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Review

Genotoxicity of ethylene oxide: A review of micronucleus assay results in human population

Manosij Ghosh[a,*], Lode Godderis[a,b,**]

[a] KULeuven, Department of Public Health and Primary Care, Centre Environment & Health, B-3000 Leuven, Belgium
[b] Idewe, External Service for Prevention and Protection at Work, B-3001 Heverlee, Belgium

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ABSTRACT

Ethylene oxide (EtO) has been categorized as "*carcinogenic to humans* (*Group 1*)" by the IARC. While several epidemiological studies have reported carcinogenicity and EtO-Hb formation; information from cytogenetic endpoints are rather inconclusive. In the present review, we focus on the results of eleven studies which have reported the results of micronucleus assay in EtO exposed workers. We have critically reviewed these studies based on the exposure assessment, concentration and duration, and compared the sensitivity of micronucleus assay to other reported endpoints like EtO-Hb, CA, SCE. The levels of EtO and EtO-Hb adducts in all the studies were strongly correlated to the results of SCE, but not to MN. MN were only observed in a limited number of studies with high EtO exposure (2–28 ppm 8 h-TWA) and not below the recommended concentration of <1 ppm. To further understand the effect of exposure of EtO on MN assay outcome, we propose studies with more harmonized protocol for exposure assessment and MN analysis, determination of suitable sample size and use of multiple target tissues to understand the effect of metabolite.

Contents

1. Introduction

Ethylene oxide (EtO) is a frequently produced organic chemical, used as an intermediate for production of several chemicals including ethylene glycol, ethoxylates among others. It is also commonly used in sterilization of medical instruments and devices. With an estimated global production of ~20 million tonnes, it is one of the most produced organic chemicals.

The high global production of EtO increases the risks of environmental and occupational exposure. The CAREX EU report published in 1999 estimated the number of workers exposed to EtO at 46900 [1]. According to the report, approximately 22300 workers were involved in medical, dental, other health services, while 1000 workers were involved in production of EtO. Reports from US National Occupational Exposure Survey between 1981 and 1983, estimated that approximately 270000 workers were exposed to varying concentrations of EtO [2], of which 98997 workers were associated with health services. More recent estimates of

Abbreviations: EtO, ethylene oxide; EtO-Hb adduct, ethylene oxide-haemoglobin adduct; CA, chromosomal aberrations; MN, micronuclei; SCE, sister chromatid exchanges; 8-h TWA, 8 h time weighted average.

* Corresponding author.
** Corresponding author at: KULeuven, Department of Public Health and Primary Care, Centre Environment & Health, B-3000 Leuven, Belgium.
E-mail addresses: gmanosij@gmail.com (M. Ghosh), lode.godderis@med.kuleuven.be (L. Godderis).

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CAREX-Canada [3], suggests that approximately 2400 workers are exposed to EtO at workplace, of which more than 2100 were involved in the health care and sterilization services.

Comparison of historical data from various sources including CAREX EU [1], IARC [4–6], US National Occupational Exposure Survey [2], CAREX-Canada [3] clearly indicates that exposure to workers involved in sterilization process has been much higher (1–30 ppm), compared to exposure levels during EtO synthesis (<1 ppm). Accidental exposure to EtO is common during and after the sterilizing cycles as well. These exposures are usually above the odour threshold of 500 ppm and are extremely hazardous. Several other accounts of accidental exposure to EtO have been reported [7–10] and is usually between 300 and 700 ppm.

While, acute exposures are known to cause nausea, bronchitis, pulmonary oedema; workers with chronic exposure are at risk of developing neurological disorders and cancer [11,12]. EtO is also a known alkylating (hydroxyethylating) agent, which can lead to the formation of adducts with DNA [13,14] and proteins like haemoglobin [15,16]. Several cytogenetic studies, *in vitro* and *in vivo,* have confirmed the genotoxicity and mutagenicity of EtO. Studies have also provided substantial evidence of carcinogenicity in rodents. Over the years, several epidemiological studies have associated EtO exposure with cancers in human [17,18] including gastro-intestinal and breast cancers [19,20].

Based on limited evidences of toxicity, EtO was first listed in the Fourth Annual Report on Carcinogens in 1985 "as possible human carcinogen". Considering the DNA-damaging activity of EtO and increased risk of cancer, demonstrated by epidemiological studies, the listing was revised to "*known to be a human carcinogen*" in the Ninth Report on Carcinogens in 2000 [21]. Similarly, EtO was considered by the IARC Working Groups from 1976 to 2012, and based on several evidences EtO has been categorized as "*carcinogenic to humans* (*Group 1*)".

Consequently, from an occupational perspective it is important to adequately follow-up workers exposed to EtO at regular intervals. Traditionally, the most common method for bio-monitoring is the measurement of EtO metabolites (in urine) or EtO adducts (haemoglobin and DNA). Nevertheless, cytogenetic studies provide significant information regarding EtO toxicity. It also provides possible mechanistic explanation of carcinogenesis. In this review we discuss EtO toxicity with special emphasis on the micronucleus assay in human population. The results of micronucleus assay are critically reviewed, highlighting the exposure concentrations, study design, findings and the knowledge gaps. The results are also compared to various other genotoxicity assays, thus providing an overview regarding the sensitivity of micronucleus assay in EtO bio-monitoring. Finally, the findings are summarised, and some recommendations are put forward.

2. Materials and methods

Several epidemiological studies have considered different aspects of EtO toxicity, including carcinogenicity and mortality. While many studies have included exposure measurement and EtO haemoglobin adduct formation (EtO-Hb), only a limited number of studies have addressed the cytogenetic endpoints. A literature search was therefore performed till December 2015 using PubMed and Scopus databases. Search string of "ethylene oxide" [All Fields] and "Micronucleus" [All Fields] in Pubmed returned a total of 23 results. Search string "Title-Abs-Key (Ethylene Oxide) And Title-Abs-Key (Micronucleus)" for article published in journals returned a total of 37 results in Scopus search. These search results included articles reporting MN formation in animal models and a limited number of epidemiological evidences. A manual search of these references was subsequently performed. For the interest of the present review, 11 studies were selected based on their inclusion of micronucleus assay (MN) in human population as one of the test endpoints. Additionally, some of the evaluated studies also reported results of sister chromatid exchanges (SCE), chromosomal aberrations (CA), DNA single-strand breaks (SSB), HPRT mutations.

3. Epidemiological evidence of MN formation in workers exposed to EtO

The eleven shortlisted studies on workers exposed to ethylene oxide have been described in this section. The articles were analyzed with respect to their quality and the observations from these reports are critically reviewed below and summarised in Table 1, highlighting the study population, EtO exposure, levels of EtO-Haemoglobin adducts (EtO-Hb) and the methods and results of genotoxicity assays performed. For each study reported, frequency ratio (FR = MN in exposed population/MN in control) was calculated for micronucleus assay for convenience of comparison between different studies, and has been represented in Fig. 1. Additionally, a quality score was assigned to each of the study out maximum possible score of 27 (Supplementary Table 1).

Högstedt et al. [22] investigated the effect of EtO on different cytogenetic parameters in an exposed Swedish population. They studied a group of 28 workers exposed to EtO and 20 control subjects. The workplace exposure during biological sampling was less than 1 ppm (8 h-TWA), with occasional high exposure of up to 52 ppm. The workers of two factories and controls were studied for several cytogenetic parameters including SCE in lymphocyte cells, and CA and MN in lymphocyte and bone marrow cells. Workers exposed in both the factories had higher frequency of CA than controls in lymphocyte cells. The frequency of SCE in lymphocyte however remained unaltered in the exposed and control groups. MN in lymphocyte cells revealed no significant change (FR for Factory 1 = 1.16; Factory 2: 0.75). A comparison between the cytogenetic endpoints indicated that CA was more sensitive in detecting genotoxic effects of EtO in both lymphocyte and bone marrow cells. The authors also suggested that bone marrow MN were good biomarker of EtO toxicity, however its use is limited by the difficulty of sampling.

In a later study, Högstedt et al. [23], compared the cytogenetic effects of EtO and propylene oxide exposure in a group of Swedish workers. A total number of 18 subjects (EtO exposed) were included, with mean age 30.8 ± 8.1. EtO-Hb adducts were measured and were between 1.2–10 nmol/gHb (mean- 3.3 nmol/gHb). The authors studied a total of 100 metaphase spreads to study CA. The percentage of CA in the study population was five. MN assay was performed according to the method of Hogstedt [24]. Briefly, lymphocyte cells were harvested after 72 h of culture, smear prepared and stained with May-Grunwald-Giemsa's stain. A total of 1000 cells were scored. MN frequency in the EtO exposed workers were reported to be 5.78%. A dose response study among the workers based on their EtO-Hb levels and MN frequency did not reveal significant correlation. However, since the study lacks a well-defined control population, it is extremely difficult to interpret the results of EtO exposure.

To understand the biological effect of EtO, Mayer et al. [25] evaluated a number of endpoints including EtO-Hb, SCEs, MN, CA, SSB and index of DNA repair. The study was conducted with 34 (10 male; 24 female) workers exposed to EtO and 24 control subjects. Workers were exposed to an EtO concentration of 0.1 ppm, while control population were exposed to much lower levels (below 0.02 ppm). Workplace exposure to EtO significantly increased SCE, and affected DNA repair capacity. The results SCE and DNA repair assay were strongly correlated with that of EtO-Hb levels. However CA, SSB were not affected by the workplace exposure to EtO, or smoking habits. MN formation remained unaltered amongst