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Review Article

Ethylene Oxide Exposure and Its Impact on Stroke Risk and All-Cause Mortality: A Population-Based Analysis

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Abstract

Background: Ethylene oxide (EO) is widely used in various industries and has been associated with multiple health risks. However, its impact on stroke risk and all-cause mortality in the general population remains has not been definitively established. This study aimed to investigate the association between EO exposure, stroke risk, and all-cause mortality in a population-based sample.

Methods: Data were obtained from the National Health and Nutrition Examination Survey (NHANES) from 2013 to 2018, involving 29,350 participants. After applying inclusion and exclusion criteria, 4,908 participants with detectable EO levels and complete stroke and mortality data were included in the analysis. Hemoglobin adducts of EO (HbEO) were used as a marker of exposure. Logistic regression models were used to examine the association between EO levels and stroke risk, while Cox proportional hazards models assessed the relationship between EO levels and all-cause mortality, adjusting for covariates such as age, high blood pressure, diabetes, and smoking history.

Results: Higher EO exposure levels were significantly associated with an increased risk of stroke, particularly after adjusting for covariates such as age (OR: 1.002, 95% CI: 1.001-1.003, $P<0.0001$) and additional covariates (OR: 1.001, 95% CI: 1.000-1.003, $P=0.023$). Higher EO levels were also associated with increased all-cause mortality when adjusted for age and additional covariates (HR: 1.333, 95% CI: 1.003-1.770, $P=0.047$).

Conclusion: This study provides evidence of a significant association between EO exposure and increased risks of stroke and all-cause mortality in the general population. Further research is necessary to confirm these findings using direct EO exposure measurements.

Keywords: Stroke; All-cause mortality; Ethylene oxide

Introduction

Stroke constitutes a significant global health burden, affecting one in four individuals at some point in their lifetime. It is the second leading cause of mortality and ranks third in disability among adults worldwide (1). Cardiovascular and cerebrovascular diseases are influenced by a combination of social determinants, psychological factors, genetic predispositions, and environmental factors. In particular, the pervasive use of ethylene oxide (EO) in industrial and sterilization

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applications has raised concerns about its role in increased environmental and occupational risks. Such exposures are increasingly linked to the pathogenesis of these severe health issues (2).

EO is employed in the sterilization industry and has been identified as a component of cigarette smoke. Classified as a potential exogenous toxicant with adverse effects on human health in the field of public health(3). EO forms macromolecular adducts by binding to proteins and nucleic acids, resulting in alkylation that can lead to a range of health issues, such as lymphoid and breast cancers (4, 5). Moreover, studies involving both animal models and clinical observations have documented EO's detrimental effects on the nervous system, including sensory impairment and diminished responsiveness(6). Recent evidence further supports EO's neurotoxicity, highlighting its role in the development and exacerbation of various neurological conditions (7).

In this study, we used the hemoglobin adduct of EO (HbEO), a sensitive and specific marker widely used in clinical trials(8), to analyze the association between EO exposure and stroke.

Prior research has documented a significant link between ethylene oxide (EO) exposure and a spectrum of health conditions, including asthma, kidney stones, and various cancers (5, 9, 10). Given the widespread use of EO and its known adverse effects, it is crucial to investigate its association with health outcomes like stroke and mortality. Although measuring associated health risks in the general population is challenging due to indirect exposure and variability in individual EO exposure, using HbEO provides a reliable biomarker that has been validated in numerous studies to reflect cumulative exposure levels. Establishing such associations requires the use of reliable exposure markers and appropriate study designs to overcome these challenges and to justify the investigation of such health outcomes.

We aimed to investigate the previously unexplored association between stroke and EO exposure. Earlier cross-sectional analyses have shown that hemoglobin adducts of EO correlate closely with serum lipid profiles, known predictors of stroke risk (11). Therefore, we used data from the National Health and Nutrition Examination Survey (NHANES) conducted between 2013 and 2018 to investigate the association between EO exposure and stroke.

Materials and Methods

Study Population

The NHANES evaluates the health status and potentially hazardous habits/history of the non-institutionalized US civilian population, managed by the US National Center for Health Statistics (NCHS), a part of the Centers for Disease Control and Prevention (CDC). The survey protocol was approved by the NCHS Institutional Review Board, with detailed survey designs documented and publicly available (12).

In this study, data from three NHANES cycles (2013-2014, 2015-2016, and 2017-2018) were combined, focusing on adults aged no less than 20 years. This age range was chosen to concentrate on adult health conditions and to reduce biases from the underrepresentation of institutionalized seniors. The inclusion and exclusion criteria for the study were as follows: 1) Participants with detectable levels of EO levels and reported stroke status available are included; 2) Participants with incomplete covariate data, including age, sex, race, high blood pressure, diabetes, and smoking history, are excluded.

Exposure Assessment

EO exposure was indirectly measured using HbEO, a validated biomarker of EO exposure(8). HbEO reflects both endogenous and exogenous exposure to EO, allowing for an estimate of cumulative exposure levels. Although direct EO exposure measurement is ideal, the use of HbEO has been validated in clinical and occupational studies as a reliable proxy. The limitations of using an indirect marker are discussed in the results and discussion sections.

Univariate Analysis on Confounding Factors

The confounding factors considered included age, race, body mass index (BMI), high blood

pressure, diabetes, alcohol consumption, and smoking history. These covariates were chosen based on prior literature suggesting their potential influence on stroke risk. To explore the univariate relationship between each covariate (i.e., potential confounding factors) and stroke, a logistic regression model was employed. Stroke history was derived from the MCQ questionnaire, which provides self-reported data on various medical conditions. The covariates analyzed included:

1) Age: The age of participants at the time of screening was recorded in years, with those aged 80 and above coded as 80.
2) Race/Ethnicity: Participants were categorized as Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, Non-Hispanic Asian, and Other Race (including multi-Racial).
3) Body Mass Index (BMI): BMI was determined by dividing the participant's weight in kilograms by the square of their height in meters (kg/m^2), with the result rounded to one decimal place.
4) Alcohol History: For the 2013-2014 and 2015-2016 cycles, alcohol history was defined as having consumed at least 12 alcoholic drinks in a lifetime; for the 2017-2018 cycle, it was defined as having ever consumed any kind of alcohol.
5) High Blood Pressure: Participants reported whether they had a history of high blood pressure.
6) Diabetes: A self-reported diagnosis of diabetes was used to determine diabetic status.
7) Smoking History: Smoking history was defined as having smoked at least 100 cigarettes in their lifetime.

Each covariate was analyzed individually to determine its univariate association with stroke using logistic regression. This initial analysis helps identify potential factors that may influence stroke risk before conducting multivariable analyses.

Statistical Analysis

Logistic regression models were used to examine associations between EO levels and stroke risk, and Cox proportional hazards models assessed the relationship between EO levels and all-cause mortality. Covariates adjusted for included age, high blood pressure, diabetes, and smoking history.

Logistic Regression Analysis of EO Levels and Stroke Risk

Logistic regression was used to analyze the association between hemoglobin adducts of ethylene oxide (EO) and stroke risk, with EO measured in pmol/g. Participants with EO levels below detection limits (8.2 pmol/g for 2013–2016 and 12.9 pmol/g for 2017–2018) were excluded. For continuous EO levels, univariable analysis (Model LR1C) and multivariable-adjusted analyses (Models LR2C and LR3C) were conducted. Model LR2C adjusted for age, a major stroke risk factor, while Model LR3C further adjusted for high blood pressure, diabetes, and smoking history to evaluate EO's independent impact. EO levels were also categorized into high vs. low groups (median split) for analysis. Univariable (Model LR1B) and multivariable-adjusted models (Models LR2B and LR3B) were applied similarly, with adjustments for age, high blood pressure, diabetes, and smoking history to comprehensively assess EO's influence on stroke risk.

Cox Proportional Hazards Model on Continuous EO Levels

Cox proportional hazards regression models were employed to assess the association between continuous EO levels and all-cause mortality. Three separate models were constructed to systematically assess this relationship:

1) Model Cox1C: This unadjusted model examined the direct association between continuous EO levels and all-cause mortality without any covariate adjustments, without incorporating any covariate ad-

justments. This baseline model aimed to provide an initial estimate of the relationship.

2) Model Cox2C: This model adjusted for age, considering the age-related risk of mortality, to assess whether the association between EO and mortality was independent of age.
3) Model Cox3C: This fully adjusted model included additional covariates: high blood pressure, diabetes, and smoking history, in addition to age, to evaluate the independent effect of EO on all-cause mortality after accounting for these potential confounders.

All-cause mortality was used as outcome of the abovementioned models, with the hazard ratio (HR) and 95% confidence intervals (CIs) reported for each model. The statistical significance was assessed using p-values. This stepwise approach enabled a detailed examination of the relationship between EO exposure and mortality, both before and after adjusting for key confounding factors, offering insights into the role of EO as a potential determinant of health outcomes.

Cox Proportional Hazards Models Comparing High vs. Low Groups

Similarly, Cox proportional hazards regression models were used to assess the association between high vs. low EO groups and all-cause mortality. The analysis was conducted using three distinct models:

1) Model Cox1B: This unadjusted model compared the mortality risk between high and low EO groups without adjusting for any covariates.
2) Model Cox2B: This model adjusted for age to determine whether the observed association between EO levels and mortality was independent of age.
3) Model Cox3B: This fully adjusted model included additional covariates—high blood pressure, diabetes, and smoking history, alongside age—to assess the independent effect of EO on all-cause mortality.

The use of both unadjusted and adjusted models allowed for a stepwise exploration of the relationship between EO exposure and mortality, ultimately providing insights into whether the observed associations were driven by EO exposure itself or confounded by established risk factors. This approach highlights the potential role of EO as an environmental determinant of mortality, while accounting for other critical health determinants.

Results

Participant Selection and Data Preparation

Overall, 29,350 participants from NHANES were surveyed during the period from 2013 to 2018. Following the inclusion and exclusion criteria, the initial selection included participants with detectable EO levels (N=6,905) and available stroke data (N=28,242). Participants with unknown stroke status (N=6) or missing stroke data (N=1,981) were excluded, resulting in 4,918 participants for univariate analysis (204 with a history of stroke and 4,714 without a history of stroke). Information of age, sex and race were available for all the included participants (Fig. 1). Further refinement excluded participants with missing data on high blood pressure (N=4), diabetes (N=4), and smoking history (N=2), leading to a final dataset of 4,908 participants (204 with stroke and 4,704 without stroke) (Fig. 1). Twenty participants without mortality data were excluded only for the survival analysis.

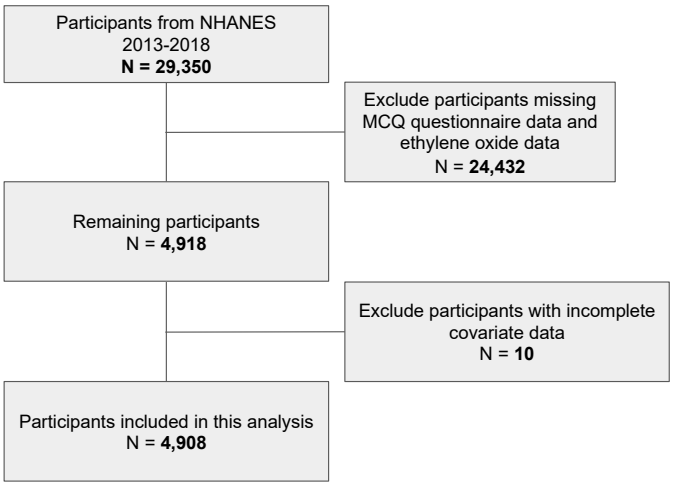

Fig. 1: Flow diagram of the inclusion and exclusion criteria from 2013 to 2018 National Health and Nutrition Examination Survey (NHANES)

Baseline Characteristics of Participants

An overview of the baseline characteristics of participants was presented in Table 1, together with significant levels determined by the univariate analysis, stratified by stroke status. The mean age of participants with a history of stroke was 67.0 yr (±13.25), while the mean age of those without stroke was 49.0 yr (±17.32). Age in years was significantly related to the stroke outcome (*P*<0.0001). Among the stroke group, 47% were female, compared to 51% in the non-stroke group (*P*=0.236).

Table 1: Baseline characteristics of study population

Variables	Participants with stroke (n = 204)		Participants without stroke (n = 4714)		P value
	Available data (n)	n (%)	Available data (n)	n (%)	
Age, years (median ± SD)	204	67.0 ± 13.25	4714	49.0 ± 17.32	<0.0001
Sex, female	204	95 (47)	4714	2395 (51)	0.236
Race	204	-	4714	-	0.504
Mexican American	-	19 (9)	-	731 (16)	-
Other Hispanic	-	17 (8)	-	497 (11)	-
Non-Hispanic White	-	88 (43)	-	1724 (37)	-
Non-Hispanic Black	-	57 (28)	-	965 (20)	-
Non-Hispanic Asian	-	10 (5)	-	615 (13)	-
Other Race - Including Multi-Racial	-	13 (6)	-	182 (4)	-
BMI, m/kg2	191	29.2 ± 7.25	4661	28.2 ± 7.11	0.167
Alcohol assumption history, yes	109	87 (80)	2242	1631 (73)	0.106
High blood pressure, yes	204	147 (72)	4710	1670 (35)	<0.0001
Diabetes, yes	204	64 (31)	4710	638 (14)	<0.0001
A history of smoking, yes	204	125 (61)	4712	2021 (43)	<0.0001

The racial distribution among participants with stroke showed 43% Non-Hispanic White, 28% Non-Hispanic Black, 9% Mexican American, 8% Other Hispanic, 5% Non-Hispanic Asian, and 6% Other Race (including Multi-Racial). In the non-stroke group, 37% were Non-Hispanic White, 20% Non-Hispanic Black, 16% Mexican American, 11% Other Hispanic, 13% Non-Hispanic Asian, and 4% Other Race (including Multi-Racial). No significant association was observed between racial distribution and stroke status (P=0.504).

The average BMI was similar between the two groups, with participants with stroke having an average BMI of 29.2 (±7.25) compared to 28.2 (±7.11) in those without stroke (P=0.167). Alcohol consumption history was reported by 80% of the stroke group and 73% of the non-stroke group (P=0.106).

High blood pressure was significantly more common in participants with stroke (72%) than those without stroke (35%) (P<0.0001). Similarly, diabetes prevalence was higher in the stroke group (31%) compared to the non-stroke group (14%) (P<0.0001). A history of smoking was reported by 61% of participants with stroke and 43% of those without stroke (P<0.0001). These four significant covariates were selected to adjust the EO association with stroke.

Association Between EO and Stroke: Continuous EO Variable

The results of logistic regression analyses were illustrated in Table 2 examining the association between EO levels, treated as a continuous variable, and the risk of stroke. The models show the odds ratios (OR) with 95% confidence intervals (CI) and corresponding P-values for different adjustments.

1) Model LR1C: The unadjusted model shows that each unit increase in EO is associated with a slightly higher stroke risk (OR: 1.001, 95% CI: 1.000–1.002, P=0.031).
2) Model LR2C**:** After adjusting for age, the association strengthens (OR: 1.002, 95% CI: 1.001–1.003, P<0.0001), highlighting age as a key factor influencing the EO-stroke relationship.
3) Model LR3C**:** With additional adjustments for high blood pressure, diabetes, and smoking history, EO remains significantly associated with stroke (OR: 1.001, 95% CI: 1.000–1.003, P=0.023).

Table 2: Association Between Continuous EO Levels and Stroke Risk

Variable	OR (95% CI)	P value
Model LR1C	1.001 (1.000–1.002)	0.031
Model LR2C	1.002 (1.001–1.003)	<0.0001
Model LR3C	1.001 (1.000–1.003)	0.023

These findings suggest a statistically significant but modest association between higher EO levels and an increased risk of stroke, even after adjusting for multiple important confounding factors. The persistence of this association, despite adjustments for age, high blood pressure, diabetes, and smoking history, highlights the robustness of the observed relationship. Such findings underscore the potential role of EO exposure as an environmental risk factor for stroke, warranting further investigation into the underlying biological mechanisms.

Association Between EO and Stroke: High vs. Low EO Levels

Table 3 presents the results of the logistic regression analyses evaluating the association between high versus low EO levels, stratified using a median split, and the risk of stroke. Three models were developed to examine both unadjusted and adjusted associations:

1) Model LR1B: This unadjusted logistic regression model indicated that participants in the high EO group had an odd OR of 1.280 (95% CI: 0.966–1.700) for stroke compared to the low EO group, with a P-value of 0.087. Although this association did not reach statistical significance, it suggests a trend towards higher stroke

risk among individuals with elevated EO levels.

2) Model LR2B: After adjusting for age, the logistic regression model showed an OR of 1.557 (95% CI: 1.166–2.087) for stroke in the high EO group, with a statistically significant *P*-value of 0.003. This statistically significant result underscores the critical role of age as a confounding factor and suggests that the increased EO levels might contribute to a higher risk of stroke, independent of age.
3) Model LR3B: This model incorporated additional adjustments for high blood pressure, diabetes, and smoking history, alongside age. With these covariates included, the association between high EO levels and stroke was attenuated, yielding an OR of 1.309 (95% CI: 0.969–1.772, *P*=0.081). Although this association did not achieve statistical significance, it indicates a persistent trend that warrants further investigation.

Table 3: Association Between High vs. Low EO Levels and Stroke Risk

Variable	OR (95% CI)	P-value
Model LR1B	1.280 (0.966–1.700)	0.087
Model LR2B	1.557 (1.166–2.087)	0.003
Model LR3B	1.309 (0.969–1.772)	0.081

Higher EO levels are associated with an increased risk of stroke, particularly when adjusted for age alone. However, the attenuation of the association after adjusting for additional covariates highlights the potential role of confounding factors such as high blood pressure, diabetes, and smoking history. This underscores the complexity of EO's impact on stroke risk and the importance of considering multiple confounders in the analysis. While EO exposure may be a contributing risk factor for stroke, its effects are likely influenced by a broader interplay of other health determinants.

Association of Log-Transformed EO with Stroke Risk

The association between log-transformed EO levels (pmol/g Hb) and the odds ratio (95% CI) of stroke across three logistic regression models was reported in Fig. 2.

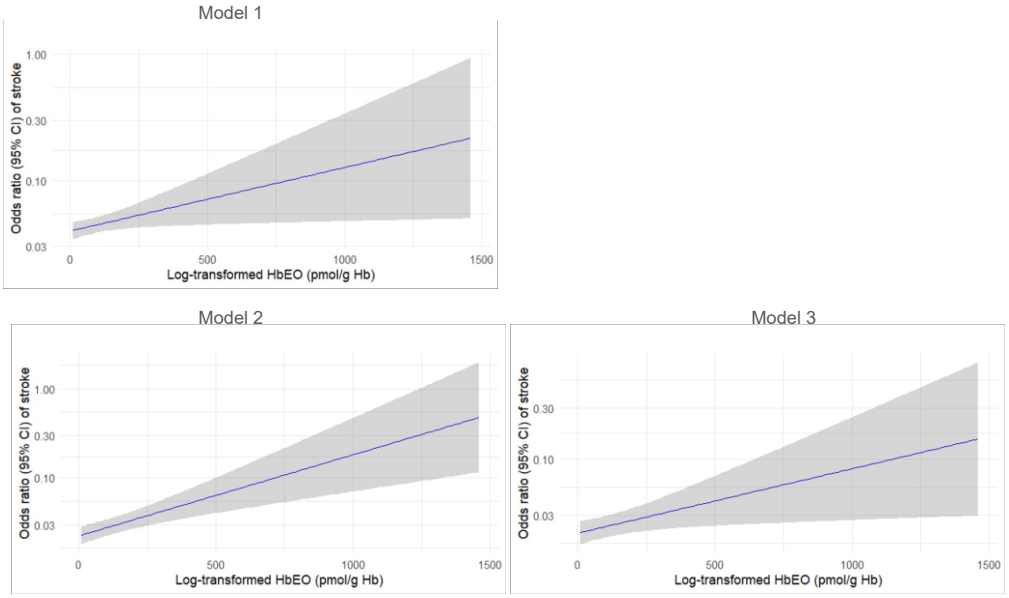

Fig. 2: Association of Log-Transformed EO with Stroke Risk

1) Model LR1C: This unadjusted model shows a positive association between log-transformed EO levels and the odds ratio of stroke. The odds ratio increases with higher EO levels, and the confidence interval widens at higher EO levels, indicating greater variability (OR: 1.001, 95% CI: 1.000–1.002, *P*=0.031). These findings provide preliminary evidence of a potential relationship between EO exposure and stroke risk, albeit with notable variability.
2) Model LR2C: In this model, adjustment for age was performed, revealing a stronger positive association between EO levels and stroke risk. The odds ratio increases more steeply with higher EO levels, and the confidence interval remains significant (OR: 1.002, 95% CI: 1.001–1.003, *P*<0.0001). This outcome suggests that the association between EO and stroke is more pronounced when the impact of age is accounted for, highlighting age as a critical factor in modifying stroke risk.
3) Model LR3C: This model further adjusts for high blood pressure, diabetes, and a history of smoking, in addition to age. Despite these additional covariate adjustments, the association between EO levels and stroke risk remained positive, though slightly attenuated compared to Model LR2C, with a significant confidence interval (OR: 1.001, 95% CI: 1.000–1.003, *P*=0.023). The relationship between EO and stroke risk is robust, even after accounting for multiple important risk factors.

The shaded areas in each graph represent the 95% confidence intervals, providing a visual depiction of the precision of the estimated odds ratios at different levels of EO. The consistent positive association across all three models underscores the potential link between higher EO levels and increased stroke risk, even after adjusting for key cofounding factors such as age, high blood pressure, diabetes, and smoking history. EO exposure may serve as an environmental risk factor for stroke, warranting further investigation into the underlying biological mechanisms.

Association of Continuous EO Levels with All-Cause Mortality in the General Population

The association between continuous EO levels and all-cause mortality in the general population was evaluated using Cox proportional hazards models (Table 4). Three distinct models were developed to evaluate the unadjusted and adjusted hazard ratios (HRs):

1) Model Cox1C: The unadjusted model showed no significant association (HR: 1.000, 95% CI: 0.999–1.001, *P*=0.894).
2) Model Cox2C: After adjusting for age, a significant association emerged (HR: 1.002, 95% CI: 1.001–1.003, *P*=0.005), suggesting that age plays a critical role in the EO-mortality relationship.
3) Model Cox3C: Further adjustment for high blood pressure, diabetes, and smoking history, in addition to age, resulted in a hazard ratio of 1.000 (95% CI: 1.000–1.002), *P*=0.184). This finding indicates that the association between EO levels and all-cause mortality was no longer statistically significant after full adjustment, suggesting that the observed age-adjusted association might have been influenced by these additional covariates.

Table 4: Cox Proportional Hazards Model for All-Cause Mortality in the General Population Using Continuous EO Levels

Variable	OR (95% CI)	P-value
Model Cox1C	1.000 (0.999–1.001)	0.894
Model Cox2C	1.002 (1.001–1.003)	0.005
Model Cox3C	1.000 (1.000–1.002)	0.184

While a significant association between EO levels and all-cause mortality exists when the analysis is adjusted solely for age, this association diminish-

es after additional adjustments for high blood pressure, diabetes, and smoking history. This attenuation underscores the potential role of these confounding factors in mediating the observed relationship between EO exposure and mortality. The findings emphasize the importance of considering a broad spectrum of covariates to accurately determine the independent effect of EO exposure on health outcomes, and they suggest that the initial association observed in age-adjusted models may partly reflect the influence of other health determinants.

Association of High vs. Low EO Levels with All-Cause Mortality in the General Population

The association between high versus low EO levels, stratified by a median split, and all-cause mortality in the general population was evaluated using Cox proportional hazards models (Table 5).

Table 5: Cox Proportional Hazards Model for All-Cause Mortality in the General Population Using EO as High vs Low groups using median split

Variable	OR (95% CI)	P-value
Model Cox1B	1.177 (0.894–1.550)	0.244
Model Cox2B	1.524 (1.155–2.011)	0.003
Model Cox3B	1.333 (1.003–1.770)	0.047

Three models were developed to evaluate the unadjusted and adjusted HRs:

1) Model Cox1B: The unadjusted Cox model indicated that participants in the high EO group had a hazard ratio of 1.177 (95% CI: 0.894–1.550, *P*=0.244) for all-cause mortality compared to the low EO group, suggesting no significant association between high EO exposure and all-cause mortality in the unadjusted model.
2) Model Cox2B: After adjusting for age, high EO levels were significantly associated with increased mortality risk (HR: 1.524, 95% CI: 1.155–2.011, *P*=0.003), highlighting age as a key confounder.
3) Model Cox3B: With additional adjustments for high blood pressure, diabetes, and smoking history, the association remained significant (HR: 1.333, 95% CI: 1.003–1.770, *P*=0.047), indicating that the relationship between high EO levels and mortality risk is robust.

Higher EO levels, as categorized by a median split, are associated with an increased risk of all-cause mortality, particularly when adjusted for age and additional. confounding factors such as high blood pressure, diabetes, and smoking history. The significant association observed in Model Cox3B emphasizes that EO exposure may serve as an important environmental risk factor for mortality, necessitating further investigation to better understand its implications for public health.

Discussion

This study explored the association between EO exposure and stroke risk using data from the NHANES database. Elevated EO levels correlate with a heightened risk of stroke, highlighting EO as a potential environmental hazard. Furthermore, we uncovered a link between EO exposure and all-cause mortality in the general population, adding to the growing body of evidence on EO's toxicity and its broader health impacts.

EO is a recognized environmental contaminant, and all individuals carry some level of endogenous EO formed via metabolic processes, such as bacterial activity in the gastrointestinal tract or liver-based ethylene metabolism (13). Beyond these baseline levels, EO exposure can be considerably higher in certain occupational settings, including food processing, spice production, and medical device sterilization, placing workers—particularly those in sterilization departments—at greater risk (14). The deleterious health effects of EO are well established; it is genotoxic and mutagenic, with studies linking human EO exposure to increased incidences of breast cancer and various hematologic malignancies (4). The U.S. Environmental Protection Agency (USEPA) identified EO as a probable human carcinogen as early as 1985 and has since reported significant excess

cancer risks surrounding major sterilization facilities (15). A Swedish occupational cohort further supports these concerns, reporting elevated mortality, primarily from tumors and circulatory diseases, in workers with high EO exposure (16). While these studies underscore EO's carcinogenic and systemic effects, none have directly examined whether EO exposure also influences stroke risk. Addressing this gap, our study suggests that EO exposure may be implicated in cerebrovascular disease, expanding the scope of EO's potential adverse health effects.

The connection between EO exposure and stroke development is not yet fully understood. Existing evidence implicates EO in the promotion of cardiovascular disease through pathways involving inflammation, oxidative stress, and disruptions in fatty acid metabolism(17). These processes likely contribute to endothelial dysfunction and the progression of atherosclerosis—critical drivers of cerebrovascular pathology. By directly fostering vascular damage, EO-induced inflammation and oxidative stress provide a mechanistic basis for the observed link between EO exposure and stroke risk. Experimental evidence further supports these links: acute exposure to ethylene chlorohydrin (ECH), a key EO byproduct, impairs mitochondrial fatty acid elongation and elevates triglyceride levels in animal models (18). By promoting lipid imbalances, these metabolic disturbances may intensify vascular injury and increase the likelihood of stroke.

Chronic EO exposure has also been linked to diminished glutathione reductase activity and heightened lipid peroxidation in the liver—hallmarks of persistent oxidative stress(11, 19). Oxidative stress is central to stroke pathogenesis, as it can directly impair endothelial integrity and promote vascular injury, ultimately leading to cerebrovascular events (20, 21). This further reinforces EO's potential role in enhancing stroke susceptibility.

Inflammatory responses and altered lipid metabolism also intersect with EO exposure. Studies show that EO can initiate organ-level inflammation in animal models, fostering conditions conducive to atherosclerosis—a critical precursor of stroke (22-24). For example, inflammatory cascade activation can elevate triglycerides and reduce HDL-C(25), impairing cholesterol clearance and exacerbating endothelial damage. Together, these inflammatory and metabolic derangements act synergistically to amplify the vascular insults that culminate in stroke.

Human data further substantiate this concern, in a study of 3,448 American adults, higher levels of EO exposure were found to be significantly correlated with elevated HbA1c levels and an increased prevalence of diabetes (26). Since diabetes accelerates atherosclerosis and endothelial dysfunction—key processes in stroke pathogenesis—EO-induced metabolic dysregulation provides yet another avenue by which EO may heighten stroke risk.

Collectively, EO exposure may affect multiple biological pathways that converge on increased cardiovascular risk, including stroke. By comparing our results with prior studies that explored EO's impacts on inflammation, oxidative stress, lipid metabolism, and glucose regulation, we provide a comprehensive understanding of the mechanisms by which EO exposure may contribute to stroke risk. The broader implications of this research emphasize the need for stringent measures to limit EO exposure, particularly in occupational settings, to mitigate its adverse health effects.

This study incorporates several methodological strengths that enhance the reliability and relevance of the findings. First, by utilizing NHANES—a nationally representative dataset with rigorous quality control protocols—our results hold broad generalizability. Second, the employment of HbEO as a validated and precise biomarker ensures that EO exposure levels are captured accurately, strengthening the foundational exposure-outcome relationship. Third, our investigation examines EO exposure and its health implications using multiple analytical approaches, including logistic regression and Cox proportional hazards models, each with progressive adjustments for key confounders. This stepwise, multifaceted analytical framework not only provides a more nuanced understanding of the

EO-stroke association but also clarifies how EO may influence all-cause mortality under varying levels of covariate control. Finally, as one of the earliest cross-sectional explorations into the link between EO and stroke, our study fills a critical gap in the literature and encourages future longitudinal and mechanistic research.

Limitations

Nevertheless, our study has several limitations. The small number of cerebrovascular-related deaths (28 cases among 4,908 participants) constrained our analysis to all-cause mortality, limiting the granularity of our findings. As a cross-sectional study, causal relationships cannot be definitively established, underscoring the need for future longitudinal and cohort studies to confirm these associations. Additionally, despite extensive covariate adjustments, residual confounding may persist due to unmeasured factors. Our ability to fully assess occupational EO exposure was further restricted by the lack of detailed occupational histories in NHANES, highlighting the importance of incorporating industrial hygiene measurements, exposure matrices, or biomonitoring data in future research.

To more rigorously address residual confounding in future research, advanced causal inference methods—such as propensity score matching, inverse probability weighting, and the use of directed acyclic graphs (DAGs)—may facilitate a more systematic identification and adjustment of unmeasured confounders. These techniques, combined with more extensive longitudinal data collection, could better delineate the temporal and causal pathways linking EO exposure to stroke risk.

Conclusion

Statistically significant correlations were observed between stroke and EO level. Independent replication in a prospective cohort is now needed to confirm the associations reported. If the associations reported here are confirmed in independent studies, it should give impetus to work to identify the mechanisms of action linking long-term, low-dose EO exposure to adverse outcomes in humans. Given the substantial negative effects on adult health that may be associated with elevated EO and also given the potential for reducing human exposure, our findings merit further research into the human health effects of this chemical.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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