Int J Seed Spice, 14(1), June, 2024: 1-15 DOI: https://doi.org/10.56093/IJSS.v14i1.1

Analytical methods and regulatory perspectives on residual ethylene oxide and its derivatives in food products

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Received: 27 August 2024
Revision: 29 September 2024
Accepted: 21 October 2024

Citation
Talaviya, H., Korat, H., Hasan, M., Mahatma, M.K. and Tripathi, K.P. 2024. Analytical methods and regulatory perspectives on residual ethylene oxide and its derivatives in food products. *Int J Seed Spice,* 14 (1): 1-15

DOI
https://doi.org/10.56093/IJSS.v14i1.1

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Abstract

Ethylene oxide (EtO), a crucial industrial chemical, has diverse applications ranging from the production of ethylene glycol to its use as a sterilizing agent for medical equipment and a fumigant for food and textiles. However, its potential health risks, particularly carcinogenicity, have raised significant concerns. This review explores the synthesis, chemical properties, and metabolism of EtO, focusing on its transformation into 2-chloroethanol (2-CE). Here we described the industrial uses of EtO and the implications of its residues in food products, particularly spices, which have recently faced regulatory scrutiny in various global markets. The review highlights the challenges in accurately detecting EtO and 2-CE residues, presenting various analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and headspace-GC-MS. Additionally, we examine the regulatory landscape, noting the disparity in maximum residue limits (MRLs) across different regions and the need for harmonization. The aim of this review is to provide a comprehensive understanding of the health risks posed by EtO, particularly in the context of food safety, and to examine the current methods for detecting its residues. Additionally, the purpose is to address the inconsistencies in regulatory frameworks across different regions and recommend harmonization efforts to ensure safer food practices. The review emphasizes the importance of developing standardized analytical methods and provides recommendations for international regulatory bodies to ensure food safety.

Keywords: Ethylene oxide, 2-chloroethanol, Ethylene, maximum residue limits, Food, Spices

Introduction

Ethylene oxide was synthesized in 1859 by Charles-Adolphe Wurtz, a French chemist, through a reaction between 2-chloroethanol and a base (potassium hydroxide). In World War I, it gained industrial utility because used as a precursor for coolants such as ethylene glycol and a chemical weapon gas known as mustard gas (Dever *et al*., 2000; Yue *et al.*, 2012). BASF (Badische Anilin-und Sodafabrik, Germany) established the ethylene oxide plant in 1914 by using of chlorohydrin method and this replaces by the direct oxidation of ethylene (Lucky *et al*., 2022; Kilty and Sachtler,1974). Currently, direct oxidation is widely used for the production of ethylene glycol, glycol ethers, and ethanolamine as well as fumigants for food and textiles to control pests and sterilizing agents for medical equipment. (Mendes *et al*., 2007). The man-made chemical ethylene oxide (EtO, or EO for short) is colourless and exists as a gas at room temperature (above 10 °C). It is a cyclic ether and the simplest epoxide: a three-membered ring consisting of one oxygen atom and two carbon atoms. It is precarious and rapidly converts in the environment to 2-chloroethanol, among other substances (Luttrell, 2008). Due to its instability, it does not persist for long in the environment. As the conversion of EtO to 2-chloroethanol (2-CE) happens relatively quickly, only 2-CE is usually detected. Small amounts of EtO are also produced when tobacco is burned. Very small amounts of it can be found in nature (Dever *et al*., 2000). EtO is produced in the human body from the oxidation of ethylene, and biological processes producing endogenous ethylene have been identified, such as lipid peroxidation, methionine and heme oxidation, and the metabolic activity of intestinal bacteria (Kilty and Sachtler,1974). However, the contribution of these processes to internal levels of ethylene or ethylene oxide has not been directly quantified (Arshad and Frankenberger (2002). EtO residue analysis in spices and seed spices is essential for ensuring consumer safety, regulatory compliance, and especially global trade**.** EtO, a fumigant used for sterilization, can leave behind harmful residues that pose significant health risks, including cancer and reproductive issues. By conducting EtO residue analysis, food producers can demonstrate their commitment to food safety, protect public health, and maintain consumer trust. Additionally, EtO residue analysis helps ensure compliance with international regulations, which often require certification of EtO-free products safety (Stupák *et al*., 2021; Tateo and Bononi, 2006). This is particularly important for the global trade of spices and seed spices, as importing countries often have strict requirements regarding food.

Chemical Properties and Industrial Uses of Ethylene Oxide (EtO)

Characteristics: it is a colourless gas at room temperature with a simple three-membered ring structure containing one oxygen atom and two carbon atoms. It is highly unstable and readily converts to 2-chloroethanol (2-CE) in the environment (Luttrell, 2008) (table 1 and fig 1).

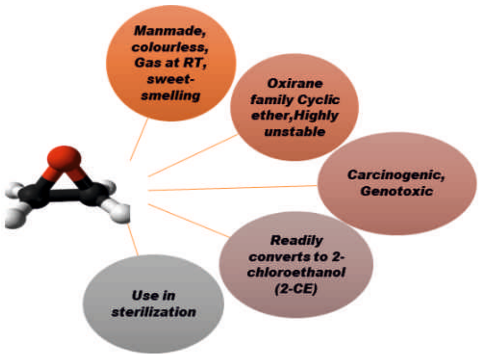


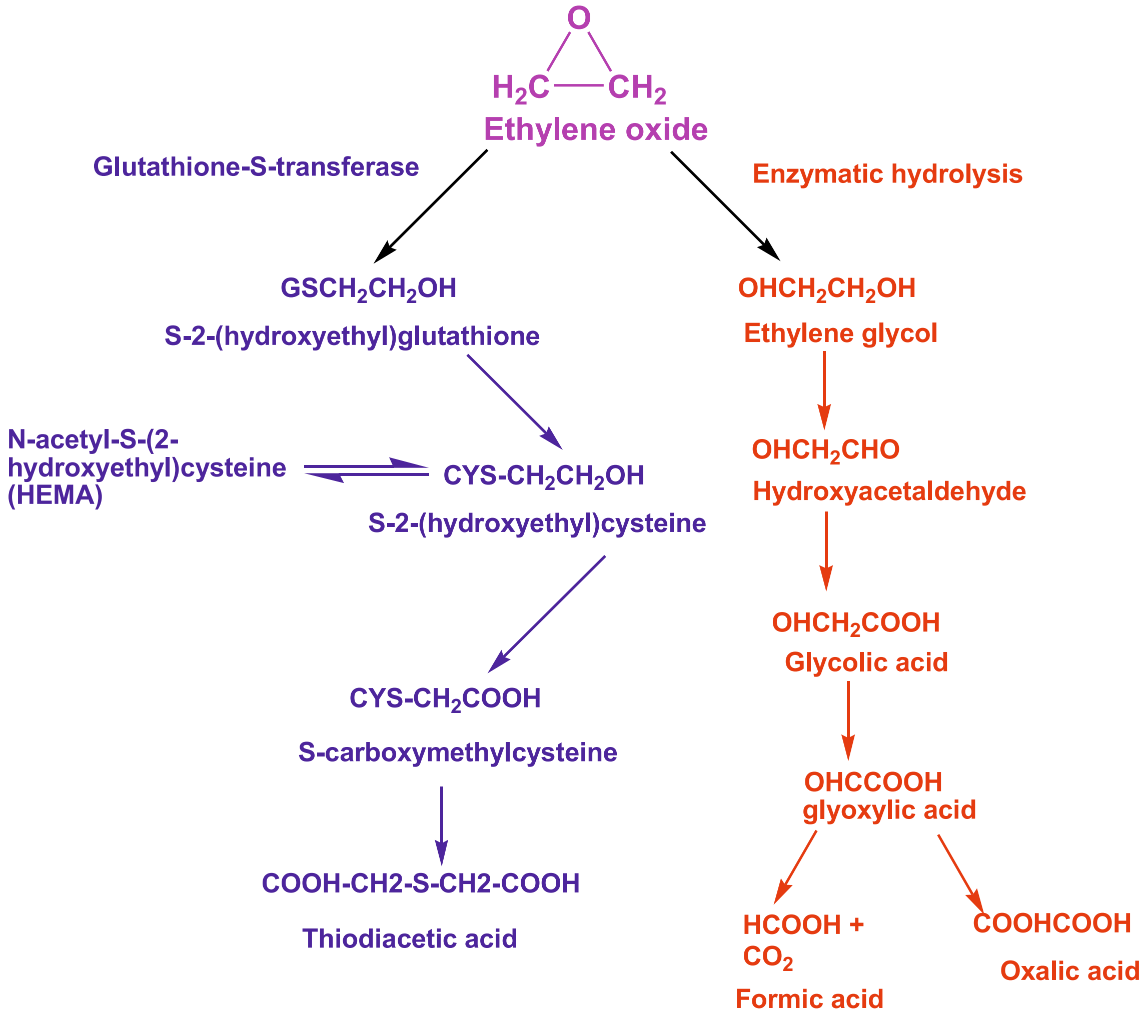
Fig.1: Generalized information about ethylene oxide

Table 1. Chemical properties of Ethylene oxide

Empirical formula	C_2H_4O
Molar mass	44.05 g/mol
Appearance	Colorless gas
Boiling point	10.4–11.0 ºC
Water solubility	Miscible

Ethylene Oxide Metabolism

1. ***Hydrolysis***: EtO is converted into ethylene glycol through both enzymatic (epoxide hydrolase) and non-enzymatic hydrolysis. Ethylene glycol is further metabolized into glycolic acid, glyoxalic acid, formic acid, carbon dioxide, and oxalic acid.
2. ***Conjugation with Glutathione***: EtO reacts with glutathione (GSH) to form S-(2-hydroxyethyl) glutathione. This metabolite can undergo further modifications, including acetylation to form N-acetyl-S-(2-hydroxyethyl) cysteine (HEMA) or conversion to S-2-(hydroxyethyl)cysteine and subsequently S-carboxymethylcysteine and thiodiacetic acid.



Scheme 1: Metabolism of ethylene oxide

Key enzymes involved were Epoxide hydrolase which catalyzes the hydrolysis of EtO to ethylene glycol and Glutathione-S-transferase helps catalyze the conjugation of EtO with glutathione.

Metabolic Pathways: The various metabolic pathways of EtO, including enzymatic and non-enzymatic hydrolysis, glutathione conjugation, and subsequent breakdown of metabolites into various end products. Understanding the metabolism of EtO is crucial for assessing its toxicity and potential health risks. the different pathways involved in EtO detoxification and the formation of potentially harmful metabolites. The relative contributions of enzymatic and non-enzymatic hydrolysis to EtO metabolism can vary among species. The efficiency of glutathione conjugation can influence the detoxification process and the levels of toxic metabolites formed.

Ethylene oxide (EtO) has recently raised concerns in the context of Indian spices, including cumin. In 2024, Singapore and Hong Kong halted sales of some spices produced by Indian companies MDH and Everest due to suspected elevated levels of ethylene oxide—a cancer-causing pesticide. The US Food and Drug Administration (FDA) is also investigating products from these brands for potentially containing the pesticide. The European Union (EU) has discovered the same cancer-causing substance in samples of chilli peppers and peppercorns from India. This development warrants attention, especially considering the popularity and trust associated with these spice brands. Both MDH and Everest insist that their products are safe, but regulatory scrutiny continues. Ministry of Health, Government of India claims the country has strict Maximum Residue Limits (MRLs) standards, but the FDA highlighted inadequate standards at an Indian spice plant in 2022. According to researcher Narasimha Reddy Donthi, the contamination is likely a post-harvest residue. As a result, it could have lasting effects, especially if the EU rejects Indian spice consignments on quality grounds (https://scroll.in/article/1070032/why-indias-food-norms-fail-to-detect-pesticide-in-spices). The United States Environmental Protection Agency (USEPA) and the International Agency for Research on Cancer (IARC) have designated EtO as a human carcinogen based on environmental risk assessment data, specifically through inhalation exposure (https://saudigazette.com.sa/article/642874/World/Asia/Indian-spices-face-heat-over-global-safety-concerns).

Carcinogenicity is associated with long-term exposure to EtO gas in the environment. Furthermore, exposure to EtO gas affects the nervous system, causing symptoms such as headaches, dizziness, and nausea. All the health effects, including reported cases of cancer, associated with EtO are based on studies of long-term inhalation exposure. It appears that most information available on the adverse effects of EtO on humans comes from occupational studies of workers exposed during EtO production and/or its use in sterilization and not from oral consumption (Mendes *et al.*, 2007). The results of a study using spices treated with EtO, such as paprika or onion, showed that EtO-treated spices are not genotoxic (Barna 1982). EtO is slightly toxic when given orally in water or corn oil, with an LD50 of 250–350 mg/kg for rats and mice. By the inhalation route, EtO is moderately toxic, with an LC_{50} (1 hr) of 1460 ppm in rats and 835 ppm in mice (https://timesofindia.indiatimes.com/blogs/voices/what-you-need-to-know-about-ethylene-oxide/).

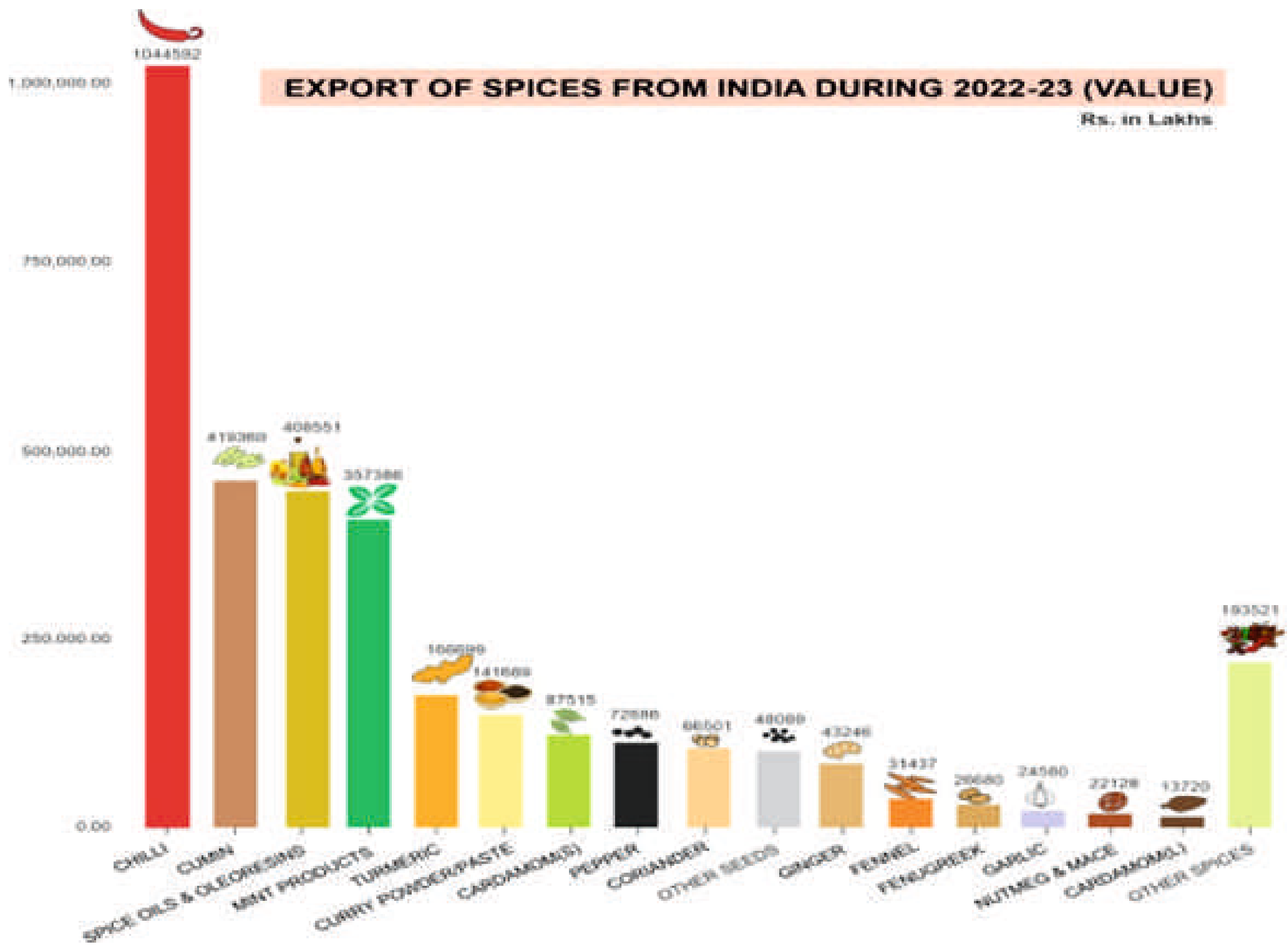


Fig.2: Export of spices from India

Ethylene Oxide is primarily used as a chemical intermediate to produce glycol ethers, acrylonitrile, ethoxylates, ethylene glycol, and polyether polyols, which are utilized in various downstream industries. The growing demand for these derivatives from end-user industries is propelling the overall market globally. The Ethylene Glycol segment dominates the global market due to its extensive use in the automotive, packaging, and pharmaceutical industries. Ethylene Glycol is widely used in the production of polyester fibres, polyethylene terephthalate (PET) resins, and automotive antifreeze (Chemicals Outlook: Global ethylene and MEG set to struggle in H2 2023 amid oversupply, weak demand). Furthermore, the increasing global population, particularly in emerging countries, is leading to a surge in demand for personal and healthcare products, driving the demand for EtO and is expected to boost the market in the coming years. As a result of these factors, the EtO market is projected to reach 42 million tonnes by 2032 (Expert Market Research: Ethylene Oxide Market Size, Price, Report, Outlook 2024-2032; Ethylene Oxide Market Size, Growth, Analysis & Forecast 2032 (chemanalyst.com)).

Health Concerns and the Regulatory Landscape

- ***Carcinogenicity*:** Inhalation exposure to EtO is classified as carcinogenic (cancer-causing) by the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (USEPA) based on environmental exposure studies.
- The Environmental Protection Agency stated in 2018 that "evidence in humans indicates that exposure to ethylene oxide increases the risk of lymphoid cancer and, for females, breast cancer."
- ***Regulatory Disparity*:** Regulations regarding EtO residues in food vary significantly across countries, highlighting the lack of international consensus.

o ***No International Maximum Residue Limit (MRL)*:** Each country establishes its own policies.

o **Examples:**

- **US and Canada:** more lenient regulations with MRLs of 7 mg/kg (ppm) for EtO and a much higher 960 mg/kg for 2-CE.
- **EU and UK:** stricter regulations with a uniform MRL of 0.1 mg/kg for the combined sum of EtO and 2-CE expressed as EtO.
- **Singapore:** MRL of 50 mg/kg for spices, significantly higher than the EU and much closer to the US/Canada levels.
- **Hong Kong:** zero tolerance for EtO residues.

- ***Analytical Challenges*:** Accurately measuring trace levels of EtO in food is complex. Discrepancies between laboratories analyzing the same samples highlight these analytical challenges.

o ***Focus on 2-CE*:** Recent research suggests that 2-CE, a breakdown product of EtO, is the primary analyte detected in processed ingredients suspected of EtO treatment. Native EtO might not be present at all.

A maximum residue level (MRL) is the highest level of pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (good agricultural practice). Some countries depend on the Codex Alimentarius Committee on Pesticide Residues to determine maximum residue limits (MRLs), while others prefer to establish their own standards (priority list of contaminants for evaluation by JECFA, 2023). MRLs have been recommended by the Joint Food and Agricultural Organization/World Health Organization Meeting on Pesticide Residues (JMPR). No international MRL is available for EtO and 2-CE residues in food products, each country has its policy regarding this matter.

The ethylene oxide analysis at trace levels in foods is highly complex due to the absence of certified reference materials for all food items. Various analytical approaches have been reported to quantify ETO, with confirmatory analysis typically involving gas chromatography coupled with mass spectrometry. Recent studies have conclusively shown that 2-chloroethanol (2-CE), and not ETO, is the predominant analyte detected in ingredients suspected to have been treated with ETO. It is imperative to develop an official method for the analysis of ETO in different food matrices to avoid false positive results. Workers in facilities processing certain herbs and spices are undoubtedly at a higher risk of exposure to ETO. However, the contribution to cancer risk from the consumption of low levels of ETO residues in spices is unequivocally deemed unlikely to be significant. Levels of ETO decrease with time, and there is little or no residue remaining when the food is eaten (Bessaire *et al.*, 2023); Jensen, 1988; BfR 2020, 2021; WHO, 1985; US EPA, 1996).

The low boiling point of ETO (10–11 °C at 760 mm Hg) means that ETO will not linger when used on produce at the farm or in storage facilities. United States Environment Protection Agency (EPA) (US EPA 2012) and EFSA (EFSA 2022) reported conflicting results on the genotoxicity of 2-CE. Fowles, *et al.*, (2001), found that the potential cancer risks associated with consuming ETO in raw spices in New Zealand are insignificant, taking into account the average spice intake of 2.8 kg per year. Nevertheless, further research is required to ascertain whether 2-CE presents a possible carcinogenic risk when consumed with spices and establish a dose-response correlation.

Analytical Techniques for Detecting Residual Ethylene Oxide and Its Derivatives in Food Products

Ethylene oxide is recommended for use as a sanitizer for spices and seasonings, emphasizing its role in ensuring the microbiological safety of food products (Rushing, 2006). Commercial food processors utilize ethylene oxide to eliminate bacteria and molds from spices, underlining its significance in food safety practices (Mladenovska *et al.*, 2021). Ethylene oxide is commonly employed for fumigating and disinfecting spices and other food items, further emphasizing its role in food processing and preservation (Wang, 2024).

As mentioned earlier, in the presence of chlorine ions, ethylene oxide (EtO) usually converts into its derivative, ethylene chlorohydrin (ECH). Using GC-MS without a derivatization method, the limit of detection (LOD) for ECH was determined to be 20 μg kg^{-1} by spiking an untreated pepper matrix with EtO. The limit of quantification (LOQ) was assumed to be 100 μg kg^{-1}, which is five times the LOD. The method's reliability was verified through recovery and repeatability tests, with average recovery values ranging from 60% to 70% (CV% = 9.6–5.5) for concentrations between 100 and 500 μg kg^{-1} (Tateo and Bononi, 2006).

To quantify ethylene oxide from a food matrix, researchers utilized gas chromatography coupled with mass spectrometry (GC-MS/MS) (Patil *et al.*, 2023; Wenio, 2023). GC-MS/MS is commonly used for the analysis of ethylene oxide residues in various food samples such as sesame, cumin, wheat, tea, spices, herbs, and dehydrated fruits. For the quantification of EtO, the sample extraction was conducted with acetonitrile at temperatures below 10°C, followed by cleanup using dSPE with C18 and primary secondary amine sorbents. Analysis was performed using GC-MS/MS with selected reaction monitoring. This method achieved a limit of quantification of 10 ng/g for both EtO and 2-CE, effectively minimizing matrix effects. The recoveries of EtO and 2-CE ranged from 74% to 120% at concentrations of 10, 20, and 50 ng g^{-1}, with precision RSDs of less than 12%, in compliance with the SANTE/11312/2021 guidelines for analytical method validation. (Patil *et al.*, 2023).

Wenio *et al.* (2023) have developed a novel GC-MS/MS method that is simple, fast, and efficient for the

Table 2. MRL level of different countries

Country/Region	Substance	MRL (mg/kg)	Notes
USA	EtO & 2-CE	7 & 960	Tolerances and exemptions for pesticide chemical residues in food
Canada	EtO & 2-CE	7 & 960	MRL search engine
EU & UK	EtO & 2-CE	0.1	Uniform MRL (sum of ethylene oxide and 2 - chloroethanol expressed as EtO)
EU	Nuts, Oil Fruits, Oil Seeds	0.05	Regulation (EU) 2015/868
Singapore	Spices	50	SFA MRL
Hong Kong	Spices	0	Zero tolerance

Comparison with EU MRL (0.1 mg/kg for EtO& 2-CE):

- Singapore's MRL is 500 times higher.
- USA and Canada's MRLs are 70 times higher.

Source:https://timesofindia.indiatimes.com/blogs/voices/what-you-need-to-know-about-ethylene-oxide

Table 3. Analysis of ethylene oxide by GC-MS

Topic	Information
Analytical Challenges	ETO analysis in foods is complex; diverse methods by agencies and countries, lack of certified reference materials
Accuracy and Discrepancies	Uncertain accuracy; discrepancies in ETO amounts reported between laboratories
Analytical Approaches	Various methods to quantify ETO and 2-CE, often using GC-MS
Sample Preparation	ETO converted to 2-CE before GC-MS analysis
Direct Measurement Methods	Solid-phase microextraction, QuEChERS procedure
Conversion Methods	ETO converted to 2 -CE under acidic conditions, extracted with ethyl acetate, and analyzed by GC-MS
Predominant Analyte	2-CE is the main analyte in processed ingredients treated with ETO
False Positives	Conversion methods may lead to artefactual detection of native ETO (false positives)
Risk Assessment	In absence of native ETO, focus on 2-CE for ETO risk assessment in foodstuffs
Documentation by Laboratories	German BfR and other labs report detection of only 2 -CE in items treated with ETO
Standardization Recommendation	Official methods by bodies like CEN, AOAC, and ISO needed to avoid false positives
Occupational Exposure	Higher ETO exposure in workers in hospitals or herb/spice processing facilities
Cancer Risk Viewpoint	US EPA and WHO consider low ETO residues in spices unlikely to significantly increase cancer risk
ETO Residue Levels	ETO levels decrease over time as they evaporates or breaks down, potentially leaving little to none in consumed food

Source: Bessaire *et al.*, 2023; Jensen, 1988; BfR 2020, 2021; WHO, 1985; US EPA, 1996)

Table 4. Quantification Methods of Ethylene Oxide and Its Derivative 2-Chloroethanol in Various Food Matrices

Matrices	Analytical Instrument	Targeted Compound	Special treatment for extraction improvement	Quantification limits	Reference
Ginger, oregano, chili, red paper cumin, and coriander	Headspace-GC	EtO	--	1 mg kg^{-1}	Woodrow *et al.*, 1995
Herbs and Spices	GC-MS	ECH	Ultrasonic bath	0.3 mg kg^{-1}	Bononi *et al.*, 2014
Ground pepper, paprika and sesame	GC-MS/MS	EtO and 2-CE	dSPE & Acid hydrolysis	0.02 and 0.01 mg kg^{-1} for EtO and 2-CE, respectively	Stupák *et al.*, 2021
Sesame and spice samples	GC-MS/MS	EtO and 2-CE	QuEChERS	EtO; 0.025 & 2-CE; 0.005 mg kg^{-1}	Cucu *et al.,* 2022
Sesame, cumin, wheat, tea, spices, herbs, and dehydrated fruits	GC-MS/MS	EtO and 2-CE	dSPE	0.01 mg kg^{-1} for EtO and 2-CE	Patil *et al.*, 2023
Sesame seeds, guar gum, wheat, and tomatoes	GC-MS/MS	EtO and 2-CE	--	0.03 mg kg^{-1} for EtO and 2-CE	Wenio, 2023
Cumin, ashwagandha, chilli powder, turmeric powder, guar gum, locust bean gum, and ginger powder	Headspace-GC-MS/MS	EtO and 2-CE	dSPE	0.01 mg kg^{-1} for EtO and 2-CE	Nerpagar *et al.*, 2023

derivatization of ethylene oxide to 2-chloroethanol and the extraction of 2-chloroethanol from plant-derived products. This method is particularly effective for products with varying water content, including low-water, oily, and high-water products. The validation criteria, including homogeneity, sensitivity, accuracy, and precision, comply with the guidelines outlined in the document SANTE/11312/2021.

For accurate quantification of ethylene oxide, it is crucial to consider the extraction of the compound from the food matrix. Studies emphasize the significance of sample preparation and analysis conditions for precise quantification of compounds in complex matrices (Heroult *et al.*, 2014). The development and validation of analytical methods are essential to ensure the reliability and accuracy of quantification results (Patil *et al.*, 2023; Czyzycki *et al.*, 2012).

Furthermore, the choice of materials for the analysis can impact the quantification process. For example, the use of poly (ethylene oxide)-block-poly (propylene oxide)-block-poly (ethylene oxide) (Pluronic F-127) as a gel matrix can influence the sensitivity and detection of compounds like ethylene oxide (Sąsiadek *et al.*, 2021). The composition of the matrix can affect the interactions between the analyte and the detection system, thereby influencing the quantification process.

Accurate quantification of ethylene oxide in food matrices can be achieved by employing advanced analytical techniques such as GC-MS/MS, optimizing sample preparation methods, and considering the impact of the matrix composition on the quantification process. Rigorous method development and validation procedures are essential for ensuring precise quantification of ethylene oxide in food matrices.

Risk Assessment Considerations

Fig.3: Risk assessment of ethylene oxide

Unresolved Issues and the Need for Harmonization

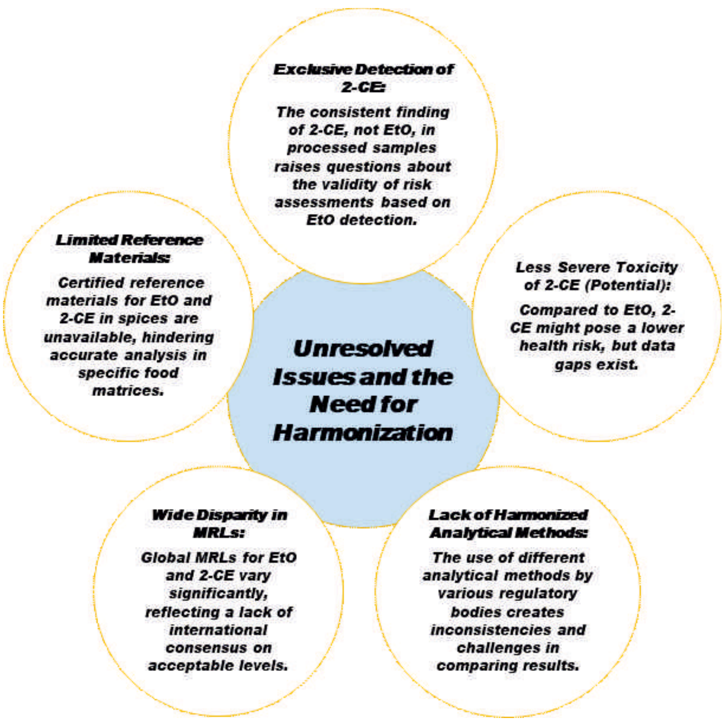
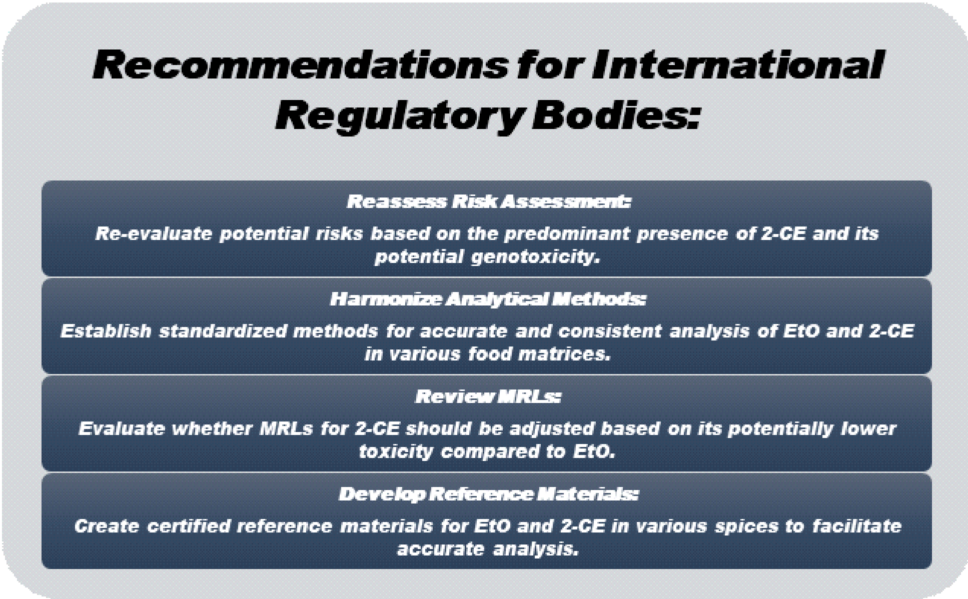


Fig.4: Unresolved Issues and the Need for Harmonization in ethylene oxide

Recommendations for International Regulatory Bodies



Regulatory bodies of Spices in India

Spices Board of India: This is a flagship regulatory body set up by the Government of India to monitor, develop, and promote Indian spices

a) The Spices Board works under the Ministry of Commerce and Industry, Government of India. It acts as a link between the Indian exporter and the importers abroad. The board has a state-of-the-art testing laboratory at its headquarters in Kochi and regional laboratories in Mumbai, Chennai, Delhi, Tuticorin, Kandla, and Guntur. Through these laboratories, the Spices Board conducts mandatory quality checks for spices exported from India.

b) **Food Safety and Standards Authority of India** (FSSAI): FSSAI is the primary authority regulating food businesses in India

In the spice industry, it's crucial to obtain an FSSAI license as it assures the safety and quality of the food products being handled. This license reflects the commitment to maintaining high standards in the business. FSSAI has developed a guidance document on Food Safety Management System (FSMS) for Spice Processing.

c) Saffron Production and Export Development Agency (SPEDA)**:** Government of India has notified the formation of an exclusive Committee to be known as 'Saffron Production and Export Development Agency (SPEDA) for the overall development of the Saffron industry in the state of Jammu & Kashmir. The Agency will be headquartered in Srinagar with Commerce Secretary, Govt. of India and Chief Secretary of J&K as Co-Chairman. The Agency shall consist of members representing the Ministry of Commerce & Industry, State Govt. of J&K, Ministry of Agriculture, Spices Board, ICAR, other related Central / State organizations and various stakeholders of the Industry viz. growers, traders and exporters of Saffron. SPEDA will assist and encourage the creation of appropriate infrastructure for processing, packing, warehousing, and research and also establish a quality evaluation laboratory for Saffron. SPEDA shall act as a subordinate agency under the Ministry of Commerce and Industry in the Central Government and function under the overall authority, supervision and control of the Spices Board.

d) Directorate of Arecanut and Spices Development (DASD): The Directorate of Arecanut and Spices Development (DASD) was established on 1st April 1966 at Calicut in Kerala as a subordinate office under the Ministry of Agriculture, Government of India. It was created to oversee the development of spices and areca nut at the national level. The DASD is responsible for the development of spices, arecanut, a large group of aromatic plants, and betel vine grown in the country at the national level. It coordinates all the development activities implemented by different agencies like State Government Departments, State Agricultural Universities (SAU), ICAR Institutes, etc. It is responsible for coordinating and monitoring programs under the Mission for Integrated Development of Horticulture (MIDH) to promote the holistic growth of the horticulture sector.

e) Export Inspection Council: (EIC) of India is an organization that ensures products meet the safety and quality requirements of importing countries. The EIC was established by the Government of India in 1963 to help India's export trade grow.

f) Indian Institute of Spices Research (IISR): The Indian Institute of Spices Research (IISR) is an autonomous organization engaged in agricultural research related to spices in India. It is a constituent body of the Indian Council of Agricultural Research (ICAR) and is located in Moozhikkal, Silver Hills, Kozhikode, Kerala. The IISR started as a Regional Station of the Central Plantation Crops Research Institute (CPCRI), Kasaragod in 1976, focusing on research on spices. It was later upgraded to the Indian Institute of Spices Research on 1st July 1995. "The IISR has launched the KisanSeva Kendra, a first-of-its-kind outlet for the sale of bio inputs of all ICAR institutes. This facility supports small and marginal farmers across the country by facilitating easy access to ICAR technology-based farm bio inputs. The institute has developed a new granular lime-based Trichoderma formulation, integrating Trichoderma and Lime into a single product, making the application easier for the farmers. 'The IISR has also released a high-yielding variety of black pepper named 'IISR Chandra. This variety has a long spike, compact setting, and bold berries, and can yield 7.5 kg of pepper per vine.

g) Agricultural and Processed Food Products Export Development Authority (APEDA):The Agricultural and Processed Food Products Export Development Authority (APEDA) is an Indian Apex-Export Trade Promotion Active government body. Established by the Government of India under the Agricultural and Processed Food Products Export Development Authority Act passed by the Parliament in December, 1985, APEDA operates under the Ministry of Commerce and Industry. The Authority has its headquarters in New Delhi.

h) **Codex Committee on Spices and Culinary Herbs** (CCSCH): The CCSCH is a subsidiary body of the Codex Alimentarius Commission, which is a joint initiative of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).It was formed in 2013. The Codex Alimentarius Commission is responsible for setting international food standards to ensure the safety, quality, and fairness of food trade. India is its member since 1964.

Preventive measures for EtO contamination

To maintain the quality of spices and spice products, exporters must ensure that these items are free from EtO and its metabolites at every stage of the supply chain. Exporters should recognize EtO as a hazard and integrate critical control points to prevent its presence in their Hazard Analysis Critical Control Points (HACCP) and Food Safety Plan (FSP) within their Food Safety Management System (FSMS). It is essential for exporters to refrain from using EtO as a sterilizing or fumigating agent in spices and to carry out tests for EtO contamination in raw materials, processing aids, packaging materials, and finished goods. In case EtO is detected at any stage of the supply chain, exporters should conduct a thorough root cause analysis and implement appropriate preventive measures to avoid future occurrences, while maintaining records of these actions. Furthermore, exporters should ensure that transporters, storage/warehouses, packaging material suppliers, and all relevant entities refrain from using EtO at any stage of the process.

Alternate methods of Sterilization

Exporters of spices are encouraged to use alternate methods of sterilization as suitable. a) Steam Sterilization b) Irradiation (not applicable to organic products under NPOP) c) any other methods approved by FSSAI.

Table 5. MRLs of EtO for spices & herbs specified in the Commission regulation (EU) 2015/868

S.N.	Seed Spices	EtO (sum of EtO & 2 chloro-ethanol expressed as EtO) in mg/kg
1.	Anise	0.10
2.	Caraway	0.10
3.	Celery	0.10
4.	Coriander	0.10
5.	cumin	0.10
6.	Dill	0.10
7.	Fennel	0.10

Process of Fixation of Codex MRL on Spices by India

Maximum residue limits (MRLs) and other regulations are thus non-tariff measures that affect international trade (Beghin and Schweizer, 2021; Santeramo and Lamonaca, 2019; Swinnen, 2016) and have the potential to be used as a form of protectionism (Li and Beghin, 2017).

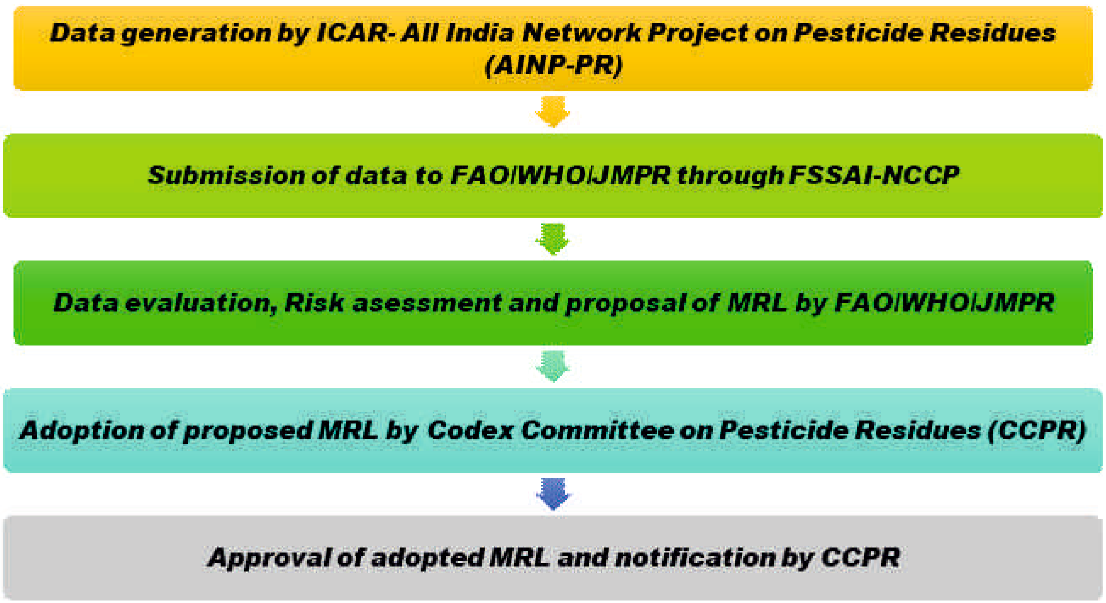


Table 6. MRLs of EtO for spices & herbs specified in the Commission regulation (EU) 2015/868

Spice	Canada (mg/kg)	EU-MRLs (mg/kg)	Great Britain (mg/kg)	Norway (mg/kg)	Switzerland (mg/kg)	Thailand (mg/kg)
Pepper (Chillies Yellow)	7	0.02	0.01	N.D.	< LOQ	0.02
Ginger (Dry)	-	-	-	0.02	0.02	0.01
Turmeric (Dry)	-	-	-	7	0.02	0.10

Ethylene oxide hazard information

GHS Code	Hazard Statement	Pictogram
H220	Extremely flammable gas	
H280	Contains gas under pressure; may explode if heated	
H302	Harmful if swallowed	
H315	Causes skin irritation	
H319	Causes serious eye irritation	
H330	Fatal if inhaled	
H331	Toxic if inhaled	
H335	May cause respiratory irritation	
H340	May cause genetic defects	
H350	May cause cancer	
H402	Harmful to aquatic life	

Source: Ethylene oxide - American Chemical Society (acs.org)

**Globally Harmonized System of Classification and Labeling of Chemicals.

Conflicts of Interest: The authors declare no conflicts of interest.

References

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Ethylene Oxide. U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. 1990.https://www.atsdr.cdc.gov/toxprofiles/tp137.pdf

Allemang, A., Lester, C., Roth, T., Pfuhler, S., Peuschel, H., Kosemund, K. and O'Keeffe, L. 2022. Assessing the genotoxicity and carcinogenicity of 2-chloroethanol through structure activity relationships and in vitro testing approaches. *Food Chem Toxicol.*, 168:113290.

Arshad, M. and Frankenberger, W.T. 2002. Ethylene in Agriculture: Synthetic and Natural Sources and Applications. In: Ethylene. Springer, Boston, MA

Barna, J. 1982. Dominant lethal tests of spices treated with ethylene oxide. *Mutat Res Environ Mutagen.*, 97(3):170-180.

Beghin, J.C. and Schweizer, H. 2021. Agricultural trade costs. *Appl Econ Perspect Policy.*, 43(2):500–30.

Bessaire, T., Eriksen, B., Laborie, S., Mujahid, C., Mottier, P., Delatour, T. and Stroheker, T. 2023. Confirmation of the full conversion of ethylene oxide to 2-chloroethanol in fumigated foodstuffs: possible implications for risk assessment. *Food Addit Contam Part A.*, 40(1):81-95.

Bononi, M., Quaglia, G. and Tateo, F. 2014. Identification of ethylene oxide in herbs, spices and other dried vegetables imported into Italy. *Food Addit Contam* Part A., 31(2):271-275.

Chemical outlook Society Reviews: Ethylene glycol: properties, synthesis, and applications

Cucu, T., David, F. and Devos, C. 2022. Analysis of ethylene oxide and 2-chloroethanol in food products: challenges and solutions.,18(8):10-16

Czyzycki, M., Wróbel, P., Szczerbowska-Boruchowska, M., Ostachowicz, B., Węgrzynek, D. and Lankosz, M. 2012. The perspective of new multi layer reference materials for confocal 3d micro x ray fluorescence spectroscopy. *X-Ray Spectro.*, 41(4):273-278.

Dever, J.P., George, K.F., Hoffman, W.C. and Soo, H. 2000. Ethylene oxide. *Kirk Othmer Encyclopedia of Chemical Technology*.

Ethylene oxide - American Chemical Society.2019. Ethylene oxide November 18, 2019, 'Im in the midst of an air pollution controversy. What molecule am I? https://www.acs.org/molecule-of-the-week/archive/e/ethylene-oxide.

Ethylene Oxide Market Size, Growth, Analysis & Forecast 2032 (chemanalyst.com)

Expert Market Research: Ethylene Oxide Market Size, Price, Report, Outlook 2024-2032

Fowles, J., Mitchell, J. and McGrath, H. 2001. Assessment of cancer risk from ethylene oxide residues in spices imported into New Zealand. *Food Chem Toxicol.,* 39(11):1055-1062.

Heroult, J., Nischwitz, V., Bartczak, D. and Goenaga Infante, H. 2014. The potential of asymmetric flow field-flow fractionation is hyphenated to multiple detectors for the quantification and size estimation of silica nanoparticles in a food matrix. *Anal Bioanal Chem.*, 406(16): 3919-3927.

_https://saudigazette.com.sa/article/642874/World/Asia/Indian-spices-face-heat-over-global-safety-concerns

_https://scroll.in/article/1070032/why-indias-food-norms-fail-to-detect-pesticide-in-spices https://timesofindia.indiatimes.com/blogs/voices/what-you-need-to-know-about-ethylene-oxide

_https://www.fao.org/fao-who-codexalimentarius/shproxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FMeetings%252FCX-735-16%252FWDs%252Fcf16_15x.pdf

Jensen, K.G. 1988. Determination of ethylene oxide residues in processed food products by gas-liquid chromatography after derivatization. *Z. Lebensm Unters Forsch.*, 187(6):535-540.

Kilty, P.A. and Sachtler, W.M.H. 1974. The mechanism of the selective oxidation of ethylene to ethylene oxide. *Catal Rev Sci Engi.*, 10(1):1-16.

Li, Y. and Beghin J.C. 2017. Protectionism indices for non-tariff measures: An application to maximum residue levels. In: Nontariff measures and international trade. *World Scientific*. 167–178.

Lucky, C., Wang, T. and Schreier, M. 2022.

Electrochemical ethylene oxide synthesis from ethanol. *ACS Energy Lett.*, 7(4):1316-1321.

Luttrell, W.E. 2008. Ethylene oxide. *J Chem Health Safety.*, 15(6):30-32.

Mendes, G.C., Brandao, T.R. and Silva, C.L. 2007. Ethylene oxide sterilization of medical devices: a review. *American J Infect Cont.,* 35(9):574-581.

Mladenovska, I., Bektashi, N., Andonovic, B., Spasevska, H., Sandeva, I., Arizanova, M. and Reka, A. 2021. Development of a novel microbiological method for detection of gamma irradiated spices. *Macedonian J Chem Chem Eng.*, 40(2):213.

Nerpagar, A., Langade, N., Patil, R., Chiplunkar, S., Kelkar, J. and Banerjee, K. 2023. Dynamic headspace GC-MS/MS analysis of ethylene oxide and 2-chloroethanol in dry food commodities: a novel approach. *J Environ Sci Health, Part B*, 58(11):659-670.

Patil, R., Langade, N., Nerpagar, A. and Banerjee, K. 2023. Development and validation of a residue analysis method for ethylene oxide and 2-chloroethanol in foods by gas chromatography tandem mass spectrometry. *Acs Agric Sci Tech.*, 3(3):287-295.

Rushing, J. 2006. Methods to ensure microbiological safety of organically produced medicinal plants: a review. *Hort Sci.*, 41(2):292-295.

Santeramo F.G. and Lamonaca, E. 2019. The effects of non-tariff measures on agri-food trade: a review and meta-analysis of empirical evidence. *J Agric Econ.,* 70(3):595–617.

Sąsiadek, E., Jaszczak, M., Skwarek, J. and Kozicki, M. 2021. Nbt-pluronic f-127 hydrogels printed on flat textiles as uv radiation sensors. *Materials*, 14(12):3435.

Stupák, M., Filatova, M., Kocourek, V. and Hajšlová, J. 2021. Gas chromatography tandem mass spectrometry analysis of ethylene oxide: an emerged contaminant in seeds and spices. *Advances Food Beve Anal.*, 34(10):5-10

Swinnen, J. 2016. Economics and politics of food standards, trade, and development. *Agric Econ.,* 47(1):7–19.

Tateo, F. and Bononi, M. 2006. Determination of ethylene chlorohydrin as marker of spices fumigation with ethylene oxide. *J Food Compo Anal*, 19(1):83-87.

U.S. Environmental Protection Agency. Evaluation of the Inhalation Carcinogenicity of Ethylene Oxide (CASRN 75-21-8) in Support of Summary Information on the Integrated Risk Information System (IRIS). National Center for Environmental Assessment, Office of Research and Development. Washington, DC. EPA/635/R-16/350Fa. 2016. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/1025tr.pdf

US EPA (United States Environmental Protection Agency), 1996. Memorandum from Leung Cheng, Health Effects Division, to Vivian Prunier Special Review Branch: Ethylene Oxide. Case No. 2275. Analytical Method, Residue Persistence in Herbs and Spices and Walnuts, and Cooking Study Dated March 28, 1996.

Wang, J. 2024. Association between ethylene oxide exposure and osteoporosis: a cross-sectional nationwide study of the us population. (preprint 2024 Research Square)

Wenio, I. 2023. A fast method for determination of ethylene oxide using gas chromatography coupled with mass spectrometry GC-MS/MS. *Appl Sci*, 13(13):7480.

WHO, 1985. (World Health Organization). Environmental Health Criteria 55: Ethylene Oxide. International Programme on Chemical Safety. WHO, Geneva

Woodrow, J.E., McChesney, M.M. and Seiber, J.N. 1995. Determination of ethylene oxide in spices using headspace gas chromatography. *J Agric Food Chem*, 43(8):2126-2129.

Yue, H., Zhao, Y., Ma, X. and Gong, J. 2012. Ethylene glycol: properties, synthesis, and applications. *Chem Soc Rev.*, 41(11):4218-4244.