SUMMARY OF THE THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INCREASED GENOTOXIC SUSCEPTIBILITY OF BREAST EPITHELIAL CELLS TO ETHYLENE OXIDE

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1. INTRODUCTION, LITERATURE REVIEW

1.1. Ethylene oxide

Ethylene oxide (ETO) is one of the most important epoxide compounds that is widely used in the industry. ETO is a well-known mutagen; its carcinogenicity is confirmed by animal studies and suggested by human epidemiological investigations. The International Agency for Research on Cancer (IARC) has found evidence on the carcinogenic property of ethylene oxide and classified it as "carcinogenic to humans" (Group 1).

1.1.1. Characteristics, exposure

Ethylene oxide (C_2H_4O) is a colourless gas with ether-like sweet odour. It has high reactivity due to its cyclic ether molecular structure. The highly flammable ETO is a widely used intermedier of the chemical industry. Based on its antimicrobial property ETO is applied as a cleaner, disinfectant and sterilizer. The food industry uses it for the fumigation of food products, and the health care for the sterilization of medical equipment.

Human exposure can be experienced predominantly by inhalation in occupational settings, occurring mainly during ethylene oxide production in chemical industry, use in plastic industry, ETO sterilization in food, medical and pharmaceutical industry, and in the sterilization units of hospitals. Operators of ETO gas sterilizers are typically exposed to ETO levels between 0.1–20 mg/m^3 8-hour-time-weighted-average in hospitals. The highest exposure levels (even several thousand mg/m^3) can be encountered at the end of process when the door of sterilizer is opened and the gas is released, as well as when the cylinders are changed; and it can be attributed to improper design, maintenance and use of the equipment. The air concentration of ETO was observed in a range of 5–150 mg/m^3 in the resting room of nurses of a Hungarian county hospital, where the gas sterilizer was run anomalously between 1976–1992.

1.1.2. Acute and chronic health effects

Ethylene oxide has considerable acute toxicity. It can strongly irritate the skin and mucus membranes with local acute effect. Systemically it often causes general symptoms, in serious

cases incoordination and speaking difficulty may develop. High doses of ETO induce pulmonary oedema and narcosis.

Chronic ETO exposure results in cataract formation. It can cause chronic inflammation of the respiratory tract, dermatitis and skin sensitization. Chronic exposure may lead to damage of the liver, peripheral and central nervous system. Embryotoxicity and teratogenicity are late toxic effects of ETO. The frequency of abortions increases among females exposed to ethylene oxide at the workplace; decreased male fertility has also been observed.

1.1.3. Genotoxicity, mutagenicity, carcinogen effects

Ethylene oxide is a directly acting alkylating agent, similarly to other epoxide compounds. Due to its high reactivity, ETO easily forms adducts with cellular macromolecules, such as DNA and proteins. ETO can alkylate the amino acid side chains of some proteins (e.g. haemoglobin), as well as the bases (purine) and phosphate groups of DNA. The modification affects predominantly N-7-guanine resulting in the formation of N-7-alkyl(hydroxyethyl) guanine. As a consequence, the glycosyl bound between the guanine base and the deoxyribose ring becomes destabilized leading to spontaneous base loss. Furthermore, the activity of N-glycosylases specialised to modified bases also contributes to the increasing number of apurinic sites.

The genotoxic and mutagenic properties of ETO has been demonstrated by several *in vitro* tests**,** *in vivo* animal and human epidemiological studies. ETO provided positive results in the *Salmonella typhimurium* Ames reverse mutation assay with some strains. It was found to induce DNA strand breaks, hypoxantine(guanine) phosphoribosyl transferase (*HPRT*) gene mutations and sister-chromatid exchange in cell cultures; *HPRT* mutations, chromosome aberrations, micronucleus formation and sister-chromatid exchange in animals *in vivo*; and DNA strand breaks, chromosome aberrations and sister-chromatid exchange in humans.

Although, inhaled ethylene oxide induced several types of cancer in animal experiments, epidemiological studies provided inconclusive findings about its carcinogenic properties in humans. The potential role of ETO exposure in the development of breast cancer has been suggested by Norman *et al.*, however, this hypothesis is still questionable. Besides its acknowledged potency in causing leukaemia and lymphoma, some studies identified additional types of tumours induced by ETO in humans, such as breast cancer, while others yielded negative results.

An unusual cluster of cancer cases, including several cases of breast cancer, has been identified among health care workers of the Markhot Ferenc Hospital in Eger. On the basis of

the increased incidence of neoplastic diseases, Tompa *et al.* conveyed the suggestion that it might be related to occupational exposure to ETO from an inappropriately run gas sterilizer. Elevated levels of chromosome aberrations and sister-chromatid exchange could be detected in the peripheral blood lymphocytes of exposed individuals. The hypothesized etiological role of ETO in breast cancer induction was also supported by an epidemiological study based on indirect standardisation from Kardos *et al.*, who demonstrated excess mortality rates (SMR: 251–273%) with strong statistical significance in the exposed hospital cohort.

1.1.4. Occupational health aspects of carcinogenicity

In virtue of the Chemical Safety Act 2000 (XXV.), the 25/2000. EüM-SzCsM Regulation defines maximal concentration (MK) level to the chemicals with stochastic effect – typically carcinogens that no-observed-adverse-effect-level cannot be established for –, which must not be exceeded at workplaces for any time. MK level for ETO is 1,8 mg/m^3 (1 ppm) in Hungary. The binding occupational exposure level (permissible exposure limit, PEL) for ethylene oxide set by the US. Occupational Safety and Health Administration is the same as that applied in Hungary (1,8 mg/m^3), however, the maximum exposure level (MEL) in the United Kingdom is higher (10 mg/m^3).

1.2. Comet assay

The measurement of DNA damage in individual cells was realised by Östling and Johanson who developed the single-cell gel electrophoresis assay (comet assay). Further improvement of the comet assay was carried out by Singh *et al.* by introducing alkaline conditions of DNA unwinding and electrophoresis allowing the detection of not only double- but also single-strand breaks and alkali labile sites (apurinic, apyrimidinic sites and phosphotriesters). In the last decade, several versions of the technique were developed for the identification of various types of DNA damage (e.g. oxidative).

1.2.1. Method, application

The alkaline version of the comet assay is a sensitive and simple method for detecting DNA single and double strand breaks and alkali labile sites. After the cytoplasm of examined cells embedded in agarose on microscope slides is lysed in alkaline solution with high salt and

detergent content, the double helix structure of DNA in the remaining nuclei is allowed to unwind and break up under alkaline conditions. In this way, single-strand breaks, even alkali labile lesions, capable of being transformed into single-strand breaks at high pH, become detectable. During electrophoresis, the intact large DNA molecule remains sedentary in the low voltage electric field, while the damaged, broken peaces migrate towards the anode. The size of the evolved „tail" (the extent of migration) is proportion to the level of DNA damage.

A useful application of the alkaline comet assay is the field of genetic toxicology. Several studies used the method to investigate the genotoxic properties of various chemicals *in vitro* and *in vivo*. A great advantage of the assay is its sensitivity and that only a few cells and a small quantity of test chemical are needed. Due to its sensitivity, the comet assay also proved to be a useful technique in epidemiological studies, mainly for the detection of DNA damage in cells from samples of individuals exposed to genotoxic agents.

1.2.2. Genotoxicological studies with hexavalent chromium

The *in vitro* model for investigating the genotoxic effect of ethylene oxide was developed in our previous study with hexavalent chromium (Cr^{VI}). Cr^{VI}, classified by the IARC as "carcinogenic to humans", exposes humans predominantly by inhalation. Since the principal biological target is the respiratory tract, a confluent layer of human lung cells provided adequate *in vitro* model for the investigation of the genotoxic effect of Cr^{VI} exposure. We characterised the DNA damaging effect of Cr^{VI} in two cell populations, i.e. in cultures of peripheral lymphocytes and alveolar type II pneumocytes (A549 human epithelial lung carcinoma cell line; ECACC No. 86012804). The treatment of cells with non-cytotoxic concentrations of sodium dichromate resulted in a concentration-dependent increase of the genotoxic effect as measured by the alkaline version of the comet assay. The lowest concentrations of sodium dichromate that resulted in a statistically significant (p<0.01) increase of the DNA damage level were 50 μM in lymphocytes and 10 μM in pneumocytes. Endonuclease (formamidopyrimidine glycosylase) pre-treatment that makes the comet assay able to detect oxidative type DNA damage increased the sensitivity of detection in pneumocytes 10-fold, suggesting a role for DNA base oxidation in the mechanism of DNA damage induced by hexavalent chromium. The plateau observed at high concentrations of the dose-response curve (1 mM) suggests the formation of DNA-DNA cross-links induced by Cr^{VI} exposure. The model developed for investigating the genotoxicity of hexavalent chromium proved to be adequate for the investigation of ethylene oxide.

2. OBJECTIVES

The objective of our study was to investigate the genotoxic effects of ethylene oxide *in vitro* in primary and secondary cell cultures.

The sensitivity of breast epithelial cells was characterised by comparing the sensitivity of known and hypothesized target cells of ethylene oxide and also that of cell types not identified as targets, in order to answer the question whether ethylene oxide exposure can be responsible for breast cancer development.

In the study we investigated:

1. Is it possible to set up an *in vitro* laboratory model, by which the DNA damage induced by treatment with ethylene oxide can be measured by means of the comet assay in human cell cultures?

2. Is there a statistically demonstrable difference in the susceptibility of the various cell types studied that can be characterised by the dose-response relationship of genotoxicity?

3. Are the breast epithelial cells similar to those cells that are susceptible to the genotoxic effect of ethylene oxide?

3. MATERIALS AND METHODS

3. 1. Cell cultures

Human lymphoblasts (HL-60 human promyelocytic leukaemia cell line; ECACC No. 93021013) were cultured in RPMI 1640 medium supplemented with 20% foetal bovine serum (FBS). The adherent cells were grown as a monolayer: the human breast epithelial cells (BT-474 human epitheloid ductal breast carcinoma cell line; ATCC No. MTB-20) in RPMI 1640 medium (supplemented with 20% FBS), and the human keratinocytes (HaCaT spontaneously transformed human keratinocyte cell line; Deutsches Krebsfroschungszentrum, Heidelberg) and cervical epithelial cells (HeLa human epitheloid cervical carcinoma cell line; ECACC No. 93021013) in DMEM medium (supplemented with 10% FBS). All cell lines were cultured in 25 cm^2 plastic cell culture flasks in the humidified 5% CO_2 atmosphere of an incubator at 37°C.

Human blood samples were repeatedly obtained from the same healthy, non-smoking male volunteer and processed immediately. After separation, peripheral lymphocytes were suspended in RPMI 1640 medium supplemented with 10% FBS.

The Trypan-blue exclusion test was used throughout the study to assess cell viability by detecting membrane integrity. The viability of isolated cells was found to be always over 90%. The passages and all other handling of cells were carried out in a class II microbiological safety cabinet with appropriate hygiene measures.

3.2. Ethylene oxide exposure

Stock solutions of ethylene oxide (1 M) were freshly prepared before each experiment by weighing liquid state ETO (boiling-point 10.4°C) into PBS (pH 7.5) in glass screw-cap vials with septa at 4°C using a gas-tight pre-cooled syringe. Dilutions were made from the stock solution in the culture media.

The cells were treated with ethylene oxide and further processed in a system that we developed earlier for the *in vitro* characterisation of genotoxic properties of hexavalent

chromium. The applied concentrations (0–1000 μM) were defined after pre-testing the cytotoxic effect of ETO with the Trypan-blue exclusion test. During the cytotoxicity studies the proportion of viable cells was found to be always >85% in the 0–1000 μM concentration range.

Lymphoblasts and peripheral lymphocytes were treated at a cell density of 2 x 10^5 cells/ml medium in plastic tubes at 37°C for 1 hour. The adherent breast epithelial cells, keratinocytes and cervical epithelial cells were seeded in 12-well plates and grown to confluence before treatment with ethylene oxide in medium at 37°C for 1 hour.

After treatment and removal of the medium, adherent cells were scraped up from the wells to avoid trypsin induced DNA damage. The medium was separated by centrifugation from the cells treated in suspension. All cell cultures were then washed and re-suspended in serum-free medium at a cell density of 1000 cells/μl for use in the comet assay.

Each experiment was repeated 3–6 times. Cell viability was determined before and after treatment by use of the Trypan-blue exclusion test and the proportion of dead cells did never exceed the values found in the investigation of cytotoxicity.

3.3. Comet assay

For the assessment of genotoxicity, the alkaline version of the comet assay was used with the following modifications: 1% normal melting-point agarose (NMA) was cast on the surface of a one-sided fully frosted microscope slide, and then after solidification removed in order to provide better surface adhesion and to substantially reduce background intensity. Then a new 1% NMA layer was cast on the surface. Cells (~10000–15000) were embedded in 0.75% low melting-point agarose (LMA) applied on the 1% NMA layer and covered with a top 0.75% LMA layer. After solidification, slides were placed in a cold lysis solution overnight. Further steps were made in a darkroom at yellow light in the absent of sunlight. After the lysis of cytoplasm, the unwinding of DNA and the breaking off of abasic lesions was allowed for 20 minutes in an alkaline electrophoresis buffer (pH>13). The electrophoresis was conducted in the same buffer in a horizontal electrophoresis tank for 20 minutes at room temperature by applying an electric current of 0.8 V/cm (300 mA). Slides were then immersed in a neutralization buffer for 3×5 minutes, dried and stained with ethidium bromide solution. Duplicated slides were used for each sample.

Digitalisation and analysis of the fluorescent images was carried out with a Zeiss Axioplan fluorescent microscope equipped with a 50 W mercury-arc lamp, connected to a black-and-white intensified charge-coupled device camera and a comet image-analysis system (Metasystems, Germany). Applying a multi-bandpass filter system, the samples were excited at 570 nm. Images of 50 randomly selected nuclei/slide (100 images/sample) were digitally captured at 400× magnification, using a 40× NA 0.75 dry objective.

DNA damage was measured and expressed as tail DNA (% of intensity) and tail length (μm), from which tail moment values (tail moment = tail DNA content (%)×tail length) were automatically calculated with the 'Comet Imager 1.2' software. After correction to the background intensity, the measurement programme of the image-analysis software set the intensity curve for the whole image and then the intensity of the head was determined by mirroring the curve to the point of highest intensity. The difference of the curves provided the tail intensity. In case the automatic measurement process did not work properly due to the asymmetric shape of the comet head, manual image-analysis was allowed by identifying the area of the head manually after marking out the object and adjusting the threshold of intensity.

3.4. Statistical analysis

Mean values of DNA damage induced by various doses of ethylene oxide in repeated experiments were compared to the baseline data of untreated cells by Student's *t*-test, since the means followed normal distribution (determined with the Skewness and Kurtosis test for normality). Significance was accepted at a 5% significance level ($p<0.05$).

4. RESULTS

4.1. Ethylene oxide cytotoxicity

The viability of investigated cell types was found to be over 85% in the 0–1000 μM concentration range of ethylene oxide measure by Trypan-blue test, but it was significantly reduced at 5 mM. We could deduct to the viability of cells from the results of the comet assay, too, since it is an experimental phenomenon that tail DNA (TD) increases considerably in a dead (apoptotic) cell population (cell death is probable at 60%–80% and unambiguous over 80%). Based on this observation, we could also conclude that opposed to the range of 0–1000 μM, treatment with a 5 mM concentration was absolutely cytotoxic in breast epithelial cells (TD 82.68%) and lymphoblasts (TD 88.24%), and partially in the other cells types.

4.2. Ethylene oxide genotoxicity

The genotoxic effect of ethylene oxide in various human cell lines was analysed by comparing the dose-response curves of DNA-damage parameters. A statistically significant increase of tail moment (TM), tail DNA and tail length (TL) values was observed in the 0–100 μM concentration range, which could be statistically confirmed by demonstrating a significant increase compared with the baseline values measured in untreated cells.

Similar dose-response curves were characteristic for the cultures of keratinocytes and cervical epithelial cells. Only a minimal elevation could be observed up to a treatment concentration of 50 μM ethylene oxide compared with the DNA damage levels of untreated cells (in keratinocytes TM 1.94, TD 12.88%, TL 13.9 μm; in cervical epithelial cells TM 2.17, TD 11.6%, TL 11.75 μm); and a considerable increase was only detectable in the 50–100 μM range. The maximum damage observed in the studied concentration range was the smallest for these cultures in comparison with that of other cell types studied (about 2-fold increase in TM, 1.5-fold in TD and TL values). A statistically significant increase of DNA damage was induced by 50 μM ethylene oxide in tail moment and tail DNA values (TM 2.39 and 2.54,

$p<0.05$; TD 15.78% and 12.08%, $p<0.05$, respectively), and by 100 μM in tail length values (14.76 μm, $p<0.05$ and 19.6 μm, $p<0.01$, respectively).

Low levels of DNA damage were characteristic in untreated human peripheral lymphocytes, compared with the other cell types (TM 1.23, TD 8.57%, TL 10.12 μm). Dose-response curves of the parameters measured were close to linear and the values showed the highest increase in the 0–100 μM concentration range among the studied cell types (more than 4-fold in TM, 2-fold in TD and TL values). The lymphocytes had significantly increased DNA damage measured in tail moment and tail DNA after treatment with 20 μM ethylene oxide (TM 2.11, $p<0.001$; TD 11.09%, $p<0.01$), and after 50 μM treatment when damage was expressed as tail length values (TL 17.59 μm, $p<0.05$).

Breast epithelial cells and lymphoblasts showed similar increase of DNA damage related to the levels of untreated cells (in breast epithelial cells TM 2.77, TD 12.01%, TL 16.74 μm; in lymphoblasts TM 2.04, TD 13.19%, TL 13.28 μm), induced by exposure to ethylene oxide. The increase of tail moment in the 0–100 μM concentration range was quite substantial (3-fold); the tail DNA and tail length values also produced a similar (more than 1.8-fold) increase in both cell types. Compared to the untreated cells, significant elevation of the DNA damage level could be observed in both cell lines treated with 20 μM ethylene oxide considering the tail moment and tail length parameters (TM 3.33 and 3.09, $p<0.05$; TL 21.24 μm and 18.72 μm, $p<0.05$, respectively). The tail DNA values of breast epithelial cells and lymphoblasts increased significantly after treatment with 50 μM ethylene oxide (TD 20.26%, $p<0.01$ and 18.21% $p<0.05$, respectively).

5. DISCUSSION

Ethylene oxide is a well-known mutagen and carcinogen; however, the number of *in vitro* studies regarding its genotoxic effect is limited compared with that of other carcinogenic compounds of similar importance. Only one study investigated the DNA-damaging effect of ethylene oxide by means of the – among others –comet assay so far. However, the subjects of exposure were *in vitro* cultures of human fibroblasts that can not be considered as targets of ETO genotoxicity and the results were visually evaluated. The primary goal of using the method was not the characterisation of DNA damage but the examination of its distribution among the cells.

The aim of our studies was to investigate the DNA-damaging effect of ethylene oxide *in vitro* in human breast epithelial cells, lymphoblasts, peripheral lymphocytes, keratinocytes and cervical epithelial cells by characterising cytotoxicity and dose-response relationships. Selection of cell types was determined by practical aspects of human exposures. The reason for investigating lymphoblasts was the demonstrated phenomenon that human exposure to ETO induces predominantly leukaemia and lymphoma. The examination of breast epithelial cells was motivated by the hypothesis that these cells may also be targets of ETO exposure, which was assumed by the observed relationship between the breast cancer cases among health care workers of the Markhot Ferenc Hospital in Eger and their exposure to ethylene oxide. Peripheral lymphocytes were selected in the study because this easily obtainable cell population is used for monitoring early response to genotoxic effects in practice. Keratinocytes and cervical epithelial cells provided base of comparison for the study, since development of skin or cervical cancers in relation with ETO exposure had not been reported yet.

The site specificity of ethylene oxide as a carcinogen is the subject of intense speculation. The most frequently observed types of neoplasm associated with exposure to ETO are haematological malignancies. Based on epidemiological findings, the development of breast cancer induced by occupational exposure to ethylene oxide has been suggested by Norman *et al*., and Steenland *et al*. have also found recently some evidence of a positive dose-response relationship between ETO exposure and breast cancer mortality. However, other studies have not supported this assumption. Müller and Bertók have published data supporting the role of

ethylene oxide in the development of breast cancer in Hungary. This presumption was later confirmed by the findings of epidemiological and *in vivo* genotoxicity studies, as well. The results of our investigations provide further support – although no primary evidence – that exposure to ethylene oxide may contribute to the induction of breast cancer.

We observed the induction of pronounced DNA damage in breast epithelial cells treated with 20 μM ETO, which was similar to the effect seen in lymphoblasts and in peripheral lymphocytes. Keratinocytes and cervical epithelial cells were significantly less susceptible to the effect of ETO, i.e. their tail moment and tail DNA values increased significantly only at doses ≥50 μM. Moreover, these cells had significantly increased tail length values only when they were treated with 100 μM ETO.

Our investigations found increased DNA damage at lower ethylene oxide concentrations than those reported in studies that analysed other endpoints. This could be due to the high sensitivity of the comet assay. In earlier studies, a significant increase of DNA damage was observed upon treatment of human fibroblasts with 2.5 mM ETO in the *HPRT* mutagenicity test and in the alkaline DNA unwinding method, respectively. By use of the alkaline elution technique, DNA damage was observed at 1 mM ETO exposure in human peripheral lymphocytes and at 0.5 mM in Chinese hamster ovary cells. There are no reliable human data for the inner those of ethylene oxide, the values can be estimated only by extrapolating results of animal studies. An approximate inner dose of ETO was calculated by Beliles and Parker using toxicokinetic data from animal studies. When exposed to 1.8 mg/m^3 (1 ppm) air concentration of ETO for 8 hours, the area under the concentration–time curve in the blood plasma of a person was estimated to be 18.8 μg×h/ml (426 μmol×h/l) on the basis of data from experiments with rats and 14.3 μg×h/ml (324 μmol×h/ml) on the basis of data from dogs. The extrapolation of animal data to humans has its limitations, and the internal dose does not necessarily correspond to the biologically effective dose. Nevertheless, it can be concluded from the above data that low ETO concentrations applied in our study might represent more accurately the plasma levels in exposed individuals than high doses used earlier.

The increase of DNA damage levels was highest in peripheral lymphocytes in the 0–100 μM concentration range among the cell types studied, especially when measured as tail moment (4.3-fold). It is assumed that the low DNA-damage level in untreated cells may be the reason for the pronounced increase, since the absolute values of DNA damage in the non-dividing peripheral lymphocytes are mostly less than in lymphoblasts or breast epithelial cells. The

standard deviation of mean values from repeated experiments is high compared with that of other cell types due to the varying proportions of susceptible cell groups present in the heterogeneous population of lymphocytes, which may also contribute to the considerable increase of DNA damage. The response to the genotoxic impact of ETO in the important lowest concentration range (0–50 μM), which is the most characteristic for real exposure situations, did not differ considerably between breast epithelial cells, lymphoblasts and peripheral lymphocytes, therefore similar susceptibilities for these cell types may be assumed. A significant DNA-damaging effect induced by low concentrations of ethylene oxide could not be observed in keratinocytes and cervical epithelial cells, indicating explicitly a lower susceptibility of these cells towards the genotoxic effect studied than that of the other cell types investigated. This finding was supported by the slow increase of DNA damage in the 0–100 μM dose range, as well as by the high concentrations of ETO necessary to induce a significant genotoxic effect in keratinocytes and cervical epithelial cells.

The susceptibility of human lymphoblasts and breast epithelial cells was similar. This supports the assumption that breast epithelial cells are targets of the DNA damage induced by ethylene oxide exposure, just like bone marrow cells. A great advantage of the *in vitro* model applied is the provision of controlled laboratory conditions that vary only in the treatment doses; however, the extrapolation of our results to *in vivo* conditions needs to be considered with caution, since no real exposure situations were studied. ETO is a subject for both spontaneous and enzymatic decomposition in the human body. Therefore if the respiratory system is the portal of entry, it may be assumed that the ethylene oxide concentration reaching epithelial cells in the breast – the biologically effective dose – is lower than that affecting lymphocytes and cells of the bone marrow rich in blood vessels, or the dose exposing gastric mucosa in case of gastrointestinal intake. Further *in vivo* studies are hence needed to elucidate the toxicokinetic properties of ethylene oxide.

Determination of the lowest ethylene oxide concentration that can induce significant DNA damage detectable by means of the comet assay has the occupational health importance of providing help with additional information for the establishment or modification of occupational exposure limits.

The comet assay is a sensitive however, not specific method for the detection of DNA damage. The different types of damage cannot be distinguished by the results of measurements, only deduced indirectly. Certainly, this method can also not specify the genotoxic agent; therefore the consideration of possible confounders has great practical importance. Notwithstanding, detection of DNA damage by means of the comet assay in

human peripheral lymphocytes, isolated from the blood of workers exposed to ethylene oxide or other DNA damaging agents, may become a helpful component in the biomonitoring of early response to genotoxic insults, contributing to a better assessment of individual risk.

6. CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

1. Ethylene oxide induces dose-dependent DNA damage detectable by means of the comet assay in human breast epithelial cells, lymphoblasts, peripheral lymphocytes, keratinocytes and cervical epithelial cells in the concentration range of 0–1000 μM in the absence of cytotoxicity.
2. The susceptibility of human breast epithelial cells, lymphoblasts and peripheral lymphocytes towards the genotoxic impact of ethylene oxide is not different essentially and significantly higher than that of keratinocytes and cervical epithelial cells.
3. Human breast epithelial cells, similarly to lymphoblasts and peripheral lymphocytes, may be target cells of exposure to ethylene oxide.
4. Detection of DNA damage induced by ethylene oxide in peripheral lymphocytes by means of the comet assay offers a potential biomarker applicable in occupational health investigations.
5. The cell lines used in our studies provide suitable *in vitro* models to further investigation of the genotoxic properties, effect mechanisms of ethylene oxide.

Recommendations:

1. Further studies can be carried out for the thorough characterisation of ethylene oxide genotoxicity with the applied cell lines as *in vitro* models.
2. Detailed elucidation of the toxicokinetic properties of ethylene oxide is necessary for the accurate extrapolation of *in vitro* results to true exposures.
3. Further studies are required to assess the feasibility of using the comet assay to early detection of DNA damage induced by occupational genotoxic exposures.

7. PUBLICATIONS

In extenso publications used in the thesis

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*First three authors contributed equally to this work

IF: 1.538

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