Chen *et al*. (2007), and Chen *et al*. (2010), but higher space velocity slightly increased ethylene selectivity for La-HZSM-5 in the study by Ouyang *et al*. (2009) [8,15,23]. Other changes in reaction conditions include the presence of water in the reactor in the form of humidity, which stabilizes AgPW catalyst but reduces ethylene selectivity in the study by Gurgul et al. that compared catalytic ability under 9% humidity with 2% humidity (2011) [29]. Matachowski *et al*. (2012) also reported higher stability of AgPW catalyst in 10% humidity, but showed results for higher ethylene selectivity and higher ethanol conversion [36]. Wang *et al.* (2011) found that the optimal ethanol feed mass fraction for Zn-Mn-Co-HZSM-5 was actually 34.4% while Petroleum Processing and Petrochemicals (2010) reported an optimal ethanol feed mass fraction of 74% for La-P-HZSM-5, significantly different values despite both catalysts being modified versions of HZSM-5 [37,38]. However, the two optimization studies and two humidity studies provide insights into whether or not water in the ethanol feed deactivates catalysts, and whether facilities must be added to remove water before the ethanol can be fed into the reactors. For all catalysts reviewed, increasing temperature increases ethylene selectivity up to an optimal reaction temperature, after which increasing the temperature reduces selectivity and stability of the catalyst.

Table 3. Effect of space velocity on catalytic ability of ethanol dehydration catalysts.

Catalyst	Reaction Condition	Condition Setting	Ethanol Conversion	Ethylene Selectivity	Reference
TiO_2/γ-Al_2O_3	Space velocity (LHSV)	52 h^{-1}	100%	98%	[15]
		234 h^{-1}	96%	97%	
SAPO	Space velocity (WHSV)	2 h^{-1}	100%	100%	[8]
		30 h^{-1}	65%	20%	
La-HZSM-5	Space velocity (LHSV)	0.5 h^{-1}	100%	97%	[23]
		25 h^{-1}	39%	100%	

Table 4. Effect of humidity on catalytic ability of AgPW catalyst.

Catalyst	Reaction Condition	Condition Setting	Ethanol Conversion	Ethylene Selectivity	GHSV	Reference
AgPW	Humidity	2% 10%	70% (470 K) 100% (470 K)	100% (470 K) 80% (470 K)	6000 h^{-1}	[36]
AgPW	Humidity	2% 9%	75% (493 K) 100% (493 K)	100% (493 K) 99% (493 K)	6000 h^{-1}	[29]

4. Industrial Concerns

In addition to research on optimizing the catalysis of ethanol dehydration, investigations in improving other aspects of producing green ethylene have also made progress. Instead of conventional methods for obtaining ethanol from fossil fuels or by synthetic gas or even newer methods such as using corn feedstock, Algenol (USA) has proposed to produce ethanol by fermentation in algae [39]. In comparison to yeast fermentation, ethanol produced by algae can be removed without killing the algae. Because algae can continuously grow and produce ethanol without being killed, the need for regeneration of the algae is eliminated, as opposed to the need to regularly replenish yeast. As a result, Algenol researchers reported that by using algae, they could produce 6000 gallons of ethanol per acre per year, a significant increase from the 400 gallons per acre per year produced by corn production. However, since Algenol is only concerned with the production of ethanol and not ethylene, a link between the production of ethanol by algae and the rest of an ethylene processing plant must be considered, whether it is storage and transport of ethanol between two separate facilities or direct feed of ethanol into the reactor.

Chen *et al.* (2007) researched the application of microscale reactors instead of conventional fixed bed reactors and found that the smaller reaction systems increased surface-to-volume ratios, mass and heat transfer capabilities, process safety, yield, and efficiency [40]. Selectivity for ethylene increased from 98.9% to 99.3% for $TiO_2/\gamma\text{-}Al_2O_3$ under identical reaction conditions, and ethylene yield increased from 0.67 g/(g-catalyst·h) to 72.7 g/(g-catalyst·h). Scaling miniature reactors to industrial proportions poses a problem, as even the slightest increase of size of the reactors will produce less favorable results, and increasing the quantity of microreactors to meet commercial demands is potentially more costly than simply using conventional fixed bed reactors.

Several other challenges must be overcome before the dehydration of ethanol to ethylene can replace steam-cracking fossil fuels to produce ethylene. Besides finding a reliable, renewable source of ethanol and developing ideal catalysts for the reaction, industrial concerns such as production cost, energy cost, catalyst regeneration, and most importantly, yield must be considered. Production costs may be lowered by increasing the space velocity of the plant, but doing so increases the optimal temperature and decreases the stability of the catalyst. An alternative is reducing the amount of equipment and machinery needed in the plant, which would also reduce the number of employees needed to operate. For energy costs, reducing reaction temperature while still achieving high selectivity is a priority, but the exiting fluid can also be used as heating fluid to recover heat and save energy. The exiting fluid is predominantly ethylene, diethyl ether, and water, and depending on the catalyst, the water may be directly fed back into the reactor with ethanol to maximize heat transfer

from water. The diethyl ether may be converted to ethylene, but that would require another facility. Instead, it can be used as a fuel to reduce energy costs as well. Catalysts with long life spans would reduce the number of times they would need to be regenerated. With catalysts such as SynDol or nanoscale HZSM-5, which have long lifespans compared to other catalysts, separate equipment for catalyst regeneration may not be necessary. This would significantly reduce production costs as well.

If the currently researched technology were applied to an industrial plant while maintaining experimental results, the plant would be able to produce as much ethylene as steam-cracking plants, except at a steep cost of farmland. The Braskem ethanol to ethylene plant, currently the only commercialized ethanol to ethylene plant, imports 462 million liters of ethanol to produce 200,000 tons of ethylene per year [14]. To produce as much ethylene as the tenth largest ethylene complex, Equate Petrochemical Company's plant (Kuwait), the plant would have to produce 1,650,000 tons of ethylene per year [5]. At 99.7% ethylene selectivity and 100% ethanol conversion for nanoscale HZSM-5, approximately the same amount of ethanol would be consumed. Using the density of ethanol at 20 °C, the following conversion can be made:

$$16{,}500 \text{ tons} \times \frac{1000 \text{ kg}}{1 \text{ ton}} \times \frac{1 \text{ L}}{0.7893 \text{ kg}} \times \frac{1 \text{ gallon}}{3.7854 \text{ L}} = 552{,}200{,}000 \text{ gallons} \quad (1)$$

Algenol's technology of producing ethanol by algae fermentation can supposedly produce 6000 gallons of ethanol per acre per year [39]. This means:

$$552{,}200{,}000 \text{ gallons} \times \frac{1 \text{ acre}}{6000 \text{ gallins}} = 92{,}000 \; acres = 370 \text{ Km}^2 \quad (2)$$

To put this in perspective, all of the U.S. Virgin Islands or a third of Hong Kong would have to be used for algae farms. This is just to compete with the tenth largest ethylene plant. To produce 141 million tons of ethylene, the projected global demand for 2012, 32,000 km^2 would have to be used. That is, an area around the size of Taiwan would be used on algae farms. A more feasible solution would be to use a mixed source ethanol feedstock—some produced by algae and some by other biomass. This way the amount of additional land needed for algae farms would be reduced, and the amount of corn or sugar cane used to produce ethanol would not cause competition with other industries, such as the food industry.

Of course, production and energy costs would have to be considered as well, in order to compare with steam cracking plants. The costs of constructing the algae facilities, transporting ethanol to the ethylene plant, purchasing or synthesizing catalysts in bulk, and heating the reactor to optimal reaction temperature are just a few of the many investments that would have to be made. A plant that produces 500,000 tons of ethylene per year would require 821,000 tons of ethanol, 22,000 tons of fuel, and a capital cost of $150 million (compare with $700 million for a cracking plant), according to Seddon [41]. The production cost of ethylene would depend mainly on ethanol prices, and currently ethanol prices are at about $910 per ton, as reported by Businessweek [42]. The International Renewable Energy Agency (IRENA) reported ethylene production costs at about $2000 per ton from corn feedstock in the U.S. ($1200 per ton from sugar cane feedstock, which is what the Braskem plant uses), while petrochemical ethylene only costs $600 to $1300 per ton [43]. Compared with the bioethylene production costs with the $1650 per ton price of ethylene reported by PRNewswire, the bioethylene plant would not gain any revenue [44]. However, using the algae technology instead of

corn feedstock and more efficient catalysts like nano-HZSM-5 would significantly reduce production costs, and may make a bioethylene plant profitable. Nanoscale and microscale HZSM-5 zeolites, the crystal size of nanoscale HZSM-5 is in the range of 50–100 nm and that of microscale HZSM-5 is in the range of 1–3 μm [25]. In addition, the high demand for ethylene and increasing public awareness of green alternatives ensures that there is a market for green ethylene, with approximately 80% of the ethylene produced by the Braskem plant was sold before its construction [14]. The bioethylene produced may also be sold with a green premium to increase profits.

High energy costs account for much of the production costs as well, and a comparative study of different ethanol to ethylene processes by Arvidsson *et al.* (2011) includes an analysis on heat recovery and conservation to potentially reduce those costs [45]. Based on a 200,000 ton ethylene capacity, simply combining an ethylene plant and an ethanol plant into one refinery instead of using stand-alone plants would reduce the minimum hot utility demand from 130.9 MW to 79.2 MW. The minimum cold utility demand would also be reduced from 195.7 MW to 141.1 MW. In addition, the integration of flue gas with the ethylene reactors of the combined bio-refinery would further reduce the utility demands to 68.0 MW (hot) and 140.4 MW (cold). Alternatively, introducing mechanical vapor recompression on the rectifier distillate was predicted to have a hot utility demand of 32.1 MW, and a cold utility demand of 102.4 MW. Lastly, the delivery of high pressure (41 bar) steam to the chemical cluster resulted in a calculated hot utility demand of 76.0 MW and a cold utility demand of 137.6 MW. A summary of results is shown in Table 5. The application of any of the theoretical biorefinery configurations would greatly reduce energy costs, with the incorporation of mechanical vapor recompression being the most efficient. In combination with the algae technology and nanoscale HZSM-5, converting ethanol to ethylene may soon become a cost-effective reality.

Table 5. Comparison of ethanol to ethylene plant configurations [45].

Configuration	Minimum hot utility (MW)	Minimum cold utility (MW)	Net electricity (MW)	Net fuel (MW)
Stand-alone EtOH	112.2	147.6	24.3	0.0
Stand-alone Ethylene	18.7	48.1	−4.4	−15.9
Biorefinery	79.2	141.1	8.0	−7.9
Bio-F [1]	68.0	140.4	8.5	7.5
Bio-MVR [2]	32.1	102.4	−15.8	−7.9
Bio-VHP [3]	76.0	137.6	17.1	−7.9

[1] Biorefinery—Flue gas integration with ethylene reactors; [2] Biorefinery—Introduction of mechanical vapor recompression on the rectifier distillate; [3] Biorefinery—Very high pressure steam 41 bar (absolute pressure) steam delivery to the chemical cluster.

5. Conclusions

Despite much advancement being made in producing ethylene from ethanol, the process is not ready to replace fossil fuel methods in meeting the world demand for ethylene. Recent successes in increasing ethylene yield and lowering reaction temperature by modifying catalysts have revealed a number of catalysts that could be applied to industry, the most favorable one being nanoscale HZSM-5, which has a 99% ethylene yield at 240 °C and a lifespan of 630 h before ethylene selectivity

decreased to below 98%. However, even with the development of catalysts such as $Ag_3PW_{12}O_{40}$ and nano-HZSM-5 achieving over 99% ethylene yield at temperatures as low as 220 °C, and algae and microreactor technology potentially reducing other production costs, bioethylene is still not profitable enough to produce industrially. Recent findings on lowering energy costs have also appeared to make ethanol dehydration more profitable, but since they were based on a 200,000 tons capacity, a larger capacity plant may not save as much on energy expenditures. As the most widely produced organic chemical in the world, ethylene is produced at the rate of over 100 million tons per year. Until ethanol dehydration plants can achieve such high yield at costs competitive with steam-cracking plants, industry will continue to use the limited fossil fuels. Thus, future researchers should conduct their research in the context of economical factors and with respect to the other steps in the process of producing ethylene from ethanol. Studies focused on the catalysis of ethanol dehydration should keep catalyst stability and lifespan in mind and should shift goals towards lowering reaction temperature instead of increasing yield, and studies on ethanol production or reactor technology should not neglect the problem of linking parts of the ethylene production chain together. In addition, studies on catalysts should include information on the effects of changing various different reaction conditions, rather than just one or two. A reaction condition that no study addressed is the effect of using impure ethanol as a feedstock. This is particularly relevant to industry, because different ethanol feedstock sources contain different impurities, which may affect a certain catalyst's catalytic ability or life span in different ways. Increasing the number of studies on algae technology may have the benefit of possibly increasing the yield of ethanol per acre of algae, and more studies on heat conservation in reactors may reduce energy expenditures needed for plant operation. Of course, one also considers simultaneously the transformation of ethylene (or ethanol) to alkanes or alkenes. It will produce products that are more valuable.

Acknowledgments

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PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 6

ANALYTICAL VERIFICATION OF THE PURITY OF ETHYLENE GENERATED BY DEHYDRATION OF ETHANOL

- Report -

RESTRAIN Generator 740

Content Analysis of Catalytic Produced Ethene

acc. to SANCO/3030/99 rev.4 (2000)

Sponsor

Restrain
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Test Facility

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Käthe-Paulus-Str. 1
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Study ID

acc. to GLP

170531DS / CGB17259

Study Completed On

12 1. DEZ. 2017

Statement of GLP Compliance

Title	RESTRAIN Generator 740 Content Analysis of Catalytic Produced Ethene
Guideline	SANCO/3030/99 rev.4 (2000)
Test Item	Ethanol 96% (Batch number: K47729671; source material for the catalytic produced ethene)
Test Systems	RESTRAIN Generator 740 (serial no. ICA740/004 and ICA740/074)
Test Facility	Noack Laboratorien GmbH Käthe-Paulus-Str.1, 31157 Sarstedt, Germany Phone: (+49) 050 66/706 70, Fax: (+49) 05066/706 789, E-mail: info@noack-lab.de

I declare that this study was conducted and reported in compliance with present OECD, EC and German principles of Good Laboratory Practice.

21.11.17
(Date)

(Jens Lange, Study Director)

Statement of the Quality Assurance Unit

Title	RESTRAIN Generator 740 Content Analysis of Catalytic Produced Ethene
Guideline	SANCO/3030/99 rev.4 (2000)
Test Item	Ethanol 96% (Batch number: K47729671; source material for the catalytic produced ethene)
Test Systems	RESTRAIN Generator 740 (serial no. ICA740/004 and ICA740/074)
Study Director:	Jens Lange

The study was verified and reported to the study director and test facility management as follows:

Inspected study phase		Inspection date	Date of report
Study plan		2017-11-02 2017-11-20	2017-11-02 2017-11-20
Experimental phase	Sampling for analysis	2017-11-27	2017-11-27
Report		2017-12-13 2017-12-14 2017-12-21	2017-12-13 2017-12-14 2017-12-21

It is confirmed that the reported results accurately and completely reflect the raw data of the study. Also methods, procedures and observations are accurately and completely described in the report.
The accordance of the study with its study plan and the principles of Good Laboratory Practice is guaranteed.

21.12.2017
(Date)

(Dr. Anja Buchholz)

Personnel Involved

Study Director	Jens Lange (Technical expert)
Technical Staff	Bastian Daldrup Alexander Nagel
Quality Assurance Unit	Dr. Anja Buchholz (Biologist) Christine Bruhnke (Biologist)
Test Facility Management	Dr. Christian Maeß (Chemist)

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Abbreviations and Definitions

A.I.	Active ingredient
Date	YYYY-MM-DD (year-month-day)
GC-FID	Gas chromatography with flame ionization detector
GC-MS	Gas chromatography with mass selective detector
QAU	Quality assurance unit
ppm (w/w)	Parts per million, correlates to the unit mg/kg

1 Summary

The content analysis of catalytic produced ethene out of Ethanol 96% (batch K47729671) by two units of RESTRAIN Generator 740 (serial numbers: ICA740/004 and ICA740/074) was performed according to SANCO/3030/rev.4 (2000) from 2017-11-27 to 2017-12-08 at Noack Laboratorien GmbH, 31157 Sarstedt, Germany.

The contents of the product ethene were determined via GC-FID on a capillary analytical column. The analytical method was validated and tested with satisfactory results in regard to linearity, accuracy (product impurities only), precision, specificity and confirmation of analyte identity during the course of the study "RESTRAIN Generator 740 - Five Batch Analysis of Catalytic Produced Ethene" with the Noack study ID 160524DS / CFB17259. Quantification was based on external standard calibration with available standard substance with peak identification based on retention time.

The analyzed products of two batches of the test system showed good homogeneity with respect to the content of the catalytic produced ethene.

For the two test systems,
the content of **Ethene** in the product
was determined to be:

ICA740/004: 94.5 ± 0.3% (w/w)

ICA740/074: 94.7 ± 1.1% (w/w)

2 Characterization of the Test Item and Systems

2.1 Properties of the Test Item

Test Item	Ethanol 96% (not denatured)
Source	Merck KGaA, 64271 Darmstadt, Germany
Product No.	100971
Batch	K47729671
Content (certified)	94.1% (m/m), 96.2% (v/v)
CAS No.	64-17-5
EC No.	200-578-6
Molecular formula	C_2H_5OH
Molecular weight	46.07 g/mol
Appearance	Colorless liquid
Density (certified)	0.808 (d 20/20)
Stability under test	Assumed to be stable during analysis; undergoes conversion by the generator
Expiry date	2021-04-30
Recommended storage	Keep container tightly closed in a dry and well-ventilated place away from heat and ignition sources.

The test item and the information concerning the test item were provided by the manufacturer.

2.1.1 Test Facility Actions (Test Item)

Receipt	2016-07-28
Identification parameter	Name, batch number, state and color
Retention sample	At least 1 g of the test item has been retained on 2016-07-28 and stored at 6 ± 2 °C.
Storage condition	Room temperature (20 ± 2 °C), protected from light in the tightly closed original container

2.2 Properties of the Test Systems

Generator name	RESTRAIN Generator 740
Serial No.	ICA740/004 (Built Nov-12) ICA740/074 (Built Sep-13)
Procedure	Ethene gas is produced via catalytic dehydration of ethanol by the generator. The reaction by-product water condensates at the typical storage temperature between 4 and 9 °C leading to the gaseous product.

The generators and the concerning information were provided by the sponsor.

2.2.1 Test Facility Actions (Generator)

Receipt	2017-05-31
Storage condition	Ambient temperature (controlled and monitored until unpacking)
Generator modifications	The air cooled metal condenser at the outlet of the catalytic oven will be replaced by a water cooled laboratory glass cooler (Liebig-condenser), as a gas tight connection is necessary for the performance of the study.

3 Methods

Test Guidelines	• SANCO/3030/99 rev.4 (method validation of the content analysis of the main product) The study was performed in compliance with GLP. For the respective guidelines please refer to the point 'Literature / References'.
Type and Purpose of the Study	Comparative analysis of the product ethene, produced by two ethene generators of the type "RESTRAIN Generator 740" with the test item ethanol as source material with respect to the concentration of the main product (ethene), as specified in part 3.2.2.

3.1 Test Procedure

Type and Frequency of Measurement	Product samples of each generator were injected in single injection and analyzed via GC-FID (analytical system).
Generator set-up	The generators were set up according to the user's manual. 2 liters of the test item (Ethanol 96%) were fed to the generator tank. The (gaseous) product was cooled down in a Liebig condenser at 6.5 ± 0.5 °C (please see 2.2). The temperature of 6.5 ± 0.5 °C was chosen to mimic realistic working conditions (4 – 9 C). The remaining (gaseous) product was sampled in the sampling bags.
Temperature monitoring	The inlet temperature of the cooling medium of the Liebig condenser was monitored in intervals of 2 minutes during the sampling procedure. A precision thermometer-data-logger (GMH 3750 GMH Messtechnik GmbH) was used.
Sample size	0.6 L of the gaseous product
Replicates	Triplicates for each of the two generators

3.2 Analytical Method

Method of determination	Analytical quantification of the product ethene was performed via GC-FID on a capillary column.

3.2.1 Analytical System

Analytical system	GC-FID-system consisting of:
Gas chromatograph	CP-3800, VARIAN
Autosampler	CTC Combi PAL, CTC ANALYTICS
Detector	flame ionization detector (FID; integrated into the GC)
Software	Galaxy Chromatography Data System, Ver. 1.9.3, VARIAN
Analytical column	PoraPLOTQ, 50 m, ID: 0.32 mm, film thickness: 10 µm, batch 9348177, AGILENT
Inlet liner	Splitless Sky Liner, single taper gooseneck w/wool, 4 x 6.5 x 78.5 mm, batch 895147BL, RESTEK

3.2.2 Reagents and Equipment

Analytical standard	Ethene, ≥99.9%, LINDE
Reagents	Nitrogen 5.0, LINDE Air
Equipment	Gastight glass syringes (100 µL, 50 mL), HAMILTON Gastight glass syringe (10 µL), SGE Tedlar® gas sampling bags with Thermogreen® LB-2 septa (0.6 L with screw-cap valve [SCV] and 2 L with push-lock valve [PLV]), SUPELCO Standard laboratory equipment

3.2.3 Conditions of Analysis

Injection mode	Headspace injection
Injection volume	1000 µL
Injector	Splitless for 1 min
Injector temperature	150 °C

GC Temperature Program

Temperature [°C]	Heating rate [°C/min]	Hold time [min]
50	0	0.2
160	3	20.0
250	30	15.0

Carrier gas (He)	1.0 mL/min
N_2 make-up flow	29 mL/min
H_2 flow	30 mL/min
Air flow	300 mL/min
Detector temperature	250 °C
Run time	~75 min
Approx. retention times	16 min

3.2.4 Method Validation

Method validation	The analytical method was validated according to SANCO 3030/99 rev.4 (2000) during the course of the study 160524DS / CFB17259. For detailed results please refer to the respective report.

3.3 Confirmation of Analyte Identity

Analyte identity — The analyte identity of the product ethene was confirmed via GC-MS in a separate run.

3.3.1 Analytical System

Analytical system	GC-MS-system consisting of:
GC	7890B, AGILENT
Autosampler	CHRONECT Robotic RTC PAL, AXEL SEMRAU
Detector	7010 Quadrupol-MS/MS, AGILENT
Software	MassHunter B 07, AGILENT Chronos 4.5.1, AXEL SEMRAU Evolution 4.6.3, GL SCIENCES
Analytical column	PoraPLOTQ, 50 m, ID: 0.32 mm, film thickness: 10 µm, batch 9348177, AGILENT
Inlet liner	AS-OPT-4011-S, OPTIC, Fritt-Liner, AXEL SEMRAU

3.3.2 Conditions of Analysis

Injection mode	Headspace injection
Injection volume	1000 µL
Injector	Optic 4
Injector temperature	200 °C

GC Temperature Program

Temperature [°C]	Heating rate [°C/min]	Hold time [min]
50	0	0.2
160	3	20.0
250	30	15

Carrier gas (He)	1.0 mL/min
Run time	~75 min
Approx. retention times	13 min

3.3.3 Conditions of Detection

Ionisation mode	Electron Impact (EI)
Ion polarity	Positive, centroid
Scan mode	MS1 scan
Scan method	m/z: 15 – 80
Scan time	0.20 sec
Detector temperature	250 °C

3.3.4 Preparation of Samples

Calibration samples

A standard at 10% (v/v) in nitrogen was prepared and used to spike actual 7 headspace vials (~21 mL, filled with air), which were used for calibration.

The calibration ranges are specified in part 8.

Test samples

For the determination of ethene, 5 µL of the generator product sample was used to spike a headspace vial (~21 mL, filled with air), corresponding to a dilution factor of 4200, prior to analysis.

4 GLP

Chronological Test Description	- Application of the test item to the generators - Preparation of product samples of each of the two generators (experimental starting) - Analysis of samples - Calculations
Dates	
Study initiation	2017-11-17
Experimental starting	2017-11-27
Experimental completion	2017-12-08
Study completion	Please see page 1
Deviations from the guidelines	None
Deviations from the study plan	A different batch of the liner was used for the GC-FID analysis due to a replacement. The column oven temperature program for the GC-FID analysis was erroneously given wrong. These deviations are considered to have no impact on quality and integrity of the study.
Archiving	The following will be retained in the archive of the test facility for the period as specified in the operative national GLP regulations (15 years): • all raw data • study plan • final report • all records performed by the quality assurance program including master schedules • sample of the test item

5 Results

5.1 Confirmation of Analyte Identity

GC-MS

The analyte identity was confirmed using GC-MS in a separate run. The mass spectra of the samples as well as standards were made and compared.
Results are provided in part 10.

5.2 Definitive Study

Table 1: **Content and Precision Determination of the Product Ethene**

Generator	[% w/w] Content in Replicate No.[1)]			Mean Content [% w/w]	SD ± [% w/w]	CV [%]
	1	2	3			
ICA740/004	94.1	94.7	94.8	94.5	0.3	0.36
ICA740/074	95.8	93.6	94.6	94.7	1.1	1.2

SD = Standard deviation
CV = Coefficient of variation
1) = Dilution factor of 4201 as well as purity and exact weight of the standard taken into account

6 Conclusions

The analyzed products of two batches of the test system showed good homogeneity with respect to the content of the catalytic produced ethene. For the two test systems, the content of Ethene in the product was determined to be: 94.5 ± 0.3% (w/w) (ICA740/004) and 94.7 ± 1.1% (w/w) (ICA740/074).

7 Literature / References

- SANCO/3030/99 rev.4, Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 (11/07/00)

- OECD Principles on Good Laboratory Practice (as revised in1997), ENV/MC/Chem(98)17, Environment Directorate, OECD, Paris, 1999

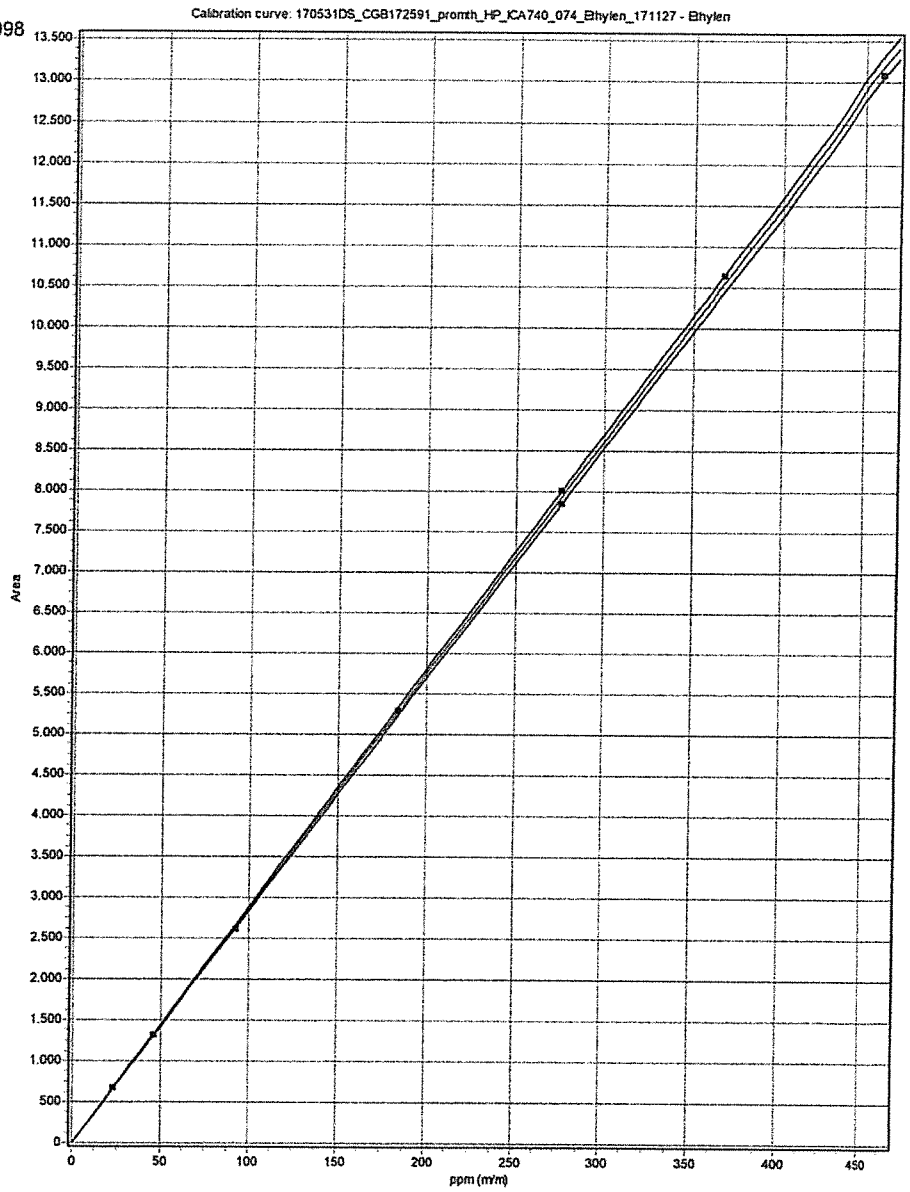
- Directive 2004/10/EC, The OECD Principles of Good Laboratory Practice (GLP)

- Principles of Good Laboratory Practice – German Chemical Law (ChemG), Annex 1

- RESTRAIN Generator 740 - Five Batch Analysis of Catalytic Produced Ethene, Noack Laboratorien GmbH, study ID 160524DS / CFB17259, Lange, 2017

8 Representative Calibration Curves and Chromatograms of Standards

Component : Ethylen
Polynom : y = b x +a
a = 0
b = 28,71152
Correlation Coef. : 0,9998
Weighting : None
Force zero : Yes



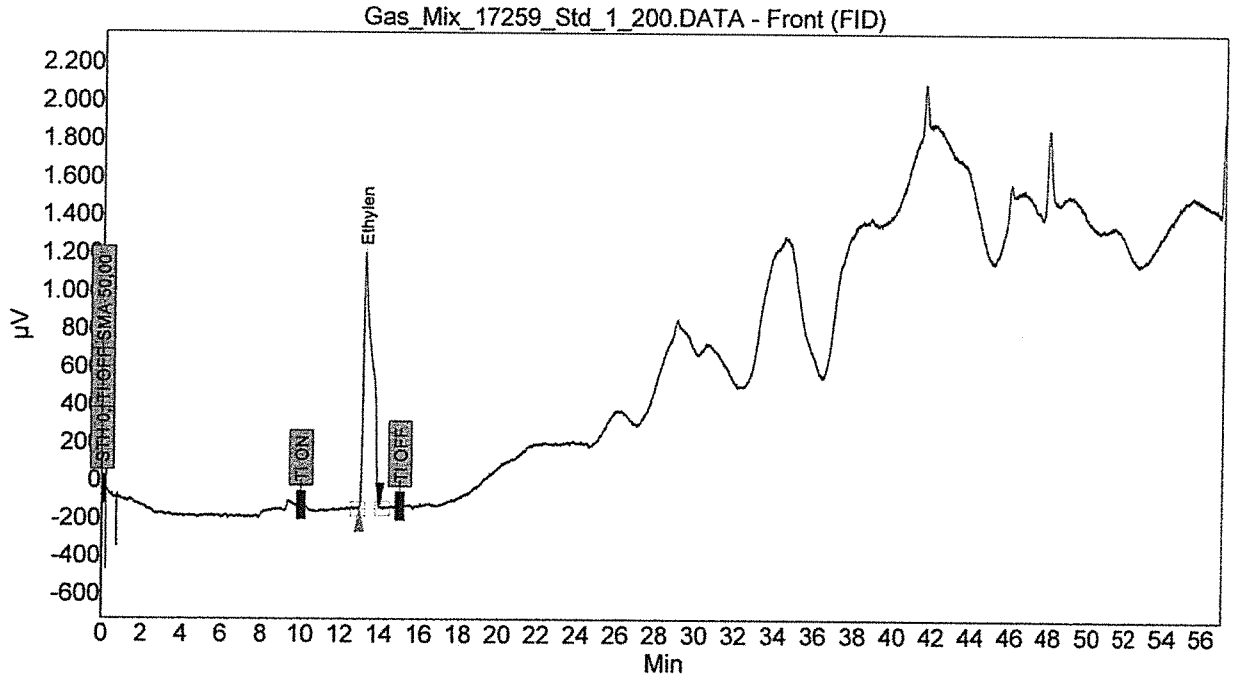
Calibration table : Ethylen

#	Used	Name	ppm (m/m)	Area	RF	Chromatogram	Date	Area (recalc)	Res %	time (Min)	Level	Username
1	1	Point 1	459.00	13089.06	0.04	Gas_Mix_17259_Std_7_200 (1)	29.11.2017 07:02:33	13178.59	0.68	13.17	7	Daldrup
2	1	Point 2	23.00	672.03	0.03	Gas_Mix_17259_Std_1_200 (1)	29.11.2017 07:03:10	660.36	1.74	13.16	1	Daldrup
3	1	Point 3	46.10	1320.06	0.03	Gas_Mix_17259_Std_2_200 (1)	29.11.2017 07:03:12	1323.60	0.27	13.20	2	Daldrup
4	1	Point 4	92.10	2610.66	0.04	Gas_Mix_17259_Std_3_200 (1)	29.11.2017 07:03:14	2644.33	1.29	13.20	3	Daldrup
5	1	Point 5	184.00	5296.10	0.03	Gas_Mix_17259_Std_4_200 (1)	29.11.2017 07:03:16	5282.92	0.25	13.20	4	Daldrup
6	1	Point 6	276.00	7848.28	0.04	Gas_Mix_17259_Std_5_200 (1)	29.11.2017 07:03:18	7924.38	0.97	13.20	5	Daldrup
7	1	Point 7	276.00	8005.23	0.03	Gas_Mix_17259_Std_5_2_200 (1)	29.11.2017 07:03:20	7924.38	1.01	13.20	5	Daldrup
8	1	Point 8	367.00	10647.09	0.03	Gas_Mix_17259_Std_6_200 (1)	29.11.2017 07:03:22	10537.13	1.03	13.17	6	Daldrup

Figure 1: **Calibration Curve and Data of the Standard Ethene**
Nominal 23.00 – 459.00 ppm (dated 2017-11-27/28)

File:Gas_Mix_17259_Std_1_200
Path: Group1\FID\171127\
Sample: N.A.
Method: 17259_PoraPLOTQ_spltless_EthOH_DEE_161219
processing Method:
CGB172591_promth_HP_ICA740_074_Ethylen_171127
Channel: Front (FID)
User: Daldrup

Sample: N.A.
Vial: 2, Rack: 1
Info: 170531DS_CGB172591_
Acquired: 27.11.2017 19:38:15
Processed: 29.11.2017 07:03:10

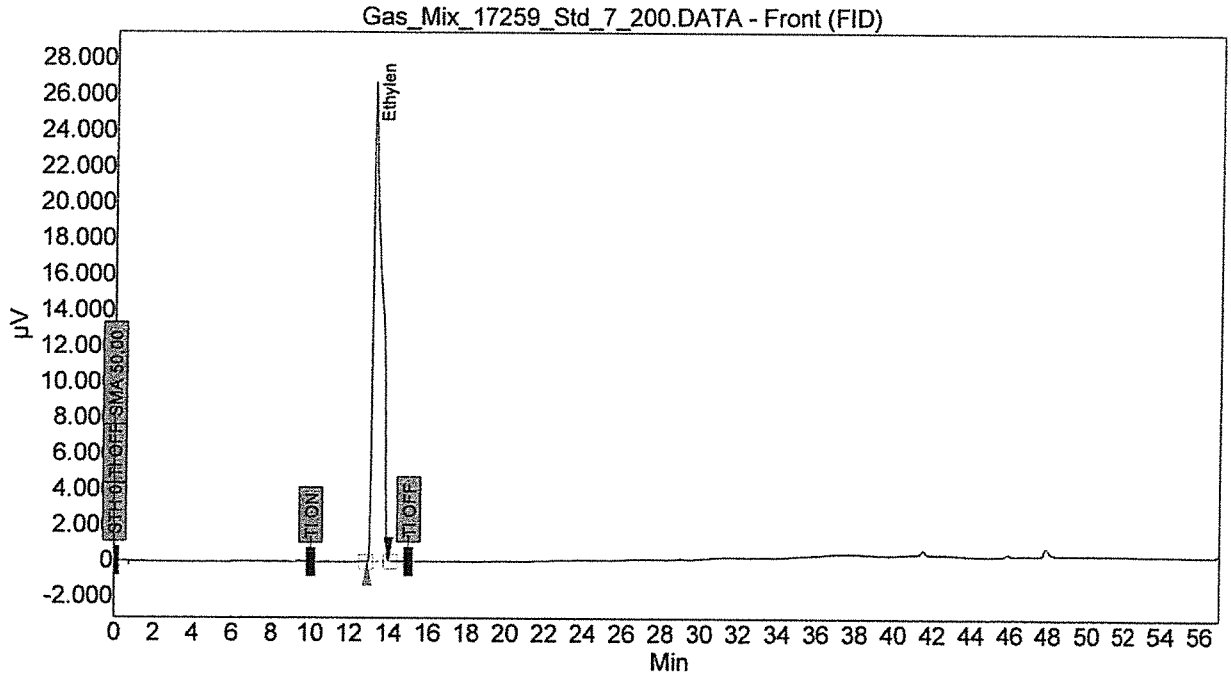


Index	Name	Time [Min]	Quantity [ppm (m/m)]	Height [µV]	Area [µV.Min]	Area % [%]
1	Ethylen	13,16	23,3696	1351,5	672,03	100,000
Total			23,3696	1351,5	672,03	100,000

Figure 2: **Chromatogram and Spectrum of the Lowest Ethene Standard**
Nominal 23.00 ppm (dated 2017-11-27)

File:Gas_Mix_17259_Std_7_200
Path: Group1\FID\171127\
Sample: N.A.
Method: 17259_PoraPLOTQ_spltless_EthOH_DEE_161219
processing Method:
CGB172591_promth_HP_ICA740_074_Ethylen_171127
Channel: Front (FID)
User: Daldrup

Sample: N.A.
Vial: 8, Rack: 1
Info: 170531DS_CGB172591_
Acquired: 27.11.2017 20:58:14
Processed: 29.11.2017 07:03:23



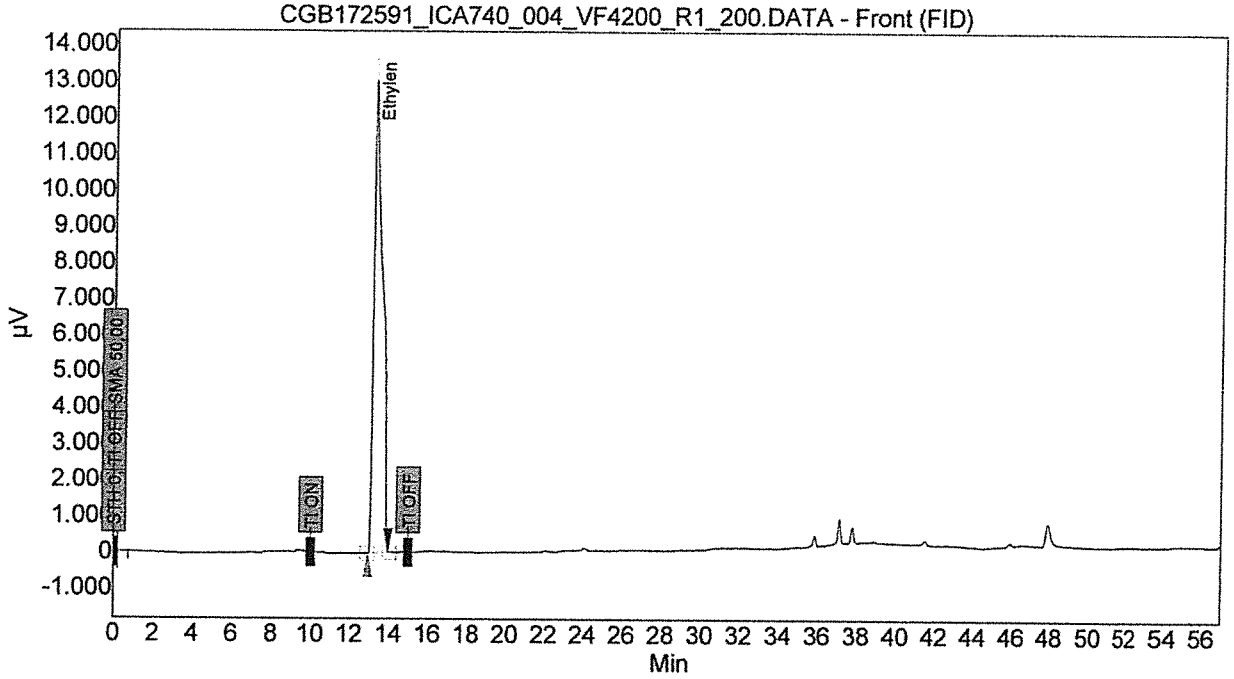
Index	Name	Time [Min]	Quantity [ppm (m/m)]	Height [µV]	Area [µV.Min]	Area % [%]
1	Ethylen	13,17	455,8818	26766,0	13089,06	100,000
Total			455,8818	26766,0	13089,06	100,000

Figure 3: **Chromatogram and Spectrum of the Highest Ethene Standard**
Nominal 459.00 ppm (dated 2017-11-27)

9 Representative Chromatograms of Test Samples

File: CGB172591_ICA740_004_VF4200_R1_200
Path: Group1\FID\171128\
Sample: N.A.
Method: 17259_PoraPLOTQ_spltless_EthOH_DEE_161219
processing Method:
CGB172591_promth_HP_ICA740_074_Ethylen_171127
Channel: Front (FID)
User: Daldrup

Sample: N.A.
Vial: 12, Rack: 1
Info: 170531DS_CGB172591_
Acquired: 28.11.2017 09:21:50
Processed: 29.11.2017 07:07:01

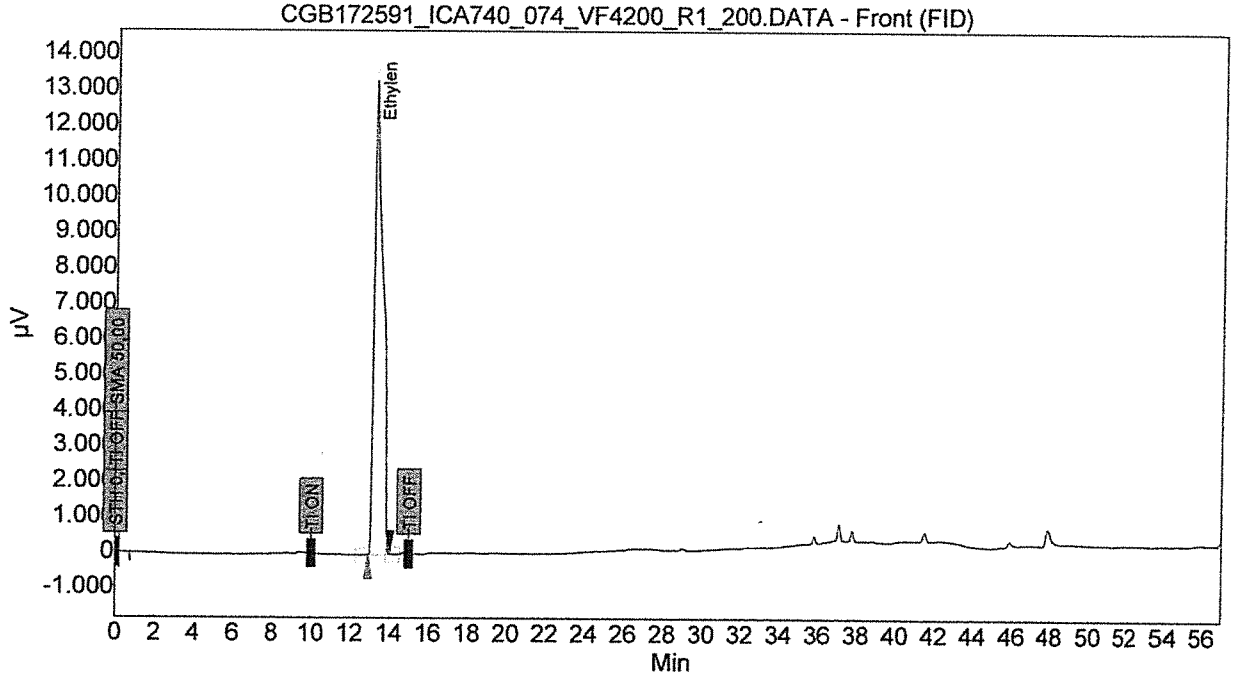


Index	Name	Time [Min]	Quantity [ppm (m/m)]	Height [µV]	Area [µV.Min]	Area % [%]
1	Ethylen	13,20	224,3240	13041,3	6440,68	100,000
Total			224,3240	13041,3	6440,68	100,000

Figure 4: **Chromatogram of Batch ICA740/004 for the Determination of the Product Ethene**
Replicate 1, dilution factor of 4201 (dated 2016-11-27)

File: CGB172591_ICA740_074_VF4200_R1_200
Path: Group1\FID\171127\
Sample: N.A.
Method: 17259_PoraPLOTQ_spltless_EthOH_DEE_161219
processing Method:
CGB172591_promth_HP_ICA740_074_Ethylen_171127
Channel: Front (FID)
User: Daldrup

Sample: N.A.
Vial: 9, Rack: 1
Info: 170531DS_CGB172591_
Acquired: 27.11.2017 23:38:21
Processed: 29.11.2017 07:06:52



Index	Name	Time [Min]	Quantity [ppm (m/m)]	Height [µV]	Area [µV.Min]	Area % [%]
1	Ethylen	13,18	228,3588	13334,0	6556,53	100,000
Total			228,3588	13334,0	6556,53	100,000

Figure 5: **Chromatogram of Batch ICA740/074 for the Determination of the Product Ethene**
Replicate 1, dilution factor of 4201 (dated 2017-11-28)

10 Confirmation of Analyte Identity

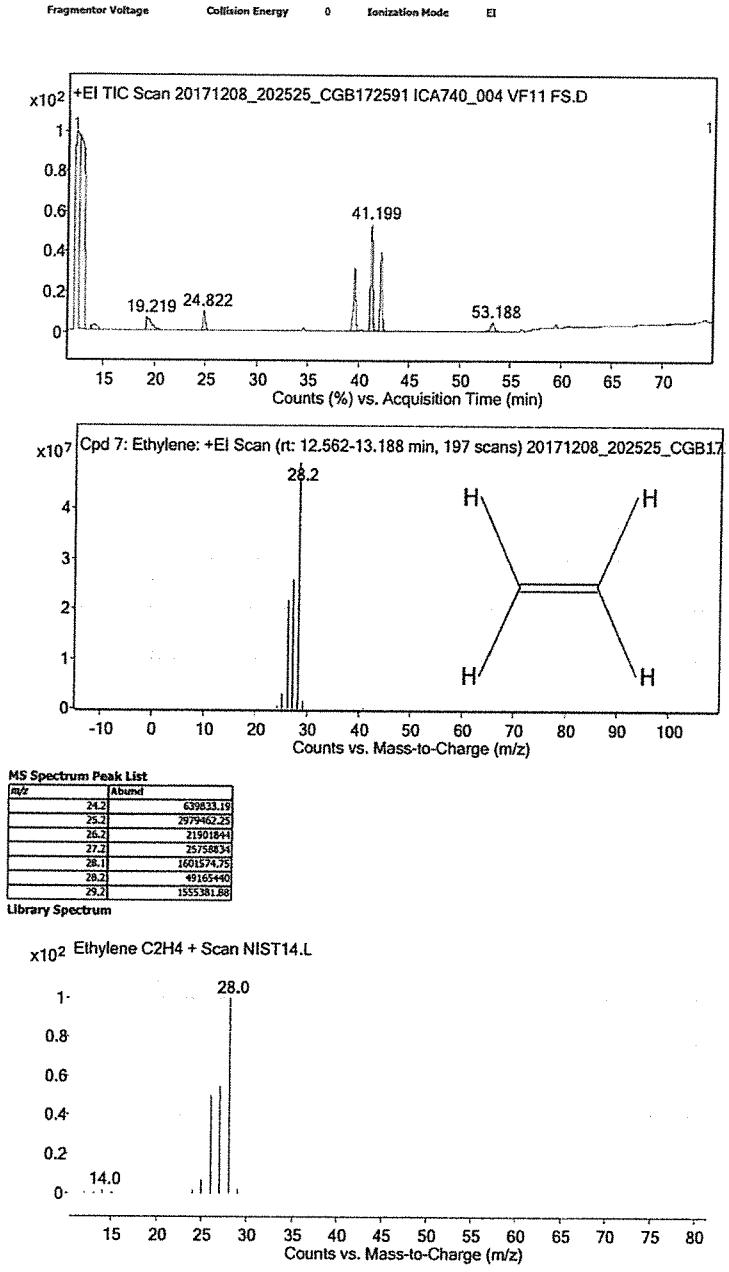


Figure 6: **MS-Chromatogram and Spectrum of the Ethylene Signal of a Generator Sample**
Including NIST library spectrum, only first signal relevant for this study (dated 2017-12-08)

11 Certificates of Analysis and Product Information

11.1 Test Item (Ethanol 96%)

Analysenzertifikat

1.00971.6010 Ethanol 96% geeignet für die Verwendung als Excipient EMPROVE® exp Ph Eur,BP
Charge K47729671

	Spezifikation		Chargenwerte	
Gehalt (m/m)	92.6 - 95.2	%	94.1	%
Gehalt (V/V)	95.1 - 96.9	%	96.2	%
Identität (IR)	entspricht		entspricht	
Aussehen	entspricht		entspricht	
Sauer oder alkalisch reagierende Substanzen	≤ 30	ppm	≤ 30	ppm
Dichte (d 20 °C/20 °C)	0.805 - 0.812		0.808	
Absorption (bei 240 nm)	≤ 0.40		0.20	
Absorption (zwischen 250nm und 260nm)	≤ 0.30		0.09	
Absorption (zwischen 270nm und 340nm)	≤ 0.10		0.02	
Das Spektrum zeigt eine stetig absteigende Kurve ohne erkennbare Peaks und Schultern	entspricht		entspricht	
Flüchtige Verunreinigungen (GC) (Acetaldehyd und Acetal)	≤ 10	ppm	≤ 10	ppm
Flüchtige Verunreinigungen (GC) (Benzol)	≤ 2	ppm	≤ 2	ppm
Flüchtige Verunreinigungen (GC) (Methanol)	≤ 200	ppm	≤ 200	ppm
Flüchtige Verunreinigungen (GC) (Summe weiterer Verunreingungen)	≤ 300	ppm	≤ 300	ppm
Flüchtige Verunreinigungen (GC) (Ausschlußgrenze)	≤ 9	ppm	≤ 9	ppm
Andere Lösungsmittel-Rückstände (ICH Q3C)	ausgeschlossen durch den Herstellprozess		ausgeschlossen durch den Herstellprozess	
Abdampfrückstand	≤ 25	mg/l	2	mg/l
Bakterien-Endotoxine	≤ 2.5	I.U./ml	≤ 2.5	I.U./ml
Gesamtanzahl aerober Mikroorganismen (TAMC)	≤ 100	CFU/g	< 10	CFU/g
Gesamtanzahl an Hefen und Schimmelpilzen (TYMC)	≤ 10	CFU/g	< 10	CFU/g
Gegen Gallensalze resistente gramnegative Bakterien (abwesend in 1 g)	entspricht		entspricht	
Candida albicans (abwesend in 1 g)	entspricht		entspricht	
Escherichia coli (abwesend in 1 g)	entspricht		entspricht	
Pseudomonas aeruginosa (abwesend in 1 g)	entspricht		entspricht	
Salmonellen (abwesend in 10 g)	entspricht		entspricht	
Staphylococcus aureus (abwesend in 1 g)	entspricht		entspricht	

Rückstände von Metallkatalysatoren oder Metallreagenzien gemäß EMEA/CHMP/SWP/4446/2000 können nicht vorhanden sein.

Entspricht Ph Eur, BP

Datum der Prüfung (TT.MM.JJJJ) 06.04.2016
mindestens verwendbar bis (TT.MM.JJJJ) 30.04.2021

Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt (Germany): +49 6151 72-0
EMD Millipore Corporation - A division of Merck KGaA, Darmstadt, Germany
290 Concord Road, Billerica, MA 01821, USA, Phone: (978) 715-4321

Seite 1 von 2

Figure 7: **Certificate of Analysis of the Test Item Ethanol 96%** (page 1 of 2)

Analysenzertifikat

1.00971.6010	Ethanol 96% geeignet für die Verwendung als Excipient EMPROVE® exp Ph Eur,BP
Charge	K47729671

Dr. Ute Volkwein
Verantwortlicher Laborleiter Qualitätskontrolle

Dieses Dokument wurde maschinell erstellt und ist ohne Unterschrift gültig.

Figure 7: **Continuation (page 2 of 2)**

11.2 Analytical Standard

→ Produktdatenblatt

THE LINDE GROUP

Linde

Ethen 3.0

EINGEGANG
13. Aug. 20

Reinheit in %: ≥ 99,9

Nebenbestandteile, ppm:

N_2	≤ 100
O_2	≤ 20
sonstige KW	≤ 1000

Angaben sind als ideale Volumenanteile (= Molanteile) zu verstehen

Lieferarten:

MINICAN

Rauminhalt, [Liter]	Füllmenge, ca. [kg]	Fülldruck, ca. [bar]	Gesamtgewicht, mit Füllung ca. [kg]	Aussen-Ø, ca.[mm]	Gesamtlänge ca. [mm]
1	0,013	12	0,150	80	270

Stahlflasche

Rauminhalt, [Liter]	Füllmenge, ca. [kg]	Fülldruck, ca. [bar]	Gesamtgewicht, mit Füllung ca. [kg]	Aussen-Ø, ca.[mm]	Gesamtlänge ca. [mm]
2	0,7	120	7	200	300
10	3,7	120	20	140	970
50	18,5	120	86	229	1640

Flaschenbündel

Rauminhalt, [Liter]	Füllmenge, ca. [kg]	Fülldruck, ca. [bar]	Gesamtgewicht, mit Füllung ca. [kg]	Maße ca. (H x L x B)[mm]
600	222	120	1320	1900 x 980 x 770

Weitere Lieferarten auf Anfrage.

Sicherheit: EG-Sicherheitsdatenblatt unter www.linde-gas.de/direkt

Umrechnungszahlen:

m^3 Gas (15°C, 1 bar)	l flüssig bei T,	kg
1	2,074	1,178
0,482	1	0,568
0,849	1,761	1

Linde AG
Linde Gases Division, Seitnerstr. 70, D-82049 Pullach
Telefon: 018 03.850 00-0*, Telefax: 018 03.850 00-1*, www.linde-gas.de

* 0,09 Euro pro Minute aus dem dt. Festnetz | Mobilfunk bis 0,42 Euro pro Minute. Zur Sicherstellung eines hohen Niveaus der Kundenbetreuung werden Daten unserer Kunden wie z.B. Telefonnummern elektronisch gespeichert und verarbeitet.

Stand 21.04.2008

Figure 8: **Product Information of the Standard Ethene**
(page 1 of 2)

→ Ethen 3.0 2 von 2

Kennzeichnung:	Flaschenschulterfarbe/ Umlaufender Farbstreifen bei Bündeln	Rot RAL 3000
	Aufkleber:	Ethen 3.0
	Ventilanschluss:	W 21,80 x 1/14 LH nach DIN 477 Nr. 1
Eigenschaften:	verdichtetes Gas, hochentzündlich	
	Chemisches Zeichen:	C_2H_4
	Molare Masse:	28,054 g/mol
	Relative Dichte bezogen auf trockene Luft (15°C, 1 bar):	0,974
	Kritische Temperatur:	282,65 K (9,5 °C)
	Siedetemperatur bei 1,013 bar (T_s):	169,43 K (-103,72 °C)
Anwendungen:	Brenngas Hochgeschwindigkeits-Flammspritzen Reifung der Früchte wichtiger Ausgangsstoff zur Herstellung von Kunststoffen	
ıfalls verfügbar:	Ethen 3.5 Ethen 4.5 Ethen flüssig 3.0 Gemische mit anderen Gasen in genau definierten Zusammensetzungen	

ungsausschluss: Alle Angaben des Produktdatenblattes entsprechen dem gegenwärtigen Wissensstand. Die Linde AG prüft und aktualisiert die Informationen ständig und behält sich das Recht vor, Änderungen oder Ergänzungen der bereitgestellten Informationen vorzunehmen. Trotz aller Sorgfalt können sich Daten inzwischen verändert haben. Eine Haftung oder Garantie für die Aktualität, Richtigkeit und Vollständigkeit der zur Verfügung gestellten Informationen kann daher nicht übernommen werden. Jeder Anwender trägt selbst die Verantwortung dafür, dass alle relevanten gesetzlichen Bestimmungen eingehalten werden und dass die hier beschriebenen Produkte für seine Einsatzzwecke geeignet sind. Die Angaben auf diesem Produktdatenblatt sind keine vertraglichen Zusicherungen von Produkteigenschaften. Die Vervielfältigung von Informationen, Texten, Bildern oder Daten bedarf der vorherigen Zustimmung der Linde AG.

Linde AG
Linde Gases Division, Seitnerstr. 70, D-82049 Pullach
Telefon: 018 03.850 00-0*, Telefax: 018 03.850 00-1*, www.linde-gas.de
* 0,09 Euro pro Minute aus dem dt. Festnetz | Mobilfunk bis 0,42 Euro pro Minute. Zur Sicherstellung eines hohen Niveaus der Kundenbetreuung werden Daten unserer Kunden wie z.B. Telefonnummern elektronisch gespeichert und verarbeitet.

Änderungen vorbehalten
Stand 21.04.2008

Figure 8: **Continuation (page 2 of 2)**

12 GLP Certificate of Noack Laboratorien GmbH

Gewerbeaufsicht in Niedersachsen

Staatliches Gewerbeaufsichtsamt Hildesheim

Gute Laborpraxis / Good Laboratory Practice

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Abs.1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Noack Laboratorien GmbH
Käthe-Paulus-Str. 1
31157 Sarstedt
DEUTSCHLAND

Noack Laboratorien GmbH
Käthe-Paulus-Str. 1
31157 Sarstedt
GERMANY

Prüfungen nach Kategorien / Areas of Expertise (gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)

1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	1 - physical-chemical testing
4 - Ökotoxikologische Prüfungen zu Bestimmung der Auswirkungen auf aquatische und terrestrische Organismen	4 - environmental toxicity studies on aquatic and terrestrial organisms
5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung	5 - studies on behaviour in water, soil and air; bioaccumulation
6 - Prüfungen zur Bestimmung von Rückständen	6 - residue studies

Ort / Place	Datum der Inspektion / Date of Inspection (Tag.Monat.Jahr / month.day.year)
Sarstedt	**07. – 10. Juni 2016 & 13. Juli 2016 /**
Sarstedt	**Jun 07th – Jun 10th, 2016 & Jul 13th, 2016**

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Hildesheim, 03.01.2017

Staatliches Gewerbeaufsichtsamt Hildesheim
Im Auftrage

Bahn

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 7

PREVIOUS ACCEPTANCE OF ETHYLENE FOR USE IN ORGANIC AGRICULTURE

Ethylene

PN

RULING BODY: NOP
STATUS: Allowed With Restrictions
CLASSIFICATIONS: Processing Non-agricultural Ingredients and Processing Aids
ORIGIN: Non-Agricultural Synthetic
DESCRIPTION: Inert ingredients must be nonsynthetic or compliant with 205.601(m). For post-harvest ripening of tropical fruit and degreening of citrus.
RULE REFERENCE: NOP 7 CFR 205.605(b)(14); NOP Guidance 5023
DATE ACTIVE: 22-Feb-2023

Ethylene

PI

RULING BODY: COR
STATUS: Allowed With Restrictions
CLASSIFICATIONS: Processing Ingredients and Aids
DESCRIPTION: For post-harvest ripening of tropical fruit and degreening of citrus and to control sprouting of potatoes post-harvest in holding bins.
RULE REFERENCE: CAN/CGSB 32.311 Table 8.3
DATE ACTIVE: 14-Jul-2021

Report of the ad-hoc expert group on pesticides in organic food production, meeting on 22 – 23 January 2008
Request concerning the use of ethylene for sprouting inhibition in potatoes and onions

5.1 Recommendations
The expert group recommends ethylene to be allowed for sprouting inhibition in potatoes and onions.

5.2 Considerations Identification of substance
No issue.
Authorization in general agriculture
Authorization at Community level
Ethylene for post-harvest treatment is subject to the 4th stage review. Annex I status is pending. RMS UK proposes Annex I inclusion.

Authorizations at Member State level
Has been considered a commodity, now registered for post-harvest treatment in various MS.

Origin
Ethylene is produced by all higher plants and therefore omnipresent in nature. The ethylene (identical to the naturally occurring ethylene) used for agricultural purposes is obtained through chemical processes (see further remarks below).

Necessity
Details of use
Constant exposure of stored potatoes and onions to ethylene in low concentration inhibits sprouting.
Alternative products and methods currently allowed
Cold storage, use of varieties with high dormancy and/or caraway seed oil (for potatoes, where registered) may provide solutions in certain situations.

Necessity of requested use
A longer marketing period is important for the economic sustainability of farms.
This use of ethylene is not directly linked to the control of a pest or disease, but under Dir 91/414, this use of ethylene is considered to be plant protection. In Reg. 2092/91, products for similar uses (including sprout inhibition with caraway oil) are listed together with pesticides in Annex II B. Therefore, it is the opinion of the expert group that the criteria in Reg. 834/2007, Art. 16 are applicable in this case.

Environmental issues
Environmental fate, hazards and risks are assessed in detail during pesticide registration, and authorizations are accompanied by obligations for appropriate risk management. The expert group does not see the need to reassess these issues.
The DAR on ethylene does not raise any concerns.
Theoretically, after release from the storage rooms, ethylene could affect the vegetation, but Report of the ad-hoc expert group on pesticides in organic food production, meeting on 22 – 23 January 2008 page 13 the quantities used are negligible in comparison to natural and industrial emissions.

Human health
No concern.

Objectives and principles of organic farming: Food quality
Under conditions of prolonged storage, a higher external and internal quality can be maintained (absence of sprouts and wrinkles, composition of tubers).
If it allows storage of potatoes at higher temperatures, it would contribute to reducing the risk of formation of acrylamide during processing, frying or baking of the potatoes.

Harmonization
Historic use in EU organic farming
Yes, but not in potatoes and onions.
Use in organic farming outside the EU
Not known to the expert group.

Precedents in EU organic farming
Ethylene: Degreening of banana, khaki and kiwi, but no use for sprout inhibition.
Sprout inhibition: caraway oil (listed in Annex II B under the generic term of “plant oils”)
Further remarks
This use can allow to store potatoes and onions for a longer period and, as a consequence, to supply the market with locally produced potatoes and onions for a longer period.
Ethylene can be applied in several ways. In airtight, closed chambers injection of compressed ethylene gas is proposed in the request for degreening citrus, while heating of ethanol on a catalyst is proposed in the request for sprouting inhibition in potatoes and onions.

There are ethylene field application methods which are not allowed in organic farming (calcium carbide, ethephon). The expert group recommends to clarify in the Regulation which ways of ethylene application are allowed.

I

(Acts adopted under the EC Treaty/Euratom Treaty whose publication is obligatory)

REGULATIONS

COMMISSION REGULATION (EC) No 889/2008

of 5 September 2008

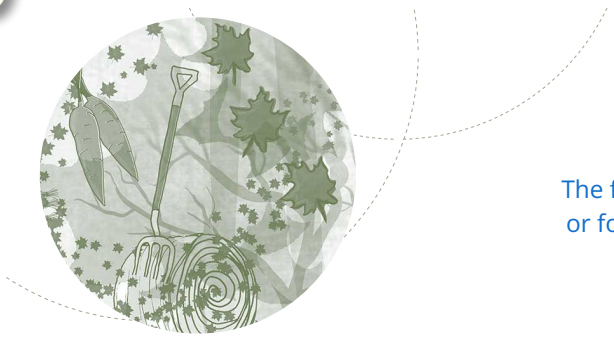
laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control

[jump to]

6. Other substances from traditional use in organic farming

Authorization	Name	Description
A	Copper in the form of copper hydroxide, copper oxychloride, (tribasic) copper sulphate, cuprous oxide, copper octanoate	Fungicide. up to 6 kg copper per ha per year For perennial crops, Member States may, by derogation from the previous paragraph, provide that the 6 kg copper limit can be exceeded in a given year provided that the average quantity actually used over a 5-year period consisting of that year and of the four preceding years does not exceed 6 kg
A	Ethylene	Degreening bananas, kiwis and kakis; Degreening of citrus fruit only as part of a strategy for the prevention of fruit fly damage in citrus; Flower induction of pineapple; sprouting inhibition in potatoes and onions
A	Fatty acid potassium salt (soft soap)	Insecticide
A	**table continues**	

PDF



OMRI Listed®

The following product may be used in certified organic production or food processing and handling in accordance with the Canadian Organic Standards.

Product

Restrain Fuel Solution Plant Growth Regulator

Company

Restrain Company Ltd.
Garos
Unit 7 The Forum
Minerva business Park
Lynch Wood
Petrborough Cambridgeshire PE2 6FT United Kingdom

Status	Category	Issue date
Allowed with Restrictions	COR: Ethylene	27-Oct-2022

Product number	Class	Expiration date
rcl-16944	Processing Ingredients and Aids	1-Dec-2023

Restrictions

For post-harvest ripening of tropical fruit and degreening of citrus and to control sprouting of potatoes post-harvest in holding bins.

Executive Director/CEO

Product review is conducted according to the policies in the current *OMRI Policy Manual©* and based on the standards in the applicable *OMRI Standards Manual©*. To verify the current status of this or any OMRI Listed product, view the most current version of the *OMRI Canada Products List©* at OMRI.org. OMRI listing is not equivalent to organic certification and is not a product endorsement. It cannot be construed as such. Final decisions on the acceptability of a product for use in a certified organic system are the responsibility of a CFIA accredited Certification Body. It is the operator's responsibility to properly use the product, including following any restrictions.

Organic Materials Review Institute
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PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 8

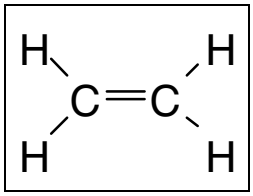
OECD REVIEW DOCUMENT INCLUDING PHYSICAL / CHEMICAL PROPERTIES OF ETHYLENE

FOREWORD INTRODUCTION

ETHYLENE

CAS N°: 74-85-1

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	**74-85-1**
CHEMICAL NAME	**Ethylene**
STRUCTURAL FORMULA	

CONCLUSIONS

Environment:
Ethylene is, due to its physical and chemical properties released mainly into the atmospheric compartment. About three quarters of atmospheric ethylene originates from natural sources, while one quarter is from anthropogenic sources. The main anthropogenic release is from burning of hydrocarbons and biomass.

It has been well documented through relevant toxicity studies that the minute amounts measured in water implies little environmental hazard to the organisms in this compartment.

In the terrestrial compartment, the vegetation has proven highly susceptible to this gas, probably through a mechanism related to the hormone function of ethylene in plants. Ethylene levels in urban areas have reached levels which inhibit growth of certain plant species. This is of concern both for decorative plants and agriculture in exposed areas. This is mainly due to incomplete burning of coal and petrol products in urban areas. The industrial emission is a minor contribution to total emissions and effects on vegetation are only expected close to production and processing plants.

Human Health:

Relevant studies have indicated a low toxicity of ethylene and no risk to human health has been identified neither from occupational exposure nor exposure of general public, either exposed directly or indirectly via the environment. However, metabolic studies in animals and man have revealed that ethylene is metabolised to ethylene oxide which is known to have carcinogenic and mutagenic effects.

RECOMMENDATIONS

No further testing of ethylene toxicity is recommended.

It is recommended to do a closer monitoring of the environmental ethylene levels in urban and polluted regions with respect to its potential intoxication of vegetation, but this goes beyond the OECD HVP Chemical Programme.

FULL SIDS SUMMARY - ETHYLENE

CAS NO: 74-85-1		SPECIES	PROTOCOL	RESULTS
PHYSICAL-CHEMICAL				
2.1	Melting Point			- 169.15 °C
2.2	Boiling Point			- 103.71 °C
2.3	Density			0.57 g/m^3 (at boiling point)
2.4	Vapour Pressure			4.27 MPa at 0°C
2.5	Partition Coefficient (Log Pow)			1.13 (calculated)
2.6 A.	Water Solubility			131 mg/l at 20 °C
B.	pH			-
	pKa			-
2.12	Oxidation: Reduction Potential			-
ENVIRONMENTAL FATE AND PATHWAY				
3.1.1	Photodegradation			Lifetime = 0.37 to 4 days best estimate = 1.45 days
3.1.2	Stability in Water			-
3.2	Monitoring Data			<u>In air:</u> Rural areas < 1 - 5 μg/m^3 Urban areas < 50 μg/m^3 Heavy traffic < 1000 μg/m^3 Occupational exposure < 5 mg/m^3 <u>In water:</u> Baseline 6.0 μg/l Polluted areas 44 μg/l
3.3	Transport and Distribution		Calculated (Fugacity Level 1 type)	In Air 99.99915 % In Water - % In Sediment - % In Soil - % In Biota - %
3.5	Biodegradation			<u>Biodegradation in water:</u> Aerobic: $T_{1/2}$ = 1 - 28 days Anaerobic: $T_{1/2}$ = 72 - 112 days
ECOTOXICOLOGY				
4.1	Acute/Prolonged Toxicity to Fish	Orange spotted sunfish various fish Fathead minnow		LC_{50} (1 hr) = 22 mg/l QSAR values: LC_{50} (4 days) = 50-119.5 mg/l NOEC (28 days) = 13mg/l

CAS NO: 74-85-1		SPECIES	PROTOCOL	RESULTS
4.2	Acute Toxicity to Aquatic Invertebrates	*Daphnia manga*		QSAR value: EC_{50} (48 hr) = 53 - 152.9 mg/l
4.3	Toxicity to Aquatic Plants e.g. Algae	*Selnastrum capricornutum*		Growth inhibition: EC_{50} (72 hr) = 40 mg/l Growth rate inhibition: EC_{50} (72 hr) = 72 mg/l NOEC (72 hr) = 13.9 mg/l
4.5.2	Chronic Toxicity to Aquatic Invertebrates	*Daphnia manga*		NOECs (16 d) = 37.4 mg/l
4.6.1	Toxicity to Soil Dwelling Organisms			-
4.6.2	Toxicity to Terrestrial Plants	Peas, potatos, oats, cotton Cattleya orcid		EC_{50} (24 hr) = 8 - 700 μg/m³ EC_{50} (24 hr) = 2.3 μg/m³
(4.6.3)	Toxicity to Other Non-Mammalian Terrestrial Species (Including Birds)			-
TOXICOLOGY				
5.1.1	Acute Oral Toxicity			
5.1.2	Acute Inhalation Toxicity	Mice		LD_{50} = 950,000 ppm
5.1.3	Acute Dermal Toxicity			-
5.4	Repeated Dose Toxicity	Rat, SD		NOEC > 10,000 ppm
5.5	Genetic Toxicity In Vitro			
A.	Bacterial Test (Gene mutation)	*E.Coli*		neg. (With metabolic activation) neg. (Without metabolic activation)
B.	Non-Bacterial In Vitro Test (Chromosomal aberrations)	CHO cells	OECD TG 473	neg (With metabolic activation) neg. (Without metabolic activation)
5.6	Genetic Toxicity *In Vivo*	Mouse	Micronuc. test	neg
		Rat	Micronuc. test	neg
5.7	Carcinogenicity	Rat		2 years inhalation study negative
5.8	Toxicity to Reproduction (Inhalation administration)	Rat	OECD TG 421 Inhalation	NOEL = 5,000 ppm (General toxicity) NOEL = 5,000 ppm (Repro. Tox.)
5.9	Developmental Toxicity/ Teratogenicity			-
5.11	Experience with Human Exposure			Work place exposure ≤ 4 mg/m³ Peak levels ≤ 50 mg/m³

SIDS INITIAL ASSESSMENT REPORT

1. IDENTITY

Name (IUPAC): Ethylene

CAS number: 74-85-1

Molecular formula: CH_2CH_2

Molecular Weight: 28.05

Other names: Ethene, acetene, bicarburetted hydrogen, olefiant gas, elayl.

Even the high volume industrial product is of high purity (about 99.9%). The main impurities are methane and ethane. There are no impurities or additives known to represent any risk of toxicity.

Ethylene is gaseous with a boiling point of -104 ^{o}C at atmospheric pressure. Ethylene is stored in the liquid state under high pressure or at low temperatures.

Ethylene has a solubility in water of 131 mg/l at 20 ^{o}C. The log Pow value of 1.13 (calculated) indicates only a slightly higher solubility in octanol than in water and no potential for bioaccumulation is indicated.

Classification and labelling of ethylene focus on the flammability and the explosive properties. Within EU ethylene is classified as extremely flammable and should be labelled according to Fx, R12 and correspondingly according to S2-9-16-33.

2. GENERAL INFORMATION ON EXPOSURE

Ethylene is the petrochemical produced in largest quantities worldwide. For 1996 the total production volume is estimated to be 83,000,000 tonnes. EU has a production capacity of 18,200,000 tonnes. More than 95% of the annual commercial production of ethylene is currently based on steam cracking of petroleum hydrocarbons.

About 80 % of the ethylene consumed in US, Western Europe and Japan is used for production of ethylene oxide, ethylene dichloride, linear low density and high density polyethylene. Significant amounts are also used to make ethylbenzene, alcohols, olefins, acetaldehyde and vinylacetate. Minor quantities are used as anaesthetic gas, for fruit ripening and for welding and cutting metals.

Industrially produced ethylene is, due to the physical state of the product, kept in closed systems during both production, storage and in further processing. Often production plants utilizing ethylene as raw material are situated close to where ethylene is produced, however large quantities are also traded internationally. Ethylene is transported by sea in gas tankers in liquid form. On land transport mainly by pipelines. Even if the gas is kept in closed systems, transport implies a risk for environmental exposure. In cases when the transport devices maintain the pressure by cooling, the cooling systems may fail, giving higher pressure and leakage through safety valves.

Ethylene is ubiquitous in the environment, arising from both natural and man made sources. Major natural sources are emissions from vegetation of all types, where it functions as a plant hormone.

The main anthropogenic sources are from combustion of gas, fuel, coal and biomass. The highest exposure concentrations to humans are due to ethylene from car motors. The total global ethylene emission has been estimated to be 18-45 · 10^6 t/y, of which approximately 74 % is released from natural sources and 26 % from anthropogenic sources. Emission from fuel oil combustion is estimated to 1.54·10^6 t/y, equal to approximately 4 % of total global emissions.

2.1 Production volumes, uses and release

Production plants and processing plants for ethylene are often sited together. A medium sized production plant has a capacity of ca. 400 000 tons/y and production runs 365 days/y. Fraction released corresponds to ca. 0.06 % of the production. As plants for processing and use of ethylene are, as already mentioned, assumed to be directly connected to the production site by pipelines, these activities do not contribute significantly to the emissions.

3. ENVIRONMENT

3.1 Environmental Exposure

3.1.1 General Discussion

Fugacity calculation using a six compartment global reference model shows that emitted ethylene almost exclusively is distributed to air. The most recent methods give a calculated lifetime of 1.45 days for ethylene in the atmosphere, this is the sum of all relevant loss pathways. Other calculations gave lifetimes in the range of 0.37-4 days. Biodegradation in water is however slower with half-lives in the range of 1-28 days, and even slower under anaerobic conditions where half-lives are 3 to 112 days.

No bioaccumulation is expected as the calculated Log P_{ow} is low (log P_{ow} = 1.13) and the vapour pressure is high. This is confirmed in the fugacity calculation below.

3.1.2 Predicted Environmental Concentration

Air
Environmental levels will depend on both anthropogenic and natural sources in the surroundings, but highest levels are measured in urban areas and this is considered due to combustion of fuel, coal and gas. Ethylene concentrations in ambient air at rural and remote sites worldwide are generally in the range of < 1 - 5 mg/m^3. In urban and indoor air contaminated with combustion products, ethylene concentrations typically range from a few up to about 50 mg/m^3. However, in extreme cases values above 1000 mg/m^3 have been measured in heavy traffic.

Local environmental concentration in air due to industrial production and use (production and use usually coexists) can be examplified by a refinery with a production capacity of 365 000 tons/y. Production is continous with emissions 365 days/y. Local emissions at the site have been measured to be 24 kg/h equal to 210 tons/y or 0.057 % of total production and use. According to TGD(96) this gives a local concentration of 160 µg/m^3 at a distance of 100 m from the source.

From these observations, the maximum Predicted Environmental Concentration is estimated to be regionally ($PEC_{regional}$) 5 mg/m^3 and locally (PEC_{local}) for traffic 1000 mg/m^3 and for industrial production and use 160 µg/m^3, and general urban areas 50 µg/m^3.

Water
The marine aquatic level of ethylene has been measured in a set og 452 samples. This gave a baseline (average) concentration of 6.0 mg/l, but increasing up to 44 mg/l in heavily exposed areas.

Environmental partitioning

A fugacity level I calculation, using a six compartment (air, water, soil solids, suspended sediments and fish) model has been conducted using the global reference model of OECD. Default values for the environmental parameters were not changed. Entered generic parameters were: melting point - 169.15 ^{o}C, vapour pressure 4.27 MPa, water solubility 200 g/m^3, $\log_{10}P_{OW}$ 1.13, half-life in air 56 hours, half-life in water, soil and sediment 672 hours. This gave the following distribution:

in air	99.99915 %,
in water	$8.27 \cdot 10^{-4}$ %,
in soil solids	$9.88 \cdot 10^{-6}$ %
in sediment solids	$2.20 \cdot 10^{-7}$ %
in suspended sediments	$6.87 \cdot 10^{-9}$ %
in fish	$5.58 \cdot 10^{-10}$ %

This means that for all practical purposes, emitted ethylene is distributed to air only.

The fate of atmospheric ethylene emitted from natural and anthropogenic sources has been estimated by several methods. The most recent published estimates indicate that about 85.4 % was destroyed in the troposphere by reaction with OH radical, and 14.5 % in the reaction with O_3. The remaining 0.07 % was transported into the stratosphere. The atmospheric lifetime of ethylene was estimated to be 1.45 days. Other estimates of lifetime of ethylene in the atmosphere due to the combined effect of O_3 reaction with ethylene and $\cdot$OH reactions are in the range of 0.37 and 4 days.

3.2 Effects on the Environment

3.2.1 Aquatic effects

There are limited experimental data on the toxicity of ethylene to fish and invertebrates. A rather old study concluded that ethylene concentrations above 22 mg/l might be lethal to orange-spotted sunfish after 1 hour exposure. Calculated values (QSAR) indicate LC_{50} values from 50 to 120 mg/l for different fish species after 4 days exposure. Calculated NOEC for fish after 28 days exposure (Fathead minnow) is 13 mg/l.

Calculated LC_{50} values for Daphnia range from 53 - 153 mg/l. NOEC after 16 days was 37.4 mg/l.

In order to estimate the toxicity of ethylene to aquatic plants, a growth inhibition test to the alga *Selenastrum capricornutum* was undertaken. Nominal ethylene concentration in the growth medium ranged from 8.2 to 131 mg/l. During the 72 hr exposure period there was a loss of ethylene in the range of 64-91 %, however in calculation of results the mean measured ethylene concentration was used. EC_{50} for the growth inhibition based on biomass was calculated to be 40 mg/l. Based on the specific growth rate (m), the 0 - 72 hr EC_{50} was calculated to be 72 mg/ml. The highest NOEC was 13.9 mg/l.

The results agree fairly well with the calculated (QSAR) EC_{50} value for growth of algae after 48 hours exposure of 122.5 mg/l.

This is a rather important finding, since it demonstrates that the aquatic algae do not show the high susceptibility to ethylene as terrestrial plants. This coincides with ethylene having no function as a plant hormone in aquatic algae, which are single cell organisms.

3.2.2 Terrestrial effects

Available data show that higher plants are highly susceptible to ethylene. Ethylene is a natural plant hormone produced by plants at all stages in growth in varying amounts. The susceptibility of a plant is also dependant on stage of growth. Ethylene plays an important role in flowering, fruit ripening,

senescence and abscission. Exposure of high concentrations of ethylene can have deleterious effects on plants if it occurs at the wrong time for plants. Adverse effects that may occur are inhibition of photosynthesis and growth, epinastic curling and shedding of flowers and leaves. Among the more sensitive agricultural or horticultural crops are peas, potatoes and oats where retardation effects were observed at concentrations in the range 8-50 mg/m^3. The most susceptible non-woody plant reported are African marigold which reacts epinasty at 1.2 µg/m^3 (0.001 ppm) and Cattleya orchid with septal tissue collapse at 2.3 mg/m^3 (0.002 ppm) ethylene for 24 hours. The table below summarizes some of the observed effects described in literature. The observations are grouped according to the seriousness of effects.

Summary table of effects of ethylene exposure to vascular plants. Exotic and tropical plants are not included. Epinasty=leaf curling, Abcission=loss

Effects	exposure time	Concentration µg m^{-3}
1) None or small long term effects:		
Epinasty, Lemon		25-50
Epinasty, tomato	3-4 h	46
Epinasty, *Chenopodium*		60
Epinasty, Potato	16 h	60
2) Effects that may cause long term effects		
Inhib growth, sweet pea, (NOEC)	2 d	12
Abscission flower, Carnation	2d	58
Inhibition of photosynth. Pea (NOEL)	2 h	115
Abscission flower, Snapdragon	1h	575
3) Long term effects:		
Decreased amount flowers, Oats	100d	8
Growth inhibition, Potato	28 d	27
Yield reduction, Tomato	28 d	50
Growth retardation, Pea		116
Yield reduction, Garden cress (30 %)	14 d	115
Yield reduction, Cotton	30 d	700

3.2.3 Other effects

E. coli B bacteria were treated with ethylene by passing the gas through a bacterial suspension at constant rate for 10 minutes. After exposure, the suspensions were plated on agar medium and incubated for 24 hours at 37℃. Survival of colonies from gas treated cells was 79 ±1.3 % of controls. The survival of the *E. coli* Sd-4 strain after the same treatment was 84.2 ±1.6 % compared to controls. It was concluded that treatment seemed to have little if any effect on the survival of both bacteria strains.

3.3 Initial Assessment for the Environment

3.3.1 Aquatic compartment

The solubility of ethylene in water is comparatively low (200 mg/l at 15 ^{o}C), and the observed levels even lower. A baseline level of 6.0 mg/l has been measured, and even in heavily exposed areas the observed levels are only about 38-44 mg/l. Compared to the no effect concentrations for fish and algae of about 13 mg/l this gives a PEC/PNEC of $3.4 \cdot 10^{-3}$ and no reason for concern.

3.3.2 Terrestrial compartment

When evaluating the terrestrial compartment, ethylene is present in air, but not in other compartments. The predicted environmental concentrations have been estimated to be $PEC_{regional}$ = 5 mg/m³ and PEC_{local} = 50 mg/m³ (burning/urban), 160µg/m³ (industrial) and 1000µg/m³ (heavy traffic).

$PEC_{regional}$ is on the same level as those effecting susceptible plants (2.3 µg/m³), however the observed effects are not of long term significance. PEC_{local} (burning/urban) of 50 µg/m³ is above the level known to affect agricultural plants (8-50 µg/m³) after long exposure periods. This is a problem localised to urban areas and locations with much traffic or burning of fossil hydrocarbons. In these areas, ethylene from fuel combustion is known to interact with other air pollutants like SO_2, NO_X, ozone etc. The importance of agriculture in such areas are low. PEC/PNEC for local industrial emissions are also high, with most ratios being greater than 1 when comparing with effect concentrations in the summary table in section 3.2.2. However it applies only for within 100 m of site and as for urban areas the agricultural importance in these areas are low. With ethylene exposure concentrations of 1000 ppm as is the case for areas with heavy traffic, even quite short exposure periods may give long term effects. The awareness of the intoxication of plants in urban areas with ethylene is not new, but the present assessment further focuses on this problem.

4. HUMAN HEALTH

4.1 Human Exposure

Ethylene was in general use as an anaesthetic for many years. It has been replaced by more modern anaesthetics, mostly due to the high explosion risk. Today, elevated exposure of humans is limited to a low number of workers at ethylene production plants, and those involved in transport of ethylene.

4.1.1 Occupational exposure

Personal and stationary monitoring of ethylene in a company where ethylene was used for controlling the ripening of bananas showed air concentrations to be in the range of 0.02-3.35 ppm (0.02 - 3.85 mg/m^3). In a study on exposure of firefighters, samples taken during the "knockdown" phase of a fire showed a concentration of 46 ppm (53 mg/m^3) ethylene.

A study was carried out among workers at a Swedish petrochemical plant in order to assess the amounts and effects of ethylene exposure. The study was carried out in two parts, part one in 1989 and part two in 1993. Eight workers exposed to high levels of ethylene (4 mg/m^3) and 3 workers exposed to lower levels (0.1 -0.3 mg/m^3) were compared to nine controls exposed to 0.01 mg/m^3. Part two of the study, which included four workers, was designed to more accurate determine exposure level which had a mean of 4.5 mg/m^3.

4.1.2 Consumer exposure

No consumer products contain or release ethylene, thus consumer exposure is not relevant.

4.1.3 Indirect exposure via the environment

Humans will be exposed to ethylene in the air which has been estimated to maximum
$PEC_{regional}$ = 2 mg/m^3 and PEC_{local} = 50 mg/m^3. In-door air is anticipated also to contain increased levels of ethylene.

4.2 Effects on Human Health

a) Mode of action of the chemical, toxicokinetics and metabolism

No clear evidence of toxic effects of exposure of humans or animals to ethylene has been reported. However, it has been shown in studies, both in animals and in humans that inhaled ethylene can be metabolised to ethylene oxide. This metabolism is of concern since ethylene oxide is a potent alkylating agent, a carcinogen and a genotoxicant, and hence more toxic than ethylene. About 5 - 10 % of ethylene inhaled by rats has been reported to be converted to ethylene oxide, depending upon the concentration of ethylene in the inhaled air.

Part of the ethylene oxide formed from ethylene has been shown to react with nucleophilic sites in DNA as well as in haemoglobin. The extent of adduct formation with haemoglobin has been used to monitor the ethylene exposure in animals and in humans after occupational exposure. The oxidation of ethylene to ethylene oxide and subsequent alkylation of DNA and proteins identifies a possible mechanism of potential toxic effects of ethylene in humans.

Epidemiological as well as experimental data concludes that ethylene oxide is a carcinogen, and this is also the conclusion of the IARC working group. Thus ethylene oxide is classified as a carcinogen.

It has been demonstrated that ethylene is acute hepatotoxic to rats pre-treated with polychlorinated biphenyl (PCB) probably due to the induction of hepatic mixed function oxidases which catalyse the oxidation of ethylene to ethylene oxide. This indicate that combined exposure to inducers of mono-oxygenases and ethylene may cause a health hazard in humans.

b) Acute toxicity

The acute toxicity of inhaled ethylene is low, but very high concentrations may cause asphyxia due to oxygen displacement. The lethal concentration for mice in air is estimated to be 950,000 ppm (1093 g/m^3). When male rats were exposed to 10, 25 or $57 \cdot 10^3$ ppm (11.5, 28.8 or 65.6 g/m^3) for 4 hours, all groups showed increased serum pyruvate and liver weight.

c) Repeated dose toxicity

The toxicity of ethylene has been tested in a 90 days inhalation study on 4 exposed and one control groups of 30 rats (15 males, 15 females). The animals were exposed 6 hours/day 5 days/week for 13 weeks. The different groups were exposed to 0; 300; 1,000; 3,000 or 10,000 ppm (0, 345, 1150, 3450 or 11,500 mg/m^3). There were no differences between controls and treated rats with respect to total weights, weight change, food consumption, haematology, clinical chemistry, gross pathology or histopathology. Male rats in the control, 300 ppm and 10,000 ppm groups showed red deposits or red discharge around the nose, whereas the males in the 1000 ppm group had red deposits around the eyes. Amongst the female rats, a red deposit was observed around left eye of one 300 ppm rat and alopecia around both ears of one 1000 ppm rat. Compared with the controls, the liver weights in several groups of exposed rats were significantly lower. There was however, no dose response relationship for this weight reduction and the cause was unknown. Ethylene appeared to have a low toxicity in rats when administered up to 10,000 ppm (11,500 mg/m^3). This is considered a no effect level (NOEL) for the 90 days study.

In a small explorative study, where a group of six male Sprague-Dawley albino rats were exposed to a continuous flow of 60% (600,000 ppm) ethylene in oxygen as inhalation for 6 days, effects could be seen on several haematology parameters. There was a significant reduction in thrombocyte count (-19.3%) and leukocyte count (-48.2%). A reduction was also seen in the bone marrow cellularity (-30%). With this very high concentration, clear signs of toxicity were seen. However, the relevance of these studies to human health is unclear since very high doses were used.

d) Reproduction developmental toxicity

The potential effects of ethylene inhalation on rat reproduction and on growth and development of the offspring has been studied in a combined reproduction/development toxicity screening test, conducted according to GLP. Four groups of rats (10 females and 10 males per group) were dosed by head only inhalation for 6 hours daily with: air only (control); 200, 1000 or 5000 ppm of ethylene (corresponding to 0, 230, 1150 or 5750 mg/m^3). This dosing regime was calculated to give about 80, 400 and 2000 mg/kg/day of ethylene for the three dosed groups respectively. The test substance was administered to parent animals for two weeks prior to mating, during the mating period and until the day prior to necropsy of the males (minimum 28 days) or until day 20 of gestation for the females. The females were allowed to litter and rear their offspring to day 4 *post-partum*, when they and their offspring were killed.

Morbidity, mortality, clinical condition, weight and food intake were observed throughout the study, and mating was carefully observed. For each female, litter data and also observations for each offspring were recorded. At termination of the study, all animals were subject to macroscopic examination for structural or pathological changes. Ovaries, testes and epididymides of the control and high dose animals were subject to a histopathological examination.

There were no deaths attributable to the test article, and body weight gain was not adversely affected during the pre-pairing, gestation or lactation periods. The treatment had no effect on fertility or fecundity and all females became pregnant. Litter size, sex ratio, mean pup weight and pup growth and clinical condition were not adversely affected by treatment.

Necropsy revealed no macroscopic finding suggestive of toxicity due to test substance administration. There was no evidence of any toxic effect on the testis due to test substance administration and there were no other microscopic findings suggestive of toxicity due to the exposure.

In conclusion, head-only administration of ethylene at nominal concentrations of 200; 1,000 or 5,000 ppm was without evidence of toxicity or adverse effects on male and female reproductive performance, fertility, pregnancy, maternal and suckling behaviour and growth and development of the offspring from conception to Day 4 post-partum. The highest dose was concluded to be a no effect level (NOEL) for the reproduction/development screening test in rats.

e) Genetic toxicity

Bacterial test *in vitro*
Ethylene at atmospheric concentrations up to 20 % gave no indication of mutagenic potential when tested i one strain of *Salmonella typhimurium* in the presence or absence of a liver metabolic activation system (S9) (Ames test). Previous testing with four *Salmonella* strains in the presence and absence of S9 have also given negative results. Ethylene also showed no genotoxic activity in *Escherichia coli*.

Non-bacterial *in vitro* test
The effect of ethylene on chromosome structures was tested in an *in vitro* cytogenetics assay using duplicate cultures of CHO cells. The test was conducted according to GLP. Treatments covering a broad range of doses, separated by narrow intervals, were performed both in the absence and presence of metabolic activation system (rat liver post-mitochondrial fraction) from Aroclor 1254 induced animals. The highest dose level used, approximately 280.5 mg/l, was equivalent to a concentration of 10 mM.

A preliminary range-finding study was performed to investigate the toxic effects of ethylene on CHO cells. In this study, treatment in the absence and presence of S9 was for 3 hours only followed by a 17 hour recovery period prior to harvest (3+17). The dose levels for the main study were selected by evaluating the effect of ethylene on mitotic index. The treatment regimes used in the range-finding study were used in the main study. Chromosome aberrations were analysed at three consecutive dose levels. No mitotic inhibition (reduction in mitotic index) was observed at the highest concentration chosen for

analysis (280.5 mg/l) in either the absence or presence of S-9. Treatment of cultures with ethylene in the absence and presence of S-9 resulted in frequencies of cells with structural aberrations that were similar to, and not significantly different from, those seen in concurrent negative control cultures. Frequencies seen in treated cultures fell within the normal range.

It is concluded that ethylene did not induce chromosome aberrations in cultured Chinese hamster ovary cells exposed to a concentration of 280.5 mg/l in the absence and presence of S9.

Genetic toxicity *in vivo*
Ethylene did not induce micronuclei formation in bone marrow cells of rats or mice exposed to up to 3000 ppm (3500 mg/m^3) for 6 h/day, five days/week for four weeks.

f) Carcinogenicity

The potential carcinogenicity of ethylene has been tested in a two years study with rats (Fischer 344 inbred). In the study, 960 rats were randomly divided into 4 groups of 120 animals of each sex and exposed 6 hr/day, 5 days/week to 0 (control), 300, 1000 and 3000 ppm (0, 345, 1150 or 3450 mg/m^3) for up to 24 months.

During the course of the study there were observations of hair loss, deposits on and around the nose and eyes and gross eye abnormalities, but there were no obvious differences among the treatment groups.

There was an overall increase in the number of animals exhibiting gross tissue masses for the test groups as compared with the control group, although this trend was not statistically significant. The spontaneous mortality (15.7 %) was roughly equal in all treated groups. The final body weights and total weight changes for treated males were higher than those in the control groups, but no dose-related pattern was seen.

There were no statistically significant difference among any of the treatment groups on any of the haematology, blood chemistry or other parameters investigated. No gross or histopathologic tissue changes attributable to the effects of the test material were observed in any of the treated rats. The summary reports only few findings which could indicate any carcinogenic effect of the treatment. However, based on the rate of formation of the carcinogen ethylene oxide and its possible role in ethylene toxicity, the study did not have statistical power to detect an increased frequency of tumour formation.

The latest evaluation by the IARC working group (1994) concludes that there is inadequate evidence in humans and in experimental animals for the carcinogenicity of ethylene. Overall, ethylene was evaluated as not classifiable as to its carcinogenicity to humans.

g) Human data

The inhalation pharmacokinetics of ethylene was investigated in human volunteers at atmospheric concentrations of up to 50 ppm by gas uptake in a closed spirometer system, and the uptake, exhalation and metabolism could be described by first-order kinetics [64]. The clearance due to uptake was low, only 5.6 %, while the rest was only exhaled without entering the blood stream. Clearance due to metabolism was 36 % of systematically available ethylene. The biological half-life of ethylene was 0.65 hours. The alveolar retention of ethylene at steady state was calculated to be 2 %. The low uptake rate of ethylene was considered due to its low solubility in blood.

There have been two preliminary but independent reports of increased miscarriage rates among women working in the petrochemical industry. Elevated ethylene concentrations were mentioned as a possible reason, but this has not been confirmed. No firm conclusions can be drawn from these reports.

A preliminary study found no increase in lung cancer incidence in 31 workers exposed to ethylene (at unspecified levels) at a US petrochemical factory. However, due to the limited number of exposed workers in this study no conclusions regarding ethylene not causing cancer can be drawn.

A study of workers at an US petrochemical plant found that an increased risk of developing brain cancer was associated with exposure to (unspecified levels of) a number of chemicals including ethylene. However, the investigators were unconvinced that the association reflected a causal relationship.

4.3 Initial Assessment for Human Health

Assessment from acute and repeated dose toxicity studies
It is assumed that the maximum occupational exposure is 5 mg/m^3 and the maximum exposure to the general population via the environment is 50 mg/m^3. The NOEL of 11,500 mg/m^3 in the 90 days inhalation study in rats gives safety margins of 2300 and $2.3 \cdot 10^5$, which indicates no hazard concern.

Assessment from the reproduction/development toxicity study in rats
No signs of reproductive or development toxicity of ethylene was observed in the inhalation test with rats. The highest concentration used (5750 mg/m^3) is taken as the NOEL and this gives a safety margin of 1150 for occupational exposure and $1.15 \cdot 10^5$ for general exposure indicates minimal hazard concern.

Assessment from genetic toxicity and carcinogenicity studies
Ethylene has been shown to be without genetic toxicity in bacterial test *in vitro*, in mammalian cells *in vitro* and in the mouse micronucleus test *in vivo*. Also a full two years carcinogenicity study has been conducted in rats. As all the tests are negative, there is no reason to indicate that ethylene should imply a high risk of genetic toxicity or carcinogenicity to humans. However, the fact that ethylene is metabolised, both in humans and experimental animals, to the carcinogenic genotoxicant ethylene oxide is of some concern.

Assessment from mode of action and mechanism of toxicity
In the case of ethylene, a possible mechanism for a toxic potential in humans has been identified, but few signs of toxicity have been observed. This is related to the fact that ethylene gives rise only to minute doses of ethylene oxide. The maximum conversion of ethylene to ethylene oxide in humans is estimated to 4 %, while about 1 % has been measured.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Ethylene is, due to its physical and chemical properties released mainly into the atmospheric compartment. About three quarters of atmospheric ethylene originates from natural sources, while one quarter is from anthropogenic sources. The main anthropogenic release is from burning of hydrocarbons and biomass.

It has been well documented through relevant toxicity studies that the minute amounts measured in water implies no environmental hazard to the organisms in this compartment.

In the terrestrial compartment, the vegetation has proven highly susceptible to ethylene, probably through a mechanism related to the hormone function of ethylene in plants. Ethylene levels in urban areas have reached levels which inhibit growth of certain plant species. This is of concern both for decorative plants and agriculture in exposed areas. This ethylene is not due to emission of ethylene from ethylene production, but rather to incomplete burning of coal and petrol products.

Relevant studies on ethylene have indicated a low toxicity and no risk to human health has been identified neither from occupational exposure nor exposure of general public, either exposed directly or indirectly via the environment. Metabolic studies in animals and man have revealed that ethylene is metabolized to ethylene oxide which is known to have carcinogenic and mutagenic effects. However, IARC has in its evaluation in 1994 concluded that there is inadequate evidence both in humans and in experimetal animals for the carcinogenicity of ethylene.

5.2 Recommendations

No further testing of ethylene toxicity is recommended.

It is recommended to do a closer monitoring of the environmental ethylene levels in urban and polluted regions with respect to its potential intoxication of vegetation, but this goes beyond the OECD HVP Chemical Programme.

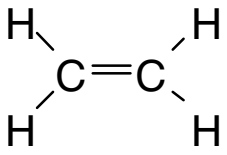
6. REFERENCES

References are not given in the SIDS initial assessment report. It is referred to those given in the Full SIDS Dossier.

SIDS DOSSIER ON ETHYLENE

Summary of Responses to the OECD Request for Available Data on HPV Chemicals

SIDS PROFILE

1.01 A	CAS NO.	**74-85-1**
1.01 C	CHEMICAL NAME	Ethylene
1.01 G	STRUCTURAL FORMULA	
	OTHER CHEMICAL IDENTITY INFORMATION	
1.5	QUANTITY	Millions metric tonnes per year: (capacity for 1996) Norway: 0.4 World: 83.0
1.7	USE PATTERN	Chemical industry; as raw material for synthesis of chemicals, petrochemicals and resins. Minor quantities used for fruit ripening and as anaesthetic gas.
1.9	SOURCES OF EXPOSURE	Fuel, coal and gas combustion. Leakage from chemical industry. Rural areas: < 1 - 5 $\mu g/m^3$ (0.9 - 4.3 ppb) Heavy traffic areas: up to 1.0 mg/m^3 (0.9 ppm) Petrochemical plants: up to 5 mg/m^3 (4.3 ppm)
ISSUES FOR DISCUSSION (IDENTITY, IF ANY)	No further testing required	

1. GENERAL INFORMATION

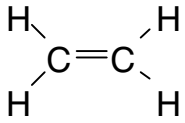
A. **CAS number:** 74-85-1

B. **Name** (IUPAC): Ethylene

C. **Name** (OECD): Ethylene

F. **Molecular formula:** CH_2CH_2

G. **Structural formula:**



H. **Substance group:** Industrial chemical; as raw material for synthesis of chemicals, petrochemicals and resins.

J. **Molecular Weight:** 28.05

1.02 OECD INFORMATION

A. **Sponsor Country:** Norway

B. **Lead Organisation:**
Norwegian Pollution Control Authority (SFT),
P.O. Box 8100 Dep.,
N-0032 Oslo
NORWAY

Contact person:
Marit Kopangen

Tel.: +47 22 573400
Fax.: +47 22 676706

C. **Name of responder:**
Noretyl ANS,
Petrochemical division,
Norsk Hydro ANS,
N-0240 Oslo
NORWAY

1.1 GENERAL SUBSTANCE INFORMATION

A. **Type of Substance:** Organic, hydrocarbon

B. **Physical state** (at 20 °C and 1.013 hPa): Gaseous

C. **Purity:**

1) High purity : > 99.9 %
2) Commercial purity : about 99.9 %

1.2 SYNONYMS

Ethene, acetene, bicarburetted hydrogen, olefiant gas, elayl.

1.3 IMPURITIES

Western Europe product, (ppm range)**:**
Methane + ethane (50-200), propylene and heavier (7-200), CO_2 (2.2-50), H_2(0.1-10), O_2 (0.6-10), acetylene (1.4-10), total sulphur (1-10), water (0.6-20) and CO (0.15-10) [3].

1.4 ADDITIVES

None known.

1.5 QUANTITY

More than 1,000,000 tonnes per annum.
Capacity for 1996 [2]:
Norway: 405,000 tonnes
World: 83,000,000 tonnes

1.6 LABELLING AND CLASSIFICATION

EEC: Fx, R12 (Extremely flammable).
S 2 (Keep out of reach of children.
S 9 (Keep container in well-ventilated place)
S 16 (Keep away from sources of ignition - No smoking)
S 33 (Take precautionary measures against static discharges)

Norway: F, R13 (Extremely flammable liquid gas)
S 9-16-33

According to IARC Monograph Volume 60, (1994):
Ethylene: The agent is not classifiable as to its carcinogenicity to humans [3].

1.7 USE PATTERN

Ethylene is the petrochemical product produced in largest quantities world-wide. More than 95% of the annual commercial production of ethylene is currently based on steam cracking of petroleum hydrocarbons [4].

About 80 % of the ethylene consumed in US, Western Europe and Japan is used for production of ethylene oxide, ethylene dichloride and low density, linear low density and high density polyethylene. Significant amounts are also used to make ethylbenzene, alcohols, olefins, acetaldehyde and vinylacetate. Most of these products are further prosessed into products such as film, blow and injection moulding, extrusion coating, cable insulation and PVC. Minor quantities have been used as anaesthetic gas, for fruit ripening and for welding and cutting metals.

A. General

	Type of use:	Category:
a)	Main industrial use	Use in closed systems Chemical Industry: used in synthesis Raw material
b)	Main industrial use	Non dispersive use Agricultural Industry As fruit ripener

B. Uses in Consumer Products
Not known

1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE

No exposure limits have been recommended in most countries, but Switzerland established a time-weighted average occupational exposure limit of 11 500 mg/m^3 [3].

1.9 SOURCES OF EXPOSURE

Ethylene is ubiquitous in the environment, arising from both natural and man made sources. Major sources are as a natural product from vegetation of all types [5].
The main anthropogenic sources are from combustion of gas, fuel, coal and biomass. Maximal exposure of ethylene to humans is considered to be through fossil combustion by vehicles. The total ethylene emission from the global surface has been estimated to be 18-45 $\cdot 10^6$ t/y, of which approximately 74% is released from natural sources and 26 % from anthropogenic sources. Emission from oil combustion is estimated to 1.54 $\cdot 10^6$ t/y [5]. Ethylene produced and consumed in chemical industry is kept in closed systems and the production facility is normally next door to the factory using ethylene as a raw material. Exposure to ethylene from industrial sources are thus mainly due to uncontrolled leakage or blow outs. Such events occur at a rate of once every 2.0 $\cdot 10^6$ t/y of produced ethylene and may result in an immediate release of about 1 ton.

1.10 ADDITIONAL REMARKS

A. Option for disposal
Incineration.

B. Other remarks
No data.

2. PHYSICAL-CHEMICAL DATA

2.1 MELTING POINT

-169.15 °C [4]

2.2 BOILING POINT

-103.71 °C [4]

2.3 DENSITY

d = 0.57 g/cm^3 at boiling point [4].
Gas density at STP 1.2603 g/l [4].
Density relative to air 0.9686 [4].

2.4 VAPOUR PRESSURE

4.27 MPa at 0 °C [4].

2.5 PARTITION COEFFICIENT $log_{10}P_{ow}$

$Log_{10} P_{ow}$ = 1.13 (calculated) [6].

2.6 WATER SOLUBILITY

A. Solubility

According to Merck Index, "One volume of ethylene gas dissolves in 4 vol of water at 0°C" [7].
One volume of ethylene gas dissolves in 9 volumes of water at 25 °C [8].
Solubility: 131 mg/l at 20°C [9].
At 15 °C the solubility in water is 200 mg/l [10].

B. pH Value, pKa Value

No data available. There is no chemical evidence to suggest a reaction between dissolved ethylene and water and pH remains unchanged.

2.7 FLASH POINT

- 136.11 °C [11].

2.8 AUTO FLAMMABILITY

Autoignition temp: 543°C [7].
Ignition temp: 425-527°C [4].

2.9 FLAMMABILITY

Extremely flammable - liquefied gas.

2.10 EXPLOSIVE PROPERTIES

Explosive limits in air (0.1 MPa and 20°C) [4] :
Lower explosive limit (LEL): 2.75 vol %
Upper explosive limit (UEL): 28.6 vol %

2.11 OXIDIZING PROPERTIES

No information

2.12 OXIDATION:REDUCTION POTENTIAL

No information.

2.13 ADDITIONAL DATA

A. Partition co-efficient between soil/sediment and water (Kd).

No information

B. Other data

Conversion factor for ethylene in air:
1 ppm in air = 1.15 mg/m^3 = 912 nl/l [1,4]

Odour threshold:
Odour low: 299 mg/m^3
Odour high: 4600 mg/m^3 [12]

3. ENVIRONMENTAL FATE AND PATHWAYS

3.1 STABILITY

3.1.1 STABILITY IN AIR

The fate of atmospheric ethylene emitted from natural and anthropogenic sources has been estimated by Sawada and Totsuka, 1986 [5]. They concluded that 89 % was destroyed in the troposphere by reaction with OH radical, and 8 % in the reaction with O_3. The remaining 3 % was transported into the stratosphere. The atmospheric lifetime of ethylene was estimated to be between 2 and 4 days.

Indirect calculation of photodegradation with O_3 as a sensibilizer gave a lifetime of 9.4 days [13]. Using OH as the sensibilizer a lifetime of 2.7 days was calculated [14].

The following lifetimes are according to Howard, P.H. et al (1991) [15]: Handbook of environmental degradation rates:

		Lifetimes:
Air:	High:	3.36 days
	Low:	0.37 days

This is based upon combined, measured photoxidation rate constants for $^{\cdot}OH$ and O_3.
If the calculation procedures for organic compounds in atmosphere of Atkinson, R. (1996) [75] are used the following depletion rates are found:

		Lifetimes	
Air	Due to $^{\cdot}OH$ reaction	1.7	days
	Due to O_3 reaction	10	days
	Due to stratospheric removal	1900	days

Stratospheric removal can be calculated according to IPPC (1995) [76], assuming a similar removal of ethylene as CO.

3.1.2 STABILITY IN WATER

No data available

3.1.3 STABILITY IN SOIL

No data available

3.2 MONITORING DATA (ENVIRONMENT)

Rudolph and Johnen, [16] did more than 200 in situ measurements of ethylene and other selected Light Atmospheric Hydrocarbons during, a cruise from Puerto Madryn (Argentina) to Bremerhaven (Germany) in 1987. The measuring locations were remote with low biological activity in the surrounding ocean areas. The ethylene level, expressed as mixing ratio was in the range 10-30 ppt (12-35 ng/m^3) in the southern hemisphere and in the northern hemisphere a factor of 2 higher. The observed ethylene levels were primarily a result of oceanic emissions and the differences were indicated to be caused by changes in oceanic phytoplankton concentration.

The oceanic distribution of ethylene and other low molecular weight (LMW) hydrocarbons has been studied by Swinnerton and Lamontagne, 1974 [17]. They analyzed 452 water samples from the open ocean and near shore for LMW hydrocarbons and found a baseline (average) ethylene of: 4.8 nanoliters/litre (6.0 µg/l) . Upper values were: Mississippi R. Delta ; 35.0 nl/l (44 µg/l) and Miami dockside; 30.0 nl/l (38 µg/l).

Fuel, coal and gas combustion. Leakage from chemical industry. Rural areas: < 1 - 5 µg/m^3, heavy traffic areas: up to 1.0 mg/m^3 [1, 3].

During burning of wood (white pine) an ethylene concentration of about 50 ml/m^3 (63 mg/m^3 was measured in the smoke [18].

3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

In their study of the dynamics of atmospheric ethylene, Sawada and Totsuka, [5] estimated the following emissons of ethylene (in 10^6 t/y):

Natural:		
Terrestrial	23.3	(65.8 %)
Aquatic	2.9	(8.2 %)
Sum	26.2	(74.0 %)
Anthropogenic:		
Fuel oil combustion	1.5	(4.28 %)
Coal cumbustion	0.42	(1.20 %)
Leakage from Industri	0.03	(0.09 %)
Søppel forbrenning	0.10	(0.29 %)
Biomass burning	7.10	(20.1 %)
Sum	9.19	(26.0 %)

Total Natural + Anthropogenic = 35.4 $\cdot 10^6$ t/y

Atmospheric depletion of ethylene:
Ethylene reacts with OH radical to form an adduct which in the presence of O_2 and NO_x forms formaldehyde. The products of reaction of ethylene with O_3 are mostly CO, CO_2, H_2O and CH_2O. Some ethylene is also transported into the stratosphere [76]. Using the most recent

estimates [75] of the depletion rates (lifetime) of ethylene in the atmosphere due to these prosesses give:

:

	lifetime (days)	
Reaction with ·OH radical	1.7	
Reaction with O_3	10	
into the stratosphere	1900	
total lifetime in atmosphere	1.45	
Ethylene sinks (removal capacity, 10^6 tons/y):		
Reaction with ·OH radical	44.4	(85.4%)
Reaction with O_3	7.5	(14.5 %)
Into stratosphere	0.036	(0.07 %)
Sum	52.0	

The ethylene transported into the stratosphere will eventually react with O_3 with the production of a krøger molecule, which again may react with NO regenerating O_3. ethylene is therefore not suspected of being a potential ozone depletor.

3.3.1 TRANSPORT

Physical properties of ethylene indicate that it will rapidly move into the atmosphere from any type of release.

3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

A fugacity level I calculation, using a six compartment model (air, water, soil solids, sedimented solids, suspended sediments and fish) was conducted using the global reference model of OECD [19]. Default values for the environmental parameters were not changed. Entered generic parameters were: melting point - 169.15 °C, vapour pressure 4.27 MPa, water solubility 200 g/m^3, $\log_{10}P_{OW}$ 1.13, half-life in air 56 hours, half-life in water, soil and sediment 672 hours. This gave the following distribution:

in air	99.99915 %,
in water	$8.27 \cdot 10^{-4}$ %,
in soil solids	$9.88 \cdot 10^{-6}$ %
in sedimented solids	$2.20 \cdot 10^{-7}$ %.
in suspended sediments	$6.87 \cdot 10^{-9}$ %
in fish	$5.58 \cdot 10^{-10}$ %

This means that for all practical purposes, emitted ethylene is distributed to air only.

3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

See 3.3

3.5 BIODEGRADATION

Also a number of research orientated studies were designed to examine the oxidation/hydroxylation and epoxidation of various hydrocarbons by microorganisms isolated from soil, fresh water systems or other natural systems and pure cultures. Generally, results of these studies show that ethylene is subject to biodegradation by various microorganisms and that ethylene oxide and ethylene glycol are most likely initial degradation products [21].

Aqueous biodegradation rates have been estimated both for aerobic and anaerobic conditions [15]:

Aerobic half-life:	High:	672 hours
	Low:	24 hours
Anaerobic half life:	High:	2688 hours
	Low:	96 hours

3.6 BOD_5, COD OR RATIO BOD_5/COD

No data available

3.7 BIOACCUMULATION

Ethylene is not expected to bioaccumulate because of $Log_{10} P_{ow} = 1.13$.
BCF (Bioconcentration factor) is calculated (QSAR) to be 4 on the basis of the toxic action of nonpolar molecules in the freshwater fish Fathead minnow (pimephales promelas), exposure duration 2.00 - 304 days [22].

3.8 ADDITIONAL REMARKS

No data.

4. ECOTOXICOLOGICAL DATA

4.1 ACUTE TOXICITY TO FISH

Little is known about the acute toxicity of ethylene to fish, but the "Water Quality Criteria, California State Water Resources Control Board, 1963" [23] refers to two reports of toxicity of ethylene to Orange-spotted sunfish from 1917 [24] and 1921 [25]. The findings were the following:

Lethal conc after 1 hour :	22 - 25 mg/l [24]
Lethal conc after $\geq$ 1 hour :	22 - 65 mg/l [25]

Calculated (QSAR) values reported in the database Ecotoxicity Profile database [26]:

Fathead minnow (*Pimephales promelas*)	4 days LC_{50} 116 mg/l
Bluegill, (*Lepomis macrochirus*)	4 days LC_{50} 85 mg/l
Channel catfish, (*Ictalurus punctatus*)	4 days LC_{50} 50 mg/l
Rainbow trout, Donaldson trout, (*Onchorhynchus mykiss*)	4 days LC_{50} 55 mg/l

Calculated (QSAR) values reported by Leeuwen et. al. [27]:

Fathead minnow (Pimephales promelas)	4 days LC_{50} 120 mg/l

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

A. Daphnia

Calculated (QSAR) value reported in the database Ecotoxicity Profile [26]:

Water flea, (Daphnia magna)	48 hours	LC_{50} 53 mg/l

Calculated (QSAR) value according to Leeuwen et. al. [27]:
Daphnid 48 hours LC_{50} 153 mg/l

B. Other aquatic organisms
No data available.

4.3 TOXICITY TO ALGAE

A growth inhibition test with *Selenastrum capricornutum* was performed according to OECD 201 and conducted according to GLP guidelines in 1996 [74]. The 5 nominal test concentrations in the growth medium ranged from 8.2 to 131 mg/l. During the 72 hr exposure period there was a loss of ethylene, however the mean measured ethylene concentrations (mean of zero time and 72 h measurement) were used for calculation of growth inhibition. Actual test concentrations (mean) were therfore: 3.3, 7.8, 13.9, 32 and 58mg/l. Loss of ethylene during the 72 hr incubation period ranged from 64 to 91 %. EC_{50} for the growth inhibition based on reduction in biomass compared to control, was calculated to be 40 mg/l (95 % conf. lim.36-46 mg/l). Based on the specific growth rate (μ, the 0 - 72 hr EC_{50} was calculated to be 72 mg/l (95 % conf. lim. could not be calculated due to that the EC_{50} value was outside the range of the test). The highest NOEC was 13.9 mg/l. The results agree fairly well with QSAR calculation for *Selenastrum capricornutum* which gave an EC_{50} after 48 hour value of 122.5 mg/l [27].

4.4 TOXICITY TO BACTERIA

E.coli bacteria were treated with ethylene by passing the gas through a bacterial suspension at constant rate for 10 minutes. After 24 hours exposure, the suspensions were plated on agar medium and incubated for 24 hours at 37 ℃. Survival of colonies from gas treated cells was 79 ± 1.3 % of controls. The survival of the *E. coli* Sd-4 strain after the same treatment was 84.2 ±1.6 % compared to controls. It was concluded that treatment seemed to have little if any effect on the survival of both bacteria strains [28].

4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS

4.5.1 CHRONIC TOXICITY TO FISH

Calculated (QSAR) value reported in the database Ecotoxicity Profile [26]:
Fathead minnow, (Pimephales promelas) 32 days MATC 15.3 mg/l

Calculated (QSAR) value according to Leeuwen et. al. [27]:
Fathead minnow, (Pimephales promelas) 28 days NOEC 13 mg/l

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Calculated (QSAR) value according to Leeuwen et. al. [27]:
Daphnia 16 days NOEC 37.4 mg/l

4.6 TOXICITY TO TERRESTRIAL ORGANISMS

4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS

No data available

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

A large and diverse literature exists on the effects of ethylene on vascular plants, including several hundred observations of ethylene exposure and effects. This is mainly due to the fact that ethylene acts as a plant hormone, regulating a whole range of different reactions. Most of these reactions can be categorised as growth regulation and include such effects as defloration, ripening, inhibition of elongation, leaf loss and senescence [9,11, 29, 30, 31, 32]. While most of these effects are non reversible, they do not all constitute effects that reduce a plants fitness nor growth and reproduction. One may categorise the effects into 3 groups based on assumed long term effects, where long term effects are associated with reduced fitness, growth or reproduction. In the table below exotic and tropical plants have been excluded in order to present data that give a more realistic view of risks associated with exposure in industrial areas.

Summary table of effects of ethylene exposure to vascular plants. Exotic and tropical plants are not included. Epinasty=leaf curling, Abcission=loss

Effects	exposure time	concentration $\mu g \cdot m^{-3}$	Ref
1) None or small long term effects:			
Epinasty, Lemon		25-50	[77]
Epinasty, tomato	3-4 h	46	[9]
Epinasty, *Chenopodium*		60	[9]
Epinasty, Potato	16 h	60	[9]
2) Effects that may cause long term effects			
Inhib growth, sweet pea, (NOEC)	2 d	12	[77]
Abscission flower, Carnation	2d	58	[77]
Inhibition of photosynth. Pea (NOEL)	2 h	115	[77]
Abscission flower, Snapdragon	1h	575	[33]
3) Long term effects:			
Decreased amount flowers, Oats	100d	8	[77]
Growth inhibition, Potato	28 d	27	[77]
Yield reduction, Tomato	28 d	50	[77]
Growth retardation, Pea		116	[9]
Yield reduction, Garden cress (30 %)	14 d	115	[77]
Yield reduction, Cotton	30 d	700	[9]

Among the more sensitive agricultural or horticultural crops are peas, potatoes, tomatoes and oats where retardation effects were observed at concentrations in the range 8-50 $\mu g/m^3$ (7-40 ppb) . The most susceptible non-woody plant reported, African marigold reacts with leaf epinasty (downward curling of leaves at 1.16 $\mu g/m^3$ (1.0 ppb) ethylene [9], the Cattleya orchid, reacts with sepal tissue collapse (loss of flower) at 2.3 $\mu g/m^3$ (2.0 ppb) after ethylene exposure for 24 hours [33].

4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)

No data

4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)

No data

4.8 BIOTRANSFORMATION AND KINETICS IN ENVIRONMENTAL SPECIES

No data

4.9 ADDITIONAL REMARKS

No data

5. TOXICITY

5.1. ACUTE TOXICITY

5.1.1 ACUTE ORAL TOXICITY:

Not relevant. Ethylene is a gas with a low boiling point (-103.71 °C).

5.1.2 ACUTE INHALATION TOXICITY

The acute toxicity of ethylene is low, but very high concentrations may cause asphyxia due to oxygen displacement. The lethal ethylene concentration in air to mice is thus estimated to be 950,000 ppm. [34].

When male rats were exposed to 10, 25 or $57 \cdot 10^3$ ppm for 4 hours, all groups showed increased serum pyruvate and liver weight [35]. Non of the studies were GLP.

5.1.3 ACUTE DERMAL TOXICITY

Not relevant. Very little ethylene is likely to be absorbed through the skin because of ethylene's low solubility in fat and low boiling point.

5.1.4 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION

No information

5.2 CORROSIVENESS/IRRITATION

5.2.1 SKIN IRRITATION/CORROSION

There is no evidence to suggest that the liquid ethylene gas is irritant, but it might cause frost injuries.

5.2.2 EYE IRRITATION

There is no evidence to suggest that the liquid ethylene gas is irritant, but it might cause frost injuries.

5.3 SKIN SENSITISATION

No data.

5.4 REPEATED DOSE TOXICITY

The toxicity of ethylene has been tested in a 90 days inhalation study on 4 exposed and one control groups of 30 rats (15 males, 15 females) [36]. The animals were exposed 6 hours/day 5

days/week for 13 weeks. The exposure groups were T-I: 300 ppm, T-II: 1,000 ppm, T-III: 3,000 ppm and T-IV: 10,000 ppm. The study was not conducted according GLP, but the study held high scientific standard and a quality assurance statement was issued. There were no differences between controls and treated rats with respect to total weights, weight change, food consumption, haematology, clinical chemistry, gross pathology or histopathology. Male rats in the control, T-I and T-IV groups showed red deposits or red discharge around the nose, whereas the male T-II had red deposits around the eyes. Amongst the female rats, a red deposit was observed around left eye of one T-I rat and alopecia around both ears of one T-II rat. Compared with the controls, the liver weights in several groups of exposed rats were significantly lower. There was, however, no dose response relationship for this weight reduction and the cause was unknown. Ethylene was not toxic to rats when administered under a stratified regimen of exposure up to 10,000 ppm.

In an explorative non-GLP study, where a group of six male Sprague-Dawley albino rats (50-60 g) were exposed to a continuous flow of 60% ethylene in oxygen as inhalation for 6 days, effects could be seen on several haematology parameters [37]. There were significant reductions in thrombocyte count (-19.3%) and leukocyte count (-48.2%). A reduction was also seen in the bone marrow cellularity (-30%).

During chronic tests on rats (newborn) exposed to a concentration of 2.62 ppm (continuous as inhalation) for 90 days, a delay in coat appearance, dentition, eye opening and circulation hypotension, cholinesterase activity inhibition, subordination disruption were reported [38]. There were no information on the quality of the study.

In rats treated by inhalation with a concentration of 100 ppm for 70 days, a change in the reflex nerve impulses, a decrease of cholinesterase activity and a reduction of the blood pressure were observed [39]. There were no information on the quality of the study.

5.5 GENETIC TOXICITY IN VITRO

A. Bacterial test

Ethylene at atmospheric concentrations up to 20 % gave no indication of mutagenic potential in Salmonella typhimurium in the presence or absence of a metabolic activation system (Ames test) [40]. The study was not conducted according to GLP, and only one (TA 100) of the four bacterial test strains recommended in the guidelines was tested. Previous testing with the full range of Salmonella strains in the presence and absence of a metabolic activation system have also given negative results [41, 42]. Ethylene showed no gentoxic activity in Escherichia coli. [28].

B. Non-bacterial in vitro test

The effect of ethylene on chromosomes was tested in an in vitro cytogenetics assay using duplicate cultures of CHO cells [71]. The methodology in this study complies with GLP and the OECD Test Guideline 473, "Genetic Toxicology: In vitro Mammalian Cytogenetic Test". Treatments covering a broad range of doses, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S9) from Aroclor 1254 induced rats. The highest dose level used, approximately 280.5 mg/ml, was equivalent to a concentration of 10 mM, corresponding to about 25 % of ethylene.

Due to the explosive properties of the test article when mixed with air, it was not possible to achieve the maximum concentration required by the Regulatory Guidelines using air as carrier gas. Nitrogen was therefore used as carrier gas, which allowed higher doses to be achieved. There are, however, technical problems associated with continuous treatment in a nitrogen atmosphere, and short (3 hour) pulse treatments were the only practical option.

A preliminary range-finding study was performed to investigate the toxic effects of ethylene on CHO cells. In this trial, treatment in the absence and presence of S9 lasted for 3 hours only followed by a 17 hours recovery period prior to harvest (3+17). The dose levels for the main study were selected by evaluating the effect of ethylene on mitotic index.

The treatment regimes used in the range-finder were repeated in the main study. Chromosomal aberrations were analyzed at three consecutive dose levels. No mitotic inhibition (reduction in mitotic index) was observed at the highest concentration chosen for analysis (280.5 μg/ml) in either the absence or presence of S9.

Appropriate negative (carrier gas) controls were included in the test system in both experiments under each treatment condition. Untreated controls were also included in the main study. The proportion of cells with structural aberrations in the negative and untreated cultures fell within historical solvent control ranges. 4-Nitroquinoline 1-oxide and cyclophosphamide were employed as positive controls in the absence and presence of liver S9 respectively. Cells receiving these were sampled in the main study, 20 hours after the start of treatment; both compounds induced statistically significant increases in the proportion of cells with structural aberrations.

Treatment of cultures with ethylene in the absence and presence of S9 resulted in frequencies of cells with structural aberrations that were similar to, and not significantly different from, those seen in concurrent negative controls. Frequencies seen in treated cultures fell within the normal range.

It is concluded that ethylene did not induce chromosome aberrations in cultured Chinese hamster ovary cells exposed to a concentration of 10 mM (25 %) in the absence and presence of S9.

5.6 GENETIC TOXICITY *IN VIVO*

The effects on micronucleus formation in bone marrow cells of rats and mice have been studied following ethylene inhalation [43]. Each group consisted of 10 animals of each of the two species and they were dosed with concentrations of 0; 40; 1,000 and 3,000 ppm for 6 hours/ day, 5 days a week for 4 weeks. An ethylene oxide control group with both species was exposed using the same conditions at a concentration of 200 ppm. Bone marrow was collected approximately 24 hours after the final exposure. Ethylene did not produce, statistically significant, exposure related increases in the frequencies of micronucleated polychromatic erythrocytes in the bone marrow of either rats or mice, while ethylene oxide exposure resulted in signifcant increases in the frequencies in both species. It is not stated if the study was conducted according to GLP.

Absorption, distribution, elimination of ethylene and formation of haemoglobin and DNA adducts were studied in rats after inhalation of 300 ppm ethylene for 12 hours/day for 3 consecutive days [44]. DNA adduct formation was measured in liver and lymphocytes and haemoglobin adducts determined in erythrocytes. The adduct formation with ethylene was compared to other alkenes and adduct formation decreased with increasing number of carbon atoms in the molecule. This was an explorative study not conducted according to GLP.

Alkylation of 7-guanine was measured in DNA from liver spleen and testis of mice 14 hours after exposure by inhalation of ^{14}C-ethylene at an initial concentration of 11 ppm for 8 hours [45]. The degree of alkylation was much higher in the liver than in the other tissues. This study was an explorative non-GLP study.

5.7 CARCINOGENICITY

The potential carcinogenicity of ethylene has been tested in a two years study with rats (Fischer - 344 inbred) [46]. The study was conducted prior to OECD Guideline 451 for carcinogenicity testing (1981), but still the study comply with this guideline except for some minor points. In the study, 960 rats were randomly divided into 4 groups of 120 animals of each sex and exposed 6 hr/day, 5 days/week to 0(control); 300; 1,000 and 3,000 ppm for up to 24 months.

During the course of the study there were observations of hair loss, deposits on and around the nose and eyes and gross eye abnormalities, but there were no obvious differences among the different treatment groups.

There was an overall increase in the number of animals exhibiting gross tissue masses for the test groups as compared with the control group, although this trend was not statistically significant. The spontaneous mortality (15.7 %) was roughly equal in all treated groups. The final body weights and total weight changes for treated males were higher than those in the control groups, but no dose-related pattern was seen.

There were no statistically significant differences among any of the treatment groups on any of the haematology, blood chemistry or other parameters investigated.

No gross or histopathologic tissue changes attributable to the effects of the test material were observed in any of the treated rats. The summary reports only few findings which could indicate any carcinogenic effect of the treatment, but lacks a conclusion at this point.

In a publication from the carcinogenicity study [41], it was concluded that the results provided "no evidence that ethylene at these concentrations causes chronic toxicity or is oncogenic in Fischer - 344 rats". However, this publication and the summary have later been criticised [47] since they do not discuss the mononuclear cell leukaemia described in the full report. It was claimed that the number of animals affected (out of 90) rose from 12 and 8 in the male and female control groups to 21 and 11, respectively in the groups receiving 3,000 ppm. On the other hand, it has been stated that mononuclear cell leukemia may occur in F344 rats at a background incidence > 75 %, and that a further increase in exposed animals is difficult to interpret with respect to human cancer development.

When the carcinogenic risk of ethylene was evaluated by the International Agency for Research on Cancer (IARC) in 1979 [1], no data were available to the working group on the carcinogenicity or mutagenicity of the substance in animals and humans. In supplement 7 published in 1987 [48] it is still summarised that no adequate data were available and ethylene is stated to be not classifiable as to its carcinogenicity to humans. The latest evaluation of ethylene by the IARC working group (1994) concludes that there is inadequate evidence in humans and in experimental animals for the carcinogenicity of ethylene [3]. Overall, ethylene was evaluated as not being classifiable as to its carcinogenicity to humans.
In the Ecotoxicity Profile database it is stated to be no information in the QSAR system which would suggest that this chemical is a potential carcinogen or mutagen [26].

In another recent evaluation of ethylene as a cancer risk factor it was concluded that it was a risk factor of concern [49]. This conclusion was based on the observed metabolism of ethylene to ethylene oxide, a compound which has been shown to be both mutagenic and carcinogenic. The linearity hypothesis for dose response relationship can not be applied in this case, since there is a saturation of the metabolism of ethylene. The findings from administration of high doses to animals can thus not be extrapolated to the human exposure level.

The carcinogenic potential of ethylene has also been reviewed in the BIBRA Bulletin [50]. This review concludes also on the basis of metabolic production of ethylene oxide that it is timely with

a detailed reconsideration of the possible carcinogenic risks of inhaling ethylene. The evaluation also calls for re-evaluation of the need for a specific industrial limit of ethylene.

5.8 TOXICITY TO REPRODUCTION

The potential effects of ethylene inhalation on male and female rat reproduction and on growth and development of the offspring has been studied [70]. The experimental study was carried out according to GLP (OECD Guideline 421; Reproduction/Development Toxicity Screening Test).

Four groups of rats (10 females and 10 males per group) were dosed by head only inhalation for 6 hours daily; air only (control); 200; 1,000 or 5,000 ppm of ethylene (corresponding to 0; 230; 1,150 or 5,750 mg/m^3). This dosing regime was calculated to give about 80; 400 and 2,000 mg/kg/day of ethylene for the three dosed groups respectively. Since the uptake from the lungs most likely is in the range of 5-10 % , the absorbed dose probably was substantially less than the figures given above.

The test material was administered to parent animals for two weeks prior to mating, during the mating period and until the day prior to necropsy for the males (minimum 28 days) and until day 20 of gestation for the females. The females were allowed to litter and rear their offspring to day 4 post-partum, when they and their offspring were killed.

Morbidity, mortality, clinical condition, weight and food intake were observed throughout the study, and mating was carefully observed. For each female, litter data and also observations for each offspring were recorded. At termination of the study, all animals were subject to macroscopic examination for structural or pathological changes. Ovaries, testes and epididymides of the control and high dose animals were subject to a histopathological examination.

There were no deaths attributable to the test article, and body weight gain was not adversely affected during the pre-pairing, gestation or lactation periods. The treatment had no effect on fertility or fecundity and all females became pregnant. Litter size, sex ratio, mean pup weight and pup growth and clinical condition were not adversely affected by treatment.

Necropsy revealed no macroscopic finding suggestive of toxicity due to test article administration. There was no evidence of any toxic effect on the testis due to test substance administration and there were no other microscopic findings suggestive of toxicity due to test article administration.

In conclusion, head-only administration of ethylene at nominal concentrations of 200; 1,000 or 5,000 ppm was without evidence of toxicity or adverse effects on male and female reproductive performance, fertility, pregnancy, maternal and suckling behaviour and growth and development of the offspring from conception to Day 4 post-partum.

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

It is referred to the experimental study [70] carried out according to the OECD Guideline 421; Reproduction/Development Toxicity Screening Test. The study is summarised under point 5.8 above.

5.10 OTHER RELEVANT INFORMATION

A. Specific toxicities (neurotoxicity, immunotoxicity etc.)
No data

B. Toxicodynamics, toxico-kinetics

Cowles, A.L. et al [51], studied the uptake and distribution of four inhalation anaesthetics in dogs. In a series of 21 experiments, 13 large mongrel dogs were ventilated with a constant concentration of ethylene (1.4 % = 12 g/m^3) and three other inhalation anaesthetics. Concentrations of the anaesthetic were measured by gas chromatography in alveolar gas, arterial blood, brain, muscle and central venous blood. The average times necessary for the partial pressure of ethylene to reach 50 % of the inspired partial pressure (1.4 %) were: alveolar gas, <2.0 min; arterial blood, <2.0 min; brain, 3.7 min; muscle, 8.2 min and central venous, 5.2 min.

Biotransformation of ethylene to ethylene oxide

Ehrenberg et. al, 1977 [52] showed that ^{14}C-labelled ethylene was metabolized to ethylene oxide when administered to male CBA mice by inhalation. This metabolism is of significant concern since ethylene oxide is a potent alkylating agent, a carcinogen and a gentoxicant, and hence more toxic than ethylene. The amount of epoxide formed was quantitatively determined from the degree of alkylation of cysteine and histidine residues in haemoglobin.

In a later study from the same laboratory [45], it was shown that ethylene oxide alkylated nucleophilic sites of mouse DNA. Since the ratio between the degree of alkylation of DNA and that of haemoglobin was the same when exposed to ethylene and ethylene oxide, it was concluded that the latter was the reactive intermediate formed from ethylene in vivo. A comparison of the degrees of alkylation obtained per unit exposure of ethylene oxide and ethylene, showed that at low levels of ethylene, about 8% of the inhaled amount was metabolized to ethylene oxide. The rate of ethylene oxidation followed saturation kinetics with increasing ethylene concentration. At 218 ppm ethylene, the oxidation rate was half of the maximal rate (K_m value). It was estimated that the maximal rate of metabolism (V_{max}) of ethylene corresponds to exposure to an air level of 4 ppm of ethylene oxide.

After exposing rats to automotive engine exhaust, Törnqvist et. al., 1988 [53] identified alkylated amino acids in haemoglobin. These resulted from conversion of about 5-10 % of inhaled ethylene and propylene to their respective epoxides which again alkylated the nucleophilic sites in haemoglobin. This quantification of the fraction of ethylene to be oxidised form agreed very well with the conversion factor of around 8 % found for the mouse in the above mentioned study [45].

Results from Törnqvist and Ehrenberg in 1990, estimate that in humans, some 6 % of inhaled ethylene in mainstream smoke is converted to ethylene oxide in smokers [54] and some 3 % in non-smokers [55].

Metabolic conversion of ethylene to ethylene oxide results in the formation of adducts to DNA and proteins, and this offers a means for identifying ethylene exposure in vivo. Determination of haemoglobin adducts using the N-alkyl Edman method has proven valuable [53]. This method has been used for monitoring adduct formation after ethylene exposure from different sources [49].

Toxicity of ethylene oxide

Ethylene oxide causes dose-related increases in the incidence of gliomas, peritoneal mesotheliomas and mononuclear cell leukemias in F 344 rats and lymphomas and adenomas/adenocarcinomas of the lung, uterus, harderian gland and mammary gland in B6C3F1 mice (for a review see Walker et. al., 1990 [56]).

Epidemiologic data on ethylene oxide support the anticipation that ethylene oxide is a carcinogenic agent. When mortality and incidence of cancer in totally 733 workers exposed to

ethylene oxide were assessed, 8 cases of leukaemia and 6 cases of stomach cancer occurred, while the expected numbers were 0.8 and 0.65 respectively [57].

In vivo as well as in vitro, ethylene oxide is seen to react both with amino acid residues in proteins and with the purine bases in DNA. When mouse, human or rat erythrocytes were exposed to ethylene oxide, the main reaction products with haemoglobin were 2-hydroxyethylations of cysteines, N-terminal valine, imidazole nitrogens of histidines and carboxylic groups [58]. The main reaction product after reaction with calf thymus DNA was N-7-(2-hydroxyethyl) guanine, whereas O-6-(2-hydroxyethyl)guanine was only about 0.5 % of this. Species differences were also observed, as rat and mouse erythrocytes were more susceptible to alkylation than the human erythrocytes.

The alkylation of DNA-bases with ethylene oxide has been studied further after exposure of rats to ethylene oxide by inhalation [59, 56, 60]. The main alkylation site both in vivo and in vitro is the N-7 position in guanine, resulting in 7-(2-hydroxyethyl) guanine, and this modification is probably the reason for its carcinogenic and mutagenic effects.

The IARC working group evaluated ethylene oxide in 1994 and came to the overall conclusion that it was carcinogenic to humans [61]. This was mainly based on the evidence for carcinogenicity from experimental studies in animals.

Effects of PCB-pre-treatment on ethylene toxicity and biotransformation

It has been demonstrated that ethylene, as well as halogenated ethylenes are acute hepatotoxic in rats pretreated with polychlorinated biphenyl (PCB) [62]. The hepatotoxicity was evident as increased serum alanine-α-ketoglutarate transaminase (SAKT) and sorbitol dehydrogenase (SDH) in rats pretreated with PCB and exposed to 20,000 ppm ethylene for 4 hours. Without pretreatment with PCB, ethylene and halogenated ethylenes are not acute toxic. From these findings it was suggested that the acute toxicity was mediated through epoxide intermediates formed by hepatic mixed function oxidases induced by the PCB pre-treatment.

When rats were exposed to ethylene in a closed desiccator jar chamber, the rate of metabolic elimination of the compound is influenced by pretreatment with PCB (single dose of Aroclor 1254, 500 mg/kg in oil 6 days prior to the experiment) [63]. Biotransformation of ethylene lead to ethylene oxide which was exhaled.

The effects of PCB pre-treatment and high exposure levels of ethylene, due to induction of mono-oxygenases and increased formation of ethylene oxide, demonstrates that the toxicity of ethylene is of concern for organisms also exposed to mono-oxygenase inducers. However, it should be kept in mind that the concentrations used are far above actual exposure levels.

5.11 EXPERIENCE WITH HUMAN EXPOSURE

Ethylene was in general use as an anaesthetic for many years. It has been replaced by more modern anaesthetics, mostly due to the high explosion risk. Chronic injury in humans resulting from prolonged and repeated exposure to low concentrations of ethylene (less than 2.5 %) was not reported in "Patty's Industrial Hygiene and Toxicology (1981)" [11].

Inhalation pharmacokinetics

The inhalation of ethylene was investigated in human volunteers at atmospheric concentrations of up to 50 ppm. The uptake, exhalation and metabolism could be described by first-order kinetics [64]. The clearance due to uptake was low, only 5.6 %, while the rest was exhaled without entering the blood stream. Clearance due to metabolism was 36 % of systemic available ethylene. The biological half-life of ethylene was 0.65 hours. The alveolar retention of ethylene at steady

state was calculated to be 2 %. The low uptake rate of ethylene was considered due to its low solubility in blood.

Reproduction effects
In a preliminary study, the miscarriage rate (six out of 15 pregnancies) amongst Swedish women who had worked in the local petrochemical industry was higher than that seen in 1549 women outside the industry. Ethylene was the main product in four of the five local petrochemical plants. No data were provided on occupational levels but measurements made in areas surrounding the plants indicated that ethylene was present in concentrations up to tenfold higher than the other pollutants (propylene, ethane, propane and phenol) [65].

A brief abstract notes that there was a higher than expected rate of miscarriage and gynaecological disease among female operatives of a polyethylene plant who were exposed to ethylene concentrations in the range of about 40-60 ppm and high levels of noise [66].

Carcinogenicity
A preliminary study found no increase in lung cancer incidence in 31 workers exposed to ethylene (at unspecified levels) at a US petrochemical factory [67].

A study of workers at an US petrochemical plant found that an increased risk of developing brain cancer was associated with exposure to (unspecified levels of) a number of chemicals including ethylene. However, the investigators were unconvinced that the association reflected a casual relationship [68].

Work Place Exposure
Personal and stationary monitoring of ethylene in a company where this gas was used for controlling the ripening of bananas showed air concentrations to be in the range of 0.02-3.35 ppm (0.02 - 3.85 mg/m^3), with an estimated average concentration of 0.3 ppm (0.35 mg/m^3). In a study on exposure of fire-fighters, samples taken during the "knockdown" phase of a fire showed a concentration of 46 ppm (53 mg/m^3) ethylene, while none was detected during the "overhaul" phase [3]

A study was carried out among workers at a Swedish petrochemical plant using measurements of haemoglobin adducts formed from ethylene oxide for monitoring of ethylene exposure [69]. The study was carried out in two parts, part one in 1989 and part two in 1993. Eight workers exposed to high levels of ethylene (4 mg/m^3) and 3 workers exposed to low levels (0.1 -0.3 mg/m^3) were compared to nine controls exposed to 0.01 mg/m^3. All exposed workers showed elevated levels of haemoglobin adducts and adduct formation was dose-related. The results indicated that about 1 % of the inhaled ethylene was metabolized to ethylene oxide.

The second part of the study, which included four workers, was designed to more accurately determine the exposure levels, which turned out to have a mean of 4.5 mg/m^3. The results confirmed part one, showing that about 1 % of inhaled ethylene was metabolized to ethylene oxide and the maximum fraction to be converted was estimated to be 4 %.

The peak level of ethylene reported for human exposure is about 50 ppm (57.5 mg/m^3), while 3.5 ppm (4.0 mg/m^3) has been characterized as a high average level for longer term exposure. The conversion will then correspond to maximum 2 ppm (3.6 mg/m$^{3)}$ of ethylene oxide for the peak level and to maximum 0.14 ppm (0.25 mg/m^3) for the high averaged level. Given occupational exposure limit levels for ethylene oxide (time-weighted averages) are 1.8 mg/m^3 (Denmark, Japan, USA, Norway) and 2.0 mg/m^3 (France, Canada, Sweden) [3].

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EXTRACT FROM IRPTC LEGAL FILE

File: 17.01 LEGAL **rn : 300046**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CAN type : REG

subject	specification	descriptor
AIR	OCC	TLV

Appendix E - simple asphyxiant. Prescribed by the Canada Occupational Safety and Health Regulations, under the Canada Labour Code (administered by the Department of Employment and Immigration). The regulations state that no employee shall be exposed to a concentration of an airborne chemical agent in excess of the value for that chemical agent adopted by ACGIH (American Conference of Governmental Industrial Hygienists) in its publication entitled: "Threshold Limit Value and Biological Exposure Indices for 1985-86". The regulations also state that the employer shall, where a person is about to enter a confined space, appoint a qualified person to verify by means of tests that the concentration of any chemical agent or combination of chemical agents will not result in the exposure of the person to a concentration in excess of the value indicated above. These regulations prescribe standards whose enforcement will provide a safe and healthy workplace.
entry date: OCT 1994 effective date: 24MCH1994

amendment: CAGAAK, CANADA GAZETTE PART II, 128 , 7 , 1513 , 1994

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File: 17.01 LEGAL **rn : 301890**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CAN type : REG

subject	specification	descriptor
TRNSP LABEL PACK		CLASS RQR

Applies to ethylene or ethylene, compressed. Schedule II, List II - Dangerous Goods other than Explosives: PIN (Product Identification No.):
UN1962. Class (2.1): Flammable gas. Special provisions: 102. Passenger Vehicles: Prohibited. Passenger Ship: Prohibited. Prescribed by the Transportation of Dangerous Goods Regulations, under the Transportation of Dangerous Goods Act (administered by the Department of Transport). The act and regulations are intended to promote safety in the transportation of dangerous goods in Canada, as well as provide comprehensive regulations applicable to all modes of transport accross Canada. These are based on United Nations recommendations. The act and regulations should be consulted for details. Information is entered under the proper shipping name found in the regulations; this may include general groups of chemical substances.
entry date: OCT 1994 effective date: 02DEC1993

amendment: CAGAAK, CANADA GAZETTE PART II, 127 , 25 , 4056 , 1993

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File: 17.01 LEGAL **rn : 301891**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CAN type : REG

subject	specification	descriptor
TRNSP		CLASS
LABEL		RQR
PACK		

Applies to ethylene, refrigerated liquid. Schedule II, List II - Dangerous Goods other than Explosives: PIN (Product Identification No.):UN1038. Class (2.1): Flammable gas. Special provisions: 102. Consumer Commodity: Prohibited. Limited Quantity: Prohibited. Passenger Vehicles:Prohibited. Passenger Ship: Prohibited. Prescribed by the Transportation of Dangerous Goods Regulations, under the Transportation of Dangerous Goods Act (administered by the Department of Transport). The act and regulations are intended to promote safety in the transportation of dangerous goods in Canada, as well as provide comprehensive regulations applicable to all modes of transport accross Canada. These are based on United Nations recommendations. The act and regulations should be consulted for details. Information is entered under the proper shipping name found in the regulations; this may include general groups of chemical substances.
entry date: OCT 1994 effective date: 02DEC1993

amendment: CAGAAK, CANADA GAZETTE PART II, 127 , 25 , 4056 , 1993

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File: 17.01 LEGAL **rn : 305377**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CAN type : REG

subject	specification	descriptor
TRNSP		CLASS
LABEL		RQR
PACK		

Applies to ethylene, acetylene and propylene in mixtures, refrigerated liquid. Schedule II, List II - Dangerous Goods other than Explosives: PIN (Product Identification No.): UN3138. Class (P): Prohibited. Prescribed by the Transportation of Dangerous Goods Regulations, under the Transportation of Dangerous Goods Act (administered by the Department of Transport). The act and regulations are intended to promote safety in the transportation of dangerous goods in Canada, as well as provide comprehensive regulations applicable to all modes of transport accross Canada. These are based on United Nations recommendations. The act and regulations should be consulted for details. Information is entered under the proper shipping name found in the regulations; this may include general groups of chemical substances.

entry date: OCT 1994 effective date: 02DEC1993

amendment: CAGAAK, CANADA GAZETTE PART II, 127 , 25 , 4056 , 1993

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File: 17.01 LEGAL **rn : 402419**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CZE type : REG

subject	specification	descriptor
AIR	EMI	MXL

GENERAL EMISSION LIMIT: 150 MG/M3 (IT APPLIES TO THE SUM OF ACETONE, ALKYLALCOHOLS, BIPHENYL, 2-BUTANONE, BUTYL ACETATE, DIBUTYLETHER, DIETHYLETHER, DIPHENYLETHER, DICHLORODIFLUOROMETHANE, 1,2-DICHLOROETHYLENE, DICHLOROMETHANE, DIISOPROPYLETHER, DIMETHYLETHER, ETHYL ACETATE, ETHYLENE GLYCOL, 4-HYDROXY-4-ETHYL-2-PENTANONE, CHLOROETHANE, METHYL BENZOATE, 4-METHYL-2-PENTANOL, N-METHYLPYRROLIDONE, OLEFINS (EXCEPT 1,3-BUTADIENE), PARAFINS (EXCEPT METHANE) AND TRICHLOROFLUOROMETHANE IF THEIR MASS FLOW > 3 KG/H).
entry date: DEC 1994 effective date: 1SEP1992

title: PROVISION OF FEDERAL COMMITTEE FOR ENVIRONMENT TO ACT NO. 309 FROM 9 JULY 1991 ON AIR PROTECTION AGAINST AIR POLLUTANTS
original : SZCFR*, SBIRKA ZAKONU CESKE A SLOVENSKE FEDERATIVNI REPUBLIKY(COLLECTION OF THE LAW OF CZECH AND SLOVAK FEDERAL REPUBLIC), , 84 , 2061 , 1991
amendment: SZCFR*, SBIRKA ZAKONU CESKE A SLOVENSKE FEDERATIVNI REPUBLIKY(COLLECTION OF THE LAW OF CZECH AND SLOVAK FEDERAL REPUBLIC), , 84 , 2398 , 1992

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File: 17.01 LEGAL **rn : 402820**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CZE type : REG

subject	specification	descriptor
TRNSP		RQR

ROAD TRANSPORT OF THE SUBSTANCE IN THE QUANTITY > 1000 KG IS ALLOWED ONLY WITH THE PERMISSION GIVEN BY RELEVANT AUTHORITY.
entry date: AUG 1994 effective date: 01APR1992

title: THE DECREE OF FEDERAL MINISTRY OF TRANSPORT NO. 122 WHICH PROMULGATE THE ACT ON ROAD TRANSPORT
original : SZCSR*, SBIRKA ZAKONU CESKOSLOVENSKE SOCIALISTICKE REPUBLIKY(COLLECTION OF THE LAW OF CZECHOSLOVAK SOCIALIST REPUBLIC), , 24 , 606 , 1979
amendment: SZCFR*, SBIRKA ZAKONU CESKE A SLOVENSKE FEDERATIVNI REPUBLIKY

(COLLECTION OF THE LAW OF CZECH AND SLOVAK FEDERAL REPUBLIC),, 21 , 531 , 1992

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File: 17.01 LEGAL **rn : 402825**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : CZE type : REG

subject	specification	descriptor
TRNSP		RQR

ROAD TRANSPORT OF THE SUBSTANCE IN THE QUANTITY > 100 KG IS ALLOWED ONLY WITH THE PERMISSION GIVEN BY RELEVANT AUTHORITY (APPLIES TO LIQUEFIED SUBSTANCE).
entry date: AUG 1994 effective date: 01APR1992

title: THE DECREE OF FEDERAL MINISTRY OF TRANSPORT NO. 122 WHICH PROMULGATE THE ACT ON ROAD TRANSPORT
original : SZCSR*, SBIRKA ZAKONU CESKOSLOVENSKE SOCIALISTICKE REPUBLIKY(COLLECTION OF THE LAW OF CZECHOSLOVAK SOCIALIST REPUBLIC), , 24 , 606 , 1979
amendment: SZCFR*, SBIRKA ZAKONU CESKE A SLOVENSKE FEDERATIVNI REPUBLIKY
(COLLECTION OF THE LAW OF CZECH AND SLOVAK FEDERAL REPUBLIC),, 21 , 531 , 1992

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File: 17.01 LEGAL **rn : 503995**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : DEU type : REC

subject	specification	descriptor
AIR	OCC	MAK

Suspected of having carcinogenic potential (group IIIB). No MAK value e established.
entry date: FEB 1996 effective date: 01JUL1995

title: Maximum Concentrations at the Workplace and Biological Tolerance Values for Working Materials (Maximale Arbeitsplatzkonzentrationen und Biologische Arbeitsstofftoleranzwerte)
original : MPGFDF, Mitteilung der Senatskommission zur Pruefung gesundheitsschaedlicher Arbeitsstoffe, 31 , , , 1995

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File: 17.01 LEGAL **rn : 700636**

systematic name:Ethene

common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : IND type : REG

subject	specification	descriptor
MANUF		RQR
SAFTY		RQR
STORE		RQR
IMPRT		RQR

These rules define the responsabilities of occupiers of any industrial activity in which this toxic and hazardous substance may be involved. These responsabilities encompass: (a) assessment of major hazards (causes, occurrence, frequency); (b) measures to prevent accidents and limit eventual impairment to human health and pollution of the environment; (c) provision of relevant factual knowledge and skills to workers in order to ensure health and environmental safety when handling equipments and the foregoing chemical; (d) notification of the competent authorities in case of major accidents; (e) notification of sites to the competent authorities 3 months before commencing; (f)preparation of an on-site emergency plan as to how major accidents should be coped with;(g) provision of competent authorities with information and means to respond quickly and efficiently to any off-site emergency; (h) provision of information to persons outside the site, liable to be affected by a major accident; (i) labelling of containers as to clearly identify contents, manufacturers, physical, chemical and toxicological data; (j)preparation of a safety data sheet including any significant information regarding hazard of this substance and submission of safety reports to the competent authorities; (k) for the import of a hazardous chemical to India, importers must supply the competent authorities with specified information regarding the shipment.
entry date: SEP 1992 effective date: 27NOV1989

title: THE MANUFACTURE, STORAGE AND IMPORT OF HAZARDOUS CHEMICALS RULES.1989
original : GAZIN*, THE GAZETTE OF INDIA, 787 , , , 1989

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File: 17.01 LEGAL **rn : 1121974**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : RUS type : REG

subject	specification	descriptor
AIR	AMBI	MAC

3.0MG/M3 1X/D, 3.0MG/M3 AV/D.
entry date: SEP 1985 effective date: AUG1984

amendment: PDKAV*, PREDELNO DOPUSTIMYE KONTSENTRATSII (PDK) ZAGRYAZNYAYUSHCHIKH VESHCHESTV V ATMOSFERNOM VOZDUKHE NASELENNYKH MEST (MAXIMUM ALLOWABLE CONCENTRATIONS (MAC) OF CONTAMINANTS IN THE AMBIENT AIR OF RESIDENTIAL AREAS), 3086-84 , , , 1984

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File: 17.01 LEGAL **rn : 1122614**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : RUS type : REG

subject	specification	descriptor
AIR	OCC	MAC CLASS

CLV: 100MG/M3 (VAPOUR) HAZARD CLASS: IV
entry date: MAY 1990 effective date: 01JAN1989

amendment: GOSTS*, GOSUDARSTVENNYI STANDART SSSR(STATE STANDARD OF USSR), 12.1.005 , , , 1988

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File: 17.01 LEGAL **rn : 1123448**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : RUS type : REG

subject	specification	descriptor
AQ	SURF	MAC CLASS

0.5MG/L HAZARD CLASS: III
entry date: JUL 1990 effective date: 1JAN1989

amendment: SPNPV*, SANITARNYE PRAVILA I NORMY OKHRANY POVERKHNOSTNYKH VOD OT ZAGRIAZNENIA (HEALTH REGULATION AND STANDARDS OF SURFACE WATER PROTECTION FROM CONTAMINATION), 4630-88 , , , 1988

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File: 17.01 LEGAL **rn : 1317115**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : USA type : REG

subject	specification	descriptor
USE		RSTR
FOOD	ADDIT	RSTR
STORE		RSTR
MANUF		RSTR
PACK		RSTR

REFERS TO HOMO AND COPOLYMERS.; Summary - THIS SUBSTANCE IS INCLUDED ON A LIST OF SUBSTANCES WHICH ARE CONDITIONALLY APPROVED TO BE USED AS COMPONENTS OF THE UNCOATED OR COATED FOOD CONTACT SURFACE OF PAPER AND PAPERBOARD FOR USE WITH FOODS HAVING THE PROPERTIES OF A DRY SOLID WITH NO FREE FAT O R OIL ON THE SURFACE. THESE SUBSTANCES ARE NOT TO BE USED IN QUANTITIES WHICH EXCEED THAT REQUIRED TO ACCOMPLISH THEIR INTENDED PHYSICAL OR TECHNICAL EFFECT AND ARE SO USED AS TO ACCOMPLISH NO EFFECT IN FOOD OTHER THAN THAT ORDINARILY ACCOMPLISHED BY PA CKAGING.
entry date: NOV 1991 effective date: 1977

title: INDIRECT FOOD ADDITIVES: PAPER AND PAPERBOARD COMPONENTS; COMPONENTS OF PAPER AND PAPERBOARD IN CONTACT WITH DRY FOOD
original : FEREAC, FEDERAL REGISTER, 42 , , 14554 , 1977
amendment: CFRUS*, CODE OF FEDERAL REGULATIONS, 21 , 176 , 180 , 1988

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File: 17.01 LEGAL **rn : 1322162**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : USA type : REG

subject	specification	descriptor
CLASS	PESTI	RQR
MANUF	PESTI	PRMT
FOOD	ADDIT	RQR

CASE NAME ETHYLENE; Summary - THIS SUBSTANCE IS INCLUDED ON A LIST OF ACTIVE INGREDIENTS CONTAINED IN A PRODUCT FIRST REGISTERED BEFORE NOVEMBER 1, 1984, FOR WHICH A REGISTRATION STANDARD HAS NOT BEEN ISSUED.
PUBLICATION OF THIS LIST INITIATES AN ACCELERATED REREGISTRATION AND DATA C ALL-IN FOR PRODUCTS CONTAINING THE LISTED ACTIVE INGREDIENTS. IN PARTICULAR THE LIST INCLUDES A NUMBER OF ACTIVE INGREDIENT CASES HAVING INDIRECT FOOD OR FEED USES.
entry date: JAN 1992 effective date: 1988

title: FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT PESTICIDES REQUIRED TO BE REREGISTERED; LIST C.
original : FEREAC, FEDERAL REGISTER, 54 , 140 , 30846 , 1989
amendment: FEREAC, FEDERAL REGISTER, 54 , 140 , 30846 , 1989

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File: 17.01 LEGAL **rn : 1336046**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : USA type : REG

subject	specification	descriptor
AIR	EMI	RQR
SOIL	EMI	RQR
AQ	EMI	RQR
MANUF	EMI	RQR

; Summary - FACILITIES THAT EXCEEDED A MANUFACTURING, IMPORTATION, OR PROCESSING THRESHOLD OF 25,000 LBS OR THE USE OF 10,000 LBS FOR THIS CHEMICAL MUST REPORT TO EPA ANY RELEASES OF THE CHEMICAL (OR CATEGORY CHEMICAL) TO AIR, LAND, WATER, POTW, UNDERGROUND INJECTIO N, OR OFF SITE TRANSFER. THIS REGULATION COVERS STANDARD INDUSTRIAL CLASSIFICATION
(SIC) CODES 20-39 ONLY).
entry date: OCT 1991 effective date: 1987

title: SUPERFUND AMENDMENTS AND REAUTHORIZATION ACT, TITLE III. EPCRA SECTION 313 LIST OF TOXIC SUBSTANCES
original : CFRUS*, CODE OF FEDERAL REGULATIONS, 40 , 372 , 65 , 1988
amendment: CFRUS*, CODE OF FEDERAL REGULATIONS, 40 , 372 , 65 , 1988

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File: 17.01 LEGAL **rn : 1340625**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : USA type : REC

subject	specification	descriptor
AIR	OCC	TLV

SIMPLE ASPHYXIANT; Summary - THIS THRESHOLD LIMIT VALUE IS INTENDED FOR USE IN THE PRACTICE OF INDUSTRIAL HYGIENE AS A GUIDELINE OR RECOMMENDATION IN THE CONTROL OF POTENTIAL HEALTH HAZARDS.
entry date: DEC 1991 effective date: 1989

title: THRESHOLD LIMIT VALUES
original : ACGIH*, AMERICAN CONFERENCE OF GOVERNMENT INDUSTRIAL HYGIENISTS, , , 11 , 1989
amendment: ACGIH*, AMERICAN CONFERENCE OF GOVERNMENT INDUSTRIAL HYGIENISTS, , , 11 , 1991

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File: 17.01 LEGAL **rn : 1408383**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : EEC type : REG

subject	specification	descriptor
FOOD		RQR
GOODS		MXL
GOODS		PRMT

THE SUBSTANCE IS INCLUDED IN THE LIST OF AUTHORIZED MONOMERS AND OTHER STARTING SUBSTANCES, WHICH SHALL BE USED FOR THE MANUFACTURE OF PLASTICS AND ARTICLES INTENDED TO COME INTO CONTACT WITH FOODSTUFFS. THE USE OF THE SUBSTANCE IS SUBJECT TO THE RESTRICTIONS SPECIFIED THEREIN. PLASTIC MATERIALS AND ARTICLES SHALL NOT TRANSFER THEIR CONSTITUENTS TO FOODSTUFFS IN QUANTITIES EXCEEDING 10MG/DM2 OF SURFACE AREA OF MATERIAL OR ARTICLE OR 60 MG/KG OF FOODSTUFFS IN THE SPECIFIED CASES.
VERIFICATION OF COMPLIANCE WITH THE MIGRATION LIMITS SHALL BE CARRIED

OUT IN ACCORDANCE WITH DIRECTIVES 82/711/EEC AND 85/572/EEC.
entry date: SEP 1995 effective date: 01JAN1991

title: COMMISSION DIRECTIVE OF 23 FEBRUARY 1990 RELATING TO PLASTICS MATERIALS AND ARTICLES INTENDED TO COME INTO CONTACT WITH FOODSTUFFS (90/128/EEC)
original : OJEC**, OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, L75 , ,19 , 1990
amendment: OJEC**, OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, L90 , , 26 , 1993

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File: 17.01 LEGAL **rn : 1421775**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : EEC type : REG

subject	specification	descriptor
CLASS		CLASS
LABEL		RQR
PACK		RQR

CLASS: F+ - EXTREMELY FLAMMABLE; EXTREMELY FLAMMABLE (R 12). LABEL: F+ - EXTREMELY FLAMMABLE; EXTREMELY FLAMMABLE (R 12); (KEEP OUT OF THE REACH OF CHILDREN (S 2)); KEEP CONTAINER IN A WELL-VENTILATED PLACE (S 9); KEEP AWAY FROM SOURCES OF IGNITION - NO SMOKING (S 16); TAKE PRECAUTIONARY MEASURES AGAINST STATIC DISCHARGES (S 33).
entry date: AUG 1994 effective date: JAN1994

title: COUNCIL DIRECTIVE 67/548/EEC OF 27 JUNE 1967 ON THE APROXIMATION OF THE LAWS, REGULATIONS AND ADMINISTRATIVE PROVISIONS RELATING TO THE CLASSIFICATION, PACKAGING AND LABELLING OF DANGEROUS SUBSTANCES
original : OJEC**, OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, 196 , ,1 , 1967
amendment: OJEC**, OFFICIAL JOURNAL OF THE EUROPEAN COMMUNITIES, L 13 , , 1 , 1994

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File: 17.01 LEGAL **rn : 1601163**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : UN type : REC

subject	specification	descriptor
TRNSP		CLASS
LABEL		
PACK		

HAZARD CLASS: 2.1 = FLAMMABLE GAS. PACKING METHOD: M. (APPLIES TO REFRIGERATED LIQUID ETHYLENE). UN NO.1038.
entry date: SEP 1994 effective date: 1993

title: RECOMMENDATIONS ON THE TRANSPORT OF DANGEROUS GOODS
amendment: !UNTDG*, UN TRANSPORT OF DANGEROUS GOODS, RECOMMENDATION PREPARED BY THE COMMITTEE OF EXPERTS ON THE TRANSPORT OF

DANGEROUS GOODS, , , 19 , 1993

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File: 17.01 LEGAL **rn : 1601490**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : UN type : REC

subject	specification	descriptor
TRNSP LABEL PACK		CLASS

HAZARD CLASS: 2.1 = FLAMMABLE GAS. (APPLIES TO COMPRESSED ETHYLENE). UN UN NO. 1962.
entry date: SEP 1994 effective date: 1993

title: RECOMMENDATIONS ON THE TRANSPORT OF DANGEROUS GOODS
amendment: !UNTDG*, UN TRANSPORT OF DANGEROUS GOODS, RECOMMENDATION PREPARED BY THE COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS, , , 19 , 1993

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File: 17.01 LEGAL **rn : 1602034**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : UN type : REC

subject	specification	descriptor
TRNSP LABEL PACK		CLASS

HAZARD CLASS: 2.1 = FLAMMABLE GAS. PACKING METHOD: M. (APPLIES TO O ETHYLENE, ACETYLENE AND PROPYLENE IN MIXTURES, REFRIGERATED LIQUID, CONTAINING AT LEAST 71.5 PERCENT ETHYLENE WITH NOT MORE THAN 22.5 PERCENT ACETYLENE AND NOT MORE THAN 6 PERCENT PROPYLENE). UN NO. 3138.
entry date: SEP 1994 effective date: 1993

title: RECOMMENDATIONS ON THE TRANSPORT OF DANGEROUS GOODS
amendment: !UNTDG*, UN TRANSPORT OF DANGEROUS GOODS, RECOMMENDATION PREPARED BY THE COMMITTEE OF EXPERTS ON THE TRANSPORT OF DANGEROUS GOODS, , , 19 , 1993

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File: 17.01 LEGAL **rn : 1604990**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : IMO type : REC

subject	specification	descriptor

subject	specification	descriptor
TRNSP LABEL PACK	MARIN	CLASS

HAZARD CLASS: 2(2.1) = FLAMMABLE GAS. (APPLIES TO REFRIGERATED LIQUID ETHYLENE). UN NO. 1038.
entry date: SEP 1994 effective date: 1991

title: INTERNATIONAL MARITIME DANGEROUS GOODS CODE (IMDG CODE)
amendment: IMCOC*, IMO DANGEROUS GOODS CODE, RECOMMENDATION PREPARED BY THE MARITIME SAFETY COMMITTEE, 26-91 , , 10086 , 1991

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File: 17.01 LEGAL **rn : 1604991**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : IMO type : REC

subject	specification	descriptor
TRNSP LABEL PACK	MARIN	CLASS

HAZARD CLASS: 2(2.1) = FLAMMABLE GAS. (APPLIES TO COMPRESSED ETHYLENE). UN NO. 1962.
entry date: SEP 1994 effective date: 1991

title: INTERNATIONAL MARITIME DANGEROUS GOODS CODE (IMDG CODE)
amendment: IMCOC*, IMO DANGEROUS GOODS CODE, RECOMMENDATION PREPARED BY THE MARITIME SAFETY COMMITTEE, 26-91 , , 10085 , 1991

****

File: 17.01 LEGAL **rn : 1604992**

systematic name:Ethene
common name :ethylene
reported name :ETHYLENE
cas no :74-85-1 rtecs no :KU5340000
area : IMO type : REC

subject	specification	descriptor
TRNSP LABEL PACK	MARIN	CLASS

HAZARD CLASS: 2*2.1) = FLAMMABLE GAS. (APPLIES TO ETHYLENE, ACETYLENE AND PROPYLENE MIXTURES, REFRIGERATED LIQUID, CONTAINING AT LEAST 71,5 % ETHYLENE WITH NOT MORE THAN 22.5 % ACETYLENE AND NOT MORE THAN 6% PROPYLENE). UN NO. 3138.
entry date: SEP 1994 effective date: 1991

title: INTERNATIONAL MARITIME DANGEROUS GOODS CODE (IMDG CODE)
amendment: IMCOC*, IMO DANGEROUS GOODS CODE, RECOMMENDATION PREPARED BY THE MARITIME SAFETY COMMITTEE, 26-91 , , 10084 , 1991

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 9

SDS FOR ETHYLENE GAS

NOTE: The following SDS is for ethylene gas is a pure compressed gas with a principal hazard being flammability. This hazard is not applicable to ethylene gas in PPM amounts generated in-situ from catalytic dehydration of ethanol

SAFETY DATA SHEET

Ethylene

Section 1. Identification

GHS product identifier : Ethylene

Chemical name : ethylene

Other means of identification : Ethene; Ethene (ethylene); impure; ethylene, pure

Product type : Liquefied gas

Product use : Synthetic/Analytical chemistry.

Synonym : Ethene; Ethene (ethylene); impure; ethylene, pure

SDS # : 001022

Supplier's details : Airgas USA, LLC and its affiliates
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

24-hour telephone : 1-866-734-3438

Section 2. Hazards identification

OSHA/HCS status : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Classification of the substance or mixture : FLAMMABLE GASES - Category 1
GASES UNDER PRESSURE - Liquefied gas
SPECIFIC TARGET ORGAN TOXICITY (SINGLE EXPOSURE) (Narcotic effects) - Category 3

GHS label elements

Hazard pictograms :

Signal word : Danger

Hazard statements : Extremely flammable gas.
May form explosive mixtures with air.
Contains gas under pressure; may explode if heated.
May cause frostbite.
May displace oxygen and cause rapid suffocation.
May cause drowsiness or dizziness.

Precautionary statements

General : Read and follow all Safety Data Sheets (SDS'S) before use. Read label before use. Keep out of reach of children. If medical advice is needed, have product container or label at hand. Close valve after each use and when empty. Use equipment rated for cylinder pressure. Do not open valve until connected to equipment prepared for use. Use a back flow preventative device in the piping. Use only equipment of compatible materials of construction. Always keep container in upright position. Approach suspected leak area with caution.

Prevention : Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. Use only outdoors or in a well-ventilated area. Avoid breathing gas.

Response : IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or physician if you feel unwell. Leaking gas fire: Do not extinguish, unless leak can be stopped safely. Eliminate all ignition sources if safe to do so.

Storage : Store locked up. Protect from sunlight. Store in a well-ventilated place.

Section 2. Hazards identification

Disposal : Dispose of contents and container in accordance with all local, regional, national and international regulations.

Hazards not otherwise classified : Liquid can cause burns similar to frostbite.

Section 3. Composition/information on ingredients

Substance/mixture : Substance

Chemical name : ethylene

Other means of identification : Ethene; Ethene (ethylene); impure; ethylene, pure

Product code : 001022

CAS number/other identifiers

CAS number : 74-85-1

Ingredient name	%	CAS number
ethylene	100	74-85-1

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Occupational exposure limits, if available, are listed in Section 8.

Section 4. First aid measures

Description of necessary first aid measures

Eye contact : Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention if irritation occurs.

Inhalation : Remove victim to fresh air and keep at rest in a position comfortable for breathing. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention. If necessary, call a poison center or physician. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

Skin contact : Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. To avoid the risk of static discharges and gas ignition, soak contaminated clothing thoroughly with water before removing it. Get medical attention if symptoms occur. In case of contact with liquid, warm frozen tissues slowly with lukewarm water and get medical attention. Do not rub affected area. Wash clothing before reuse. Clean shoes thoroughly before reuse.

Ingestion : Remove victim to fresh air and keep at rest in a position comfortable for breathing. Get medical attention. If necessary, call a poison center or physician. Ingestion of liquid can cause burns similar to frostbite. If frostbite occurs, get medical attention. Never give anything by mouth to an unconscious person. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband. As this product rapidly becomes a gas when released, refer to the inhalation section.

Most important symptoms/effects, acute and delayed

Potential acute health effects

Eye contact : Liquid can cause burns similar to frostbite.

Inhalation : Can cause central nervous system (CNS) depression. May cause drowsiness or dizziness.

Section 4. First aid measures

Skin contact : Dermal contact with rapidly evaporating liquid could result in freezing of the tissues or frostbite.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Ingestion : Can cause central nervous system (CNS) depression. Ingestion of liquid can cause burns similar to frostbite.

Over-exposure signs/symptoms

Eye contact : Adverse symptoms may include the following:, frostbite

Inhalation : Adverse symptoms may include the following:, nausea or vomiting, headache, drowsiness/fatigue, dizziness/vertigo, unconsciousness

Skin contact : Adverse symptoms may include the following:, frostbite

Ingestion : Adverse symptoms may include the following:, frostbite

Indication of immediate medical attention and special treatment needed, if necessary

Notes to physician : Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

Specific treatments : No specific treatment.

Protection of first-aiders : No action shall be taken involving any personal risk or without suitable training. If it is suspected that fumes are still present, the rescuer should wear an appropriate mask or self-contained breathing apparatus. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

See toxicological information (Section 11)

Section 5. Fire-fighting measures

Extinguishing media

Suitable extinguishing media : Use an extinguishing agent suitable for the surrounding fire.

Unsuitable extinguishing media : None known.

Specific hazards arising from the chemical : Contains gas under pressure. Extremely flammable gas. In a fire or if heated, a pressure increase will occur and the container may burst, with the risk of a subsequent explosion.

Hazardous thermal decomposition products : Decomposition products may include the following materials:
carbon dioxide
carbon monoxide

Special protective actions for fire-fighters : Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Contact supplier immediately for specialist advice. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool. If involved in fire, shut off flow immediately if it can be done without risk. If this is impossible, withdraw from area and allow fire to burn. Fight fire from protected location or maximum possible distance. Eliminate all ignition sources if safe to do so.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode. For incidents involving large quantities, thermally insulated undergarments and thick textile or leather gloves should be worn.

Section 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel : Accidental releases pose a serious fire or explosion hazard. No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Shut off all ignition sources. No flares, smoking or flames in hazard area. Avoid breathing gas. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.

For emergency responders : If specialized clothing is required to deal with the spillage, take note of any information in Section 8 on suitable and unsuitable materials. See also the information in "For non-emergency personnel".

Environmental precautions : Ensure emergency procedures to deal with accidental gas releases are in place to avoid contamination of the environment. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

Methods and materials for containment and cleaning up

Small spill : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment.

Large spill : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

Section 7. Handling and storage

Precautions for safe handling

Protective measures : Put on appropriate personal protective equipment (see Section 8). Contains gas under pressure. Do not get in eyes or on skin or clothing. Avoid breathing gas. Use only with adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Do not enter storage areas and confined spaces unless adequately ventilated. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.
Use only non-sparking tools. Empty containers retain product residue and can be hazardous. Store and use away from heat, sparks, open flame or any other ignition source. Use explosion-proof electrical (ventilating, lighting and material handling) equipment.

Advice on general occupational hygiene : Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.

Conditions for safe storage, including any incompatibilities : Store in accordance with local regulations. Store in a segregated and approved area. Store away from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see Section 10). Eliminate all ignition sources. Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F). Store locked up. Keep container tightly closed and sealed until ready for use. See Section 10 for incompatible materials before handling or use.

Section 8. Exposure controls/personal protection

Control parameters

Occupational exposure limits

Ingredient name	Exposure limits
ethylene	**ACGIH TLV (United States, 3/2017).** TWA: 200 ppm 8 hours.

Appropriate engineering controls : Use only with adequate ventilation. Use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits. The engineering controls also need to keep gas, vapor or dust concentrations below any lower explosive limits. Use explosion-proof ventilation equipment.

Environmental exposure controls : Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Individual protection measures

Hygiene measures : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Eye/face protection : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: safety glasses with side-shields.

Skin protection

Hand protection : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. If contact with the liquid is possible, insulated gloves suitable for low temperatures should be worn. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.

Body protection : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product. When there is a risk of ignition from static electricity, wear anti-static protective clothing. For the greatest protection from static discharges, clothing should include anti-static overalls, boots and gloves.

Other skin protection : Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory protection : Based on the hazard and potential for exposure, select a respirator that meets the appropriate standard or certification. Respirators must be used according to a respiratory protection program to ensure proper fitting, training, and other important aspects of use.

Thermal hazards : If there is a risk of contact with the liquid, all protective equipment worn should be suitable for use with extremely low temperature materials.

Section 9. Physical and chemical properties

Appearance

Physical state : Gas.

Color : Colorless.

Odor : Characteristic.

Odor threshold : Not available.

pH : Not available.

Melting point : -169.15°C (-272.5°F)

Boiling point : -103.77°C (-154.8°F)

Critical temperature : 9.95°C (49.9°F)

Flash point : Closed cup: -135.85°C (-212.5°F)

Evaporation rate : Not available.

Flammability (solid, gas) : Extremely flammable in the presence of the following materials or conditions: oxidizing materials.

Lower and upper explosive (flammable) limits : Lower: 2.7%
Upper: 36%

Vapor pressure : @ 70°F (21.1°C) = Above critical temperature

Vapor density : 1 (Air = 1) Liquid Density@BP: 35.3 lb/ft3 (566 kg/m3)

Specific Volume (ft ^{3}/lb) : 13.8007

Gas Density (lb/ft ^{3}) : 0.07246

Relative density : Not applicable.

Solubility : Not available.

Solubility in water : 0.13 g/l

Partition coefficient: n-octanol/water : 1.13

Auto-ignition temperature : 450°C (842°F)

Decomposition temperature : Not available.

Viscosity : Not applicable.

Flow time (ISO 2431) : Not available.

Molecular weight : 28.06 g/mole

Aerosol product

Heat of combustion : -47194540 J/kg

Section 10. Stability and reactivity

Reactivity : No specific test data related to reactivity available for this product or its ingredients.

Chemical stability : The product is stable.

Possibility of hazardous reactions : Under normal conditions of storage and use, hazardous reactions will not occur.

Conditions to avoid : Avoid all possible sources of ignition (spark or flame). Do not pressurize, cut, weld, braze, solder, drill, grind or expose containers to heat or sources of ignition.

Incompatible materials : Oxidizers

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Information on toxicological effects

Acute toxicity

Not available.

Irritation/Corrosion

Not available.

Sensitization

Not available.

Mutagenicity

Not available.

Carcinogenicity

Not available.

Classification

Product/ingredient name	OSHA	IARC	NTP
ethylene	-	3	-

Reproductive toxicity

Not available.

Teratogenicity

Not available.

Specific target organ toxicity (single exposure)

Name	Category	Route of exposure	Target organs
ethylene	Category 3	Not applicable.	Narcotic effects

Specific target organ toxicity (repeated exposure)

Not available.

Aspiration hazard

Not available.

Information on the likely routes of exposure : Not available.

Potential acute health effects

Eye contact : Liquid can cause burns similar to frostbite.

Inhalation : Can cause central nervous system (CNS) depression. May cause drowsiness or dizziness.

Skin contact : Dermal contact with rapidly evaporating liquid could result in freezing of the tissues or frostbite.

Ingestion : Can cause central nervous system (CNS) depression. Ingestion of liquid can cause burns similar to frostbite.

Symptoms related to the physical, chemical and toxicological characteristics

Eye contact : Adverse symptoms may include the following:, frostbite

Inhalation : Adverse symptoms may include the following:, nausea or vomiting, headache, drowsiness/fatigue, dizziness/vertigo, unconsciousness

Skin contact : Adverse symptoms may include the following:, frostbite

Ingestion : Adverse symptoms may include the following:, frostbite

Delayed and immediate effects and also chronic effects from short and long term exposure

Section 11. Toxicological information

Short term exposure

Potential immediate effects : Not available.

Potential delayed effects : Not available.

Long term exposure

Potential immediate effects : Not available.

Potential delayed effects : Not available.

Potential chronic health effects

Not available.

General : No known significant effects or critical hazards.

Carcinogenicity : No known significant effects or critical hazards.

Mutagenicity : No known significant effects or critical hazards.

Teratogenicity : No known significant effects or critical hazards.

Developmental effects : No known significant effects or critical hazards.

Fertility effects : No known significant effects or critical hazards.

Numerical measures of toxicity

Acute toxicity estimates

Not available.

Section 12. Ecological information

Toxicity

Not available.

Persistence and degradability

Not available.

Bioaccumulative potential

Product/ingredient name	$LogP_{ow}$	BCF	Potential
ethylene	1.13	-	low

Mobility in soil

Soil/water partition coefficient (K_{OC}) : Not available.

Other adverse effects : No known significant effects or critical hazards.

Section 13. Disposal considerations

Disposal methods : The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Empty Airgas-owned pressure vessels should be returned to Airgas. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Empty

Section 13. Disposal considerations

containers or liners may retain some product residues. Do not puncture or incinerate container.

Section 14. Transport information

	DOT	TDG	Mexico	IMDG	IATA
UN number	UN1962	UN1962	UN1962	UN1962	UN1962
UN proper shipping name	ETHYLENE	ETHYLENE	ETHYLENE	ETHYLENE	ETHYLENE
Transport hazard class(es)	2.1 	2.1 	2.1 	2.1 	2.1 
Packing group	-	-	-	-	-
Environmental hazards	No.	No.	No.	No.	No.

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Additional information

DOT Classification : **Limited quantity** Yes.
Quantity limitation Passenger aircraft/rail: Forbidden. Cargo aircraft: Forbidden.
Special provisions T75, TP5

TDG Classification : Product classified as per the following sections of the Transportation of Dangerous Goods Regulations: 2.13-2.17 (Class 2).
Explosive Limit and Limited Quantity Index 0.125
ERAP Index 3000
Passenger Carrying Ship Index Forbidden
Passenger Carrying Road or Rail Index Forbidden

IATA : **Quantity limitation** Passenger and Cargo Aircraft: Forbidden. Cargo Aircraft Only: 150 kg.

Special precautions for user : **Transport within user's premises:** always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.

Transport in bulk according to Annex II of MARPOL and the IBC Code : Not available.

Section 15. Regulatory information

U.S. Federal regulations : **TSCA 8(a) CDR Exempt/Partial exemption**: Not determined
Clean Air Act (CAA) 112 regulated flammable substances: ethylene

Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs) : Not listed

Clean Air Act Section 602 Class I Substances : Not listed

Clean Air Act Section 602 Class II Substances : Not listed

DEA List I Chemicals (Precursor Chemicals) : Not listed

Section 15. Regulatory information

DEA List II Chemicals (Essential Chemicals) : Not listed

SARA 302/304

Composition/information on ingredients

No products were found.

SARA 304 RQ : Not applicable.

SARA 311/312

Classification : Refer to Section 2: Hazards Identification of this SDS for classification of substance.

SARA 313

	Product name	CAS number	%
Form R - Reporting requirements	ethylene	74-85-1	100
Supplier notification	ethylene	74-85-1	100

SARA 313 notifications must not be detached from the SDS and any copying and redistribution of the SDS shall include copying and redistribution of the notice attached to copies of the SDS subsequently redistributed.

State regulations

Massachusetts : This material is listed.

New York : This material is not listed.

New Jersey : This material is listed.

Pennsylvania : This material is listed.

International regulations

Chemical Weapon Convention List Schedules I, II & III Chemicals

Not listed.

Montreal Protocol (Annexes A, B, C, E)

Not listed.

Stockholm Convention on Persistent Organic Pollutants

Not listed.

Rotterdam Convention on Prior Informed Consent (PIC)

Not listed.

UNECE Aarhus Protocol on POPs and Heavy Metals

Not listed.

Inventory list

Australia : This material is listed or exempted.

Canada : This material is listed or exempted.

China : This material is listed or exempted.

Europe : This material is listed or exempted.

Japan : **Japan inventory (ENCS)**: This material is listed or exempted.
Japan inventory (ISHL): Not determined.

Malaysia : Not determined.

New Zealand : This material is listed or exempted.

Philippines : This material is listed or exempted.

Republic of Korea : This material is listed or exempted.

Taiwan : This material is listed or exempted.

Thailand : Not determined.

Turkey : This material is listed or exempted.

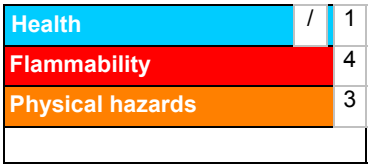
United States : This material is listed or exempted.

Section 15. Regulatory information

Viet Nam : Not determined.

Section 16. Other information

Hazardous Material Information System (U.S.A.)



Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings and the associated label are not required on SDSs or products leaving a facility under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered trademark and service mark of the American Coatings Association, Inc.

The customer is responsible for determining the PPE code for this material. For more information on HMIS® Personal Protective Equipment (PPE) codes, consult the HMIS® Implementation Manual.

National Fire Protection Association (U.S.A.)

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Copyright ©2001, National Fire Protection Association, Quincy, MA 02269. This warning system is intended to be interpreted and applied only by properly trained individuals to identify fire, health and reactivity hazards of chemicals. The user is referred to certain limited number of chemicals with recommended classifications in NFPA 49 and NFPA 325, which would be used as a guideline only. Whether the chemicals are classified by NFPA or not, anyone using the 704 systems to classify chemicals does so at their own risk.

Procedure used to derive the classification

Classification	Justification
FLAMMABLE GASES - Category 1 GASES UNDER PRESSURE - Liquefied gas SPECIFIC TARGET ORGAN TOXICITY (SINGLE EXPOSURE) (Narcotic effects) - Category 3	Expert judgment Expert judgment Expert judgment

History

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Key to abbreviations : ATE = Acute Toxicity Estimate
BCF = Bioconcentration Factor
GHS = Globally Harmonized System of Classification and Labelling of Chemicals
IATA = International Air Transport Association
IBC = Intermediate Bulk Container
IMDG = International Maritime Dangerous Goods
LogPow = logarithm of the octanol/water partition coefficient
MARPOL = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution)
UN = United Nations

References : Not available.

Section 16. Other information

Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

PETITION TO ADD ETHYLENE GENERATED ON-SITE FROM ETHANOL AS A GROWTH REGULATOR FOR POTATOES AND ONIONS IN STORAGE TO THE NATIONAL LIST OF ALLOWED SUBSTANCES FOR ORGANIC PRODUCTION

APPENDIX 10

INFORMATION ON THE UTILITY AND VALUE OF ETHYLENE IN AGRICULTURE, AND ITS PREFERENCE TO PREVIOUSLY - USED, HAZARDOUS CHEMICAL PESTICIDES.

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REVIEW

Sprout suppression on potato: need to look beyond CIPC for more effective and safer alternatives

Vijay Paul[1,3] · **R. Ezekiel**[2,4] · **Rakesh Pandey**[3]

Revised: 22 July 2015 / Accepted: 28 July 2015 / Published online: 13 August 2015

Abstract World over, potatoes are being stored at 8–12 °C (85–90 % RH). This is the most common way of long-term (up to 6 to 9 months) storage of potatoes. The benefit of storing the potatoes within the temperature range of 8–12 °C is minimum accumulation of sugars in stored potato tubers. In sub-temperate, sub-tropical and tropical countries of the world, short-term (3 to 4 months) storage of potatoes is being done by non-refrigerated traditional/on-farm methods. These short- and long-term storage methods keep the stored potatoes suitable not only for table purpose but also for processing. However, once the natural dormancy period of potato is over, the prevailing temperatures in these storage methods favour sprouting and sprout growth. Therefore, use of some sprout suppressant to check the sprout growth becomes essential under these methods of potato storage. CIPC [Isopropyl *N*-(3-chlorophenyl) carbamate] is the most wide spread and commonly used sprout suppressant on potatoes. CIPC has been in use for more than 50 years and research carried out over such a long period use of CIPC has not only enhanced our understanding of its properties and chemistry but also about the production and toxicological status of its metabolites/degradation products. Today, various safety issues and concerns have surfaced primarily due to continuous and long-term use of CIPC. This review presents an appraisal on CIPC and explains the reasons for the long-time dependence on this chemical as a potato sprout suppressant. Issues like maximum residue limit and acceptable daily intake limit are being discussed for CIPC. This article brings an update on practical aspects of potato storage, residue levels of CIPC, efficacy of CIPC as sprout suppressant and health and environmental safety issues linked with CIPC and its metabolites. The aim of this article is to find possible solutions, way outs and future plans that can make the sprout suppression of potatoes safer and more risk free.

Keywords ADIL · CIPC · Metabolite toxicity · MRL · Potato · Potato storage · Residue · Safety issues · Sprout suppressant

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Introduction

Potato (*Solanum tuberosum* L.) is an important food crop and it is being grown in nearly 150 countries. Global potato production in the year 2012 was 364.81 million tonnes wherein, the developing countries contributed for 213.74 million tonnes (FAOSTAT 2013a). World potato production has increased at an annual average rate of 4.5 % over the last 10 years. In terms of production, potato has exceeded the growth of many other major food commodities in developing countries and particularly in Asia (IPY 2008; FAO 2008). Today potato is the 4th major food crop after rice, wheat and maize. Just after the harvest, there is huge arrival of potatoes in

the market resulting in their surplus availability. In the years of higher production, there is glut in developing and economically poor countries. This is primarily due to inadequate facilities and poor infrastructure for storage, marketing and utilization of potatoes in processing. This situation usually cause distress sale by the farmers (Mehta and Ezekiel 2006; Sundaram 2011; Gautam et al. 2013). Fresh potatoes are available only for 2 to 4 months (depending on the geographical region and country). Storage of potatoes is therefore necessary to meet the requirements for the remaining period of a year. Storage of potatoes either for short-term (2 to 4 months, under non-refrigerated conditions) or long-term (5 to 9 months, under refrigerated conditions) helps in reducing the postharvest losses and gluts like situation (Ezekiel et al. 2005; Paul and Ezekiel 2013). The situations and problems pertaining to the storage of potatoes in sub-temperate, sub-tropical and tropical countries of the world are different from that of temperate countries. This is because of the fact that the harvest of potatoes in the former is followed by hot summer months whereas, in the latter harvest is followed by cool winter months.

During storage at temperatures of 8–12 °C, potatoes are treated with a sprout suppressant either once (if stored up to 5 months) or twice (if stored for 6 to 9 months). World over, isopropyl *N*-(3-chlorophenyl) carbamate (CIPC also referred as chlorpropham) is the most commonly used sprout suppressant on potatoes when stored at 8–12 °C (Smith and Bucher 2012). CIPC is primarily an herbicide which was introduced in the year 1951 (Marth and Schultz 1952). After that, its use has gradually spread in developed and then in developing countries (Sawyer and Malagamba 1987; Burton et al. 1992; Tayler et al. 1996; Ezekiel et al. 2005). Many years of research and commercial use has shown the efficacy of CIPC as a sprout suppressant on potatoes especially when they are stored at 8–12 °C. This effect of CIPC is with little or no adverse effect on quality parameters (Rastovski 1987; Tayler et al. 1996; Blenkinsop et al. 2002; Ezekiel et al. 2005; Mehta et al. 2010). Now, it is more than 50 years since the commercial use of CIPC (as sprout suppressant) started on stored potatoes (Teper-Bamnolker et al. 2010; Verhagen et al. 2011). A large number of studies carried out during this period have enhanced our understanding about CIPC, its mode of action, its metabolism in plants and animal and its fate in our environment. This article aims to update and highlight the safety and environmental issues which have surfaced due to continuous and long-term use of CIPC as potato sprout suppressant. Concerns associated with high degree of toxicity as exhibited by metabolites of CIPC (present or formed in the CIPC-treated tubers and also produced in humans on the consumption of CIPC-treated tubers) are also highlighted in this article. Based on the survey of literature and outcome of various studies this article suggests some of the possible solutions, way out and future strategies that will help in making the potato storage and use of sprout suppressant on potatoes safer and more risk free.

Different options of storing the harvested potatoes

Storage at 2–4 °C

Storage of potatoes at 2–4 °C (90–95 % RH) in cold stores is ideal for storing the seed potatoes because at this temperature there is no sprouting or sprout growth. Under this condition, potatoes remain viable for a long period and therefore these tubers can be used as seed potatoes for planting in the subsequent season. This low temperature is however not suitable for storing the potatoes meant for either table or processing purposes. Potatoes stored at this temperature start accumulating reducing sugars (glucose and fructose) and become sweet in taste (Rees et al. 1981; Sonnewald 2001). Consumers do not like to the eat potatoes that taste sweet. Storage of potatoes at 2–4 °C induces a process referred as cold-induced sweetening (CIS). CIS involves degradation of starch (main reserve material present in the potatoes) into reducing sugars (Sonnewald 2001). As a result, potatoes stored at 2–4 °C accumulate reducing sugars in them. Accumulated or higher levels of reducing sugars are also responsible for unacceptable dark brown colour on processed products (chips, crisps, French fries and flakes etc.). This browning is mainly because of a non-enzymatic reaction referred as Maillard reaction (Maillard 1912; Kyriacou et al. 2009; Everts 2012). In this way, potatoes stored at low temperature no longer remain suitable for processing (Ezekiel et al. 2003; Kyriacou et al. 2009).

Short-term storage of potatoes

For short-term storage of potatoes (up to 2–4 months), on-farm methods are practiced by the farmers in most of the potato grown countries (Mehta and Ezekiel 2010; Gautam et al. 2013). These on-farm methods are highly cost-effective and help the farmers in extending the marketing period and thereby fetching more economic benefits (Ezekiel et al. 1999; Paul and Ezekiel 2003a; Kumar and Ezekiel 2006; Mehta and Ezekiel 2010; Paul and Ezekiel 2013). Potatoes are stored in the form of heaps of potatoes or they are placed in pits of appropriate size. Storage of harvested potatoes by above methods are done either in the vicinity of the field (where the potato crop was grown) or the farmers may prefer to store the potatoes near to their house for proper monitoring and look after. Protection from the sun light and possible rains is done by covering the heap of potatoes with straw material and pits by erecting a thatched roof like structure at low height (made from locally available materials) (Paul et al.

2002a, b; Paul and Ezekiel 2004). Besides the above methods, storage of potato in a dark room as such or with provision of evaporative passive cooling, spreading tubers on floor, storing in bins, hanging potatoes in bamboo baskets are also being practiced for short-term storage (Mehta and Ezekiel 2010; Gautam et al. 2013). All the above methods fall in the category of non-refrigerated methods of storing potatoes.

Studies conducted at various centers located in India (representing sub-tropical region) indicated that the temperature inside heap and pit can be 10 to 15 °C lower than the prevailing ambient temperatures. During the storage period of 3 months, temperature and RH varies from 21 to 32 °C and 51 to 95 %, respectively (Kumar et al. 2005; Paul and Ezekiel 2005; Ezekiel et al. 2005). Weight losses due to sprouting and decay of tubers under these on-farm storage methods have been estimated from 10 to 40 % depending upon the duration, type and location of storage, variety, maturity of stored tubers, extent of protection provided to potatoes against sun light, heat of the sun and rains (Paul and Ezekiel 2003a, b; Mehta and Ezekiel 2010). As relatively higher temperature prevails during the period of storage in sub-tropical and tropical regions, these on-farm storage for 2 to 4 months helps in maintaining low levels of reducing sugars in stored tubers (Kumar et al. 2005; Gautam et al. 2013). Because of this, the tubers retain their suitability for table as well as processing purposes. Once the dormancy period (6 to 8 weeks) of harvested and stored potatoes is over, the temperature that prevails under these storage methods (in sub-tropical and tropical regions) favours sprouting necessitating the use of a sprout suppressant to check sprouting and sprout growth.

Long-term storage of potatoes

For long-term storage of potatoes (up to 9 months), storage at 8–12 °C with 85–90 % RH is the most appropriate method and this is normally followed in developed nations. This method of potato storage has become popular in developing countries as well. The basic reason behind selecting the temperature range of 8–12 °C is because of the relationship between the rate of respiration of potato tubers with the storage temperature. At the temperature range of 8–12 °C the rate of respiration of potato tuber is minimum (Burton 1989). The most significant benefit of storing the potatoes within the temperature range of 8–12 °C is that this temperature range allows the minimum accumulation of reducing sugars in stored potato tubers (Smith 1987; Ezekiel et al. 2007a, b). This thereby keeps the stored potatoes suitable for consumption (table and processed potato). However, once the natural dormancy period of tuber is over, this storage temperature of 8–12 °C is favourable for sprouting and sprout growth. Therefore, the use of a potato sprout suppressant becomes essential here.

Adverse consequences of sprouting of potatoes during storage

It is already made clear above that control of sprout growth is a key factor either for short-term or long-term storage of potato. Sprout growth contributes significantly towards the weight loss of the potato. As per an estimate by Burton (1955), respiration increases by 50 % if sprouts on tuber reach to 1 % of the tuber weight (i.e., 1 g of sprout per 100 g fresh weight of tuber). It has been revealed that the epidermis of the sprout is about 100 times more permeable to water in comparison to rest of the surface of the tuber (Burton 1955). Because of this, sprout growth equivalent to 1 % increase in the tuber surface area leads to doubling of the moisture loss from the potato tubers. Respiration as well as evaporation increase rapidly with the onset of sprouting and continuous growth of sprouts. Sprouting therefore results in rapid increase in physiological weight loss of stored tubers. Besides causing the weight loss, sprouting is also highly detrimental to the nutritional status and quality aspects of potatoes (van Es and Hartmans 1987a; Mani et al. 2014). Sprouting leads to higher rate of respiration, remobilization of storage compounds in the potato tubers mainly starch and proteins besides causing shrinkage due to loss of water (Sonnewald and Sonnewald 2014). These changes also cause deterioration in processing quality due to loss in mass, decreased turgor, structural change due to growth of sprout tissue and increase in sugar concentrations due to hydrolysis of starch (Burton et al. 1992; Davies 1990; van Es and Hartmans 1987b; Daniels-Lake et al. 2005). Potato quality parameters such as firmness and content of vitamin C are also adversely affected by sprouting (Rezaee et al. 2011). So, to reduce weight loss and other undesirable physiological and biochemical changes that can adversely affect the quality of potatoes, the use of sprout suppressants has become an integral part of potato storage and potato industry.

CIPC as an effective sprout suppressant for potatoes during short-term and long-term storage

CIPC is a selective and systemic herbicide with an ability to translocate acropetally in plant system (Ashton and Crafts 1981). It has only slight solubility in water (89 mg per litre) but it is highly soluble in organic solvents. Half-life of CIPC in soil is about 65 days at 15 °C and 30 days at 29 °C (O'Neil et al. 2006; EXTOXNET 1996). CIPC acts as a mitotic inhibitor by interfering the process of spindle formation during the cell division (Vaughn and Lehnen 1991). It is known to inhibit protein synthesis, RNA synthesis, activity of β-amylase along with suppression of transpiration and respiration and interfere with of oxidative phosphorylation and photosynthesis

(Vaughn and Lehnen 1991). CIPC is considered as the most effective sprout suppressant for potatoes. It can be converted into emulsifiable concentrate, fogging concentrate, granules and dustable powder (van Vliet and Sparenberg 1970; Corsini et al. 1979; Conte et al. 1995). It is usually applied as a post-harvest fogging treatment on stored potatoes.

Sprout suppression ability of CIPC has been found to be more effective at temperature of 15 °C or below. Its efficacy decreases at temperature higher than 15 °C (Mondy et al. 1992a; Kleinkopf et al. 1997; Ezekiel et al. 2005; Sanli et al. 2010). Research carried out in India has shown the sprout suppression ability of CIPC even at higher storage temperatures (ranging from 21 to 32 °C) although not to the same extent as observed at the storage temperature of 8–12 °C (Ezekiel et al. 2005; Mehta et al. 2007, 2010). On an average, temperature of 18–32 °C (52–88 %) and 19–27 °C (69–92 % R.H) prevails under heaps and pit methods of potato storage respectively during 90 days of storage in sub-tropical conditions when the ambient temperatures may vary between 34 and 41 °C or more (Mehta et al. 2007). Effectiveness of CIPC has been demonstrated at temperature even higher than 15 °C under traditional and non-refrigerated methods of potato storage in number of studies (Ezekiel et al. 2002; Singh et al. 2004; Chandel et al. 2008; Kumar and Ezekiel 2006; Mehta et al. 2007, 2010).

Fogging treatment of CIPC is given either once or twice depending upon the duration of storage period. The dose of CIPC is about 18 g (a.i.) tonne^{-1} of potatoes. Normally two applications are done for long-term storage of potatoes, so total CIPC applied is about 36 g (a. i.) tonne^{-1} of potatoes (Lewis et al. 1997; Ezekiel et al. 2005). In UK, the maximum amount of CIPC that can be applied to fresh market potatoes is 36 g per tonne of potatoes whereas, for processing 63.75 g per tonne of potatoes is allowed (Mohammed 2012). In addition to the use of CIPC as a sprout suppressant, it is also used as an herbicide. As per an estimate, CIPC accounts for about 11 % of the total herbicide sale world-wide (Farawela 2009).

Factors affecting the residue of CIPC in treated potatoes and processed products

Application rates of CIPC may vary depending on the storage temperature, length of storage period and method of application (Mondy et al. 1992a; Sakaliene et al. 2009). Retreatment often becomes necessary to extend sprout inhibition during storage (Corsini et al. 1979; Kleinkopf et al. 1997; Mahajan et al. 2008). Often, there is a lack of uniform distribution of applied CIPC and thereby its concentration in the tubers. For instance; after aerosol or direct spray treatment, CIPC concentrations vary significantly depending upon the location i.e., in top, middle and bottom piles of potatoes (Corsini et al. 1979; Kleinkopf et al. 1997). Potatoes stored in piles had uneven distribution of CIPC, presumably because of differential airflow within the piles (Conte et al. 1995). Potato cultivars also differ in concentration of CIPC dose which is required for the effective control of sprout growth besides being influenced by storage conditions and temperatures (Kleinkopf et al. 2003). All the factors and situations as described above play a role in leading to residue levels of CIPC in the treated potatoes during and after the period of storage.

CIPC is usually applied prior to the start of sprouting (Ravanel and Tissut 1984). Ability of CIPC to suppress the sprout growth is more if it is applied prior to sprouting. Application of CIPC to already sprouted potatoes causes the desiccation of sprouts but its ability to suppress the sprout growth is reduced. Multiple applications of CIPC may be necessary if the permitted application rate is relatively low as it is practiced by European nations (NAPPO 2013). Noel et al. (2004) evaluated the distribution of CIPC after applying it in different formulations. Study revealed that the residue levels of CIPC on the potato tuber depends more on the type of formulation applied than to any other single factor. Treatment of CIPC in the form of dust powder lead to the highest CIPC residue deposit on the potato tuber compared with emulsifiable concentrate while hot fogging showed very low residue level of CIPC. Wilson et al. (1981) observed residue levels of CIPC up to 45 mg per kg of potatoes following the aerosol treatment. Whereas, study by Mondy et al. (1992b), showed that potato tubers dipped in a 1 % emulsion of CIPC resulted in residue level up to 400 mg per kg of potatoes in the peel. This was the maximum residue level recorded in the treated tubers. Peel is known to contain maximum levels of CIPC and in this study residue examination was done just after the treatment under storage at 5 °C (losses of CIPC are minimum at this temperature). Study by Conte et al. (1995) showed presence of 10 times more residues in tubers that were treated with CIPC powder than the tubers treated with aerosol. It was reported by Mehta et al. (2010) that the residue concentration was higher immediately after the spray application of CIPC at a rate of 30 mg per kg of potatoes compared to 20 mg per kg of potatoes but at the end of storage period this difference was no longer detectable. They also recorded 10–20 times lower levels of residue in the cortex than in the peel of treated tubers. Brajesh and Ezekiel (2010) found a correlation between the number of CIPC applications and the residue of CIPC in potato tubers. CIPC was also detected in potato crisps (Lewis et al. 1996) potato chips (Nagami 1997) French fries (Lentza-Rizos and Balokas 2001) and extruded potato peels (Camire et al. 1995). There are other reports where CIPC residue was detected and determined not only in cooked and processed potato products but also in the oil which was used for frying (Ritchie et al. 1983; Nagayama and Kikugawa 1992; Ezekiel and Singh 2007; Park et al. 2009).

Factors contributing for reduction of CIPC residue in treated potatoes

It was observed that up to 45 % of applied CIPC remain present in the soil adhering to the treated and unwashed tubers (Coxon and Filmer 1985). Corsini et al. (1979) reported that the residue of CIPC in peel samples (tuber taken from a large commercial store after aerosol application) were fairly high (15–85 mg L^{-1}) whereas less than 1 mg L^{-1} was found in peeled tubers. Wilson et al. (1981) showed that washing the tubers under running water reduced CIPC concentration from 45 to 40 mg L^{-1}. By applying a more rigorous washing procedure, 88 % of CIPC was removed (level changed from 1.6 to 0.2 mg per kg of potatoes) from potatoes which were earlier treated with an emulsified solution of CIPC (0.1 %) (Tsumurahasegawa et al. 1992). Washing of potatoes with water that were treated with dustable powder of CIPC and stored for 28 days showed reduction in the residue of CIPC from 3.8 to 2.9 mg per kg. This means that 24 % of the CIPC residue leached into the water (Lentza-Rizos and Balokas 2001). Similar observations were recorded by Park (2004) and Sakaliene et al. (2009). In fact presence of CIPC was detected in water which was used for washing the potatoes treated with CIPC. Conte et al. (1995) and Lewis et al. (1996) suggested that removal of CIPC from the potato by peeling is much more effective than washing. On the other hand, Sakaliene et al. (2009) emphasized on cleaning of the surface of the treated tubers by washing to remove CIPC residues because peeling although removes the majority of the chemical but it also removes nutrients from the potato. It is important to mention here that Sakaliene and co workers also laid emphasis on commercial availability of alternative sprout control methods as well. Studies are available where large differences in the residue of CIPC in the peel, unpeeled and peeled tubers have been seen (Coxon and Filmer 1985; Mondy et al. 1992b; Brajesh and Ezekiel 2010). According to Lentza-Rizos and Balokas (2001), peeling removes approximately 91–98 % of the CIPC from the tubers.

Storage time has a substantial effect on the CIPC residue present in the potato tuber. Residue level of CIPC decreases with the duration of storage (Mondy et al. 1992a; Lentza-Rizos and Balokas 2001; Sakaliene et al. 2009; Brajesh and Ezekiel 2010). Reduction in the residue levels of different agrochemicals is also affected by the handling and processing related steps such as; washing, heating, blanching, cooking and frying besides storage duration and storage temperatures (Keikotlhaile et al. 2010; Bajwa and Sandhu 2014). Boiling the potatoes in water or cooking them by steaming resulted in reduced residue of CIPC in cooked tubers as compared to uncooked tubers due to leaching of CIPC into the cooking water (Mondy et al. 1992b). Processing has also been shown to reduce the residue of CIPC in potatoes (Lentza-Rizos and Balokas 2001; Ezekiel and Singh 2007; Sakaliene et al. 2009; Park et al. 2009; Gonzalez-Rodriguez et al. 2011).

Maximum residue limit (MRL) and acceptable daily intake limit (ADIL) for CIPC

On 1st August, 1996, a federal Re-registration Eligibility Decision (RED) for CIPC was issued to continue its use as sprout inhibitor on harvested potatoes in storage by the Environmental Protection Agency (EPA 1996). This decision allowed for a maximum residue limit (MRL; residue tolerance) of 50 mg kg^{-1} of tuber fresh weight (equivalent to 50 ppm). During the year 2002, the established allowable MRL for CIPC for fresh potatoes was reduced to 30 ppm (EPA 2002a, b). In the year 2007, the MRL for potatoes treated by CIPC was fixed at 10 ppm for human consumption across the Europe by Advisory Committee on Pesticides (ACP) (McGowan et al. 2009). While, MRL of 5 to 10 ppm was envisioned by European Union (EU) member countries (Anonymous 2002; Kleinkopf et al. 2003) and 10 ppm by European Commission (2008) and Health and Safety Executive (HSE 2009). As per the regulatory status in three NAPPO (North American Plant Protection Organization) counties (NAPPO 2013), the MRL of CIPC (applied in any form) is 15 ppm for Canada and 30 ppm for USA. These limits also impose restriction on the repeated use of CIPC. However if we wish to see in practical terms, it becomes essential to use the CIPC at least twice for storing the potatoes up to 6 to 9 months.

Actual residue level of CIPC as detected in stored potato tubers treated with CIPC ranged from 8 to 15 ppm (Lentza-Rizos and Balokas 2001; Singh and Ezekiel 2010). The acceptable daily intake limit (ADIL) with respect to CIPC for human body is reported to be 0.05 mg kg^{-1} (of body weight) day^{-1} (Anonymous 1997; Chlorpropham 2003; EFSA 2012). Residue of CIPC is the most frequently encountered chemical present in potatoes and has been regularly found in WPPR/PRC surveys since 1994. In UK, it is applied as a fog according to strict guidelines and only by suitably qualified individuals. Majority of the residue levels reported in the PRC surveys and by industry in UK were below the proposed levels, but levels on two PRC samples in 2003 reached 12 and 20 mg kg^{-1} respectively (Bradshaw and Ogilvy 2006). There are reports that some samples with exceeding MRL were withdrawn from the exporting market (Noel et al. 2004). There are two possible explanations for this 1) The highest recommended application was exceeded even more than which is necessary to control the sprouting and 2) Uneven distribution of CIPC in the piles of stored potatoes (Noel et al. 2002, 2003). If this could be a situation in UK, then the problem of higher residue levels can be expected to be more common in developing and third world countries where neither strict guidelines are followed nor the treatment is being given each time by suitably qualified individuals. Besides this, the old set up and available infrastructure for most of the potato stores may impose hindrance in uniform distribution of CIPC. There is

also a tendency of indiscriminate/overuse of CIPC to save the stored potatoes for better price and sometime for the sake of reputation of cold store owners. As per WPPR/PRC surveys since 1994, CIPC is regular and the most frequently encountered residue in potatoes. Study pertaining to whole diet by the USA Food and Drug Administration (FDA) indicated that CIPC is one of the most abundant pesticides in the diet of adults (Daniels-Lake et al. 2011).

Consumption of potatoes in different countries and its relation with ADIL of CIPC

Keeping in view the MRL of CIPC in the whole potato tuber as 5, 10 15, 30 and 50 ppm and the ADIL as 0.05 mg kg^{-1} day^{-1} (as stated above), the data generated for maximum quantity of potatoes a person can consume on daily basis is presented in Table 1. Data on overall consumption of potato (g $head^{-1}$ day^{-1}) in selected countries and regions of the world are presented in Table 2. Now taking into consideration the data presented in Tables 1 and 2 - following inferences can be drawn.

1. Highest consumption of potatoes (507 g $head^{-1}$ day^{-1}) is in Belarus (Table 1). With this much of potato consumption it appears quite obvious that MRL of CIPC in the potatoes should not go beyond 5 ppm (Table 1). But, even with MRL of CIPC as 5 ppm, consumption of potatoes @ 507 g $head^{-1}$ day^{-1} can be considered safe only for the persons with body weight of 50 kg or more (Table 1). Consumption of potatoes equivalent to the national average of Belarus by a person with a body weight of less than 50 kg will possibly expose him/her to a CIPC levels beyond the permissible limit. Next highest consumption of potato is in Ukraine (383 g $head^{-1}$ day^{-1}). With this consumption on daily basis, MRL of CIPC in the potatoes should not go beyond 10 ppm. But, even with MRL of CIPC as 10 and 5 ppm, consumption of 383 g of potatoes in Ukraine cannot be considered safe for the persons with body weight less than 70 and 30 kg, respectively (Table 1).
2. Like the above cases, nations like Poland, Estonia, Russian Federation, Kazakhstan, Malawi and Belgium consume around 300 g of potato per head on daily basis (Table 2). Here again, with the MRL of CIPC at 10 and 5 ppm, people having a body weight less than 60 and 30 kg, respectively are at the risk of taking in the CIPC beyond its permission limit (Table 1). In a similar way, for countries such as; Canada, Bosania & Herzegovina, Nepal, Azerbaijan and Germany and for special groups of nations like; Europe and European Union (where consumption of potatoes is around 200 g $head^{-1}$ day^{-1}) MRL for CIPC should not go beyond 15 ppm (Table 1). But even with the MRL of CIPC at 15, 10 and 5 ppm, people with body weight less than 60, 40 and 20 kg, respectively are at the risk of consuming higher levels of CIPC.

It is true that the quantity and the form in which the CIPC-treated potatoes are consumed (fresh and/or processed product) govern the extent to which an individual will be exposed to CIPC. From the above examples that include different nations with different food habits and in different geographical locations, it is evident that even with the lower recommended MRL of CIPC i.e., 5 and 10 ppm, people can be at risk of higher intake of CIPC. This can happen at least during certain period of year when the availability of potato is totally met from the stored and CIPC treated potatoes. Considering the MRL of 10 ppm for CIPC in potato tuber, countries with potato consumption of around 500, 300 and 200 g $head^{-1}$ day^{-1} are possibly at risk of taking in higher levels of CIPC if people with body weight of 100, 60 and 40 kg, respectively are consuming the CIPC-treated potatoes equivalent to their national average. Taking into consideration the point number 2 as stated above and the data presented in Table 1, it can also be presumed that people in countries like; Malawi, Belgium, Lithuania, Kyrgyzstan, United Kingdom (UK), Rwanda, Romania, Latvia, Ireland and Peru are at the risk level in between the countries consuming 300 and 200 g of potatoes per head on daily basis.

It is important to mention here that 1) Sweden (even with 160 g of per capita consumption per day) has imposed ban on the use of CIPC (Gomez-Castillo et al. 2013), 2) Mexico (with 37 g of per capita consumption per day), which is one of NAPPO country, has not registered any sprout inhibitor for its use on potato (NAPPO 2013), 3) In Netherlands (with 257 g of per capita consumption per day) and Switzerland (with 114 g of per capita consumption per day), S-carvone is also used as potato sprout suppressant at commercial scale and it is marketed with the trade name “TalentTM” (Gomez-Castillo et al. 2013) and 4) Sakaliene et al. (2009) is of the view that until there has been refinement of risk assessment and risk management of the CIPC residues along with an estimation of possible adverse effects of CIPC on the vulnerable groups such as infants and children, the emphasis should be placed on the use of those cultivars that can be stored successfully up to 6 months and longer without any treatment of CIPC. But, keeping in view the effectiveness, widespread use, well established commercial base and non-availability of any other sprout suppressant that can be considered equivalent to CIPC, the use of CIPC is continue. In fact, there are efforts which insist on retaining the use of CIPC in the supply chain of potatoes (Potato Council 2013) and its re-registration not only on the basis of its efficacy to control the sprouting in stored potatoes but also by telling that the use of CIPC on potatoes will continue to be deemed safe (Kippley 2012). In UK, a group named as PICSG (The Potato Industry CIPC

Table 1 Maximum quantity of potato (in gram) a person can consume on daily basis to reach up to the proposed levels of Acceptable Daily Intake Limit (ADIL) of 0.05 mg kg^{-1} of body weight day^{-1} for different Maximum Residue Limits (MRL)

Body weight of an individual (kg)	MRL (mg kg^{-1} of tuber fresh weight or ppm)				
	5	10	15	30	50
5	50	25	17	8	2
10	100	50	33	17	4
20	200	100	67	25	6
30	300	150	100	33	8
40	400	200	133	42	10
50	500	250	167	50	12
60	600	300	200	58	14
70	700	350	233	66	16
80	800	400	267	75	18
90	900	450	300	83	20
100	1000	500	333	91	22

Data in the table are generated based on the values of MRL and ADIL as documented by EPA (1996); Anonymous (1997); EPA (2002a), (2002b); Anonymous (2002); Chlorpropham (2003); Kleinkopf et al. (2003); HSE (2009); McGowan et al. (2009); EFSA (2012); NAPPO (2013)

Stewardship Group) has started a drive "Be CIPC Compliant" in 2013 (http://www.cipccompliant.co.uk/stewardship/, http://www.cipccompliant.co.uk/, http://www.fwi.co.uk/articles/22/07/2013/140121/potato-industry-launches-cipc-stewardship-plan.htm, http://www.fwi.co.uk/articles/05/09/2013/140592/new-campaign-on-the-correct-use-of-cipc-in-potato-stores.htm). This group consists of potato growers, contractors, industries (including Potato Council), processors, regulatory body, research institute etc. The main objective and plea of this group is to make efficient and best use of CIPC as sprout suppressant for stored potatoes. Today, this objective of the PICSG has in fact become more relevant and essential. This need to be implemented world over and in this article, same is being substantiated below by highlighting safety issues and concerns that are now clearly linked with the continuous and long-time use of CIPC on potatoes.

Some important safety issues and environmental concerns related with CIPC

World over, continuous use of CIPC (as a sprout suppressant on potatoes) for a period of more than 50 years and that too at commercial scale (Marth and Schultz 1952; Gomez-Castillo et al. 2013) has brought in some pertinent issues which are related directly to the safety of human, animal, water and environment. Some of such issues are described below.

- CIPC belongs to group of pesticides known as carbamates. CIPC is applied by thermal fogging and this step causes not only the thermal degradation of CIPC but also the breakdown of CIPC. Carbamates break down to aniline based derivatives which have high toxicity profile (Balaji et al. 2006). One of such breakdown product of CIPC is 3-chloroaniline (3-CA) and being aniline based derivative this is considered more polluting and highly toxic than the parent compound itself (Park 2004; Orejuela and Silva 2005; Balaji et al. 2006; Sihtmaee et al. 2010; Smith and Bucher 2012). As per Mohammed et al. (2014, 2015), 3-CA is aromatic amine and dangerous to human and environment. The potential/possible danger with respect to 3-CA can be realized from the fact that other 2 derivatives of aniline i.e., 2-chloroaniline and 4-chloroaniline are already classified as hazardous substances which are possibly carcinogenic to humans (Sihtmaee et al. 2010; Smith and Bucher 2012). The big concern over 3-CA is because it is structurally similar to 4-CA but at the same time chemical structure of CIPC is such that 3-CA and not the 4-CA is produced as one of the metabolic products (Mohammed 2012). In 2012, the European Commission recommended that both CIPC and 3-CA need to be included in the maximum residue level value (MRL) to assess the consumer exposure to pesticide residues in and on food of plant and animal origin (European Commission 2012). As per Mohammed (2012) as well, there are growing concerns not only regarding the safety profile of CIPC but for its degradation products mainly the 3-CA. It is suggested that the high temperature (300–600 °C) of the fogging machine and the contact of CIPC with the metallic surfaces (aluminum pipe of the fogger) mediate the degradation of CIPC via pyrolysis. This thermal degradation (fragmentation and/or rearrangement) is accompanied with the formation of 3-CA (Heikes 1985; Worobey and Sun 1987; Nagayama and Kikugawa 1992; Camire et al. 1995; Park et al. 2009; Przybylski and Bonnet 2009; Paiga et al. 2009). Repeating the application of CIPC during long storage periods not only lead to

Table 2 Per capita consumption of potatoes and potato products in some countries/regions of the world during the year 2011

Country/region	Consumption of potatoes and potato products (g head^{-1} day^{-1})
Belarus	507
Ukraine	383
Poland	314
Estonia	312
Russian Federation	305
Kazakhstan	296
Malawi	293
Belgium	290
Lithuania	279
UK, Kyrgyzstan,	276
Rwanda	274
Romania	272
Netherlands (Holland)	257
Latvia	254
Ireland	247
Peru	226
Canada	215
Bosnia & Herzegovina, Nepal	206
Azerbaijan	197
Germany	194
Chile	185
Finland	183
Bolivia, Czech Republic	181
Greece	179
Lebanon	178
Algeria	177
Spain	170
Luxembourg	168
Iran, Malta	167
Republic of Moldova	165
Denmark	164
Hungary, Iceland	162
Austria, Sweden	160
Portugal	159
The former Yugoslav Republic of Macedonia	158
USA, Norway	152
France	149
Lesotho, Uzbekistan	148
Slovakia	147
New Zealand	145
India	69
Europe	198
European Union	191
America	89
Oceania, Asia	76
Africa	27
Total World	95

Source: FAOSTAT (2013b)

higher levels of 3-CA but also its higher binding to the potato tuber (Mohammed 2012).

- The dietary risk of herbicide and its metabolites cannot be assessed accurately if the residues remain strongly bound to the potato. Strong binding of 3-CA to potato is already reported (Skidmore et al. 2002; Mohammed 2012). This further raises the seriousness of this toxin and its toxicological implications.
- In addition to the above said thermal degradation, microbial degradation (mediated by bacteria) of applied CIPC also results in the formation of 3-CA during prolonged storage especially in the condition of high moisture (which usually prevails in storage environment recommended and practiced for potatoes) (Wolfe et al. 1978; Kleinkopf et al. 1997; David et al. 1998; Park et al. 2009; Verhagen et al. 2011).
- 3-CA is also present as a minor manufacturing impurity/contamination in CIPC formulation (0.05 % of CIPC by weight) (Worobey and Sun 1987; Park et al. 2009). The basic reason for this is the use of 3-CA as one of the substrate along with isopropyl chloroformate for the commercial production of CIPC.
- Once CIPC enters in human/mammals, animal and plant/potato tubers, it degrades into metabolites such as 3-CA; isopropyl *N*-4 hydroxy-3-chlorophenyl carbamate; isopropyl-*N*-5-chloro-2-hydroxyphenyl carbamate; 3, 3′-dichloro azobenzene; p-methoxy-chlorpropham; 3-chloro-4 hydroxyaniline; 3-chloro-4 methoxyaniline; 1-hydroxy-2 propyl-3-chlorocarbanilate and 3′-chloroacetanilide; isopropyl N-(3-chloro-4-methoxyphenyl) carbamate; isopropyl N-(3-chloro-4-hydroxyphenyl) carbamate etc. (Davis et al. 1977; Kidd and James 1991; Carrera et al. 1998; Orejuela and Silva 2005; Balaji et al. 2006; Smith and Bucher 2012). These metabolites are reported to be cytolytic, highly toxic, carcinogenic, cause reduction in ATP synthesis, bring about modifications in cell permeability besides being pollutants (Davis et al. 1977; Heikes 1985; Worobey and Sun 1987; Worobey et al. 1987; Kidd and James 1991; Carrera et al. 1998; Balaji et al. 2006; Smith and Bucher 2012). Out of these metabolites of CIPC, 3-CA is one of the metabolites which are also produced in mammals on consumption of CIPC. Approximately 20 % of CIPC taken in by mammalian body may get metabolized into 3-CA.
- CIPC is slightly volatile (NAPPO 2013) and as described above, tuberes can also metabolize it slowly into the compounds which are more toxic than CIPC itself. This volatilization and breakdown of CIPC reduces the efficacy of CIPC in two ways 1) effective CIPC available in the tuber is reduced and 2) the metabolites produced show either little or no sprout suppression ability.
- It was noticed by Nagayama and Kikugawa (1992) that putting the CIPC in soybean oil and heating it at 180 °C give rise to a gradual decrease in CIPC with an accompanying production and increase in the levels of 3-CA. This suggests that frying of CIPC treated potatoes (during processing) results in the degradation of CIPC into 3-CA (Park 2004; Worobey and Sun 1987; Worobey et al. 1987; Park et al. 2009).
- Recent work on the kinetics of degradations of CIPC and also its metabolites (by hydrolysis, biolysis, photolysis and thermal processes) and their partitioning in air, water and soil indicated vast differences in the lab and field conditions (Smith and Bucher 2012). Under lab conditions, there is usually overestimation of degradation. This therefore necessitates for looking into the actual kinetics as a part of decision making process by the regulatory agencies in deciding the MRL and ADIL of CIPC.
- Occasionally, application of CIPC to control sprouting can fail or remains inefficient to control the sprouting. Timing of CIPC application is critical to its success in suppression of sprout growth. Late or untimely application of first or second application of CIPC produces mixed results ranging from adequate sprout inhibition to complete failure (Kleinkopf et al. 2003; Park 2004; Park et al. 2009). This situation may put the demand/pressure for additional application of CIPC and that too at still higher dose. This in turn will enhance the residue level of CIPC in the tubers.
- CIPC blocks the spindle formation and in doing so the process of cell division (mitosis) is inhibited (Ashton and Crafts 1981; Vaughn and Lehnen 1991; Kleinkopf et al. 2003). In this way, absence of cellular division prevents the sprouting. With this mode of action, CIPC in fact targets the very essential and an indispensible cellular process which is very basic and common to both, plants and animals. Besides this, CIPC also causes the alteration in cellular structure and functions.
- CIPC is very less soluble in water (89 mg per litre) and therefore it requires organic solvents (like; methanol or dichloromethane) for its application as a fogging treatment. Heavy use of these solvents not only adds to the toxicity status but also impose the risk on the personnel involved in treating/fogging application and to the immediate environment.
- As already stated above that CIPC has only limited solubility in water but even with this little solubility its residue in the washed water contaminate water bodies and environment (Park 2004). There are growing levels of contamination to the environment, soil and water bodies with the breakdown products/metabolites of CIPC is a matter of more serious concern, especially with respect to 3-CA (Angioi et al. 2005). This is due to low degradation of 3-CA. It is also important to mention here that in comparison to CIPC, solubility of 3-CA is quite high in water (5,400 mg L^{-1}).

- CIPC is among the three pesticides which has been found in the highest concentrations in the diet of the average American and comprises 90 % of the total synthetic chemical residue in US potatoes and in this way also it is going to be a health concern (Gunderson 1988; Prange et al. 1997; Daniels-Lake et al. 2011). Recent literature points out clearly that the CIPC residue left in the tuber is harmful for human body (El-Awady Aml et al. 2014) and new legislation is also limiting the use of CIPC (Cools et al. 2014). The reason for such an impact of CIPC can be understood in a more clear way from the following data. The approved limit of CIPC application per tonne of potatoes (meant for processing, during the season) is 63.75 g (Mohammed 2012). An average sized potato store of 1, 000 tonnes could potentially be treated with 63.750 kg of CIPC (Smith and Bucher 2012). Now assuming the latest MRL (the legal maximum) for CIPC as 10 mg CIPC kg^{-1} of potatoes as set by HSE (2009), about 53.750 kg of the CIPC chemical is unaccounted for in a storage season. This shows that vast amount of CIPC is lost to the store fabric, atmosphere, soil and water (Smith and Bucher 2012). With the above practice in use the buildup levels of CIPC has kept on increasing year after year.
- A few criteria have been laid for a potato sprout suppressant that can be considered as ideal by many workers (Beveridge et al. 1981a; Vaughn and Spencer 1991; Teper-Bamnolker et al. 2010). These criteria include 1) The chemical should effectively inhibit sprouting under commercial storage, 2) The chemical should have minimum effect on the quality parameters of the potatoes (weight loss, sugar content, appearance etc.), 3) There should be low toxicity of the sprout suppressant and its residues do not cause problems to humans, 4) The chemical should break down rapidly and it need to be environmentally friendly. Our updated understanding on CIPC as of today indicate it clearly that first two criteria are being met by CIPC but definitely there are problems, issues and growing concerns with respect to last two criteria.
- Isopropylphenyl carbamate (referred as IPC or propham) is also an herbicide which belongs to the same class as CIPC. Initially, it was also in use commercially to prevent sprouting (mostly in combination with CIPC) but now its application has been banned in most of the countries. IPC is also not supported in the countries of European Union (EU) due to ecological concern (Mohammed 2012).
- With respect to CIPC it is also true that among the herbicides it is very toxic to worms and relatively more harmful to birds, fishes and other aquatic animals, environment and ecosystem (Kidd and James 1991; EXTOXNET 1996; Anonymous 1997; Anonymous 2002; Kleinkopf et al. 2003; Greene and Pohanish 2005; O'Neil et al. 2006; HSE 2009; Safety Data 2009; MSDS 2009; Paul et al. 2014). Both, CIPC and 3-CA are categorized under List I and Hazardous Substances which should be avoided in ground water (EPA 2010). As per European Community Pollutant Circular No 90–55 (1990), 3-CA is recognized as a toxic water pollutant and harmful to aquatic life (David et al. 1998).
- About 60 % of the total potato production is used for human consumption and remaining 40 % is used for other purposes including animal feed, seed tubers, industry and pharmaceutical products (Topcu et al. 2010). Here it becomes important to mention that use of CIPC treated potatoes as feed may also pose health risks and safety concerns for animals as well.

In view of the above listed facts, recent understanding on toxicological aspects, potential risks and growing concerns - CIPC and its metabolites needs to be handled and used in a more judicious way because there is potential impact and implications of the CIPC and its metabolites on humans and environment. Inferences drawn above in point number 1 and 2 (although not based on actual trials and sampling) appear to be relevant and factious in view of the critical details presented above for CIPC and its metabolites.

Increasing the efficacy of CIPC as sprout suppressant and reducing its residue in potatoes

Contamination of store fabric, food chain, water/ground water bodies with CIPC and its metabolites has emerged as a serious concern. Decreasing the degradation of CIPC into its toxic metabolites and increasing the efficacy of CIPC further for its ability to suppress the sprout growth are the two possible options that can be utilized to tackle the problems associated with CIPC. It is suggested that the concentration of CIPC breakdown product, for example 3-CA, can be reduced by modifying the process of fogging. This can be done by lowering the fogging temperature and avoiding the metal pipes (used to carry CIPC fog into the potato store). The formation of 3-CA in the air samples during fogging was found to be abolished at burning temperature of 190 °C. This was in sharp contrast when usual burning temperature of 600 °C was used (Mohammed 2012). This modification however would not reduce the levels of 3-CA which is formed due to the microbial degradation and this aspect therefore need to be resolved. UK Potato Council [Sutton Bridge Experimental Unit (SBEU)] in collaboration with the University of Glasgow and others initiated the studies to improve the efficiency of sprout control by CIPC. Best practice guidelines for the most effective use of CIPC are being made available and these are also updated regularly [www.potato.org.uk and www.assuredproduce.co.uk/Aproduce/]. The guidelines usually include store layout, application methods, dose, timings of CIPC treatments, deposition and decline rates etc. In view of the current situation, enhancing the efficacy of CIPC further should be a

priority area of research. Innovative refinements in the instrumentation and delivery system will also contribute significantly in achieving the above objectives.

It is reported that large amounts of field soil if remains adhered around the harvested tubers than it can impair the distribution of the CIPC vapour. This not only reduces the efficacy of CIPC treatment as sprout suppressant but it also leads to non-uniformity in treatment (NAPPO 2013). This aspect needs to be taken care by managing the harvesting and field related practices. It has been seen that second or even third application of CIPC for satisfactory control of sprouts for long-term storage are governed primarily by factors such as cultivar or extent to which potato faced the stresses. In this direction, suitable variety selection, agronomic practices and postharvest management practices in the form of a time schedule can be of immense help in either skipping or minimizing the number of CIPC applications. It has been noticed that a single aerosol application of CIPC @ 20 to 25 g per tonne of tubers provide effective sprout control up to 9 months in variety Russet Burbank when stored at 7.2 °C (NAPPO 2013). This variety specific response need to be investigated so that we can come to know the very basis of this. If this is because of some varietal feature/s of Russet Burbank then efforts can be taken up in the direction of incorporating such feature/s into other varieties as well. Besides providing new physiological and biochemical understanding, such work will significantly contribute in reducing the residues of CIPC in the treated potatoes.

Enhancing the natural dormancy period of potato tubers from its present duration of 2 to 4 month (depending on the temperature that prevails after the harvest and variety) to 4 to 6 months or even more can also be one of the indirect approaches. This is an interesting area of work as this will prove to be highly beneficial in reducing the frequency of CIPC treatment to the stored potatoes. Further, there is a need to look for the possibility of using some carriers with CIPC for enhancing its delivery and uptake by the stored potatoes. Scientific information generated from the studies pertaining to the steps like; washing with water, soaking in solutions of salt and some chemicals (chlorine, chlorine dioxide, hydrogen peroxide, ozone, acetic acid, hydroxy peracetic acid, iprodione and detergents), peeling, trimming, blanching, boiling, frying cooking, steaming and canning etc. can assist in degrading and removing of the applied agrochemical from the edible commodities before their consumption (Sakaliene et al. 2009; Keikotlhaile et al. 2010; Bajwa and Sandhu 2014). These aspect need to be refined and standardized so that the residue levels of CIPC and its breakdown products can be reduced to the maximum possible extent.

CIPC is reported to undergo volatilization and get degraded if expose to UV radiation (Bradshaw and Ogilvy 2006). So, attempts need to be made to look into the possible use of UV radiation mediated degradation of CIPC via titanium dioxide coating (as such or with nano particles, when exposed to UV light). This method and procedure is already in use for breaking down the volatile organic compounds (VOCs) into CO_2. So, such method can be utilized to decontaminate the storage space from the unused and accumulated CIPC and its metabolites when the facility is not in use. This strategy may prove useful in reducing the overall built-up of CIPC and its metabolites due to continuous use of CIPC for number of years. Some of other novel ways of reducing the levels of residue (either CIPC or its metabolites) also need to be developed and tested for CIPC-treated tubers as well. This can then be applied either when the storage period of potatoes is over or just prior to the consumption/utilization of potatoes by processing industry.

The time laps after the CIPC treatment is known to decrease the residue level of CIPC in the tubers. So, one way that can make the use of CIPC more safe is by strictly following the schedule and regulations with respect to the time gap that need to be maintained between the last CIPC treatment and the time when the potatoes are to be sent to the market. For CIPC, such details are known but similar details are by enlarging missing for different toxic products/metabolites that are formed from CIPC and also present in the CIPC-treated potatoes. This aspect therefore needs to be investigated so that appropriate guidelines and recommendations can be made available in future. Another important area is to search for alternatives of CIPC. Considerable work has been done and is in progress. This aspect is therefore, described below in detail.

Alternatives of CIPC and possibility of integrated and effective use in combinations

Over a period of time, researchers have gradually become aware of some of the practical, technical, safety related problems and issues linked with the use of CIPC. Attempts are therefore being made to find out some alternative to CIPC that can be safer, applied more easily and also cost-effective (Sawyer and Thorne 1962; Beveridge et al. 1981a, b; Weerd 2005; Gomez-Castillo et al. 2013; Paul et al. 2014). Sprout suppression by long-chain alcohols was reported by Burton in the year 1956 (Burton 1956). The C_9 alcohols were effective in controlling the growth of sprouts however the alcohols with branched chain were found to be ineffective (Sawyer and Thorne 1962). Later on, Meigh (1969) and Burton (1989) reported that compounds containing 9–10 carbon atoms per molecule were effective in suppressing the sprout growth. Nonanol (3, 5, 5-trimethylhexan-1-ol) suppressed sprout growth but its suppressive effect was not persistent as the sprout growth was noticed again within 2–3 weeks (Burton et al. 1992). Many other chemicals including cineole and fenchone (Vaughn and Spencer 1991), lavender, sage and rosemary essential oil (Vokou et al. 1993), maleic hydrazide

(Mamani Moreno et al. 2012), short-chain alcohols, aldehydes such as salicylaldehyde, benzaldehyde, cinnamaldehyde, aliphatic aldehydes, ketones, derivative of phenoxy acetic acid (Vaughn and Spencer 1993; Paul and Ezekiel 2002), triadimefon (Paul and Ezekiel 2003c), volatile monoterpenes like 1, 8-cineole and eucalyptus oil (Vaughn and Spencer 1991; Knowles and Knowles 2007), essential oils like caraway (Hartmans et al. 1995; Oosterhaven et al. 1995; Sorce et al. 1997; Sanli et al. 2010; Teper-Bamnolker et al. 2010; Rentzsch et al. 2012; Gomez-Castillo et al. 2013), peppermint, spearmint, clove oil, mint oil (Kleinkopf et al. 2003; Rentzsch et al. 2012; Teper-Bamnolker et al. 2010; Gomez-Castillo et al. 2013), mono, di and trimethyl-naphthalenes, benzothiopenes, menthone and neomenthol (Coleman et al. 2001), mentha oil (Mehta and Kaul 2002), essential oils from fresh aerial parts of *Mentha spicata* (Chauhan et al. 2011) and formulation of essential oils from *Chenopodium ambrosioides* and *Lippia multiflora* (Owolabi et al. 2010) were tested and found to suppress the sprouting and sprout growth. Chemicals like; ethylene (Prange et al. 2005, 1997, 1998; Daniels-Lake et al. 2005), ozone (Daniels-Lake et al. 1996), glyphosate (Paul and Ezekiel 2006a, 2006b), hydrogen peroxide (Afek et al. 2000; Kleinkopf et al. 2003; Bajji et al. 2007), 1, 4-dimethyl naphthalene 1, 4-DMN (de Weerd et al. 2010; Campbell et al. 2010; Canada 2011; Potato 2012), 2, 6-diisopropyl naphthalene (2, 6-DIPN) (Lewis et al. 1997) were also tested to control the sprout growth on potatoes. Ethyl ester of 2, 4-dichlorophenoxy acetic acid (2, 4-D), ethyl ester of 2, 4, 5-trichlorophenoxy acetic acid (2, 4, 5-T), imazethapyr and glyphosate are herbicides like CIPC and they are also reported to be effective and better than lower alcohols and acetaldehyde in suppressing the sprout growth on potatoes (Burton 1989; Burton et al. 1992; Tayler et al. 1996; Paul and Ezekiel 2002; Paul and Ezekiel 2006a, b; Paul et al. 2014; Hutchinson et al. 2014). Perhaps in view of either practical problems or due to issues related to human health and environment safety further work with these herbicides as an alternative to CIPC was not taken up. In addition to above described sprout suppressants, suppression of sprout growth has also been demonstrated by the use of γ radiations (Ezekiel et al. 2008; Olsen et al. 2011; Rezaee et al. 2011; Lu et al. 2012a, b) and UV-C light (Cools et al. 2014).

Studies on the possibilities of replacing the CIPC with naturally occurring compounds showed that in spite of great deal of work on various options so far none of the option has assumed wide spread use, commercial angle and acceptability. Till today, we do not have either equivalent or better alternative than CIPC (Mohammed 2012). It is true that most of the available alternatives of CIPC provide only short-term and reversible sprout suppression and therefore they are not good candidates for long-term storage of potatoes. Lesser efficacy, frequent/multiple applications and higher cost in comparison to CIPC are the main demerits for most of the other sprout suppressants. For long-term storage, most of the alternatives need to be applied number of times and this will result in cost escalation beyond a feasible limit. David Walker, Chairman of FPSA (The Fresh Potatoes Suppliers Association), stated that alternatives of CIPC play a more significant role in sprout suppression on fresh potatoes but, CIPC is critical and there is no complete alternative solution (Potato 2013). Likewise, Director-General Richard Harris of PPA (The Potato Processor's Association) said that for the long-term storage of processing potatoes the potato industry is totally dependent upon CIPC. According to him the sector would witness devastation if potatoes are not supplied for 52 weeks of the year (Potato 2013). From the point of potato industry and the people who are directly or indirectly associated with it, utility as well as dependency on CIPC can be understood. But at the same time, it should also need to be realized that continuous and long-time dependence on only one type of sprout suppressant is not wise and that too when issues related to toxicity, acceptable residue levels and safety aspects have been raised and becoming more clear.

Use of an integrated approach to control the sprout for long-term is suggested by making use of CIPC in conjunction with other sprout suppressants by Bradshaw and Ogilvy (2006). This can help in reducing the residue levels of CIPC. An alternative to CIPC that does not interfere with wound-healing can be applied early and may prove more effective for varieties that exhibit short dormancy duration. Introduction of alternative/s will definitely help in reducing the present dose and/or frequency of CIPC treatments and this in turn will reduce the residue levels of CIPC in the tubers. In this way, CIPC and alternative sprout suppressants can offer a viable, cost-effective, safer and environmentally friendly approach. This will reduce not only the residue levels of CIPC but also the levels of its degradation products/metabolites which pose more risk to health and environment. In USA, sprouting is also managed with application of 1, 4 DMN consecutively with CIPC. The CIPC is applied first (16–22 g per tonne of potatoes one time as a single application) and then the 1, 4-DMN is applied. In comparison with the CIPC treatment alone; 1, 4-DMN is found to be effective in achieving adequate suppression of sprouts on potatoes if potatoes are previously treated with CIPC (Kleinkopf et al. 2003; Campbell et al. 2010). Now for long-term control of sprouting, in addition to the preharvest treatment of potato crop with maleic hydrazide, CIPC can be applied to the harvested potatoes during the storage (NAPPO 2013). The possibility and prospects of using preharvest foliar application of glyphosate as an additional or alternative/supplementary to CIPC as sprout suppressant are explored by Paul et al. (2014). S-carvone is another sprout suppressant. It is a natural volatile that leaves little or no residue. It is costly and therefore usually it is used in organic potato stores (Teper-Bamnolker et al. 2010; Rentzsch et al. 2012). Output and outcome of recent research towards the

refinement of instrumentation and application methodologies with worked out sequence of treatments with different sprout inhibitors (one after another) will help in reducing the application rates of CIPC.

3-Decen-2-one (an unsaturated ketone) has been found to exhibit sprout suppression ability. Presently, it is permitted in the USA as a flavouring agent in foods. Registration of this compound as a potato sprout inhibitor is underway in both Canada and the USA. This compound causes physical damage to the sprouts and provides season-long control with only a few applications. Aerosol applications will give approximately 3 to 8 weeks of sprout control depending upon variety and storage temperature. Inhibitory effect of 3-decen-2-one is not permanent and therefore the tubers will eventually re-sprout. Several other compounds like salicylaldehydes, jasmonates and farnesene have also been added to the list of compounds that can inhibit the sprouting of potato. They are effective but none of them have a lasting effect. Therefore, search for effective and viable alternatives of CIPC need to be looked and probed with new angles and ideas.

Recently, a new type of potato sprout inhibitor was discovered at Washington State University by Rick Knowles and Lisa Knowles. This patented inhibitor is also approved for commercial use in US (Knowles 2013). This chemical has been registration for its use in Canada and Europe. The inhibitor is reported to be a naturally occurring molecule and it is classified as biopesticide by EPA. As per report, the inhibitor offers safe, comprehensive long-term storage control and requires no capital investment by the consumers as it can be easily applied using existing equipment. For commercial use, the trade name given to this inhibitor is SmartBlock® and rights for its marketing is owned by AMVAC (American Vanguard Corporation). Investigations have revealed that one application of this chemical inhibits sprouting for 2 to 3 months. Two to three applications provide effective sprout suppression for full season (8 to 9 months) and that too with little residue.

Conclusions and way forward

Suitability of potatoes for long-time storage makes them one of the most important foods worldwide and in one way comparable to that of grains. It has been suggested that potatoes can be an alternative for costly cereal crops because potatoes are traded globally while cereals are not (IPY 2008; FAO 2008; Lutaladio and Castaidi 2009). Prolonged storability of potatoes and availability of different storage options enables the potato processing industry to operate round the year. With these advantages and ability of potato to get adaptive to a wide range of climatic conditions and soil types (Burlingame et al. 2009; Ghazavi and Houshmand 2010; Topcu et al. 2010), it is now realized that potato is world's single most important tuber crop with a vital role in the global food system. With these benefits it is quite obvious that in future potato is going to play a major role in contributing to food and nutrition security, poverty alleviation, environmental conservation and sustainable development.

One of the most important requirements of harvested potatoes is timely and proper storage. It is in this context that aspects associated with potato storage are very crucial. Today world over the most prevalent long-term (6 to 9 months) storage method for potatoes is at temperature of 8–12 °C (85–90 % RH) along with the use of CIPC as sprout suppressant. The time line of information generated on potato for a period of over 60 years is described and discussed in this article by covering various aspects including; postharvest management, storage problems, accumulation of reducing sugars, CIS, darkening of fried products, storage methods, CIPC as sprout suppressant, merits and demerits of CIPC and its continuous use on potatoes during storage, search for alternatives of CIPC, enhancement in our understanding on the toxicological profile of CIPC especially its metabolites/degradation products (produced at the time of its fogging due to high temperature of fogger), uptake of these products by the stored potatoes, formation of CIPC degradation products in potatoes (during storage) and in human/mammals (on the consumption of CIPC treated potatoes). The information revealed very clearly that the use of CIPC has provided the required boost and support to the potato production and potato based processing industries but at the same time it has also gradually made us over-dependent on its use as a potato sprout suppressant.

Keeping in view the versatility of potato as a crop and its diverse uses there will be further increase in the consumption of potatoes and potato products in many countries including the developed and developing countries (IPY 2008; FAO 2008). The information available till date and the data presented here on the MRL and ADIL of CIPC do point out the problems linked with the residue of CIPC and its harmful metabolites. Studies have already reported that toxicological evaluation of CIPC as tested and documented under lab conditions is an underestimation. At present, there is wide variation in the quantity of potatoes that is consumed by the people living in different countries. This thereby suggests that parameters like MRL and ADIL need to be country specific (in view of their food habits and food consumption patterns). In this context, it is important that different regulatory agencies should take initiative and relook into the criteria on which parameters like; MRL and ADIL are fixed, advocated and recommended. Fixing of these limits need to be evaluated by taking into consideration not only the CIPC but also the metabolite/s produced by CIPC. This aspect is in fact already highlighted by Gonzalez-Rodriguez et al. (2011); Smith and Bucher (2012); and European Commission (2012). Such

inclusions should not remain restricted only to one particular harmful degradation product/metabolite but to all such toxic breakdown products/metabolites which are and can be formed or present in response to the treatment of a given sprout suppressant. Breakdown products/metabolites can be formed as a result of metabolism in the treated commodity itself or later in the humans on the consumption of treated commodity. Such an approach will also be needed for other agrochemical/pesticides as well.

In future, a viable, effective and low-cost alternative to CIPC for potato sprout suppression will definitely be needed. But till then, importance needs to be given to the concept of using at least two different types of sprout suppressants (use of CIPC with another sprout suppressant, one after another in a required sequence). This aspect needs to be disseminated widely by developing suitable combination/s depending up on the need, situation, feasibility, location, cost, acceptance and preference etc. Time has come when more serious R & D is required for enhancing the effectiveness and efficacy of CIPC. Guidelines for most effective use of CIPC as introduced and also updated regularly by UK need to be introduced in other countries as well. This is important as this will help in curbing the indiscriminate use of CIPC (in terms of use of higher doses and more than the required number of applications). These changes will help in reducing the overall dose of CIPC and its residue in tubers.

The final practical output of various investigations that aimed to understand the CIS and making the potato resistant to CIS is still awaited in terms of actual practicalities and large-scale applicability across the countries. If this can be achieved, then the potatoes can be stored up to 8 to 9 months at low temperature (2–4 °C) and that too without any use of sprout suppressant. But again, this option is more energy/power dependent besides being costly. These aspects will make it less available, assessable and acceptable in developing and third world countries of the world. Therefore, it is necessary to store the potatoes with judicious and effective use of sprout suppressant taking into consideration health and environment issues.

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Using Ethylene as a Sprout Control Agent in Stored 'Russet Burbank' Potatoes

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Abstract. The effect of ethylene on tuber sprout growth and quality in potato (*Solanum tuberosum* L. 'Russet Burbank') was tested in laboratory and commercial studies for 6 and 3 years, respectively, in comparison with untreated (laboratory study) and CIPC-treated tubers (laboratory and commercial studies). In both studies, ethylene was applied continuously at 166 μmol·m^{-3} for at least 25 weeks, beginning in early December (laboratory study) or early December to early January (commercial study). In the laboratory study, ethylene delayed the appearance of sprouts for 5 to 15 weeks, compared with untreated tubers. In the ethylene-treated tubers in both studies, sprouts appeared on many eyes but most of them remained very small (<5 mm long). Longer sprouts (>5 mm) appeared after 15 weeks but did not exceed 12 and 59 mm in the laboratory and commercial studies, respectively. Sprouts on ethylene-treated tubers were more easily detached up to 6 weeks after ethylene treatment ended, compared with untreated tubers. In both studies, ethylene treatment was not associated with decay, disorder or internal sprouting problems. In both studies, the Agtron fry color [or U.S. Dept. of Agriculture (USDA) color grade] of ethylene-treated tubers was darker than CIPC-treated tubers at almost all sampling times. Continuous exposure to ethylene was an effective sprout control agent but it produced a darker fry color, compared with CIPC-treated potatoes.

As part of the effort to reduce chemical additives to our food and to the environment, researchers are trying to find an alternative to the use of CIPC (chlorpropham; 1-methylethyl-3-chlorophenyl-carbamate) to control sprout growth on potato tubers during storage (Prange et al., 1997). CIPC is among the three pesticides found in highest concentrations in the diet of the average American and comprises 90% of the total synthetic chemical residue in United States potatoes (Gartrell et al., 1986; Gunderson, 1988; Vaughn and Spencer, 1991). In addition, CIPC residues in potato products are used as a non-tariff trade barrier by some governments. The aim of our research is to find a replacement for CIPC on 'Russet Burbank' potato, which is the major cultivar used in many countries for commercial French fries. In the course of evaluating various alternatives, e.g., Daniels-Lake et al., 1996, we hypothesized that ethylene may be an acceptable replacement for CIPC.

The effect of continuous long-term exposure of potato tubers to ethylene on sprout growth and tuber quality is not well understood. It is recognized that potato tubers produce ethylene (Creech et al., 1973, McGlasson, 1969; Poapst et al., 1968). The rate is normally very low, 0.008 to 0.015 μL·kg^{-1}·h^{-1}, but can be as high as 0.1 to 0.4 μL·kg^{-1}·h^{-1} when the tubers are stressed or sprouting (Creech et al., 1973, McGlasson, 1969; Okazawa, 1974). Burton (1952) suggested that ethylene may be one of the endogenous volatile substances in potato tubers involved in sprout suppression although Burton (1989) and Rylski et al. (1974) later stated that this was incorrect. Studies using exogenous ethylene do not clearly indicate the effect of long-term exposure (>6 months), mainly because many of them are short-term studies, e.g., ≤40 d. Furthermore, some conclusions are based on responses while ethylene was applied and others are based on responses after ethylene was removed. Thus, some of these short-term studies have concluded ethylene breaks dormancy and stimulates sprout growth (Alam, 1992; Alam et al., 1994; Rosa, 1925; Vacha and Harvey, 1927), or does not hasten sprouting of dormant potatoes (Denny, 1926) and/or suppresses growth of sprouts, roots or stems (Elmer, 1932, 1936; Furlong, 1948; Huelin, 1933; Hughes et al., 1973; Metlitskii et al., 1982; Timm et al., 1986). In a review by Kader (1985), the author concludes that ethylene can have a dual effect on potato tubers: it shortens the duration of rest markedly, but inhibits the elongation of sprouts. Shortening rest would be desirable for seed potatoes but not for table or processing potatoes in the author's opinion. This conclusion is based on research by Rylski et al. (1974), which showed that ethylene treatment of nonsprouting tubers shortens the duration of rest markedly, with sprouting being promoted by short (up to 72 h) exposures. In the same study, inhibition of sprout elongation was observed when sprouting tubers were exposed continuously to ethylene over a 14-d study period. The only study examining exposure to ethylene over typical commercial storage periods of 6 months or longer was Metlitskii et al. (1982). They reported that an ethylene-generating liquid (Hydrel) suppresses sprouting and disease development in stored potatoes for more than 6 months.

Ethylene has various effects on fry color, which was not measured in any of the above studies. Haard (1971), using a 24-h ethylene treatment, concluded it darkens fry color in some cultivars but improves it in others. Parkin and Schwobe (1990) showed

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that ethylene applied for 9 weeks at 1000 $\mu L \cdot L^{-1}$ delays fry color darkening at 3 °C by slowing the conversion of sucrose to hexoses. Unfortunately, in both studies ethylene was applied at storage temperatures of 3 to 4 °C, a temperature that can induce low-temperature sweetening and darker fry color in potato tubers.

The objective of this study was to examine the effect of continuous ethylene exposure over a 25-week storage period on sprouting of cured 'Russet Burbank' potato tubers held at normal storage temperatures. In addition, possible effects on tuber quality were evaluated in terms of fry color, disease development and tuber disorders.

Materials and Methods

This study was repeated for 6 years in the laboratory, from 1990–91 (year 1) to 1995–96 (year 6), and also for 3 years in a commercial storage (1992–93, 1993–94, and 1994–95).

In the laboratory study, commercially produced 'Russet Burbank' potatoes were obtained from four separate locations, i.e., four grower replications, in New Brunswick, Canada (years 1 to 3) or two sites in New Brunswick and two in Prince Edward Island, Canada (years 4 to 6). They were suberized (≈4 to 5 weeks at 13 to 15 °C to allow wound healing) commercially before they were received in the laboratory (years 1 to 4) or after they were received (years 5 and 6). Treatments began in the first week of December in each year (mid-December in year 1), after suberization. Each grower replication was stored at 9 °C in a separate 64 L PVC barrel (IPL Ltd., Moncton, N.B.) in a temperature-controlled room for 25 weeks. Within the room there were three rows of four barrels, for control (air), CIPC and continuous 166 $\mu mol \cdot m^{-3}$ (4 $\mu L \cdot L^{-1}$) ethylene, respectively. Within each barrel, five 2-kg samples (≈10 tubers) of potatoes were stored in mesh onion bags. Following a split-plot design, the experimental factors were assigned and randomized. The three treatments (main plots) were assigned to the three rows and the four growers (subplots) were assigned to the four barrels within each row. The five removal dates were assigned to the five samples within each barrel. In the final year, each treatment was applied to a single 0.67-m^3 stainless steel cabinet with an air-tight acrylic lid and water-trough seal. The five samples from each grower replication were contained in a ventilated PVC basket, and each cabinet held four baskets, one from each grower.

All barrels were ventilated with air at 0.5 to 0.7 $L \cdot h^{-1}$ for 6 consecutive hours each day. In the final year, the cabinets were ventilated during two 6-h periods per day, interspersed by two 6-h static periods without ventilation. Each ventilation period consisted of 5.75 h at 4 $L \cdot min^{-1}$ and 15 min at 15 $L \cdot min^{-1}$. Control potatoes (air) received no additional treatment, while the CIPC treatment was applied by dipping the tubers in Sprout-Nip EC (Stanchem Inc., Etobicoke, Ont.) at a concentration of 10 $g \cdot L^{-1}$ at the start of each storage season. In 1990–91, the CIPC dip was repeated at 5-week intervals during the storage term. Ethylene gas was applied at 166 $\mu mol \cdot m^{-3}$ (133 $\mu mol \cdot m^{-3}$ in year 1) by addition to the ventilation airstream. Ethylene was measured by drawing a 1-mL headspace sample manually from each chamber and injecting into a GC (Carle Instruments, Anaheim, Calif.), equipped with a flame ionization detector, a 2.4-m stainless steel column hand-packed with 60 to 80 alumina (Chromatographic Specialties, Brockville, Ont.), with helium as the carrier gas. Detector output was processed by LabCalc software (Galactic Industries, Salem, N.H.), and the results were assessed immediately. Chamber headspace was analyzed once per week, during the middle of a ventilation cycle to verify that treatment concentrations were being maintained. In the last year ethylene was measured automatically. A sample-and-return loop circulated headspace gases from the storage chamber through a motorized valve (Valco, Valco Instruments, Houston, Texas). Each chamber was sampled sequentially, with a purge cycle between chambers. A 1-mL sample of the headspace was automatically injected into a GC (Shimadzu, Kyoto, Japan), equipped with a 1.2 × 3.2 mm packed column with 80/100 mesh Porapak P, a photoionization detector, with nitrogen as the carrier gas. Detector output was processed by PeakSimple for Windows software (SRI Inc., Torrance, Calif.). Headspace gases were analyzed 12 times every day.

Sprout development, disease and disorder incidence were evaluated at the start of each year's experiment, and at each removal date during the storage term. Sprout development was evaluated by determining sprout mass per kilogram of initial tuber fresh mass (sprout mass), the length of the longest sprout in each sample (maximum length), and the mean number of sprouts per tuber (sprout number) in two categories, small (2 to 5 mm) and large (>5 mm). Disease and disorders were assessed by estimating the percentage of the tuber affected.

To assess the fry color of the tubers (year 2 to 6), a disc 50 mm in diameter was cut from an 8-mm-thick central longitudinal slice of each tuber. The discs were deep fried for 2.5 min in canola oil (Maple Leaf Foods, Moncton, N.B.) at 190 °C, and then allowed to cool to room temperature. Fry color of each cooled disc was measured using

Table 1. Effect of storage time and treatment on mean sprout mass (sprouts >5 mm) over 6 storage years in the laboratory study.

	Sprout mass/initial tuber fresh mass ($g \cdot kg^{-1}$)		
	Storage time (weeks)		
Treatment	15	20	25
		1990–91	
Air	3.3	17.2	44.7
CIPC	0	0	0
Ethylene	0.1	0.8	4.9
		1991–92	
Air	1.4	8.3	26.2
CIPC	0	0	0
Ethylene	0.0	0.0	0.0
		1992–93	
Air	1.4	2.2	8.5
CIPC	0	0	0
Ethylene	0.0	0.0	0.0
		1993–94	
Air	4.4	12.3	24.9
CIPC	0	0	0
Ethylene	0.0	0.0	0.1
		1994–95	
Air	3.2	11.0	23.0
CIPC	0	0	0
Ethylene	0.0	0.0	0.5
		1995–96	
Air	3.6	17.4	38.5
CIPC	0	0	0
Ethylene	0.0	0.0	0.0
		All years combined (log_{10})[z]	
Air	0.56	0.99	1.36
CIPC	0	0	0
Ethylene	0.00	0.01	0.12
SE (n = 120; df = 45)[y]		0.027	
Significant effects		S***, T***, S × T***	

[z]All years, except 1990–91, which had poor ethylene control.
[y]Statistical analysis did not include CIPC treatment, which always had zeros.
***Significant at $p \leq 0.001$, where S = storage time and T = treatment.

Table 2. Effect of storage time and treatments on mean maximum sprout length over 6 storage years in the laboratory study.

	Maximum sprout length (mm)		
	Storage time (weeks)		
Treatment	15	20	25
		1990–91	
Air	49.1	157.5	301.0
CIPC	0	0	0
Ethylene	5.0	6.6	19.4
		1991–92	
Air	33.1	132.4	246.2
CIPC	0	0	0
Ethylene	0.02	3.5	7.1
		1992–93	
Air	46.0	32.1	173.2
CIPC	0	0	0
Ethylene	3.7	3.7	7.0
		1993–94	
Air	64.6	109.2	168.8
CIPC	0	0	0
Ethylene	0.5	2.7	5.9
		1994–95	
Air	32.7	110.4	156.8
CIPC	0	0	0
Ethylene	0.7	4.6	7.9
		1995–96	
Air	73.5	170.8	290.7
CIPC	0	0	0
Ethylene	0.0	0.4	11.4
		All years combined (square root)[z]	
Air	1.68	1.99	2.31
CIPC	0	0	0
Ethylene	0.21	0.56	0.94
SE (n = 120; df = 45)[y]		0.058	
Significant effects		S^{***}, T^{***}, $S \times T^{NS}$	

[z]All years, except 1990–91, which had poor ethylene control.
[y]Statistical analysis did not include CIPC treatment, which always had zeros.
[NS,***]Nonsignificant at $p \leq 0.05$ or significant at $p \leq 0.001$, respectively, where S = storage time and T = treatment.

an Agtron reflectance colorimeter (Agtron M-35-D, Agtron Inc., Sparks, Nev.), calibrated using Agtron standard reflectance discs #00 as zero and #56 as 100%.

In 1 year (1993–94), in addition to the above measurements, the strength of attachment of the sprouts to the tubers (air and ethylene treatment only) was tested after 20 and 25 weeks storage and on separate samples held after these removal dates at 20 °C for an additional 3 or 6 weeks. The force required to detach the sprout from the potato was determined by measuring the load as the sprout was detached. The base of the sprout was held in a modified alligator clip which was connected to a digital electronic balance placed above the sprout. The tuber was attached to a motorized arm which pulled the tuber downward until the sprout was detached. The balance was connected to a computer which recorded the maximum force applied to the sprout as it was detached from the tuber.

Data from each characteristic in the laboratory study were analyzed by ANOVA, using the Genstat 5 statistical program (Genstat committee, 1993). Before statistical analysis the data for all years were combined so that each year was treated as a replication. To counteract the biasing effect of numerous zero values and to accommodate large changes from one removal date to the next as sprout growth progressed, the data sets for the sprout characteristics were restricted to removal dates having measurable sprout growth, and then analyzed using either $\log_{10}$ or square-root transformations, as appropriate. Only results significant at $P \leq 0.05$ are discussed.

In the commercial storage study, two identical adjacent bulk storage buildings were used, each holding ≈3 million kg of 'Russet Burbank' potatoes. In one, the potatoes were given the normal CIPC treatment (mid-December in 1992–93 and mid-November in 1993–94 and 1994–95) and, in the other, the potatoes were treated continuously with 166 $\mu mol \cdot m^{-3}$ ethylene, (beginning on 5 Jan. in 1992–93; 14 Dec. in 1993–94 and 1 Dec. in 1994–95). Treatment continued until the last week of June (1992–93 and 1993–94) and early July (1994–95). Potatoes were processed into French fries for resale after these dates. Ethylene was delivered from a compressed gas cylinder into the ventilation system and measured weekly with a GC (HNU Systems, Newton, Mass.) equipped similarly to the Carle GC above. Each week a 10 tuber sample was collected from each treatment and fry color was determined using a method similar to the one described above, except that the color was recorded using the USDA French fry color chart (USDA grade 1 = 88 to 100 Agtron units, Grade 2 = 70

Table 3. Effect of storage time and treatments on mean number of large sprouts (>5 mm) over 6 storage years in the laboratory study.

	Sprouts per tuber (no.)		
	Storage time (weeks)		
Treatment	15	20	25
		1990–91	
Air	2.2	4.1	6.4
CIPC	0	0	0.0
Ethylene	0.2	0.2	6.7
		1991–92	
Air	1.1	3.5	5.6
CIPC	0	0	0
Ethylene	0.0	0.0	0.0
		1992–93	
Air	0.8	1.0	6.3
CIPC	0	0	0
Ethylene	0.1	0.1	0.3
		1993–94	
Air	1.0	2.4	2.8
CIPC	0	0	0
Ethylene	0.0	0.0	2.8
		1994–95	
Air	0.8	1.7	3.4
CIPC	0	0	0
Ethylene	0.0	0.2	2.3
		1995–96	
Air	1.9	2.4	3.2
CIPC	0	0	0
Ethylene	0.0	0.0	0.4
		All years combined (square root)[z]	
Air	1.04	1.45	2.03
CIPC	0	0	0
Ethylene	0.04	0.18	0.90
SE (n = 120; df = 45)[y]		0.091	
Significant effects		S^{***}, T^{***}, $S \times T^{NS}$	

[z]All years, except 1990–91, which had poor ethylene control.
[y]Statistical analysis did not include CIPC treatment, which always had zeros.
[NS,***]Nonsignificant at $p \leq 0.05$ or significant at $p \leq 0.001$, respectively, where S = storage time and T = treatment.

to 87, Grade 3 = 54 to 69, Grade 4 = 36 to 53, Grade 5 = 21 to 35, Grade 6 = 5 to 20 and Grade 7 = 0 to 4). In the first year, the sample was taken randomly from the storage pile and in the second and third years the samples were prepared at the beginning from one grower and randomly selected throughout the storage season. In 1 year (1993–94), additional 'Russet Burbank' potatoes were collected from six different commercial sources across Canada and treated similarly to the 'Russet Burbank' sample mentioned previously. These samples were removed at 7- to 8-week intervals and measured for sprout mass, sprout number and maximum length as described for the lab study.

Results

Laboratory study

Sprouting. In all years, sprouting was minimal in all treatments at 5 and 10 weeks of storage (data not shown), but increased progressively at successive removal dates. Sprout mass, maximum length and number of large sprouts per tuber (Tables 1, 2, and 3, respectively) increased dramatically in the air treatment at 15 weeks and at each subsequent removal. CIPC-treated tubers had negligible sprout production. In the ethylene treatment, the beginning of sprouting was delayed by 5 to 15 weeks, compared with the air treatment (data not shown).

Table 4. Effect of storage time and treatments on mean number of small sprouts (2 to 5 mm) over 5 storage years in the laboratory study. Data were not collected in year 1 (1990–91).

	Sprouts per tuber (no.)		
	Storage time (weeks)		
Treatment	15	20	25
		1991–92	
Air	0.7	2.0	2.2
CIPC	0	0	0
Ethylene	0.0	0.5	3.5
		1992–93	
Air	1.7	2.2	7.3
CIPC	0	0	0.1
Ethylene	0.0	0.0	0.4
		1993–94	
Air	0.0	0.0	0.2
CIPC	0	0	0
Ethylene	0.1	3.8	12.2
		1994–95	
Air	0.0	0.2	0.5
CIPC	0	0	0
Ethylene	0.0	4.4	7.9
		1995–96	
Air	0.5	0.3	0.1
CIPC	0	0	0
Ethylene	0.2	2.7	7.0
		All years combined (square root)	
Air	0.63	0.78	1.11
CIPC	0	0	0
Ethylene	0.21	1.30	2.29
SE (n = 120; df = 45)[z]		0.143	
Significant effects		S^{***}, T^{***}, $S \times T^{***}$	

[z]Statistical analysis did not include CIPC treatment, which always had zeros.

***Significant at $p \leq 0.001$, where S = storage time and T = treatment.

After sprouts appeared in the ethylene treatment, total sprout mass (sprouts >5 mm) was always less than untreated potatoes (Table 1). Total sprout mass on the ethylene-treated potatoes remained similar to the CIPC-treated potatoes for the entire storage period of 25 weeks. Except for 1990–91, when there was poor ethylene control (actual concentration was sometimes less than the desired concentration due to poor sealing), the maximum sprout mass never exceeded 0.5 $g \cdot kg^{-1}$ of initial tuber fresh mass.

Maximum sprout length of ethylene-treated potatoes increased over storage time but was always much less than untreated potatoes (Table 2). The mean value never exceeded 12 mm on ethylene-treated tubers, except for 1990–91.

The number of large sprouts (>5 mm) increased with storage time and, except for the first year, was always less in ethylene-treated potatoes than in untreated potatoes (Table 3). The mean number of large sprouts on ethylene-treated tubers never exceeded 1 per tuber after 20 weeks and after 25 weeks exceeded 1 per tuber in only 3 years.

The number of small sprouts (2 to 5 mm) increased with storage time (Table 4). It was the same in ethylene-treated potatoes as in untreated potatoes after 15 weeks. The number of small sprouts on ethylene-treated potatoes increased and exceeded untreated potatoes after 20 and 25 weeks. The mean number of small sprouts on ethylene-treated tubers exceeded one per tuber after 20 weeks in 3 of 5 years. At 25 weeks there was more than one sprout per tuber in 4 of the 5 years, reaching a mean value of over 12 per tuber in 1993–94. Although they were not counted, the number of eyes which produced sprouts also appeared to increase when the number of small sprouts increased.

Fry color. When all years were combined (Table 5), the data showed that fry color improved (lightened) with storage time, regardless of treatment. The fry color of the ethylene-treated potatoes was darker than untreated or CIPC-treated potatoes at all storage times. This difference was greatest in the first 15 weeks, and progressively narrowed to no difference between untreated and ethylene-treated potatoes after 25 weeks.

Sprout detachment. Removal force was measured after 20 and 25 weeks of storage in 1993–94. At removal, only the untreated potatoes had sprouts of sufficient size for evaluation (Table 6). After 3 and 6 weeks of holding tubers in air at 20 °C, measurable sprouts developed on the ethylene-treated tubers. Although the removal force increased in untreated and ethylene-treated tubers over the 6-week period, the sprout removal force for the sprouts on ethylene-treated tubers was consistently less than on untreated tubers.

Decay and disorders. During the 6 years of the study there was neither decay nor disorders associated with any of the treatments (data not shown).

Commercial storage study

Sprouting. In the 1 year (1993–94) when sprouts were measured, the CIPC-treated potatoes had the shortest sprouts and the smallest sprout mass until after week 22 (Table 7). Between week 22 and 29, the sprouts on the CIPC-treated potatoes had very large increases in length and mass. During the same time sprout length on ethylene-treated tubers appeared to be stable and very similar to lengths observed in the laboratory study. Although the mass of sprouts on ethylene-treated tubers increased 3-fold during this time, it remained considerably lower than sprouts on CIPC-treated potatoes at week 29.

Fry color. The fry color in the commercial trial was generally darker in the ethylene-treated tubers than in the CIPC-treated tubers in all 3 years (Fig. 1). This was similar to the trends observed

Table 5. Effect of storage time and treatments on mean Agtron fry color over 5 storage years in the laboratory study. Data were not collected in year 1 (1990–91).

	Agtron value (% reflectance)[z]				
	Storage time (weeks)				
Treatment	5	10	15	20	25
			1991–92		
Air	64.4	68.1	70.4	69.5	67.3
CIPC	64.5	65.7	67.1	74.9	75.4
Ethylene	61.2	64.9	66.5	67.7	68.8
			1992–93		
Air	64.4	67.8	68.1	70.4	66.8
CIPC	62.5	67.5	66.0	71.6	70.1
Ethylene	64.6	67.5	66.3	67.8	70.9
			1993–94		
Air	61.0	64.0	69.9	70.3	69.1
CIPC	69.1	68.0	72.9	77.5	66.2
Ethylene	56.1	56.7	64.3	66.2	69.9
			1994–95		
Air	64.7	67.4	69.7	68.5	72.4
CIPC	69.8	70.9	67.7	71.9	78.2
Ethylene	58.2	63.8	57.5	64.4	67.2
			1995–96		
Air	72.4	71.3	72.9	74.4	70.8
CIPC	70.4	73.5	73.7	77.2	74.9
Ethylene	44.9	52.1	56.7	64.2	63.1
			All years combined[y]		
Air	64.6	67.7	70.2	70.6	69.3
CIPC	67.3	69.1	69.5	74.6	75.2
Ethylene	57.0	61.0	62.2	66.1	68.0
SE (n = 300; df = 149)			0.82		
Significant effects			S^{***}, T^{***}, $S \times T^{***}$		

[z]Initial Agtron values (day 0): 68.6 (1991–92); 50.0 (1992–93); 51.5 (1993–94); 75.0 (1994–95); 82.3 (1995–96).
[***]Significant at $p \leq 0.001$, where S = storage time and T = treatment.

in the laboratory data for the same years (Table 5).

Sprout detachment. During removal of the potatoes at the end of the storage period, almost all of the sprouts on the ethylene-treated potatoes readily fell off the tubers while being handled by the loading equipment. This observation was in agreement with the data from the sprout removal force measurements in the laboratory (Table 6).

Decay and disorders. In the commercial tuber samples there were no decay or disorder differences between CIPC- and ethylene-treated tubers, with the exception of internal sprouting. There was more internal sprouting in CIPC-treated tubers, 0.538% (1993–94) and 0.697% (1994–95), compared with ethylene-treated tubers, 0.0045% (1993–94) and 0.054% (1994–95).

Discussion and Conclusions

The laboratory and commercial studies over 6 and 3 years, respectively, showed that continuous ethylene treatment at 166 $\mu mol \cdot m^{-3}$ had several consistent and repeatable effects during long-term storage of 'Russet Burbank' potatoes. In the presence of continuous ethylene, sprouts appeared later than in untreated tubers, which appears to contradict the claims of earlier authors, i.e., that ethylene shortens the duration of rest markedly (Kader, 1985; Rylski et al., 1974). This may be rationalized by explaining that our results were based on continuous exposure whereas the above conclusion was based on sprouting after ethylene-treated tubers were put in air. Although most of the treatments of Rylski

Table 6. Sprout removal force of air and ethylene treatments immediately after storage and after 3 and 6 weeks re-conditioning in air at 20 °C in the 1993–94 laboratory study.

		Removal force (N)		
		Reconditioning time (weeks)		
Storage time (weeks)	Treatment	0	3	6
20	Air	5.2	5.8	6.4
	Ethylene	---	1.9	4.0
25	Air	9.0	6.2	8.6
	Ethylene	---	1.9	3.1
SE (n = 80; df = 567)			0.48	
Significant effects			T^{***}, $T \times S^{**}$, $S \times T \times R^{*}$	

[*,**,***]Significant at $p \leq 0.05$, 0.01, or 0.001, respectively, where S = storage time, T = treatment, and R = reconditioning time.

Table 7. Effect of storage time and treatments on sprout number, mass and maximum sprout length in the 1993–94 commercial trial.

Treatment	0	Storage time (weeks) 7	15	22	29
			Sprouts/tuber (no.)		
CIPC	0	0 (0.000)[z]	0 (0.030)	0.08 (0.285)	7.97 (2.823)
Ethylene	0	0.01 (0.121)	0.03 (0.178)	1.09 (1.042)	7.83 (2.798)
SE (of square root, n = 96; df = 68)			0.124		
Significant effects			$S^{***}, T^{**}, S \times T^{*}$		
			Sprout mass/initial tuber fresh mass ($g \cdot kg^{-1}$)		
CIPC	0	0 (0.00)[y]	0.01 (0.003)	0.08 (0.033)	33.36 (1.536)
Ethylene	0	0.05 (0.020)	0.09 (0.036)	2.10 (0.492)	6.73 (0.888)
SE (of Log_{10}, n = 96; df = 68)			0.0553		
Significant effects			$S^{***}, T^{NS}, S \times T^{***}$		
			Maximum length (mm)		
CIPC	0	0 (0.00)[y]	0.3 (0.10)	3.3 (0.629)	112.8 (2.056)
Ethylene	0	1.3 (0.352)	7.0 (0.905)	35.9 (1.567)	18.3 (1.286)
SE (of Log_{10}, n = 96; df = 68)			0.1002		
Significant effects			$S^{***}, T^{***}, S \times T^{***}$		

[z]Values backtransformed from square root used in statistical analysis; square root values in brackets.
[y]Values backtransformed from Log_{10} used in statistical analysis; Log_{10} values in brackets.
[NS,*,**,***]Nonsignificant, significant at $p \leq 0.05$ 0.01, or 0.001, respectively, where S = storage time and T = treatment.

et al. (1974) were ≤72 h, they did conduct one experiment using continuous ethylene at 2 μL·L^{-1} for 40 d, which inhibited sprouting, compared with an air treatment. After the ethylene was removed on day 40, sprouts appeared and grew at a rate similar to tubers in air. Since they had concluded from their other results with 72 h treatment that ethylene shortens rest, they speculated that this 40 d treatment also terminated rest but continuous exposure somehow inhibits bud elongation. Compared with untreated potatoes, continuous ethylene in our study did not increase the number of large sprouts but it increased the number of small sprouts and tuber eyes producing sprouts. Although Rylski et al. (1974) did not observe any changes in the number of small sprouts or sprouting, it can be suggested that, as an extension of what they had speculated, continuous ethylene treatment terminates rest at the biochemical and cellular level in tuber eyes but appears to inhibit visible sprout cell differentiation and elongation.

Large sprouts that developed on the ethylene-treated potatoes after storage were much easier to detach, compared to sprouts on untreated tubers. Although ethylene is recognized as an abscission promoting agent in many crops (Reid, 1985), we believe this to be the first report of abscission enhancement in stem tissue. Furthermore, there was a residual abscission effect after ethylene treatment stopped.

In both the laboratory and commercial studies, there were no decay, disorder or internal sprouting problems in ethylene-treated tubers. The internal sprouting observed in the commercial potatoes treated with CIPC is a problem that is sometimes observed in CIPC-treated tubers and is associated with an inadequate amount of CIPC on the tubers (Hruschka and Heinze, 1967).

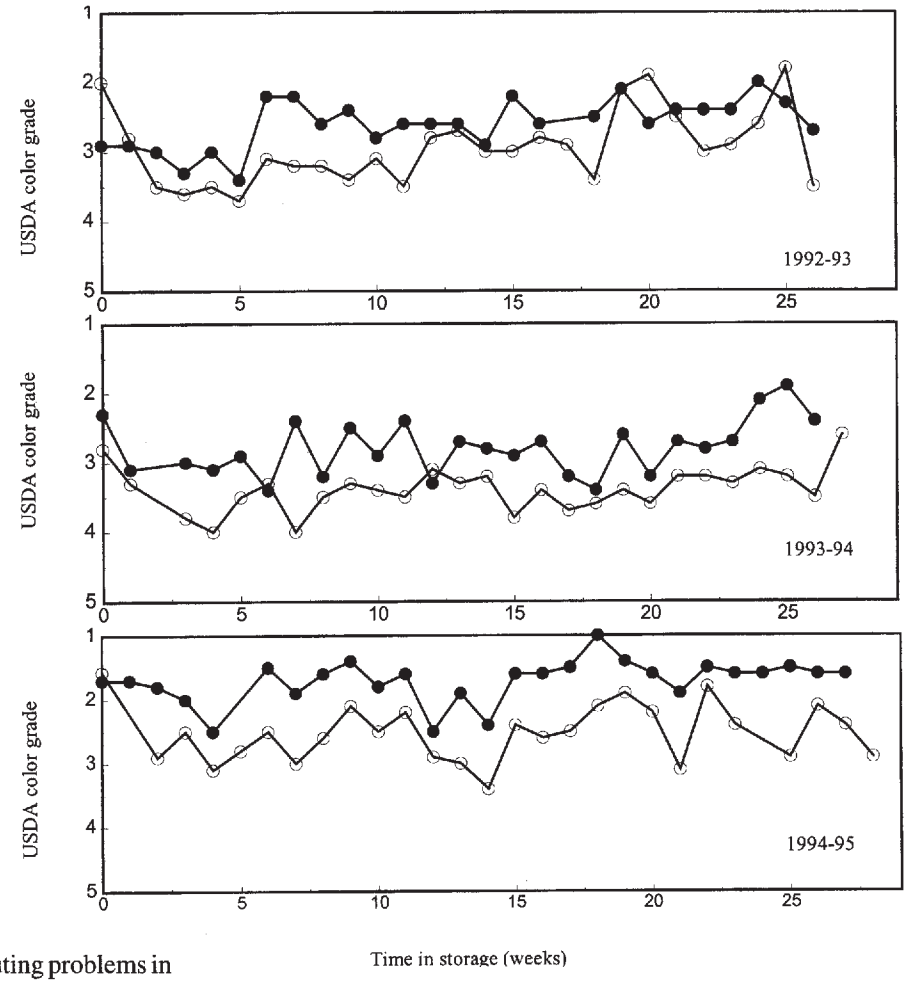

Fig. 1. USDA fry color grades of potatoes in the commercial study in three storage seasons (1992–93, 1993–94, and 1994–95) (closed circle = CIPC, open circle = ethylene).

Lulai et al. (1995) showed that several jasmonate compounds control tuber sprouting. Since reports of stimulation of internal ethylene production with the application of methyl jasmonate on tomatoes (Saniewski and Czapski, 1985; Saniewski et al., 1987b) and apples (Saniewski et al., 1987a) have been published, it is possible that the mode of action of jasmonates and other sprout control compounds may be partly related to the stimulation of internal ethylene in the tubers.

Although it was not obvious in all years in the laboratory and commercial studies, continuous ethylene in several years resulted in a drop in fry color, observable at the first sample date after ethylene was applied. This was not permanent and fry color recovered (higher Agtron values) over time. In the laboratory study, the fry color of potatoes continuously treated with ethylene was frequently similar to untreated potatoes, especially after longer storage times when the untreated potatoes were producing many sprouts. However, the fry color of ethylene-treated tubers was almost always darker than tubers treated with CIPC in the laboratory and commercial studies.

Since the results of this study showed that continuous ethylene treatment at 166 $\mu mol \cdot m^{-3}$ substantially suppressed tuber sprouting during long-term storage, the conclusion of Kader (1985) that ethylene treatment is desirable for seed potatoes but undesirable for table and processing potatoes is not entirely correct. If fry color has little or no effect on market value, e.g., table stock, continuous ethylene treatment is a viable option. The use of continuous ethylene for long-term storage of seed potatoes is also appealing since it reduced apical dominance and caused more uniform sprouting. However, it may result in excessive sprout abscission during the planting process, if sprouts are already present. For applications in which fry color is important, e.g., French fries or chips, then continuous ethylene may be a viable alternative to CIPC, under one or both of the following circumstances:
1) French fry and/or chip market consumers will accept a darker fry color as a trade-off in reducing chemical residues in the product.
2) A method is developed to minimize the darkening of fry color following ethylene exposure.

Continuous ethylene treatment could also be useful in warmer climates, even for French fry and chip potatoes, where the higher storage temperatures result in greater losses of CIPC and other commercial sprout inhibitors through volatilization.

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Ethylene inhibited sprouting of potato tubers by influencing the carbohydrate metabolism pathway

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Abstract

The aim of this study was to investigate the role of ethylene to control sprouting of potatoes by observing the effect of exogenous ethylene on carbohydrate metabolism and key enzymes. The initial time of potato tuber sprouting and sprouting index were recorded, and rate of respiration, total sugar, total reducing sugar, starch, fructose, glucose, sucrose and the activities of acid invertase (AI), neutral invertase (NI), sucrose synthase (SS), sucrose phosphate synthase (SPS), starch phosphorylase and amylase during sprouting were measured. Exogenous ethylene inhibited sprouting of potato tubers. Moreover, exogenous ethylene increased respiration total sugar, AI activity, SPS activity, SS activity, and reduced sugar and assay activity. Nevertheless, starch, glucose, fructose, NI activity and starch phosphorylase activity showed lower variation. Lower sprouting resulted into potatoes with higher levels of total sugar, total reducing sugar and glucose, and lower level of fructose and sucrose. And sprouting could be inhibited by increasing the activities of SS, SPS and AI by treatment with 199.3 μl L^{-1} exogenous ethylene. Overall, exogenous ethylene inhibited sprouting of potato tubers by influencing its carbohydrate metabolism.

Keywords: Potato tuber, Exogenous ethylene, Sprouting, Carbohydrate metabolism, Key enzymes

Introduction

Potato is one of the most important food crops in the world and is a main staple food for human consumption (Saraiva 2004), and sprout management was an important aspect of successful storage and distribution, to maintain good quality of the tubers for the intended purpose. Exogenous ethylene had been shown to inhibit potato sprouting, as described in several reports (Daniels-Lake et al. 2006, 2011). The effect of ethylene on sprouting was probably connected with changes in sucrose concentrations in

potato tubers, there might be relation between carbohydrate and ethylene. Transgenic approaches, targeted to carbohydrate pathways in the plant, have shown that modifying carbohydrate metabolism affected tuber dormancy and sprouting (Vreugdenhil 2007).

Although there had been some research about sprout-inhibitory of ethylene (Prange et al. 2005) and its effect on sugar accumulation (Zhao et al. 2015; Foukaraki et al. 2016a, b), the reports about overall effect on carbohydrate and its mechanism was rare. In this work, potato tubers were treated by solid ethylene releasing agents as a matter of convenience, sprouting index, rate of respiration, starch and sugar contents, the activity of key sugar metabolizing enzymes were determined. The aim of this work was undertaken to examine how exogenous ethylene inhibited sprouting of potato tubers by regulating the carbohydrate metabolism pathway.

Materials and methods

Chemicals

Chromatographic grade acetonitrile and ultrapure water were used for HPLC analysis. All other chemicals used were of analytical grade.

Plant material and ethylene treatments

"*Favorita*" potato stored in cold storage (about 4 °C) for 3 months, had passed dormant period, were immediately delivered to the laboratory, where tubers free of visual defects and of uniform size were selected. Potatoes were divided into three groups (about 15 kg) and packed in plastic bags, each containing the same number and three replicates of tubers.

The potatoes were treated by exogenous ethylene as follows. Three groups of potatoes were separated as control M_0 no treatment was done, M_1 and M_2 were treated by 91.7 and 199.3 μl L^{-1} exogenous ethylene, respectively. At last, the potatoes were stored at 15 °C. The sampling for detecting sugar content and key enzymes of the potatoes was done on 0, 4, 8, 12, 16, 20 and 24 days, after treatment, respectively.

Sprouting index

Buds were considered to have sprouted once a sprout of minimum length 2 mm had formed, as defined by Zhao et al. (2015). Initial germination time and sprouting indexes of all treatment were recorded at sampling dates. The method was as follows: if all bud eye of the tuber didn't germinated, it was considered as level-0; level-1 was the tuber that the germination percentage of bud eye was lower than

25 %; level-2 was the tuber that the germination percentage of bud eye was between 25 to 50 %; level-3 was the tuber that germination percentage of bud eye was higher than 50 %. Calculation formula for evaluating the sprouting index was as follow:

$$\text{Sprouting index} = \sum_{i=0}^{3} iX_i \times 100/T$$

In which: I = sprouting level; X_i = the tuber number of level-i; T = the total number of tuber in plastic bag.

Respiration

Whole tuber respiration was evaluated by CO_2 production using an infrared gas analysis in an open system (Compact Minicuvette System CMS-400, Walz GmbH, Effeltrich, Germany) as described by Hajirezaei et al. (2003). The rate of respiration was reported as mg (kg h^{-1}).

Soluble sugar determination

Total sugar, reducing sugar and starch Extraction (1): 0.25 g of potato samples was extracted using water bath at 60 °C for 20 min with 50–60 ml water. Extraction (2): 0.2 g of samples was heated for 30 min in boiling water bath with 10 ml 6 M HCl and 15 ml water. Extraction (3): 0.2 g of potato samples was heated and dissolved with 3.2 ml 60 % perchloric acid and 3 ml water.

Extraction (1) and (2) were assayed using DNS reagent for total sugar and reducing sugar, respectively, as described in Hu et al. (2008). Read the value of sugar concentration corresponding to the absorbance from the calibration curve of glucose at a wavelength of 540 nm. Starch content was estimated by the method of Men and Liu (1995). This method involves dissolving starch in perchloric acid, diluting with distilled water, reacting with iodine solution and measuring the absorbance at a wavelength of 660 nm. Total sugar, reducing sugar and starch content was reported as micromoles of glucose and soluble starch equivalents (GAE) per milligram of dry weight. All spectrophotometric assays were run on a V-1100D spectrophotometer (Mapada, China).

Fructose, glucose, and sucrose The procedure for carbohydrate determination has been previously described in detail (Olsen et al. 2003). Tuber tissue (1.5 g) was extracted in 40 mL of 80 % ethanol for 30 min by ultrasonic extraction using a SB-25-12DT ultrasonic cleaner (Scientz, China), filtered (Whatman No. 1). The extraction was evaporated under vacuum at 50 °C by RE-52AA rotary

evaporator (Puredu, China), the residue was dissolved in 10 ml of mobile phase. The final extract was filtered through a 0.45 μm pore-size membrane filter, and immediately injected to HPLC as 20 μL. High performance liquid chromatography (Shimadzu, Japan) include hypersil NH_2 column (5 μm, 250 × 4.6 mm, Dalian Elite Analytical Instruments Co. Ltd., Deaic, China) and RID-10A detector. The column and detector temperature was 35 and 40 °C. The mobile phase was acetonitrile: water (70:30, v/v) with a flow rate of 1 ml min^{-1}. Peak areas for respective sugars (fructose, glucose, and sucrose) were recorded and sugar concentration (mg g^{-1} of tissue dry weight) was calculated using standard substance.

Enzyme assays All procedures related to enzyme extraction were carried out at 4 °C or lower. Plant samples were extracted in 0.1 M PBS buffer (pH 7.5) containing 5 mM $MgCl_2$, 1 mM EDTA, 1 mM EDTA 0.1 % (v/v) ß-mercaptoethanol and 0.1 % (v/v) Triton X-100, at 4 °C, centrifuged at 10,000 rpm and 4 °C for 10 min, then the supernatant fluid into 10 ml calibration tube. The activities of SPS, SS, acid and neutral invertase were determined from the same extract.

Assay of activity acid invertase (AI) and neutral invertase (NI) The measurement of activity of AI and NI was carried out according to the Nielsen et al. (1991). Procedure with some minor modifications. The soluble AI activity was assayed by adding 50 μl reaction buffers (0.1 M pH 5.5 acetic acid buffer and 1 % sucrose) to 50 μl crude enzyme, and incubated at 34 °C for 1 h. The controlled trial is that 50 μl crude enzyme incubated at 100 °C for 10 min. The reaction was stopped by boiling the mixture for 5 min and adding 1.5 ml of 3, 5-dinitrosalicylic acid, after incubated in a water bath at 100 °C for 5 min. The mixture was set to the volume to 25 ml with distilled water. The soluble AI activity was assayed from the obtained light absorption value at a wavelength of 540 nm. The assay for NI activity was similar to that of AI except that the reaction was performed in phosphate buffer (pH 7.5). The resulting reducing sugars were estimated by Nelson-Somogyi method. Invertase activity was expressed in units of mg g^{-1} h^{-1} FM.

Assay of sucrose synthase (SS) and sucrose phosphate synthase (SPS) activity For the assay of sucrose phosphate synthase (SPS) and sucrose synthase (SS), the respective tissues were extracted following the method of Miron and Schaffer (1991). Assay mixture for SPS contained 0.1 M borate buffer (pH 8.0), 15 mM $MgCl_2$, 5 mM fructose-6-phosphate, 15 mM glucose-6-phosphate, 10 mM UDP-glucose and enzyme extract. After incubation at 30 °C for 1 h, the reaction was stopped by adding 0.2 ml of 30 % KOH and then cooled to room temperature. The sucrose formed was determined by anthrone reagent. Background was determined by adding the stopping base before adding the enzyme. The reaction mixture for SS assay was similar to SPS assay but it contained 0.06 M fructose instead of fructose-6-phosphate and was devoid of glucose-6-phosphate. The sucrose hydrolysed during SS catalyzed reaction and sucrose formed during SPS catalyzed reaction were estimated according to Vassey et al. (1991). The enzyme activities were expressed as nmol sucrose hydrolysed or formed mg g^{-1} h^{-1} FM, respectively.

Assay of starch phosphorylase activity For determination of starch phosphorylase activity (Dubey and Singh 1999), plant materials from each treatment were homogenized in 5 ml buffer containing 100 mM sodium succinate (pH 5.8), 10 % glycerinum, 1 mM EDTA, 15 mM β-mercaptoethanol, 1 mM EDTA, 5 mM $MgCl_2$ and centrifuged at 15,000 rpm for 20 min at 4 °C. The assay mixture contained 0.8 ml SDB [100 mM sodium succinate (pH 5.8), 0.1 % bovine serum albumin (w/v), 10 mM β-mercaptoethanol, 0.2 mM EDTA, 10 % glycerinum], substrate mixture [100 mM sodium succinate (pH 5.8), 5 % soluble starch (w/v), 0.1 mM glucose-1-phosphate 0.2 mM AMP] and enzyme extract to make the total volume up to 1.0 ml. The reaction was stopped after 10 min by adding 2.6 ml [2.6 g ammonium molybdate in 100 ml 14 % [v/v] sulfuric add]. The mixture was centrifuged and phosphorus content in the supernatant was estimated following the method of Fiske and Subbarow. The enzyme activity was calculated as nmol of P_i liberated mg g^{-1} h^{-1} FM.

Assay of amylase Amylase was assayed in colorimetry according to the method of Zhou (1995). The extract was performed in ice-bath. The homogenate was centrifuged at 3000 rpm for 15 min. The resulting supernatant was kept at 40 °C water-bath for 5 min following addition of 1 % starch solution, then kept boiling for 5 min after addition of DNS. The enzyme activity was measured by the absorbency of a wavelength of 525 nm, and was showed in the amount of maltose transformed in 5 min. The standard curve was obtained in the same way.

Statistical analysis The statistical analysis was carried out using SPSS 17.0 (SPSS Inc., Chicago, IL). Results were expressed as mean values ± standard deviation. Means were compared by multivariate analysis followed by the Duncan's test. A difference was considered statistically significant when $P < 0.05$.

Results and discussion

Sprouting indexes and rate of respiration

The initial germination time of tubers of M_0, M_1 and M_2 were 4, 4 and 5 days after treatment, (Fig. 1). Tubers exposed to ethylene (M_1 and M_2) exhibited lower sprouting indexes in proximal parts compared to the control (M_0) until 12 days, then, sprouting indexes of M_1 increased rapidly, and at the end of this experiment, they were higher than M_0 except for the last point (Fig. 1). After 24 days, the sprouting indexes of M_2 was the least, which was lower than that of M_1 ($P > 0.05$) and M_0 ($P < 0.05$).

Fig. 1

Sprouting indexes of potato tubers. *Each value* represents mean ± standard deviation of three replicates

Time-dependent changes in respiration rate were shown in Fig. 2 after the start of the ethylene treatment, tuber respiration rate began to increase rapidly. Respiration rates were highest at 4 days after ethylene treatment, and then slowly declined. Compared to the untreated samples, 199.3 μl L^{-1} ethylene increased respiration by 15 %, while, respiration rates was significantly reduced by 91.7 μl L^{-1} ethylene.

Fig. 2

Respiration rate of potato tubers. *Each value* represents mean ± standard deviation of three replicates

Exogenous ethylene increased tuber respiration rates, affected tuber dormancy and sprouting (Gottschalk 2011). The trend of respiration rate in the present work was almost same as the previous report of Downes et al. (2010) that onion bulb respiration rate increased immediately after being treated with ethyle (Alexopoulos et al., 2008). And the result was also in line with potato tubers at 6 °C (Foukaraki et al., 2010).

Carbohydrate metabolism

Sugar contents and starch As observed in Fig. 3a, the contents of total sugar in potato tubers of different treatments had a trend of increasing in the whole process. During first 4 days of storage the total sugar increased while, M_2 presented little decreasing between 4 and 12 days. After 24 days, the total sugar content of M_0, M_1, M_2 increased 62.73, 68.54, and 82.92 mg/g, respectively. Compared to the control, total sugar content of M_1 increased little ($P > 0.05$), but M_1 was higher than M_0 in the whole process. Exogenous ethylene of 199.3 μl L^{-1} promoted total sugar content, which was significant different from that of 12–20 days. It could be confirmed that total sugar could be strengthened by exogenous ethylene. Foukaraki et al. (2012) found that ethylene-treated tubers contained higher levels of total sugars, the present result confirmed that the increasing of total sugar be strengthened by exogenous ethylene.

Fig. 3

Total sugar (**a**), total reducing sugar (**b**), starch (**c**) content of potato tubers. *Each value* represents mean ± standard deviation of three replicates

Exogenous ethylene had remarkable effects on total reducing sugar content (Fig. 3b). After 24 days, the total reducing sugar content of M_0, M_1, and M_2 were decreased to 116.03, 100.9, 106.77 mg/g (Fig. 3 b), respectively. Compared to the control, reducing sugar content of M_1 and M_2 were lower, and there was a significant difference between them ($P < 0.05$), and the total reducing sugar content of M_1 was much lower than M_2 ($P < 0.05$). An interaction between ethylene and CO_2 in the storage atmosphere has been reported, with higher concentrations of ethylene increasing tuber reducing sugar content (Daniels-Lake et al. 2009), the reason might be the different cultivar of potatoes used in study, in which the carbohydrate could present various responses to external stimulation.

As shown in Fig. 3c, the starch content decreases gradually. During first 4 days, the starch content of all treatments increased and then there was a large reduction from 4 to 8 days. The content of starch in M_0, M_1 and M_2 reduced by 27.83, 19.23 and 37.19 %, respectively, after 24 days. At the end of storage, M_1 was higher markedly compare to M_2 ($P < 0.05$), while M_0 was much lower than M_1 and higher than M_2 ($P < 0.05$). Starch degradation had been discussed as an important event related to the induction of sprouting (Hajirezaei et al. 2003), who got that breakdown rate of starch was negatively correlated with respiration in potatoes. The results was in corroborated with previous report of Biemelt et al. (2000) showing that starch degradation was not a prerequisite for the initiation of sprouting. Considering the best sprouting inhibition effect, it could be assumed that lower starch level was beneficial for reducing the sprouting indexes. Exogenous ethylene could reduce the disappearing of total reducing sugar.

Sucrose, fructose and glucose content The changes of glucose, fructose and sucrose in tubers were shown in Fig. 4. The effect of exogenous ethylene on three kinds of monosaccharide were varied. As shown in Fig. 4a, the glucose content of M_2 was higher than the control ($P < 0.05$), while glucose content of M_1 was lower, and there was no significant difference between them. The change in glucose was similar earlier report (Frazier et al. 2006). Compared to M_0, fructose content of M_2 was declined significantly ($P < 0.05$), and M_1 was lower than M_0 ($P < 0.05$) (Fig. 4b), however, fructose content of all the three treatments changed little. In addition, significant difference could be found between any two of treatments ($P < 0.05$). It proved that higher level of exogenous ethylene could decrease fructose content. Sucrose was reported as a prerequisite signalling molecule for hormonal dormancy control

(Foukaraki et al. 2016a, b). Sucrose content in tubers was significantly decreased by exposure to exogenous ethylene, the effect of different treatment was in the order of $M_1 < M_2 < M_0$. In addition, significant difference was also observed between any two treatments ($P < 0.05$).

Fig. 4

Glucose (**a**), fructose (**b**) and sucrose (**c**) content of potato tubers. *Each value* represents mean ± standard deviation of three replicates

Ethylene might enhance the level of glucose (Foukaraki et al. 2012), and the effect reversed with increasing content of ethylene. Cools et al. (2011) observed that the levels of three sugars in onion were decreased to a variable extent on ethylene treatment which was consistent agreed with our results.

Based on the above analysis the lower sprouting development tended to present higher levels of respiration, total sugar, total reducing sugar, glucose, and lower fructose and sucrose in potatoes. In this work, the results show that 199.3 μl L^{-1} exogenous ethylene could inhibited sprouting of potato and enhanced respiration total sugar, disappearing of total reducing sugar, decrease sucrose content.

Enzymes in carbohydrate metabolism

Invertase A continuous increase with time in acid invertase (AI) activity was observed in both treatments (Fig. 5a). It can be seen that, AI activity of M_2 maintained a high level at the first 12 days, while, AI activity of M_1 was lower than the control, and there was a significant difference between them. Both M_0 and M_2 showed a decrease in the sprouting potato tubers between 12 and 16 days, while AI activity of all treatments increased upto 8 days. After 24 days, the order of AI activity in potato tubers was $M_1 > M_2 > M_0$. Neutral invertase (NI) activity in potato tubers showed a rank of $M_2 > M_0 > M_1$, there was no significant difference from the control (Fig. 5b). Higher concentrations of exogenous ethylene significantly promoted the NI activity but significantly inhibited the activity of NI when the concentrations of exogenous ethylene were lower. Figure 5c showed the activity of invertase in potato tubers during 24 days, the activity of invertase was found to be increased in all treatments, (Fig. 5c). Both the activity of M_1 and M_2 were higher than the control M_0 ($P < 0.05$), but there was no significant difference between them.

Fig. 5

Carbohydrate metabolism-related enzyme activities. *Each value* represents mean ± standard deviation of three replicates. **a** acid invertase (AI), **b** neutral invertase (NI), **c** invertase, **d** sucrose synthase (SS), **e** sucrose phosphate synthase (SPS), **f** starch phosphorylase, **g** amylase

Yao et al. (2005) pointed out that 400 mg L^{-1} ethephon raised the activities of acid and neutral invertases at late growth stage, which was therefore promoting the sucrose accumulation in the stalks. Wang and Zhang (2000) took apples as materials, spray with 300 mg L^{-1} ethephon at the early stage of the mature fruit, the results showed that compared with the control and aminooxyacetic acid treatment, the neutral invertase activity were significantly improved, and the ethylene biosynthesis in starkrimson fruit was stimulated.

Sucrose synthase (SS) and sucrose phosphate synthase (SPS) After 24 days, the SS activity of M_2 was significantly lower than M_1 ($P < 0.05$), although the amount of exogenous ethylene was elevated. Compared to M_0, SS activity of M_1 was increased significantly ($P < 0.05$), and M_2 was slightly lower than M_0 ($P > 0.05$) (Fig. 5d). In addition, significant difference could be found between any two of treatments ($P < 0.05$). In many plants, SS is known to be involved in sucrose cleavage rather than sucrose synthesis (Róth et al. 2007), it proved that higher level of exogenous ethylene could decrease activity of sucrose synthase.

The activity of sucrose phosphate synthase (SPS) was found to be increased by exposure of 199.3 µl L^{-1} ethylene during 24 days. As illustrated in Fig. 5e. SPS activity of M_2 reached the maximum at 20 days, which was significantly higher than the control, but for M_1, no significant difference from the control was observed. After 24 days, the SPS activity was observed in the order $M_2 > M_0 > M_1$, which proved that higher level of exogenous ethylene could increase activity of SPS.

Wang et al. (2013) noticed that SPS was negatively correlated with sucrose content in cane, which was contrast with findings of Lingle (1999). Pan et al. (2007) reported that four enzymes made great contribution to sucrose accumulation in sugarcane internodes, and SAI, SS and NI were negatively while SPS was positively correlated with sucrose content in cane, respectively. Verma et al. (2011) emphasized that SPS activity was positively correlated with sucrose content and negatively correlated with hexose sugars content, but SS activity was negatively correlated with sucrose content and positively correlated with hexose sugars content. Changes of sugar contents due to exogenous ethylene treatment were supported by the changes of different sugar metabolizing enzymes. Increase in SPS, SS

activity after 24 days (Fig. 5) was related decrease in sucrose content in the potato tubers resulting in a much lower sucrose content in the potato tubers of the exogenous ethylene treated than that of the control.

Starch phosphorylase (SP) The activity of starch phosphorylase were had little change (Fig. 5f), there was no specific inclining or declining rule of starch phosphrylase in the three treatments. At the first 4 days activity of starch phosphorylase of all treatments increased and reached the maximum at the 4 days, then, the activities of M_0, M_1 and M_2 were decreased during 4 to 12 days, and reached the minimum at the 12 days. After 24 days, the starch phosphorylase activity was in the order of $M_2 > M_0 > M_1$, and there was no significant difference between them. Chen and Cai (2005) also noticed that ethylene could stimulate the activities of acid invertase, amylase, sucrose synthase and sucrose phosphate synthase, inhibited the activity of amylase, while no significant effects to starch phosphorylase.

Amylase In the process of exogenous ethylene exposure, the activity of amylase increased rapidly and reached a peak, and then decreased. At the first 4 days, the amylase activity of M_0 was lower than M_1 and M_2, both of M_0 and M_2 were hightest at 8 days, while amylase activity of M_1 was highest at 12 days. After 24 days, the order of amylase activity in potato tubers was $M_0 > M_1 > M_2$.

Overall, ethylene was showed to stimulate the activities of acid invertase, amylase, sucrose synthase and sucrose phosphate synthase, inhibited the activity of amylase, while no significant effects to the neutral invertase and starch phosphorylase ($P > 0.05$).

Conclusion

Exogenous ethylene inhibited sprouting of potato, and increased respiration, total sugar, glucose, reduced fructose and sucrose. Besides, the lower sprouting development tended to present higher levels of respiration, total sugar, total reducing sugar and glucose, lower fructose and sucrose in potatoes. Sprouting could be inhibited by increasing the activities of SS, SPS and AI by treatment with 199.3 μl L^{-1} exogenous ethylene. Exogenous ethylene inhibited sprouting of potato tubers by influencing its carbohydrate metabolism.

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Exogenous ethylene inhibits sprout growth in onion bulbs

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• *Background and Aims* Exogenous ethylene has recently gained commercial interest as a sprouting inhibitor of onion bulbs. The role of ethylene in dormancy and sprouting of onions, however, is not known.
• *Methods* A cultivar (*Allium cepa* 'Copra') with a true period of dormancy was used. Dormant and sprouting states of onion bulbs were treated with supposedly saturating doses of ethylene or with the ethylene-action inhibitor 1-methylcyclopropene (1-MCP). Initial sprouting was determined during storage at 18 °C by monitoring leaf blade elongation in a specific size class of leaf sheaths. Changes in ATP content and sucrose synthase activity in the sprout leaves, indicators of the sprouting state, were determined. CO_2 and ethylene production of onion bulbs during storage were recorded.
• *Key results* Exogenous ethylene suppressed sprout growth of both dormant and already sprouting onion bulbs by inhibiting leaf blade elongation. In contrast to this growth-inhibiting effect, ethylene stimulated CO_2 production by the bulbs about 2-fold. The duration of dormancy was not significantly affected by exogenous ethylene. However, treatment of dormant bulbs with 1-MCP caused premature sprouting.
• *Conclusions* Exogenous ethylene proved to be a powerful inhibitor of sprout growth in onion bulbs. The dormancy breaking effect of 1-MCP indicates a regulatory role of endogenous ethylene in onion bulb dormancy.

Key words: Bulb dormancy, *Allium cepa*, onion, sprout growth, ethylene, CO_2 production, respiration, 1-methylcyclopropene.

INTRODUCTION

Sprouting limits the storability of onion (*Allium cepa*) bulbs. At harvest, onion bulbs are usually dormant. Depending on genotype and storage conditions sprout growth is initiated after a certain period of storage (Komochi, 1990). Hormonal control involving a gradual increase of the ratio of sprouting promoters to inhibitors may underlie the loss of dormancy with time (Gubb and MacTavish, 2002). However, the specific roles of different hormones, and especially of ethylene, in the regulation of dormancy and sprouting of onion bulbs is not known. Application of the ethylene-releasing agent ethephon to dry onions generally enhanced sprouting (Abdel-Rahman and Isenberg, 1974; Miedema and Kamminga, 1994; Benkeblia and Selselett-Attou, 1999). In contrast, application of ethephon during bulb development in the field apparently reduced sprouting during storage (Thomas and Rankin, 1982). On the other hand, treatment of dry bulbs with the ethylene-action inhibitor 1-methylcyclopropene (1-MCP) reduced sprout growth in bulbs stored at 4 °C or 12 °C, but not when stored at 20 °C (Chope *et al.*, 2007). Notwithstanding these conflicting scientific reports it has been shown in commercial onion stores that continuous application of ethylene retards sprout growth during cold storage (Johnson, 2006).

Progress in onion dormancy research has been hampered, at least in part, by inadequate experimental methodology and using inappropriate genotypes to study dormancy and sprout growth. Since dormancy of onion genotypes after harvest can vary between none and several weeks it is imperative to select a genotype with a true period of dormancy (Yasin and Bufler, 2007). Moreover, dormancy release in a population of onion bulbs, even from a single harvest, is not uniform and can stretch over several weeks (Yasin and Bufler, 2007). During bulb growth and development sprout leaf initials are formed at the apex of the compressed stem, differentiating into small sprout leaves which encircle the growing point and enclose younger leaves within (Brewster, 1994). These sprout leaves consist of a proximal leaf sheath and a distal leaf blade, separated by a pore (Fig. 1A) through which the next youngermost sprout leaf will ultimately emerge. When sprouting is initiated the leaf blade elongates but not the leaf sheath (De Mason, 1990); elongation of the leaf sheath may be delayed by several weeks or even months, depending on storage conditions (G. Bufler, unpubl. res.). Thus, by monitoring the length of the leaf blade of a specific size class of leaf sheaths initial sprout growth can be detected and tracked during the course of an experiment (Yasin and Bufler, 2007). Lang *et al.* (1987) proposed that dormancy was defined by the non-growth of leaf blades. Using this approach, it has been shown that extension of leaf blades in bulbs of *Allium cepa* 'Copra' was initiated about 8 weeks after harvest and was simultaneous with an increase in respiratory and sink activity in sprout leaves (Yasin and Bufler, 2007).

The present study was undertaken to clarify the role of ethylene in onion bulb dormancy and sprout elongation using the precise methods indicated above for tracking release from dormancy and initial sprout elongation. The effects of both exogenous ethylene and 1-MCP were investigated.

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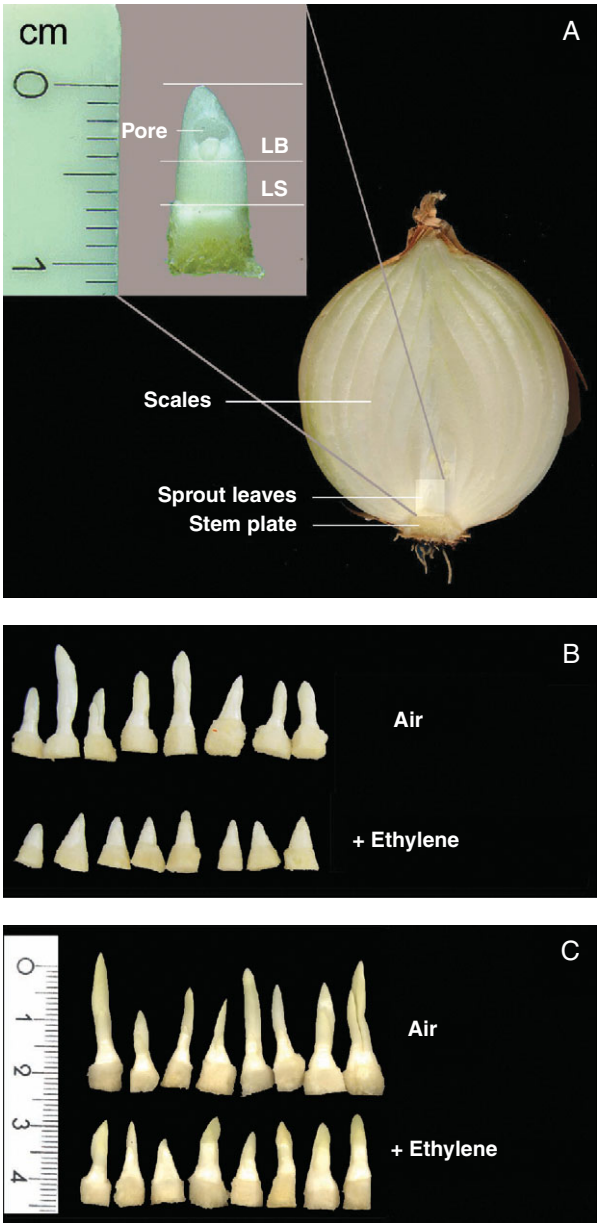

FIG. 1. Photographs and digital scans of sprout leaves isolated from onion bulbs after different treatments. (A) Longitudinal cut through a dormant onion bulb showing the stem plate, with the approximate location of sprout leaves. Insert: dormant bud with sprout leaf visible in the size class (leaf sheath length 2·0–3·5 mm) used in this study. LS, Leaf sheath; LB, leaf blade. (B) Sprout leaves (leaf sheaths 2·0–3·5 mm) isolated from onion bulbs stored at 18 °C and continuously treated with air or 7·2 ± 1·4 μL L^{-1} ethylene until 14 weeks after harvest. Ethylene treatment was started during bulb dormancy 2 weeks after harvest. (C) Sprout leaves (leaf sheaths 2·0–3·5 mm) isolated from sprouting onion bulbs after 4 weeks continuous treatment at 18 °C with air or 7·2 ± 1·4 μL L^{-1} ethylene. Ethylene treatment was started 12 weeks after harvest.

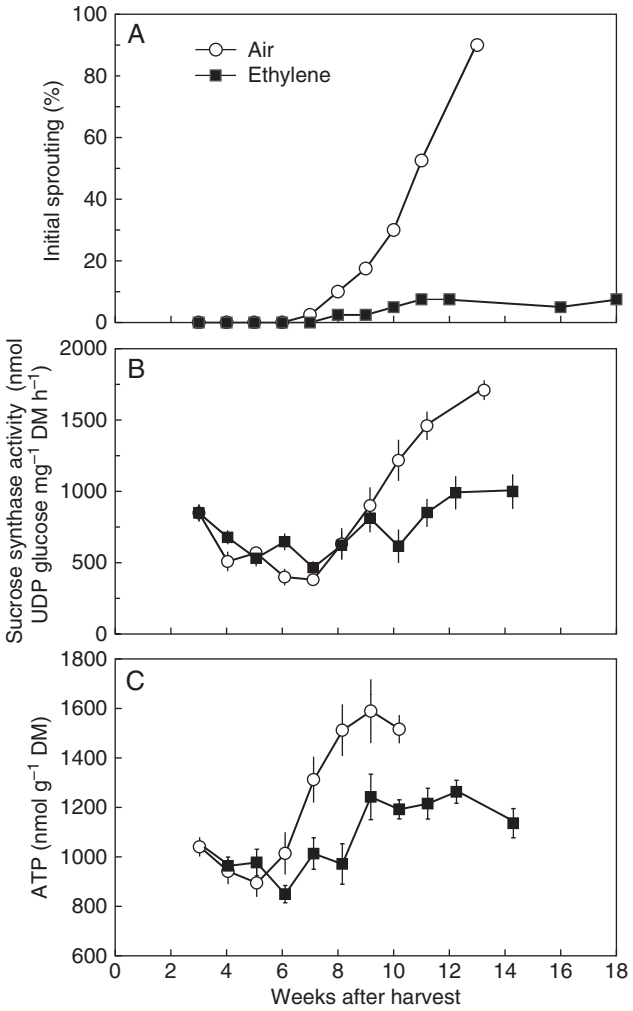

FIG. 2. Changes in (A) percentage initial sprouting, (B) sucrose synthase activity and (C) ATP content in sprout leaves of onion bulbs during storage at 18 °C in air or 7·2 ± 1·4 μL L^{-1} ethylene. Each data point represents the mean of 40 bulbs (A; $n = 40$) or ten sprout leaves (B, C; $n = 10$). Vertical bars indicate ± s.e.

MATERIALS AND METHODS

Plant material

Bulbs of *Allium cepa* L. 'Copra' were grown from seed or transplants at the Experimental Station of Horticulture, University of Hohenheim. Common agricultural practices of fertilization and plant disease control were adopted. Bulbs were harvested when 70–80 % of the plants had collapsed foliage. They were subsequently dried for 2 weeks in shallow trays in a ventilated and temperature-controlled room at 25 ± 2 °C. After drying the foliage was cut off and the bulbs stored at 18 ± 2 °C.

Treatment with ethylene during dormancy

This experiment was carried out in autumn 2006 (Fig. 2) and repeated with minor modifications in autumn 2007 (Fig. 3), producing similar results. Two weeks after harvest onion bulbs were divided between eight 60-L plastic barrels connected to a gas flow-through system. Flow rates (between 10 and 20 L h^{-1}) were adjusted manually during the course of the experiment using needle valves to keep the CO_2 concentration in each of the containers below 0·5 %. The barrels were in a temperature-controlled room (18 ± 2 °C). Four barrels were ventilated with air and the other four containers were ventilated with 7·2 ± 1·4 μL L^{-1} ethylene in 2006 and 10·6 ± 1·4 μL L^{-1} ethylene in 2007. Most known ethylene effects are saturated between

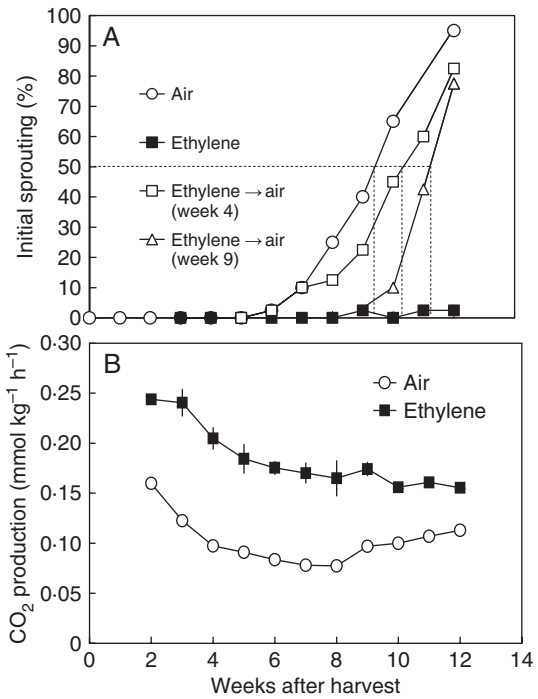

FIG. 3. Changes in (A) percentage initial sprouting and (B) CO_2 production of onion bulbs during storage at 18 °C in air or 10·6 ± 1·4 μL L^{-1} ethylene. Batches of bulbs were transferred at 4 weeks or 9 weeks after harvest from 'ethylene' into 'air'. Dashed lines indicate time of 50 % initial sprouting. Each data point in (A) represents the mean of 40 bulbs ($n = 40$); each data point in (B) represents the mean of four storage barrels ($n = 4$). Vertical bars indicate ± s.e. In the case of the air treatment error bars are too small to be visible.

5 and 10 μL L^{-1} (Abeles *et al.*, 1992). The slight fluctuation in the ethylene concentration in each season was due to the ethylene source during the treatment period; however, the actual ethylene concentration was identical in each of the four barrels. Ethylene was supplied from a compressed nitrogen/ethylene gas mixture subsequently mixed with air. The ethylene concentration in the gas lines entering and leaving the storage barrels was analysed in 1-mL gas samples at weekly intervals by gas chromatography using a Shimadzu GC-6A (Duisburg, Germany) equipped with a flame ionization detector, activated Al_2O_3 in a 1·4-m stainless steel column, and nitrogen as the carrier gas. Ethylene concentration in the air supply was below detection (ethylene detection limit approx. 0·005 μL L^{-1}). Similarly, the CO_2 production of the onion bulbs was determined at weekly intervals by analysing 1-mL gas samples using gas chromatography using a Shimadzu GC-3BT equipped with a thermal conductivity detector, activated charcoal in a 0·7-m stainless-steel column, and helium as the carrier gas. Relative humidity inside the storage room and storage barrels was kept below approx. 80 % and 90 %, respectively.

Depending on the experiment, at certain time points, batches of bulbs were transferred from air to ethylene treatment and vice versa.

Treatment with ethylene during sprouting

This experiment was carried out only in autumn 2006. Sprouting bulbs, previously stored for 12 weeks at 18 ± 2 °C were placed at the same temperature in 60-L plastic barrels and connected to the flow-through system as described above. Measurement of sprout leaf elongation and CO_2 and ethylene concentration was carried out at weekly intervals as described above.

Treatment with 1-MCP

This experiment was carried out in autumn 2004 following a preliminary experiment in 2003 which had similar results. There were two dates of treatment in 2004, 2 and 4 weeks after harvest. Since there was no significant difference between the two dates of treatment only data from the second date of treatment (2004) are presented. Four weeks after harvest, onion bulbs were placed at 20 °C in four sealed 50-L plastic containers. The weight of bound 1-MCP (SmartFresh® powder; a.i. 0·14 %) required to obtain 0·25 μL L^{-1} in a 50-L container was placed in a 10-mL glass syringe and sealed with a septum. After injecting 0·8 mL of warm water and dissolving thoroughly, the entire contents of a syringe (gas and liquid) were injected into each of the four 50-L plastic containers. Control bulbs were enclosed in another four 50-L plastic containers, but without receiving any further treatments. After 5 h incubation onion bulbs from each treatment were removed from the containers and stored in plastic bins at 18 ± 2 °C.

Bulb sampling

At weekly intervals, 40 bulbs were sampled from each treatment (ten bulbs from each of four 60-L plastic containers). After dissecting the centre of the bulbs, excising the appropriate size of sprout leaves and measuring leaf blade length (see below), sprout leaves still attached to a small part of the stem plate (smaller than can be seen in Fig. 1A) were individually frozen in liquid nitrogen, lyophilized and stored at −30 °C until use.

Determination of initial sprouting

Initial sprout growth was determined as previously described (Yasin and Bufler, 2007). When isolating appropriate sprout leaves for freezing in liquid nitrogen outer sprout leaves with leaf sheaths >3·5 mm in length were excised and discarded; only sprout leaves with leaf sheaths between 2·0 mm and 3·5 mm in length (Fig. 1A) were used to monitor leaf blade length using calipers. At harvest (70–80 % foliage collapsed) bulbs were assumed to be dormant. A one-tailed confidence interval ($n = 40$; $P = 0·05$) of the mean leaf blade length from 40 randomly chosen bulbs at harvest or 1 week after harvest was used to define the exclusion limit for dormant bulbs; i.e. bulbs at subsequent sampling dates containing sprout leaves (size class of leaf sheaths 2·0–3·5 mm) with leaf blade lengths exceeding the calculated exclusion limit were denoted as 'initially sprouting' and hence used to calculate percentage initial sprouting. Exclusion limits in 2004, 2006 and 2007 were determined as 4·3 mm, 5·3 mm and 4·8 mm, respectively.

Determination of sucrose synthase activity

Sucrose synthase activity (E.C.2·4·13) was extracted and determined as described previously (Yasin and Bufler, 2007). Lyophilized sprout leaves of individual bulbs were powdered in liquid nitrogen and extracted on ice (100 μL mg^{-1} tissue; 50 mM Hepes–KOH, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, 2·5 mM DTT, 0·05 % TritonX-100, pH 7·5). After centrifugation, an aliquot of the supernatant was dialysed in a micro-dialyser capsule equipped with a Zellutrans dialysis membrane (MWCO 12 000–14 000; Carl Roth GmbH, Karlsruhe, Germany) against buffer (50 mM Hepes–KOH, 5 mM $MgCl_2 \cdot 6H_2O$, 2·5 mM DTT, pH 7·5). Sucrose synthase activity in the extract was determined in the cleaving direction as described by Pak *et al.* (1995). Assay conditions have been optimized to yield maximum enzyme activities. Sucrose synthase activity was expressed on a dry-matter basis as nmol UDP-glucose mg dry $matter^{-1}$ h^{-1}. Sucrose synthase activities represent mean values of sprout leaves isolated from ten individual bulbs ($n = 10$).

Extraction and determination of ATP and ADP

Adenine nucleotides were extracted on ice from powdered lyophilized sprout leaves (10–20 mg) using 5 % (w/v) trichloroacetic acid containing 2 mM EDTA (Yasin and Bufler, 2007). ATP contents were analysed by a luminometric method using an ATP monitoring kit (BioThema AB, Handen, Sweden) based on the firefly luciferase reaction. ATP contents represent mean values of sprout leaves isolated from ten individual bulbs ($n = 10$).

Statistical analysis

Standard error of means (s.e.) are based on measurements of ten individual bulbs ($n = 10$) in the case of sucrose synthase activity and ATP content, and on four storage barrels ($n = 4$) in the case of CO_2 production rate. Percentage initial sprouting is derived from the calculation of confidence limits ($n = 40$, $P = 0·05$) of the blade length of dormant bulbs as described above. When appropriate, treatment means of blade lengths ($n = 40$) were subjected to Student–Newman–Keuls test following ANOVA.

RESULTS

Ethylene treatment

Initial sprouting of previously dormant onion bulbs stored in air started about 7 weeks after harvest and reached almost 100 % 13 weeks after harvest (Fig. 2A). In contrast, 92·5 % of dormant onion bulbs continuously treated with 7·2 μL L^{-1} ethylene did not sprout up to 18 weeks after harvest when the experiment was discontinued (Figs 1B and 2A). Sucrose synthase activity in sprout leaves of air-stored bulbs increased simultaneously to the initiation of sprout growth (Fig. 2B), indicating increased sink activity in this bulb part (Pak *et al.*, 1995; Yasin and Bufler, 2007). Increase of ATP content, another indicator of initiated sprout growth (Yasin and Bufler, 2007), also occurred in the sprout leaves of air-stored bulbs when sprouting was initiated (Fig. 2C). Continuous ethylene treatment, however, largely reduced these increases in sucrose synthase activity and ATP content in sprout leaves (Fig. 2B, C).

The sprouting-inhibiting effect of ethylene disappeared when ethylene was removed, regardless of the time of removal (Fig. 3A). If ethylene treatment was short (2 weeks) and supplied during dormancy (between 2 and 4 weeks after harvest), sprout growth was initiated at about the same time as for air-treated bulbs, though the time to reach 50 % sprouting bulbs was increased by about 1 week (Fig. 3A). If ethylene treatment was extended to 9 weeks after harvest and then stopped, initial sprout growth was detectable 10 weeks after harvest (Fig. 3A). In contrast to its inhibiting effect on sprouting, ethylene significantly stimulated CO_2 production; 10·6 μL L^{-1} ethylene approximately doubled the CO_2 production rate of dormant onion bulbs, although this effect diminished during later stages of the treatment (Fig. 3B). If ethylene treatment of onion bulbs was started 12 weeks after harvest when sprouting was fully initiated it also inhibited sprout growth significantly (Table 1 and Fig. 1C) and stimulated CO_2 production, both in a reversible manner (data not shown). Ethylene-enhanced CO_2 production is a common phenomenon in vegetative tissue (Abeles *et al.*, 1992).

1-MCP treatment

Onion bulbs produce very low amounts of ethylene (air-stored bulbs in these experiments: between 0·002 and 0·005 μL kg^{-1} h^{-1}). To check a possible role of endogenous ethylene in onion bulb dormancy, onion bulbs were treated with the ethylene action inhibitor 1-MCP (Sisler and Serek, 1997). Treatment of dormant bulbs 4 weeks after harvest with 1-MCP caused premature sprouting; initial sprouting of untreated control bulbs started 7 weeks after harvest, whereas in bulbs treated with 1-MCP sprout growth was inititated 5 weeks after harvest (Fig. 4A). This hastening effect of 1-MCP on sprout growth initiation corresponded to a premature increase of sucrose synthase activity 6 weeks after harvest (Fig. 4B). However, the proportion of bulbs sprouting increased more slowly in the 1-MCP treatment than in the

TABLE 1. *Blade lengths of sprout leaves isolated from sprouting bulbs after various times of treatment, starting 12 weeks after harvest, with ethylene (7·2 ± 1·4 μL L^{-1}) or air at 18 °C (leaf sheath length 2·0–3·5 mm)*

	Blade length (mm)	
Days of treatment	Air	Ethylene
0	7·8[a]	–
14	10·3[b]	8·2[a]
28	(10·8 b)*	8·1[a]

Each value represents the mean of 40 bulbs ($n = 40$).

Numbers followed by the same letter are not significantly different ($P = 0·05$; Student–Newman–Keuls test).

*Exact monitoring of leaf blade length at a defined leaf sheath length becomes invalid at this stage of sprout development in air because leaf sheath elongation has started (see Materials and Methods). This is indicated by an apparently stagnating blade length at a fixed sheath length even though sprout leaf growth is in progress.

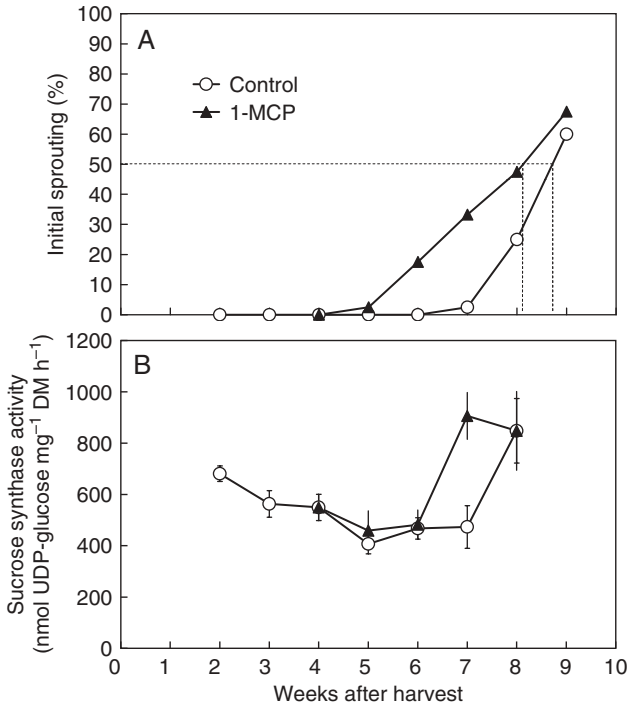

FIG. 4. Changes in (A) percentage initial sprouting of onion bulbs and (B) sucrose synthase activity in sprout leaves after treatment with 1-MCP or untreated (control). Treatment with 1-MCP was 4 weeks after harvest. Dashed lines indicate 50 % initial sprouting. Each data point represents the mean of 40 bulbs (A; $n = 40$) or ten sprout leaves (B; $n = 10$). Vertical bars indicate $\pm$ s.e.

control; bulbs treated with 1-MCP reached 50 % initial sprouting 3·2 weeks after the start of sprouting (week 5) in contrast to 1·7 weeks for control bulbs (week 7; Fig. 4A).

DISCUSSION

These results indicate that ethylene has at least two effects on onion bulbs after harvest: (1) it inhibits sprout elongation and (2) it interferes with dormancy. While the two effects are experimentally entangled it is important to note that each is related to an entirely different physiological process.

Exogenous ethylene inhibited sprout growth of onion bulbs when supplied during sprouting (Table 1) and when supplied during dormancy (Figs 2A and 3A). It is suggested that in both cases ethylene action was primarily on sprout leaf elongation and not on dormancy as could be supposed in the latter case. This view is supported by the observation that after stopping ethylene ventilation during dormancy (week 4) the increase in percentage of sprouting bulbs occurred at the same time as in air-stored bulbs, i.e. at the end of natural dormancy (Fig. 3A). Thus, it seems that the presence of ethylene prevented sprout growth after the bulbs were released naturally from dormancy. Translated into the terminology of Lang *et al.* (1987) this means that in the course of continuous ethylene treatment endogenous bulb dormancy was superseded by ethylene-induced eco-dormancy. As soon as the restraint imposed by exogenous ethylene was removed sprout growth could ensue.

Ethylene is known to inhibit growth of stems, leaves and roots (Abeles *et al.*, 1992). For example, inhibition of potato sprouting by continuous application of ethylene has long been known (Rylski *et al.*, 1974) and has found commercial application (Prange *et al.*, 1998). The nature of growth inhibition of onion sprout leaves by ethylene is not known, except that ethylene inhibits leaf blade elongation (Fig. 1B, C). Recently, it has been demonstrated that growth of dark-grown arabidopsis seedlings is inhibited by ethylene concentrations as low as 0·2 nL L^{-1} (Binder *et al.*, 2004) and its implications for ethylene signalling were discussed (Chen *et al.*, 2005). Ethylene production of onion bulbs during dormancy and initial sprout growth was very low (between 0·003 and 0·005 μL kg^{-1} h^{-1}) but is reported to increase during storage (Abdel-Rahman and Isenberg, 1974). Whether endogenous ethylene is involved in the control of sprout growth during later stages of sprout development, e.g. when sprout leaves press through the tightly closed neck of the bulb, would be an interesting aspect to investigate.

1-MCP has been a powerful tool to demonstrate ethylene effects in various stages of plant development (Huber, 2008). Treatment of dormant onion bulbs with 1-MCP caused breaking of dormancy 2 weeks before natural dormancy release was detectable (Fig. 4A). However, the dormancy-breaking effect of 1-MCP was not very strong, as indicated by the relatively slow increase in percentage of sprouting bulbs compared with untreated bulbs. Possibly only some of the bulbs responded to 1-MCP owing to the non-uniform sprouting behaviour of onion bulbs. On the other hand, a 2-week exposure of dormant onion bulbs to 10·6 μL L^{-1} exogenous ethylene did not affect the duration of dormancy compared with air-treated bulbs (Fig. 3A). It seems, therefore, that relatively low concentrations of endogenous ethylene may be somehow involved in the regulation of dormancy but relatively high concentrations of exogenous ethylene are not. If, however, the ethylene treatment was extended to 9 weeks after harvest, the time to reach 50 % initial sprouting was shorter (2 weeks versus 3 weeks) than for the air treatment (Fig. 3A). In this case all or most of the bulbs may have exited endogenous dormancy 9 weeks after harvest, only prevented from sprout leaf elongation by the presence of ethylene. Like onion bulbs, potato tubers are low producers of ethylene. In developing potato microtubers continuous treatment with inhibitors of ethylene action also resulted in premature sprouting, suggesting a critical role of endogenous ethylene in tuber dormancy (Suttle, 1998). A 3-d exposure of potato tubers to 2 μL L^{-1} ethylene, however, reduced the length of dormancy significantly (Rylski *et al.*, 1974). From these and other data it was concluded that, depending on the concentration and duration of exposure, exogenous ethylene can either hasten or delay tuber sprouting (Rylski *et al.*, 1974; Suttle, 2004). At present a similar conclusion cannot be drawn for onion bulb dormancy.

Although there may be some similarities in the dormancy physiology of onion bulbs and potato tubers, onion bulb dormancy is much less investigated. Nonetheless, the results in this study clearly indicate that exogenous ethylene suppresses sprout growth of onion bulbs by inhibiting leaf blade elongation. The role of ethylene in dormancy control,

however, is far from clear. Endogenous ethylene may be involved in maintenance of bulb dormancy as suggested by the dormancy breaking effect of 1-MCP. It seems likely, however, that ethylene may be one factor in a complex interaction of growth substances still to be identified.

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Ethylene Inhibits Sprouting of Onion Bulbs during Long-term Storage

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Abstract. **The protrusion of sprouts is a major cause of loss of dry onions (*Allium cepa* L.) during extended storage. Sprouting is conventionally suppressed by treating onions with maleic hydrazide (MH) before harvest. More recently, ethylene was reported to inhibit sprout growth in stored onions, but commercial use of ethylene has been limited. Therefore, the objective of this study was to assess the effectiveness of ethylene on sprout suppression of five commercial cultivars of onions during storage and compare its effectiveness with and without MH treatment. Onions treated with MH were stored for up to 9 months at 1 °C in atmospheres with and without ethylene in a series of experiments conducted over six seasons. Differences in sprout elongation and protrusion were observed during storage in air among the five cultivars. Storage in ethylene was effective in inhibiting sprout elongation and root growth of onion bulbs in all cultivars, with concentrations of 7 $\mu L \cdot L^{-1}$ ethylene being more effective than 1 $\mu L \cdot L^{-1}$ ethylene. Delaying application of ethylene by 4 months was less effective in inhibiting sprout elongation than continuous treatment. Ethylene provided greater sprout suppression than MH treatments alone and could serve as a replacement for MH.**

Onions (*Allium cepa* L.) are a popular vegetable consumed throughout the world. In the United States, onions are second only to potatoes among all vegetables sold, comprising more than 10% of total vegetables at retail based on weight (U.S. Department of Agriculture Economic Research Service, 2020). To meet demand throughout the year, onions must be stored for extended periods of time to ensure a constant supply to retail markets. Effective storage at 0 °C and 65% to 75% relative humidity (RH) can maintain good-quality bulbs for ≥5 months (Adamicki, 2016). A significant loss of marketable onions occurs during extended storage due to sprouting from dormancy break and, to a lesser extent, from decay, softening, and rooting (Adamicki, 2005).

Dormancy is the temporary suspension of visible growth of any plant organ containing a meristem and can be classified into three categories (Lang, 1987; Rohde and Bhalerao, 2007). Endodormancy initiates when the signal to suspend growth originates from the organ of interest. Paradormancy occurs in the organ of interest due to signals originating from an external organ. Ecodormancy is in response to unfavorable environmental conditions. Endo- and ecodormancy in postharvest onion bulbs are the primary regulators for arrested growth (Pak et al., 1995). Growers harvest onions once they complete the bulbing phase as highlighted by the cessation of growth and senescence of aboveground foliage. This signals that the bulbs have transitioned to an endodormant state. Bulbs transferred to cold storage remain endodormant for up to 3 weeks, after which, the bulbs break endodormancy to resume preharvest levels of cellular division and are capable of rooting (Pak et al., 1995), increased respiration (Ward and Tucker, 1976), and expansion of sprout leaves near the center of the bulb (Yasin and Bufler, 2007). The rate and timing of the resumption of growth varies significantly among cultivars but can be delayed by holding onions at 0 to 2 °C (Adamicki, 2005).

Storage can be extended by forcing onion bulbs into an ecodormant state through exposure to threshold low temperatures or from the preharvest application of chemical inhibitors such as maleic hydrazide (MH) (Adamicki, 2005; El-Otmani et al., 2003; Ilić et al., 2011). MH is a growth inhibitor that suppresses terminal meristem activity and internodal elongation

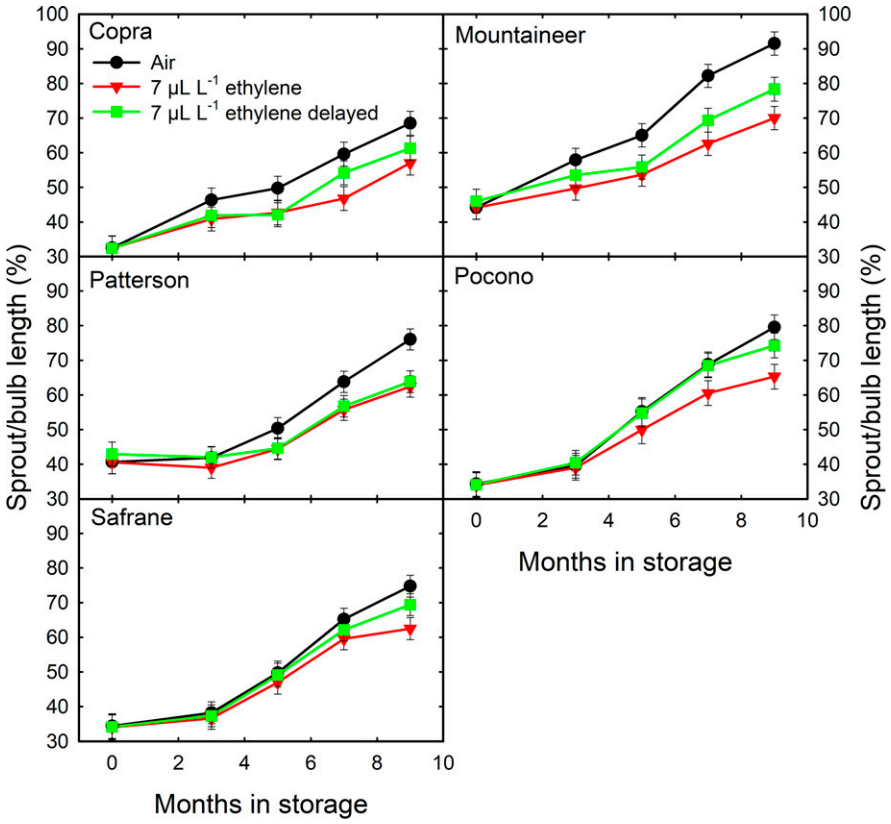

Fig. 1. The % sprout length relative to the bulb height of 'Copra', 'Mountaineer', 'Patterson', 'Pocono', and 'Safrane' onions from the 2015 to 2020 seasons during storage at 1 °C for up to 9 months in air, 7 $\mu L \cdot L^{-1}$ ethylene or air for 4 months, followed by 7 $\mu L \cdot L^{-1}$ ethylene (delayed). Onions were treated with maleic hydrazide in the field before harvest. Error bars represent 2 × SE.

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of plants (Naylor and Davis, 1950). Alternative approaches for the long-term storage of produce need to be identified as the use of chemicals in food production comes under increased scrutiny.

Recently exposure of onions to ethylene during storage was reported to reduce sprouting (Bufler, 2009; Ohanenye et al., 2019), and in Europe, the use of ethylene in commercial storage is being promoted as a method of sprout suppression. In North America, the use of ethylene to inhibit sprouting of onions has received little attention. It has also not been reported how the effectiveness of ethylene inhibition on sprouting compares to that of MH treatments. Further, whether the combination of MH and ethylene has an additive effect on the reduction of sprouting has not been researched. The objective of this study was to assess the effectiveness of ethylene on the suppression of sprouting of onions during long-term cold storage and whether it can supplement or replace the more conventional MH treatment.

Material and Methods

Onions. For a series of experiments conducted over six seasons, onions were commercially harvested from production fields from 2015 to 2020. A total of five yellow onion cultivars were assessed, which included 'Copra' (Bejo Seeds Inc., Geneva, NY; three seasons), 'Mountaineer' (American Takii, Inc., Salinas, CA; four seasons), 'Patterson' (Bejo Seeds Inc.; five seasons), 'Safrane' (Bejo Seeds Inc.; four seasons), and 'Pocono' (Seminis Vegetable Seeds Inc., Tifton, GA; two seasons). Unless stated otherwise, onions were treated with 2.26 kg·ha^{-1} MH (Arysta LifeScience Canada Inc., Guelph, ON). Treatment occurred in the field when 40% to 50% of the tops had fallen 10 to 14 d before harvest. Onions were harvested in mid-September to early October. After harvest, bulbs were cured for 10 to 14 d under a continuous flow of warm dry air. The air temperature was held at 27 to 29 °C for ≈3 d and then lowered to 21 to 24 °C with an RH of 40%. After curing, onions were cooled to ambient temperature with 65% to 70% RH and then transferred to commercial cold storage at 0 to 1 °C and 70% to 75% RH until being placed under experimental storage conditions in late November. For each storage treatment, onions of each cultivar were sampled from three fields. Onions were sampled from different locations within a field in instances where less than three fields of a specific cultivar were available.

To test whether continuous ethylene treatment during storage could replace MH treatments, onion samples were manually harvested in the 2018 and 2019 seasons from sections of fields that were not sprayed with MH as well as adjacent sprayed sections. Cultivars included Pocono (2019) and Safrane (2018 and 2019). Onion samples (22.7 kg) were placed in mesh bags, transferred to a bin, and cured with the rest of the crop.

Storage conditions. Onions were stored at the Kentville Research and Development Center (Kentville, Canada) from December through August in custom-made top-loading 0.34-m^3 stainless steel CA chambers. A tray containing 500 g of $CaCl_2$ was placed in the bottom of each chamber to control RH. Onions were sorted to remove any visibly defective onions and ≈3.6 kg of samples, each comprising 20 to 30 onions, were placed in mesh bags. Four mesh bags (one for each evaluation) for each cultivar/field were placed into bushel plastic bins and bins were placed in storage chambers, which each held four bins and represented a treatment replication. The chambers were flushed with air at 2 L·min^{-1} for 2 to 3 d before establishing ethylene atmospheres. For ethylene treatments, compressed ethylene gas was mixed with air in a manifold to achieve the desired concentration before introduction to the designated chamber. Chambers were flushed with 2 L·min^{-1} of air or ethylene in air on a schedule of 6 h on/6 h off to maintain a constant ethylene concentration and flush out CO_2. Ethylene concentration was monitored every 2 h using an automated sampling system interfaced with a Shimadzu gas chromatograph with a photoionization detector (GC-8A; Shimadzu Scientific Instruments, Columbia, MD) and an Alumina F1 60/80 mesh packed column (Chromatographic Specialties Inc., Brockville, ON). Chambers were held in a 1 °C cold room. Actual temperature and RH were monitored in chambers using data loggers and temperature in chambers averaged 1.8 ± 0.4 °C and RH averaged 71.6 ± 6.8%.

Treatments. Each season, onions were stored in air or 7 µL·L^{-1} ethylene for up to 9 months. Abeles et al. (2012) reported that saturating effects of ethylene were considered to occur at ethylene concentration between 5 and 10 µL·L^{-1}, and Bufler (2009) reported that 7 µL·L^{-1} ethylene was effective in inhibiting onion sprouting. Therefore, 7 µL·L^{-1} ethylene was chosen as a standard ethylene treatment concentration and was applied each season.

A delayed ethylene treatment was applied in the 2015, 2016, 2017, 2018, and 2019 seasons, where onions were held in air for the first 4 months and then held in 7 µL·L^{-1} ethylene for the remainder of storage starting at the beginning of April. To explore the effectiveness of a lower ethylene concentration, in the 2015 season, onions were held in 1 µL·L^{-1} ethylene for the entire storage period. In the 2018 and 2019 seasons, onions with and without MH treatment were stored in air, 7 µL·L^{-1} ethylene, or air for 4 months followed by 7 µL·L^{-1} ethylene (delayed). In the 2020 season, to assess potential shelf life after storage, 'Patterson' and 'Mountaineer' onions were stored in air or 7 µL·L^{-1} ethylene, and onions were evaluated immediately after storage or after an additional 4 weeks at ambient conditions, which averaged 20.5 ± 0.9 °C and 65.0 ± 12.2% RH.

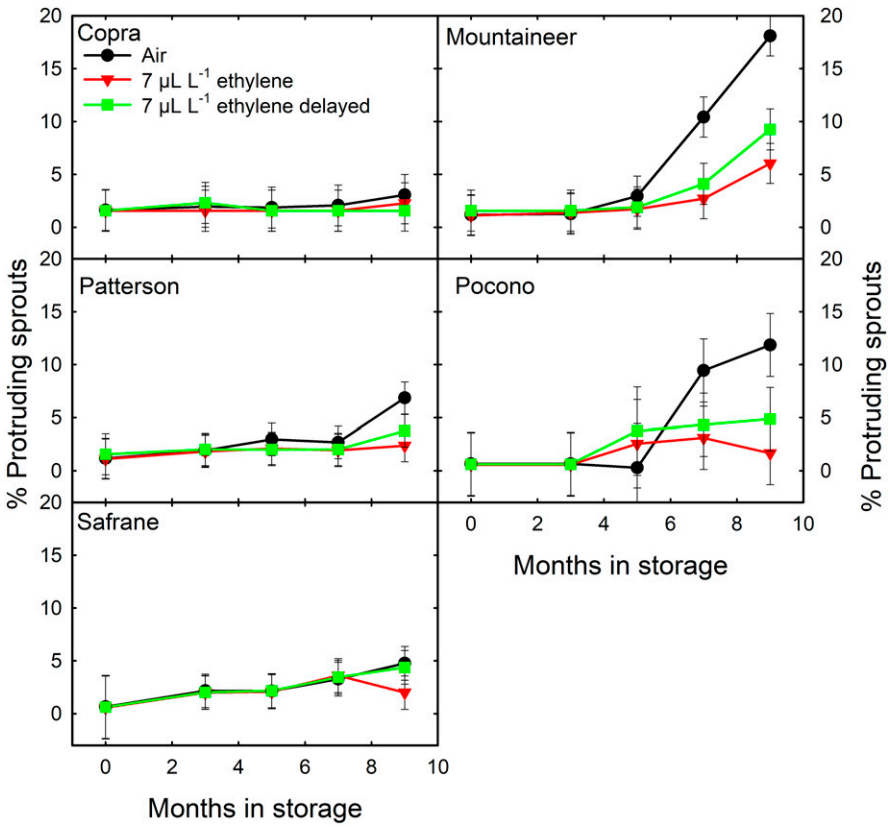

Fig. 2. The % bulbs with protruding sprouts of 'Copra', 'Mountaineer', 'Patterson', 'Pocono', and 'Safrane' onions from the 2015 to 2020 seasons during storage at 1 °C for up to 9 months in air, 7 µL·L^{-1} ethylene or air for 4 months followed by 7 µL·L^{-1} ethylene (delayed). Onions were treated with maleic hydrazide in the field before harvest. Error bars represent 2 × SE.

Onion evaluation. Onions were removed from storage for evaluation after 0, 3, 5, 7, and 9 months for each season. At each removal, a subsample of the onions consisting of a mesh bag containing ≈3.6 kg of onions was removed from the treatment chambers and onion bulb quality was assessed. Upon removal, onions were placed under plastic to prevent condensation and held overnight to warm to room temperature. Onions were then weighed to determine weight loss and assessed for the presence of decay and protruding sprouts. Decay caused by both fungal and bacterial pathogens was variable among onion cultivars, fields, and seasons and included *Fusarium* basal rot, neck rot, sour skin, and soft rot. Some decay was not apparent until bulbs were cut. Any amount of decay was considered unacceptable, and decayed bulbs were counted and discarded. To assess sprout growth, onion bulb height was measured from stem plate to shoulder and the onion was then cut longitudinally, and sprout length was measured from stem plate to tip of the sprout. Bulbs expressing rooting were counted in the 2018 and later seasons. Desiccation of bulbs was not observed, except for those expressing substantial sprouting.

Statistical analysis. For each season the experimental design for storage was a latinized block, where each block comprised one of the three fields that served as replicates for each cultivar, and storage chambers were assigned to each of the storage treatments. Each of the four totes within a chamber contained four bags of onions, one for each of the four storage removals. Onions in a tote represented one cultivar for one field. Data were analyzed by analysis of variance using Genstat for Windows 21st Edition (VSN International, Hemel Hempstead, UK). A meta-analysis of the combined data from the 2015 to 2020 seasons was conducted using the restricted maximum likelihood procedure in Genstat to provide a robust assessment of the continuous and delayed 7 $\mu L \cdot L^{-1}$ ethylene treatments. A mixed-model analysis was used with a meta-structure on seasons. A random model of Year/Chamber.Rep was used with a fixed model of storage treatment × cultivar × removal. Large residuals were removed before analyses and F probabilities <0.05 were considered significant.

Results and Discussion

Sprout elongation in air. At the beginning of storage, % sprout/bulb length ranged from 32.6% in 'Copra' to 44.2% in 'Mountaineer' based on the meta-analysis of onions stored over six seasons (Fig. 1). Initial sprout length of 'Pocono' and 'Safrane' was similar to that of 'Copra', whereas that of 'Patterson' was similar to 'Mountaineer'. The % sprout/bulb length of all cultivars increased during 9 months of storage, although there appeared to be a delay in elongation during the first 3 months of storage in 'Patterson' and to a lesser degree in 'Pocono' and 'Safrane' onions. This delay in elongation suggests that the duration of endodormancy varied among the cultivars in this study. The meta-analysis revealed a significant interaction between onion cultivars and storage time ($P < 0.001$). After 9 months of storage, sprout length increased 132% in 'Pocono', followed by 'Safrane' (117%), 'Copra' (110%), 'Mountaineer' (107%), and 'Patterson' (87%). Protruding sprouts were not apparent among bulbs from most cultivars until 7 months of storage, when air-stored 'Mountaineer' and 'Pocono' onions both had more than 9% bulbs with visible sprouts (Fig. 2). After 9 months of storage, the percentage of onions with protruding sprouts was greatest in air-stored onions of the cultivar 'Mountaineer' (18.1%), followed by 'Pocono' (11.9%), 'Patterson' (6.9%), 'Safrane' (4.8%), and 'Copra' (3.1%).

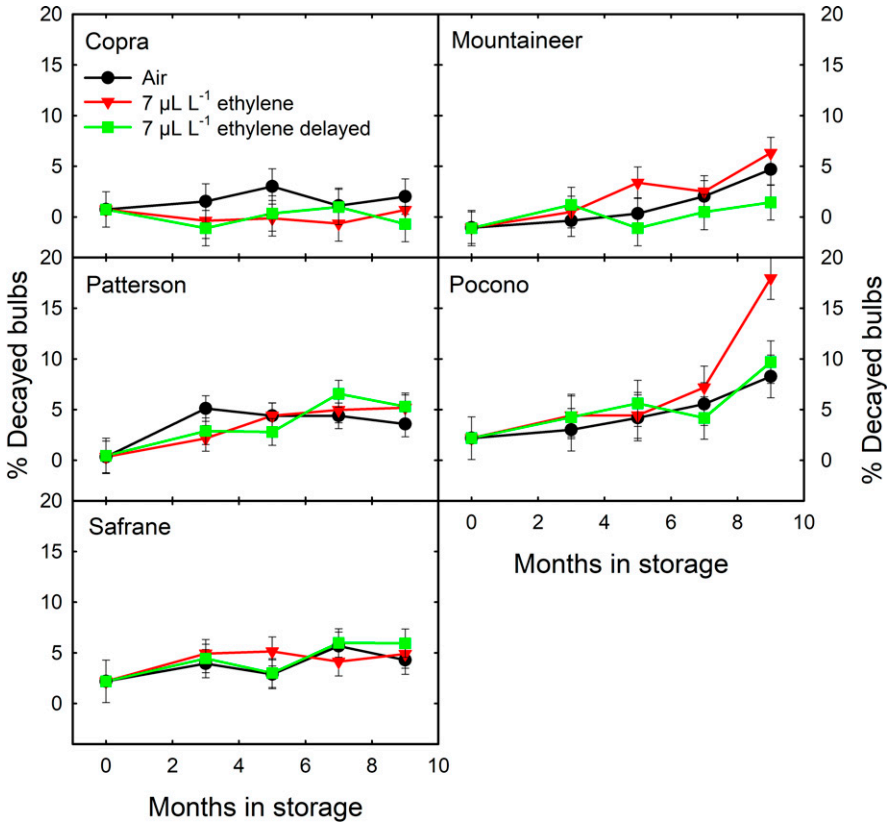

Fig. 3. The % bulbs with decay of 'Copra', 'Mountaineer', 'Patterson', 'Pocono', and 'Safrane' onions from the 2015 to 2020 seasons during storage at 1 °C for up to 9 months in air, 7 $\mu L \cdot L^{-1}$ ethylene or air for 4 months, followed by 7 $\mu L \cdot L^{-1}$ ethylene (delayed). Onions were treated with maleic hydrazide in the field before harvest. Error bars represent 2 × SE.

Sprouting following prolonged storage varied substantially among onion cultivars (Aoba, 1955; Grevsen and Sorensen, 2004; Miedema, 1994). Aoba (1955) reported the cultivar-dependent rate of sprout elongation among three cultivars commenced after 1 to 2 months of normal storage conditions. In a review of onion dormancy, Komochi (1990) concluded the rate of sprout elongation was more important than the length of endodormancy in determining sprout resistance of onions. Brewster (1987) described sprout elongation as a continuous process that accelerated with storage time. We observed that after 3 months of storage, sprout elongation increased at a constant rate ranging from 3.2% to 6.5% per month among the cultivars. 'Copra' had the lowest rate of sprout elongation as well as the least number of bulbs with protruding sprouts.

Ethylene. Sprout growth was reduced in all onions stored in 7 $\mu L \cdot L^{-1}$ ethylene atmospheres compared with onions stored in air (Fig. 1).

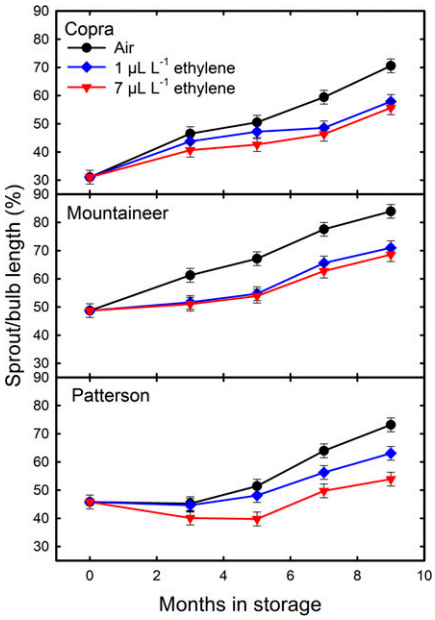

Fig. 4. The % sprout length relative to the bulb height of 'Copra', 'Mountaineer', and 'Patterson' onions from the 2015 season during storage at 1 °C for up to 9 months in air, 1 $\mu L \cdot L^{-1}$ ethylene, or 7 $\mu L \cdot L^{-1}$ ethylene. Onions were treated with maleic hydrazide in the field before harvest. Error bars represent 2 × SE.

After 9 months of storage, the % sprout/bulb length of onions stored in 7 $\mu L \cdot L^{-1}$ ethylene averaged 16.4% to 23.5% less than air-stored onions in the five cultivars studied. Storage in 7 $\mu L \cdot L^{-1}$ ethylene also reduced sprout emergence, which after 9 months of storage was 26.1%, 66.7%, 65.5%, 86.1%, and 58.2% less than air stored 'Copra', 'Mountaineer', 'Patterson', 'Pocono', and 'Safrane' onions, respectively (Fig. 2). This inhibition of sprout growth was previously reported for 'Copra' onions held in 7 or 10 ppm ethylene at 18 °C (Bufler, 2009) and 'Sherpa' onions held in 10 $\mu L \cdot L^{-1}$ ethylene at 1 °C (Alamar et al., 2020; Ohanenye et al., 2019).

When the application of 7 $\mu L \cdot L^{-1}$ ethylene was delayed until the end of the fourth month of storage, sprouting was delayed similarly to that observed in the continuous 7 $\mu L \cdot L^{-1}$ ethylene treatment in 'Patterson' but to a lesser degree in other cultivars (Figs. 1 and 2). After 9 months of storage, the % sprout/bulb length was 7.5%, 11.9%, 13.7%, and 11.1% greater in 'Copra', 'Mountaineer', 'Pocono', and 'Safrane' onions subjected to the delayed ethylene treatment compared with those held in continuous 7 $\mu L \cdot L^{-1}$ ethylene (Fig. 1). The lack of difference in 'Patterson' between the delayed and continuous ethylene treatment may be a result of the endodormancy observed during the first 3 months of storage that was not observed in the other cultivars. The delayed ethylene treatment was also less effective compared with the continuous treatment in suppressing sprout emergence of most cultivars (Fig. 2). After 9 months of storage, onions subjected to the delayed ethylene treatment averaged 3.2%, 1.4%, 3.2%, and 2.4% more bulbs with protruding sprouts in 'Mountaineer', 'Patterson', 'Pocono', and 'Safrane', respectively, than in onions subjected to ethylene throughout storage, whereas differences in 'Copra' were <1% ($P < 0.001$). Bufler (2009) also observed that a delayed ethylene treatment reduced sprout elongation and suggested that although the presence of ethylene inhibited elongation, this inhibition was lost when onions were removed from the ethylene atmosphere.

Decay was unaffected by ethylene treatment (Fig. 3). With the exception of 'Pocono', decayed onions comprised <7% in all treatments and storage times. After 9 months of storage in 7 $\mu L \cdot L^{-1}$ ethylene, decay of 'Pocono' bulbs comprised 18% of the bulbs compared with 8.3% of bulbs in air or 9.7% of bulbs held in the delayed ethylene treatment. Ethylene treatments had no significant effect on fresh weight loss during storage. After 9 months of storage, weight loss averaged 8.0%, 7.6%, and 8.8% in air, continuous 7 $\mu L \cdot L^{-1}$ ethylene, and delayed 7 $\mu L \cdot L^{-1}$ ethylene treatments, respectively.

Ethylene concentration. In the 2015 season, continuous treatment with 1 $\mu L \cdot L^{-1}$ ethylene was compared with 7 $\mu L \cdot L^{-1}$ ethylene for three onion cultivars (Fig. 4). Differences in % sprout/bulb length had a significant interaction among cultivars and treatments ($P = 0.006$), with reduction of sprout elongation by the 1 $\mu L \cdot L^{-1}$ ethylene treatment being less in 'Patterson' onion than those of 'Copra' or 'Mountaineer'. After 9 months of storage, onions held in 1 $\mu L \cdot L^{-1}$ ethylene had 4.0%, 3.5%, and 17.9% greater % sprout/bulb length than those held in 7 $\mu L \cdot L^{-1}$ ethylene in 'Copra', 'Mountaineer', and 'Patterson' onions, respectively. The effect of ethylene treatments on protruding sprouts or decay did not differ significantly. Because these preliminary results indicated that 1 $\mu L \cdot L^{-1}$ ethylene was less effective in inhibiting sprout elongation than 7 $\mu L \cdot L^{-1}$ ethylene, later seasons continued to focus on the 7 $\mu L \cdot L^{-1}$ ethylene treatment. Although no other studies report direct comparisons of ethylene concentrations on sprout inhibition, Bufler (2009) treated 'Copra' onions with 7.2 or 10.6 $\mu L \cdot L^{-1}$ ethylene in different seasons and observed a similar inhibition of sprout growth.

Maleic hydrazide. In the 2018 and 2019 seasons, onions were harvested both with and without MH treatment and subjected to storage in air or ethylene to assess whether ethylene treatments could be an effective replacement for MH. Sprouting of 'Safrane' onions was reduced by both MH and ethylene treatments (Fig. 5). Air-stored onions treated with MH had 22% lower % sprout/bulb length than onions not treated with MH after 9 months of storage. A greater reduction in % sprout/bulb length was observed in onions held in continuous 7 $\mu L \cdot L^{-1}$ ethylene in both MH treated and untreated onions, which were 40% and 35% lower than untreated air-stored onions after 9 months of storage, respectively. However, when ethylene treatment was delayed 4 months, sprout growth was greater in onions that were not treated with MH than in those that received the MH treatment.

The number of onions with protruding sprouts reflected these effects on sprout growth. MH treatment reduced the number of bulbs with protruding sprouts in 'Safrane' onions stored in air and the delayed ethylene treatment. However, onions subjected to continuous ethylene had <2% bulbs with protruding sprout throughout storage regardless of MH treatment ($P = 0.013$). Rooting of bulbs was also inhibited by both MH and ethylene treatments. The combined treatment of MH and continuous 7 $\mu L \cdot L^{-1}$ ethylene totally inhibited root formation in 'Safrane' onions. Those treated with only MH or continuous 7 $\mu L \cdot L^{-1}$ ethylene had 8% or 13% of bulbs having roots after 9 months of storage, respectively. In contrast, more than 58% of untreated air-stored onions developed roots after 9 months. When ethylene was delayed, 38% of onions not treated with MH developed roots ($P = 0.002$). Similar results were reported in 'Sherpa' onions where storage in 10 $\mu L \cdot L^{-1}$ ethylene at 1 °C for 35 weeks resulted in 18% of bulbs forming roots as compared with 63% of air stored bulbs (Cools et al., 2011). Decay and weight loss of stored onions were not significantly affected by the MH or ethylene treatments.

In contrast to 'Safrane' onions, sprouting and root growth of untreated 'Pocono' onions from the 2019 season remained low during storage.

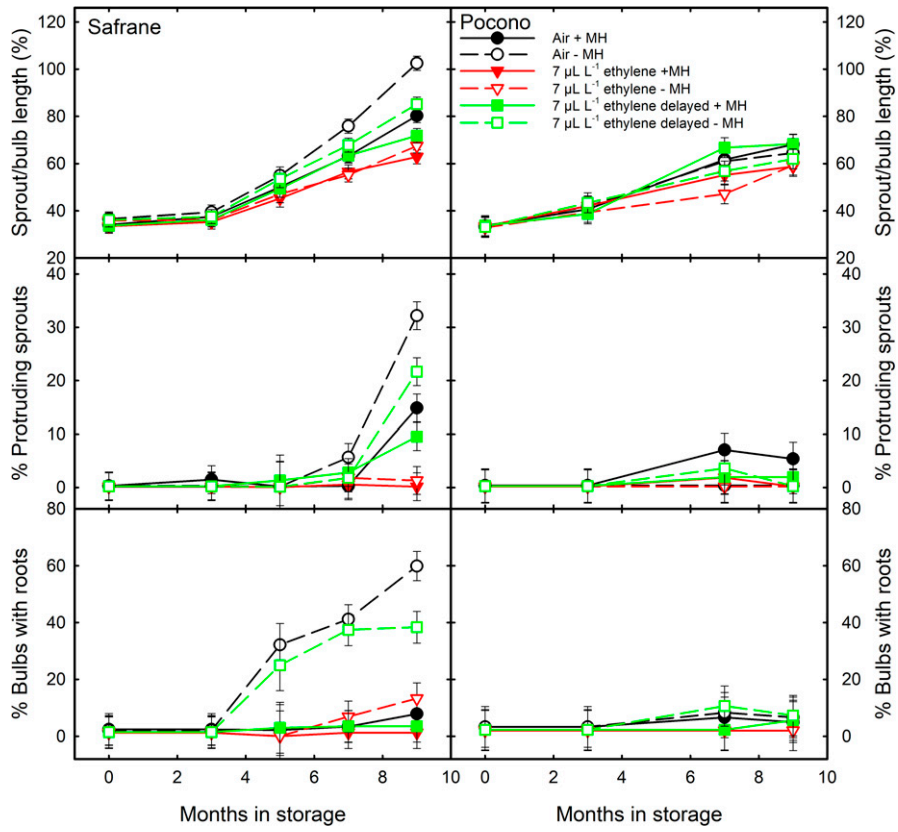

Fig. 5. The effect of preharvest maleic hydrazide (MH) treatment on % sprout length relative to the bulb height, % bulbs with protruding sprouts, and % bulbs with roots of 'Pocono' and 'Safrane' onions from the 2018 and 2019 seasons during storage at 1 °C for up to 9 months in air, 7 $\mu L \cdot L^{-1}$ ethylene, or air for 4 months, followed by 7 $\mu L \cdot L^{-1}$ ethylene (delayed). Error bars represent 2 × SE.

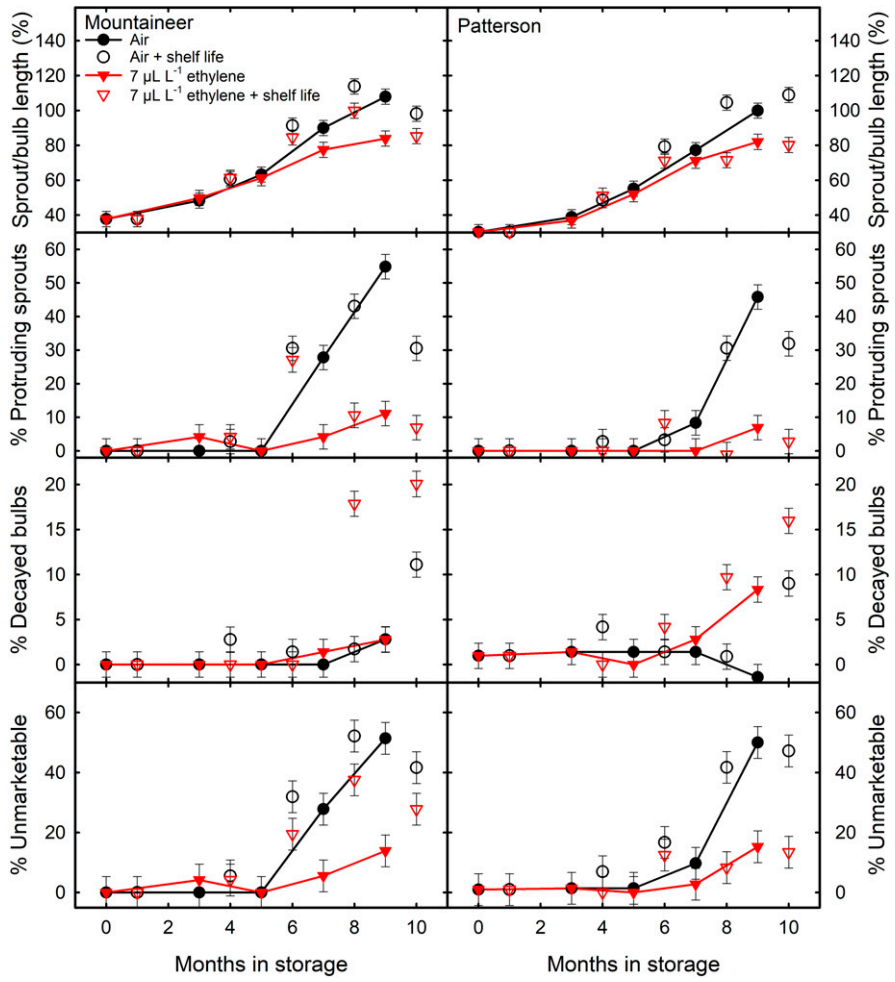

Fig. 6. The % sprout length relative to the bulb height, % bulbs with protruding sprouts, % bulbs decayed, and % unmarketable bulbs of 'Mountaineer' and 'Patterson' onions from the 2020 season during storage at 1 °C for up to 9 months in air or 7 µL·L^{-1} ethylene with or without an additional 4 weeks in air at 20 °C (shelf life). Onions were treated with maleic hydrazide in the field before harvest. Error bars represent 2 × SE.

No significant differences between onions treated with MH or ethylene treatments on sprout growth, rooting, or decay were observed (Fig. 5).

Shelf life. In the 2020 season, quality retention of MH treated 'Mountaineer' and 'Patterson' onions following storage in air or continuous 7 µL·L^{-1} ethylene was determined after an additional 4 weeks in air at room temperature, which averaged 20 °C. After three months of storage, the additional four weeks of ambient storage did not significantly increase unmarketable onions, which averaged less than 6% and 7% of 'Mountaineer' and 'Patterson' onions, respectively (Fig. 6). However, when onions stored for ≥5 months were removed from 1 °C storage and held an additional 4 weeks, the percentage of unmarketable onions increased significantly as a result of increased sprout elongation ($P < 0.001$), sprout protrusion ($P < 0.001$), and bulb decay ($P < 0.001$). Unmarketable onions were significantly less in onions held in 7 µL·L^{-1} ethylene than those held in air for both 'Mountaineer' and 'Patterson' due to a reduction in sprout elongation ($P = 0.017$) and protrusion ($P = 0.014$). After 7 and 9 months of storage, the percentage of bulbs with protruding sprouts did not increase significantly in the onions held in 7 µL·L^{-1} ethylene during the additional 4-week shelf-life treatment. In contrast, onions stored in air had 24% to 33% more protruding sprouts than onions that had been stored in 7 µL·L^{-1} ethylene. However, after 7 or 9 months of storage, onions stored in ethylene developed 7% to 16% more decayed bulbs after 4 weeks at 20 °C than air stored onions. In 'Patterson' an increase in decayed bulbs (8%) was also observed immediately after 9 months of storage. Similarly, in the meta-analysis of onion cultivars stored over six seasons, incidence of decay of 'Pocono' onions increased after 9 months of storage in 7 µL·L^{-1} ethylene (Fig. 3). Further assessment of residual effects of ethylene treatment during different ambient shelf-life conditions and durations should be conducted to further assess commercial benefits of ethylene treatments.

Greater levels of both fungal and bacterial decay after prolonged storage in ethylene became apparent after ambient storage and could be a result of ethylene-induced increase in decay susceptibility of the onions. Ethylene stimulates senescence in fruit and vegetative tissues of plants resulting in increased susceptibility to decay (Saltveit, 1999). Previous studies report ethylene enhanced decay incidence and severity in nonclimacteric fruit. The use of ethylene to de-green oranges [*Citrus sinensis* (L.) Osbeck] increased stem-end rot caused by *Diplodia natalensis* P. Evans (Brown and Lee Hyoung, 1993) and severity of gray mold in strawberries (*Fragaria ×ananassa* Duch.) inoculated with *Botrytis cinerea* Pers.:Fr. (El-Kazzaz et al., 1983). Similarly, removing ethylene from the storage environment reduced strawberry fruit susceptibility to gray mold (Wills and Kim, 1995) and peduncle rot caused by *Penicillium* in stored pineapples (*Ananas comosus* L.) (Sabater-Vilar et al., 2018). In contrast, continuous exposure of stone fruit and grapes (*Vitis vinifera* L.) to ethylene did not increase the incidence or severity of brown rot (*Monilinia fructicola*) or gray mold (Palou et al., 2003), whereas ethylene induces resistance in *Nicotiana benthamiana* to *Botrytis* (Chagué et al., 2006). The prolonged exposure to ethylene in our study may negate any potential beneficial effects in mitigating decay. Ethylene-induced decay appeared to be cultivar dependent and was not observed until after ≥8 months of storage and shelf-life evaluation. Additional assessments may be needed to evaluate whether this observed effect would be a significant concern under commercial onion storage and marketing.

Conclusion

Storage of onions in atmospheres containing ethylene appears to be an effective technology to prolong storage life with acceptable quality being maintained for up to 7 months in all cultivars assessed in this study. An atmosphere of 7 µL·L^{-1} ethylene was effective in prolonging storage life of MH treated onions by inhibiting sprout elongation and root growth. Although differences in sprout elongation were observed among the five cultivars evaluated in this study, ethylene was effective in reducing sprout elongation in all cultivars. Delaying the application of ethylene by 4 months also delayed sprouting but was not as effective as the continuous treatment. Ethylene provided greater benefit than could be obtained by MH treatments alone and could serve as a replacement for MH. For maximum effectiveness, ethylene exposure must be maintained throughout storage. Furthermore, the residual effectiveness of sprout inhibition on onions stored in 7 µL·L^{-1} ethylene without MH requires further evaluation.

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