OCCUPATIONAL EXPOSURE TO ETHYLENE OXIDE IN WOMEN STERILISING STAFF WORKING IN GAUTENG PROVINCE, SOUTH AFRICA: EXPOSURE ASSESSMENT AND ASSOCIATION WITH ADVERSE REPRODUCTIVE OUTCOME

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A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy (Medicine)

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CONTENTS

DECLARATION

I, Florentina Daniela Gresie-Brusin, declare that this thesis is my own unaided work and it is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg.

It has not been submitted before for any degree or examination at this or any other university.

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_____Day of_____2005

ETHICS APPROVAL

The protocol of this study was approved by the Committee for Research on Human Subjects (Medical) of the University of the Witwatersrand, Johannesburg (protocol number M02-05-11).

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SEARCHING FOR ETHYLENE-OXIDE RELATED TOPICS

Papers concerned with ethylene oxide exposure and its relationship with health effects in general and reproductive health effects in particular have been identified through searching medical websites (www.pubmed.com) and developmental and reproductive toxicology websites (http://toxnet.nlm.nih.gov, www.cdc.gov, www. osha.gov, www.epa.gov). A comprehensive list of key words and phrases was used: ethylene oxide; ethylene oxide and health effects; ethylene oxide and health effects and humans; ethylene oxide and reproductive effects.

The lists of papers returned by the web search were used to select the key articles in the relevant journals of occupational, environmental medicine and public health and the key publications in these fields. These articles and publications were then searched for at the libraries of the National Institute for Occupational Health and the University of the Witwatersrand, Faculty of Health Sciences.

Textbooks of occupational and environmental medicine, obstetrics-gynaecology, public health, epidemiology and medical statistics were also consulted.

PRESENTATIONS ARISING FROM THIS STUDY

1. ***Occupational exposure to ethylene oxide in women sterilising staff working in Gauteng province, South Africa: exposure assessment and association with adverse reproductive outcome.*** Presented at the Annual Research Day, School of Public Health, the University of the Witwatersrand, Johannesburg, May 2004
2. ***Occupational exposure to ethylene oxide in women sterilising staff working in Gauteng province, South Africa: exposure assessment and association with adverse reproductive outcome.*** Presented at the Research Forum, the National Institute for Occupational Health, Johannesburg, May 2004
3. ***Occupational exposure to ethylene oxide during pregnancy and association with adverse reproductive outcome.*** Presented at the 17th Congress of the International Society for Environmental Epidemiology, Johannesburg, 13-16 September 2005

ABSTRACT

Ethylene oxide is used widely in hospitals as a gaseous sterilant for heat-sensitive medical items, surgical instruments and other objects and fluids that come into contact with biological tissues. Although ethylene oxide is recognised as a reproductive toxicant in humans, so far few studies have been carried out to investigate the association between exposure to ethylene oxide and the occurrence of adverse reproductive outcomes (Hemminki *et al* 1982 and 1983; Rowland *et al*, 1996; Yakubova *et al*, 1976). The results of these studies suggested that ethylene oxide is capable of causing reproductive dysfunction and that further research is needed in order to understand its effects on reproductive health.

This study investigated the association between exposure to ethylene oxide during pregnancy and adverse reproductive outcome in women sterilising staff working in sterilising units using ethylene oxide in Gauteng province, South Africa.

The study had the following objectives: 1) to describe the extent and nature of ethylene oxide use in sterilising units operational in medical facilities in Gauteng; 2) to assess the current exposure to ethylene oxide in sterilising units in Gauteng; 3) to collect information on the last recognised pregnancy using a questionnaire; 4) to assess the validity of the information on the evolution and outcome of the last recognised pregnancy collected by the means of the questionnaire; 5) to assess the association between occupational exposure to ethylene oxide during pregnancy and adverse reproductive outcome.

The study population was represented by singleton pregnancies that: 1) occurred in women currently working in sterilising units using ethylene oxide in Gauteng province, South Africa; 2) were the last recognised pregnancy occurring in these women after the 1st January 1992; 3) occurred while the mother was employed. The adverse reproductive outcome was defined as the occurrence of any the following: spontaneous abortion, still birth, pregnancy loss (spontaneous abortion or still birth), low birth weight and combined adverse reproductive outcome (spontaneous abortion, still birth or low birth weight).

The study enrolled 68.8% of the medical facilities in Gauteng that were using ethylene oxide to sterilise medical equipment. The majority of the employees working in the sterilising units included in the study were women (96.6%) and they were employed in one of the following jobs: technician (operator), instrument packer and cleaner.

Most of the sterilising units participating in the study used ethylene oxide sterilisation daily and only 15.4% of them reported that the employees operating the ethylene oxide steriliser used protective clothing. Recorded levels of ethylene oxide were provided by 46.2% of the sterilising units; they were all bellow 0.25 ppm (the South African long-term exposure limit for occupational exposure to ethylene oxide is 5 ppm). Changes in ethylene oxide sterilisation equipment and or technology were reported by 42.3% of the sterilising units and they were all engineering control measures aimed at reducing exposure to ethylene oxide.

Measurements of the current levels of ethylene oxide were performed at the time of the study by the National Institute for Occupational Health using hydrobromic acid-coated petroleum charcoal tubes connected to calibrated Gilian pumps through which air containing ethylene oxide was drawn. The samples were analysed by the Analytical Services of the National Institute for Occupational Health. A total of 418 samples were collected (100 blank samples, 97 personal samples and 221 static samples). Quality control was ensured by the following methods: 1) verification by an Approved Inspection Authority; 2) collection of duplicate samples; 3) collection of blank samples.

These measurements showed that exposure to ethylene oxide still occurred in sterilising units (ethylene oxide was detected in 9 out of the 10 public hospitals) and that the employees most exposed are the ones working with the ethylene oxide steriliser (technician or operator).

There were 113 women working in the sterilising units enrolled in the study who had been pregnant after the 1st January 1992; 109 of them agreed to participate in the study and to complete the questionnaire.

Information on exposure to ethylene oxide during pregnancy was obtained from three sources: walk-through survey, questionnaire-collected data and measurements of the levels of ethylene oxide in sterilising units at the time of the study.

Information on the evolution and outcome of these pregnancies was gathered from the mother using a questionnaire.

The questionnaire collected demographic data, reproductive history, medical data, risk factors for the adverse reproductive outcome (environmental and occupational exposures, lifestyle), and data regarding the evolution and outcome of the last recognised pregnancy. The questionnaire also collected detailed information on the job held at the time of the last recognised pregnancy (if the woman was working with ethylene oxide, she was asked to provide a complete list of daily tasks she was performing). Prior to administration, the questionnaire was tested on a small sample of working women.

The validity of the questionnaire-collected information on the evolution and outcome of the last recognised pregnancy was assessed by comparing this information against medical records (considered the "gold standard"). The assessment showed that mothers' recall was accurate for the following variables: medical facility were the pregnancy was recorded, date of the reproductive event, gestation length, vital status of the newborn, number of foetuses, child gender, disease/medical problems during pregnancy and treatment received during pregnancy. There was an error in the mothers' reporting of the birth weight of their babies. The possible misclassification of outcome resulting from this error was shown to be non-differential (the proportion of subjects misclassified on outcome did not depend on exposure). Therefore, this misclassification could bias the effect estimate towards the null value or it could not produce any bias at all.

The analysis carried out to detect possible associations between exposure to ethylene oxide and adverse reproductive outcomes included 98 of the initial 109 pregnancies on which information had been collected (11 pregnancies were excluded from the analysis for the following reasons: 2 were multiple pregnancies, 4 were conceived before 1st January 1992 and 5 were conceived while the mother was not employed).

Amongst the 98 singleton pregnancies included in the analysis, 19 were classified as exposed and 79 as unexposed to ethylene oxide.

The relative risk for spontaneous abortion was RR=16.63 (95%CI=1.97-140.42; p=0.004), for stillbirths RR=3.47 (95%CI=0.63-19.01; p=0.18), for pregnancy loss RR=6.24 (95%CI=1.95-19.93; p=0.003), for low birth weight RR=0.61 (95%CI=0.09-4.30; p=0.51) and for combined adverse reproductive outcome RR=2.09 (95%CI=1.00-4.36; p=0.06).

No confounders were detected for any of the associations between exposure to ethylene oxide and the adverse reproductive outcomes under study.

For the association between exposure to ethylene oxide and combined adverse reproductive outcome the analysis detected three effect modifiers: paternal age (father aged 40 or older at conception), passive smoking and maternal age (mother aged 35 or older at conception).

In conclusion, this study, the first in South Africa on ethylene oxide exposure and adverse reproductive outcomes, confirmed the widespread use of ethylene oxide, exposure to this agent in public sector hospitals and associations between exposure to ethylene oxide and spontaneous abortion and between exposure to ethylene oxide and pregnancy loss (either spontaneous abortion or stillbirth).

Moreover, the study provided data on reproductive outcomes in employed women (on which scant data are available in South Africa) and added information on the validity of self-reported pregnancy data relative to medical records.

The findings of the study support the conclusions of the previous studies that had suggested that exposure to ethylene oxide during pregnancy could lead to adverse reproductive outcomes. The study detected no associations between exposure to ethylene oxide and stillbirth, low birth weight or between exposure to ethylene oxide and combined adverse reproductive outcome.

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ABBREVIATIONS

BEL	Biological Exposure Limit
CARO	Combined Adverse Reproductive Outcome
CBC	Complete Blood Count
CDC	Centers for Disease Control and Prevention (USA)
CSD	Central Sterilising Department
EPF	Early Pregnancy Factor
ETO	Ethylene Oxide
hCG	Human Chorionic Gonadotrophin
IARC	International Agency for Research on Cancer
IgE	Immunoglobulin E
IPCS	International Programme on Chemical Safety
LBW	Low Birth Weight
NIOSH	National Institute for Occupational Safety and Health (USA)
OEL-CL	Occupational Exposure Limit – Control Limit
OEL-RL	Occupational Exposure Limit – Recommended Limit
OSHA	Occupational Safety and Health Administration (USA)
PL	Pregnancy Loss
ppm	parts per million
REL	Recommended Exposure Limit
SAB	Spontaneous Abortion
SB	Stillbirth
SCE	Sister Chromatid Exchange
SMR	Standardised Mortality Ratio
TLV	Threshold Limit Value
TORCH	(T)oxoplasmosis, (O)ther agents, (R)ubella, (C)ytomegalovirus, (H)erpes simplex
TWA (TWA8)	Time Weighted Average (8 hours)
WHO	World Health Organisation

1. INTRODUCTION

In this chapter the literature on the use of ethylene oxide and its health effects is reviewed and methodological issues related to reproductive epidemiology are discussed.

Health care is a labour intensive occupational setting and, in most countries, health care workers constitute a major sector of the workforce. Health care workers represent diverse educational, social and ethnic levels and are usually predominantly female. Workers in health care are generally viewed as "providers", not "workers", yet they are exposed to a remarkable array of physical, chemical, biological and psychosocial hazards. A National Occupational Hazards survey conducted by NIOSH identified 179 known skin and eye irritants and 135 carcinogenic, mutagenic, or teratogenic agents present in hospitals. In the USA injury rates in hospitals are twice the average for other service industries, about the same as for blue-collar workers. Many anaesthetic, antineoplastic and sterilising agents used in health professions are toxic and potentially hazardous to the reproductive process. Among sterilants, ethylene oxide has been classified as mutagen, teratogen and human carcinogen and it is a particularly deceptive chemical because of its high-odour threshold (Ahlborg and Hemminki, 1995; Omenn and Morris, 1984; Yassi and Warshaw, 1998).

Ethylene oxide is a colourless flammable gas with characteristic ether like odour. It is used in the manufacture of the ethylene glycol (used as anti-freeze and as an intermediate for polyester fibres, films and bottles), non-ionic surface-active agents (used for home laundry detergents and dishwashing formulations), glycol ethers (used for surface coatings) and ethanolamines (for soap, detergents and textile chemicals). It is used as a pesticide fumigant in libraries, museums, beekeeping, spice and seasoning fumigation, animal and plant quarantine, transportation vehicle fumigation and diary packing. It is also used to sterilise agricultural products and to hasten maturation of tobacco leaves (Harrison, 1997; Landrigan, 1992).

Ethylene oxide is used widely in hospitals as a gaseous sterilant for heat-sensitive medical items, surgical instruments and other objects and fluids that come into contact with biological tissues (IARC, 1994).

As a sterilant, ethylene oxide is used in two general forms, 100% and mixtures, both of which are distributed as pressurised liquids. Small cartridges of 100% ethylene oxide containing enough for one sterilisation cycle are used in negative pressure sterilisers; cartridges are

placed inside the steriliser intact and then automatically punctured after the door is sealed. Mixtures of ethylene oxide (10-12%) and various fire and explosion-resistant diluents (e.g., carbon dioxide, various chlorofluorocarbons) are supplied in large cylinders for use in larger positive pressure sterilisers; external plumbing delivers the ethylene oxide mixture from the cylinder to the steriliser chamber. Most new sterilisers are manufactured with built-in local exhaust ventilation and other safety features (LaMontagne[1] and Kelsey, 1998).

For years, the methods used in the chemical sterilisation of instruments or other surgical materials have carelessly and needlessly put many health care workers at risk. Not even rudimentary precautions were taken to prevent or limit exposures. For example, it was the common practice to leave the door of the steriliser partially open to allow the escape of excess ethylene oxide, or to leave freshly-sterilised materials uncovered and open to the room air until enough had been assembled to make efficient use of the aerator unit (Stellman, 1998).

Ethylene oxide has a wide range of adverse health effects: dermatological, ocular, respiratory, cardiovascular, digestive, neurological, haematological and carcinogenic effects, together with effects on mortality. Two studies (Hemminki *et al* 1982 and 1983; Rowland *et al*, 1996) suggested that ethylene oxide is also capable of causing reproductive dysfunction and that further research is needed in order to understand these effects of ethylene oxide.

1.1. OCCUPATIONAL EXPOSURE TO ETHYLENE OXIDE IN STERILISING STAFF

Used primarily in the sterilisation of the non-disposable equipment, chemical sterilants such as ethylene oxide are effective because they interact with infectious agents and destroy them. Alkylation, whereby methyl or other alkyl groups bind chemically with protein-rich entities such as the amino groups in haemoglobin and DNA, is a powerful biological effect. In intact organisms, this may not cause direct toxicity but should be considered potentially carcinogenic until proven otherwise. Ethylene oxide itself is a known carcinogen and is associated with a variety of adverse health effects (Stellman, 1998).

Ethylene oxide came into widespread use as a sterilant in hospitals in the 1950s. It is used in a wide variety of health care and laboratory research settings, including medical products manufacturing, hospitals (including essentially all acute care hospitals), outpatient clinics (e.g., dental, podiatry) and veterinary clinics and hospitals. Workers in central supply, dental operatories and surgical suites who use ethylene oxide are at risk of potential exposure. The National Occupational Exposure Survey conducted by NIOSH in the USA between 1981 and 1983 indicated that 270,000 workers were potentially exposed to ethylene oxide. Of this

number, 22% were estimated to be exposed to ethylene oxide and 78% to materials containing ethylene oxide (IARC, 1994; LaMontagne[1] and Kelsey, 1998).

The typical source of ethylene oxide exposure in the hospital environment is through the operation of sterilising equipment. Unless good engineering controls and good work practices area used, workers in these settings may encounter relatively high concentrations of ethylene oxide over relatively brief periods (US Department of Health and Human Services, Public Health Services; CDC; NIOSH, 1988).

1.1.1. Sterilisers Used in Medical Facilities (NIOSH, 1989)

All gas sterilisers consist of an enclosed space where items are exposed to ethylene oxide for a sufficient period to sterilise them. The two types of gas sterilisers used in health care facilities are (1) automatic, general-purpose sterilisers that are supplied by compressed-gas cylinders or single-dose cartridges and (2) sterilisers that use glass ampoules.

- *Automatic, General-Purpose Sterilisers*

Most hospital gas sterilisers may be classified as automatic, general-purpose sterilisers. They are constructed of metal and feature a gasketed door that locks when the steriliser is in operation.

The majority of these units are pressurized during sterilisation and use 12% ethylene oxide (by weight) and 88% dichlorodifluoromethane (refrigerant-12) supplied in compressed-gas cylinders.

Other automatic, general-purpose sterilisers are not pressurized but operate below atmospheric pressure throughout the entire cycle and use small, single-dose cartridges of 100% ethylene oxide.

For the automatic, general-purpose sterilisers, the basic sterilisation cycle consists of the following phases:

1. an initial chamber evacuation, humidification and ethylene oxide charging phase,
2. a dwell period during which sterilisation takes place and
3. a final chamber evacuation phase that may include aeration.

Both pressurized and non-pressurized sterilisers may have vacuum purges at the end of the cycle.

Most items are sterilised at 130°F (54.4°C) for about 2.5 hours; heat-sensitive items are sterilised at 100°F (37.8°C) for about 5 hours. Aeration typically takes 12 hours.

The pressurized sterilisers use a water-sealed vacuum pump to evacuate the chamber. To prevent siphoning from the drain, plumbing codes require an air gap where the discharge line from the pump empties into the floor drain.

The single-dose cartridge sterilisers that use 100% ethylene oxide are fitted with a vented vacuum pump driven by compressed air. The discharge line is usually vented outside the building through the roof or an exterior wall.

For all automatic, general-purpose sterilisers, a buzzer indicates the completion of the basic cycle. At this point, the operator allows the steriliser's within-chamber aeration to continue uninterrupted through the aeration mode.

For loads that must be transferred to undergo aeration, the operator unloads the steriliser and transfers the sterilised items to an aerator. This transfer may be done immediately at the end of the final evacuation cycle, or the operator may open the steriliser door slightly and leave the area for approximately 15 min (the "door-cracked" period) before returning to transfer the load. Some sterilisers are fitted with a catch that permits the door to remain partially open for the "door-cracked" period. Once the door is fully opened, the load is removed from the steriliser. In most cases, baskets used to transfer items are manually pulled from the shelves of the steriliser chamber and carried to the aerator, where they are set on shelves. Sterilised items may also be individually transferred to the aerator by hand, or they may be transported to the aerator in a cart.

- *Sterilisers Using Glass Ampoules*

For ethylene oxide sterilisers that use glass ampoules, the operator places wrapped or packaged items inside a plastic bag lining the container box. The operator then hand-breaks a single-use glass ampoule of ethylene oxide and inert stabilizers inside the liner bag. (The ampoule is encased in a plastic sheath and contained in a small, sealed plastic bag when it is broken.) The liner bag is then quickly collapsed to expel excess air and is sealed with a twist-tie. The container box is shut immediately to enclose the liner bag, which rapidly inflates as the ethylene oxide diffuses through the plastic sheath and the sealed plastic bag. During the sterilisation period, ethylene oxide also diffuses through the liner bag and the container box into the room air. After 12 hours, the container and the liner bag are opened and the items are removed for aeration.

1.1.2. Occupational Exposure to Ethylene Oxide

Occupational exposure to ethylene oxide tends to be much higher in health instrument manufacture and in hospitals than in the chemical processing industries. In health instrument

manufacture and in hospitals, the control of employee exposures may be more difficult due to the greater potential for workers to have physical contact with ethylene oxide gas (Greenberg *et al*, 1990). Hospitals account for greater than half of all potentially ethylene oxide-exposed workers in various industries. In sterilisation facilities, ethylene oxide exposure sources can be categorised in three groups (Mortimer and Kercher, 1989):

- Those with potential for creating immediately dangerous ethylene oxide exposures of a 1000 ppm or more as the result of an accident or incident. They include the ethylene oxide container for the steriliser (i.e., cylinder or cartridge), the steriliser itself and leaks developed from the steriliser door gasket;
- Those that might occasionally emit enough ethylene oxide to create exposures greater than 5 ppm. They include the steriliser evacuation system, the opening of the steriliser door at the completion of the cycle and the sterilised load transfer procedure;
- Those which might cause exposures of a few ppms or less, including opening the aerator door during aeration procedure and cleaning the interior of the steriliser chamber.

Other possible exposures to ethylene oxide can be due to improper ventilation at the steriliser door; improperly or unventilated air gap between the discharge line and the sewer drain; removal of items from the steriliser and transfer of a sterilised load to an aerator; improper ventilation of aerators and aeration areas; incomplete aeration of items; inadequate general room ventilation; passing near steriliser and aerators during operation. Ethylene oxide concentrations near malfunctioning or improperly designed equipment may reach hundreds of mg/m^3 of air for brief periods (IARC, 1994). Unnecessary exposures to ethylene oxide commonly occur due to rush requests from surgery or other departments for sterile equipment that has not been adequately aerated (LaMontagne *et al*, 1992).

It should be emphasized that the exposure of hospital workers to ethylene oxide tends to be of a short-term and intermittent nature with the likelihood of exposure to short-term (5-120 min) concentrations of about 100 mg/m^3 (55.6 ppm) and to peak concentrations of up to 1800 mg/m^3 (1000 ppm) following the opening of sterilisation chambers (Florack and Zielhuis, 1990; IPCS, 1985; LaMontagne *et al*, 1993).

In a study evaluating the determinants of workplace exposures to ethylene oxide, LaMontagne and Kelsey (2001) assessed the following three outcome variables in relation to the exposure event: exceeding the action level, exceeding the excursion limit and uncontrolled accidental ethylene oxide releases. The survey took place in 90 Massachusetts hospitals using one of the following types of ethylene oxide: only 100% ethylene oxide, a mixture of 12% ethylene oxide and 88% Freon (or substitute propeller), therefore referred to as 88:12, or both (100%

ethylene oxide and 88:12). For the outcome of exceeding the action level, the use of a combined steriliser-aerator was the only potential determinant to remain significantly inversely related to the outcome after adjusting for frequency of ethylene oxide use and action level monitoring. The authors found that the use of 88:12 or both 88:12 and 100% ethylene oxide in a given hospital was strongly related to exceeding the excursion limit. After adjusting for frequency of ethylene oxide use, only ethylene oxide type was significantly related to the occurrence of accidental exposures.

Hori *et al* (2002) investigated the level of personal exposure to ethylene oxide and the environmental concentration of ethylene oxide in sterilisation facilities of five Japanese hospitals. In most stationary samples, the ethylene oxide concentration was less than the threshold value limit (TLV) of 1 ppm during working hours. Out of a total of 38 samples, three (collected in two different hospitals) exceeded 1 ppm at night. One stationary sample collected in the cylinder room of one of the hospitals was also above 1 ppm. Instantaneous sampling was also performed in or in front of the sterilisers when the doors were open. In one of the hospitals, levels of 40-200 ppm were detected at the moment when sterilised materials were taken out from the steriliser. In the other four hospitals, the concentration of ethylene oxide in front of the steriliser when the door was open ranged from not detectable levels to 10 ppm. All personal samples showed personal exposure levels below TLV-TWA (1 ppm). The levels of ethylene oxide exposure in workers not operating the ethylene oxide steriliser were all below the detection limit.

1.1.3. Exposure Prevention

1.1.3.1. Ethylene Oxide Substitution

Substitution of a hazardous material with a thoroughly evaluated less hazardous material is the most efficacious method of controlling a workplace hazard (Landrigan and Baker, 1991).

Some, but not all hospital sterilisation needs can be met with alternatives, such as vapour phase hydrogen peroxide (VPHP) and various gas plasma technologies (such as the Plaslyte system using gaseous peracetic acid or Sterrad systems using low-temperature hydrogen peroxide gas plasma, LT-HPGP) (Keene, 1999; LaMontagne[2] and Kelsey, 1998).

Low temperature hydrogen peroxide gas plasma sterilisation systems have been shown to effectively sterilise heat-sensitive, metal and non-metal instruments. The efficacy of these systems is comparable to the one of ethylene oxide sterilisation with the added advantage of safety and immediate availability of the sterilised instruments for use (Kyi *et al*, 1995).

Although not yet in widespread use, these technologies are beginning to be evaluated by healthcare facilities as safer more environmental friendly alternatives to ethylene oxide processes (Keene, 1999).

1.1.3.2. Ethylene Oxide Exposure Monitoring

a) Air Monitoring

According to the South African Regulations for Hazardous Chemical Substances (Regulations for Hazardous Chemical Substances, 1995), assessment of potential exposure to a hazardous chemical substance in the work place should be made at intervals not longer than two years to determine if any employee may be exposed by any route of intake.

If the assessment indicates that any employee may be exposed, the employer shall ensure that monitoring is carried out and that the exposure shall be controlled.

There are two major approaches to air monitoring for determination of airborne contaminant levels: 1) *personal sampling*, where a collection device is placed near to the breathing zone of the worker. It measures exposures at the point nearest to the actual entry of airborne contaminants and therefore the measurements are more likely to represent actual potential exposures; 2) *area monitoring*, which employs fixed or mobile sampling stations in the work area.

When the airborne contaminant is a gas, sampling may be accomplished by any of the following methods (Fowler, 1997; Moeller, 1998):

1. *Active collection*, by drawing a measured volume of air through a collection system that is then analysed;
2. *Collection in an evacuated container* (such as inert plastic bags, glass bottles, stainless steel cylinders) used to carry a sample of air to a convenient site for analysis;
3. *Passive collection*, with a dosimeter that attracts gas molecules by diffusion from the atmosphere;
4. *Collection in a colour-sensitive medium* in a device in which colour change is proportionate to the concentration of the contaminant and which can be read directly;
5. *Direct evaluation through direct-reading instruments* sensitive to one or several atmospheric gases.

Essentially, air samplers using an active collection device (methods 1 and 2) consist of a filter or sorbent collector, an air mover or fan to pull the air and associated contaminants through the collector and a means of controlling the rate of flow. The collection medium depends on the physical and chemical properties of the materials to be collected and analysed, gases being

generally collected via solid sorbents and liquid reagents. Once a sample has been collected, its identification and quantification commonly require laboratory chemical or physical analysis. These methods are more sensitive and can be used to determine lower concentrations than other approaches mentioned.

Passive dosimeters offer the advantage of not requiring a suction source to draw air through the collection device and are thus more acceptable to workers since the need for carrying a pump is avoided.

The direct-reading devices (both instrumental and colour change) provide a more rapid (immediate) result and are useful when an immediate hazard must be assessed.

Sampling Strategies

An industrial hygiene evaluation starts with a preliminary survey, which will reveal process points at which there is potential for exposure. It will be the basis for the design of a sampling programme, providing the information needed to decide what, when and where to sample. A walk-through survey of the premises permits observation of all the plant operations. During the walk-through survey job activities are reviewed with plant personnel to learn where workers are deployed and exactly how they carry out their job assignments. Control measures, too, are reviewed. Consideration must be given to whether the situations are set up for continuous or intermittent exposure and the potential for exposure to uncommon episodes, which may or may not be predictable (Cohen, 1998).

The next step is choosing the sampling procedure. A reasonable, efficient sampling strategy is to sample the worker with the highest risk of exposure. A maximum-risk worker should be selected and sampled for each operation that poses a risk of exposure. Samples should be obtained during periods of maximum ethylene oxide concentration for comparison with ceiling standards. Periods of maximum ethylene oxide concentration should be determined by using all available knowledge about the area, workers and process being sampled (NIOSH, 1989).

Ethylene Oxide Sampling and Analysis

Three types of monitoring are generally used for ethylene oxide: direct reading instruments (e.g. infrared analysers), samples collected on activated charcoal for subsequent analysis and passive dosimeters.

- *Portable infrared analysers* are direct-reading instruments that may be used for area monitoring of ethylene oxide concentrations. These instruments may not be accurate at

ethylene oxide concentrations below 1 ppm (1.8 mg/m^3) because they are sensitive to high humidity and may produce false readings.

NIOSH method 3702 employs a direct-reading technique using a portable gas chromatograph with a photoionisation detector. Samples are collected by drawing a known volume of air into a gas-sampling bag. Use of a sampling bag allows sampling times ranging from a few seconds to 8 hours. The working range of this method is 0.001 to 1000 ppm in relatively noncomplex atmospheres such as those in the steriliser areas of hospitals (NIOSH, 1989).

- *Activated charcoal tubes* are used to determine exposure for the entire sampling period (an 8-hr day, for example).

NIOSH method 1614 and *OSHA method 50* determine ethylene oxide in workplace air by packed column gas chromatography (GC) with an electron capture detector (ECD). The sample is adsorbed on hydrobromic acid-coated charcoal and adsorbed with dimethylformamide. The sample is then derivatized to 2-bromoethylheptafluorobutyrate for analysis. This method has a detection limit of 0.0006 ppm (1 μg) ethylene oxide per sample and the working range is from 0.05 to 4.6 ppm. This method is applicable to short-term (10-min) samples (IARC, 1994; NIOSH, 1989; OSHA, 1985).

- *Passive dosimeters* are generally worn on a worker's lapel; after chemical analyses, they can provide a semi-quantitative indication of exposure.

b) Biological Monitoring

Biological monitoring is the measurement of a chemical, its metabolite, or a non-adverse biochemical effect in a biological specimen (e.g., blood, urine, exhaled air) for the purpose of assessing exposure (Checkoway *et al*, 1989; Rosenberg and Harrison, 1997).

Biological monitoring data can provide estimates of organ doses (in other words, they are indicators of how much of a given contaminant has been taken into the body and the accompanying dose) and may also be used to estimate environmental concentration when the latter cannot be measured directly (Checkoway *et al*, 1989; Moeller, 1998).

Exposure to chemical carcinogens and mutagens can be monitored by

- Measuring their interaction with critical cellular macromolecules (DNA and proteins). This reaction results in the formation of DNA or protein adducts or both, which can be detected in human tissues and fluids, and

- Assessing visible chromosome damage that can be detected by structural chromosome alterations, chromosome numerical changes, sister chromatid exchange (SCE), or as micronucleated cells (Osterloh and Bezman Tarcher, 1992).

Determination of Ethylene Oxide-Haemoglobin Adducts Levels

Ethylene oxide acts as an alkylating agent in biological systems, reacting directly – and virtually irreversibly – with carboxyl, amino, sulfhydryl and hydroxyl groups on organic molecules, including the purine and pyrimidine bases, amino acid and proteins. Ethylene oxide binds covalently to DNA, principally at the N-7 position of guanosine to form N-7-hydroxyethyl guanine. It also binds histidine in haemoglobin to form N-3-(2-hydroxyethyl)-histidine (Landrigan, 1992).

Measurement of chemically stable haemoglobin adducts of genotoxic compounds allows a time-integrated determination of variable and intermittent exposure at low concentrations and is therefore suitable for monitoring individual exposure to such chemicals (Boogaard *et al*, 1999). Time-integrated exposure to ethylene oxide can readily and reliably be assessed by measurement of the concentration of their N-terminal valine adducts in haemoglobin from a small blood sample. There is a good correlation between the airborne concentrations of ethylene oxide and these adduct levels detected in the blood of workers occupational exposed to low concentrations of ethylene oxide (Boogaard *et al*, 1999).

Measurement of ethylene oxide-haemoglobin adducts levels may be used to monitor exposure that has occurred over the life span of red blood cells (i.e., over the past 120 days). In experimental studies almost linear dose-response relationships have been observed between airborne concentration of ethylene oxide and the resulting formation of haemoglobin adducts (Landrigan, 1992; Sarto *et al*, 1991).

The correlation between airborne concentrations of ethylene oxide and the level of haemoglobin adducts allows the calculation of tentative biological exposure limits (BEL, defined as a value that corresponds to the average level of exposure to an occupational exposure limit over 4 months, 5 days/week, 8 hours/day) for ethylene oxide (Boogaard *et al*, 1999).

Several methods have been reported for the determination of N-(2-hydroxyethyl) adducts in haemoglobin, with cysteine, valine and histidine: a radioimmunological technique; a modified Edman degradation procedure with gas chromatography/mass spectrometry; a gas chromatography method with selective ion monitoring mass spectrometry; and a gas chromatography/electron capture detection method (IARC, 1994; Van Sittert *et al*, 1993).

Popp *et al* (1994) investigated the DNA damage and sister chromatid exchange in the lymphocytes of 25 hospital workers exposed to ethylene oxide and of a group of 25 controls standardized by smoking habits, sex and age. The peak levels of ethylene oxide measured during the first 8 minutes after opening of the sterilisation unit ranged from 0.5 to 417 ppm. Using the alkaline filter elution method, the authors found increased DNA-protein cross-links in the exposed group compared with the control group.

Angerer *et al* (1998) analysed the relationship between workplace ambient air concentration of ethylene oxide and the concentration of N-2-hydroxyethylvaline in the globin of 12 male exposed workers engaged in sterilisation of disposable medical equipment. Environmental concentration of ethylene oxide ranged from 0.2 to 8.5 ppm, with a level of approximately 7 ppm being detected during unloading of the sterilised material and a peak exposure ranging between 6.5-6.8 ppm. These levels of exposure were correlated[*] with the results of biological monitoring, confirming the relationship between the ethylene oxide concentration in the air and the amount of N-2-hydroxyethylvaline in human globin.

Cytogenetical Monitoring

Ethylene oxide has been shown to produce a range of cytogenetical changes, including chromosome aberrations, increased frequency of sister chromatid exchange (SCE) and micronuclei. Although cytogenetical monitoring has not been used widely to assess ethylene oxide exposure in industry, it holds promise as a potential means for categorizing exposures in epidemiologic studies (Landrigan, 1992; Vainio *et* al, 1983).

Laurent *et al* (1984) evaluated the cytogenetical consequences of on-the-job exposure to ethylene oxide by using sister chromatid exchange methodology. Sister chromatid exchanges cytologically represent a four-strand exchange in the DNA in a somatic cell. Because alkylating agents behave as carcinogens as well as mutagens, the analysis of the SCE rate should be a good indirect measurement of mutation and therefore an indication of a "genetic risk". The study investigated the cytogenetical effects of exposure to high levels of ethylene oxide comparing the rate of SCE in 25 workers exposed to ethylene oxide for the last two years to the levels of SCE in 22 controls. The estimated quantities of ethylene oxide inhaled by the workers in the exposed group while unloading the steriliser or removing sets from ventilation area ranged from 3.8 mg to 60.5 mg. The total amount of ethylene oxide inhaled over the two-year period ranged from 532 mg to 5802 mg. The findings of the study showed

[*] The authors did not specify the value of the correlation coefficient.

that occupational exposure to high levels of ethylene oxide increased the SCE rates ($p<0.001$) and that this effect may be cumulative and persistent.

Sarto *et al* (1987) investigated the induction of sister chromatid exchanges (SCE) and DNA repair capability (measured as unscheduled DNA synthesis, UDS) in the peripheral lymphocytes of sanitary workers exposed to ethylene oxide compared to controls matched for sex, age and smoking habits. The measured levels of ethylene oxide were 1.84 ppm (expressed as TWA). The authors found a statistically significant ($p<0.002$) increase in the SCE frequencies in the exposed group compared to the control group. They also found that the most important factor inducing SCE was the concentration of ethylene oxide, expressed as time-weighted average (TWA).

Richmond *et al* (1985) studied the health effects of ethylene oxide on employees of the American Supply Corporation, which used ethylene oxide for sterilisation of disposable medical devices. The exposed group consisted of employees exposed to ethylene oxide for periods between 1 and 10 years. The measured air concentrations of ethylene oxide ranged from 1 to 40 ppm (TWA), with short-term excursion levels usually below 75 ppm. The effects of exposure to ethylene oxide in the study group and the control group were assessed by medical examination and cytogenetical evaluation. The most distinctive change was the increased number of cells containing chromosomal aberrations (i.e., triradial and quadriradial chromosomes) that occurred in the exposed group, primarily among workers exposed to 100% ethylene oxide. Based on their findings, the authors recommended that protection of employees exposed to ethylene oxide should include a cytogenetical screening program conducted on a periodic basis.

Van Sittert *et al* (1985) analysed several biological parameters (haemoglobin alkylation, chromosome changes, haematological profile, lymphocyte activation, serum immunoglobulin concentration) in a group of plant workers engaged in the manufacture of ethylene oxide and in a control group matched by age and smoking habits. The workers exposed to ethylene oxide had been working at the plant for periods between 1 and 14 years. Atmospheric concentrations of ethylene oxide were usually below the detection limit (<0.05 ppm), with levels up to 8 ppm occasionally recorded. The authors did not find statistically significant differences in any of the biological parameters investigated between the group of workers exposed to ethylene oxide and the control group.

In the study previously mentioned (see *Determination of Ethylene Oxide-Haemoglobin Adducts Levels)*, Popp *et al* (1994) found the SCE frequencies in the exposed group significantly reduced compared with the control group standardized for smoking habits, sex

and age. In the authors' opinion, this result might be due to an unsatisfactory standardization of the control group with respect to sex, age and smoking habits, that may not exclude great differences between the groups with respect to other personal data and lifestyle factors or differences in certain parameters of biological effect monitoring, especially enzyme polymorphisms.

1.1.3.3. Exposure Limits

In South Africa, the use of ethylene oxide is regulated by the Occupational Health and Safety Act: Regulations for Hazardous Chemical Substances (Regulations for Hazardous Chemical Substances, 1995). These regulations set two types of exposure limits for a hazardous chemical: OEL-CL (occupational exposure limit – control limit) and OEL-RL (occupational exposure limit – recommended limit).

Exposure limits are expressed as airborne concentrations averaged over a specified period of time. The long-term exposure limit (8-hour time weighted average, TWA8) is intended to control effects that require prolonged or accumulated exposure by restricting the total intake by inhalation over one or more work shifts. Short-term exposure limits (usually 15 minutes) are applied to substances whose effects are likely to be seen after brief exposures occurring once or repeatedly.

An OEL-CL is the maximum concentration of an airborne substance, averaged over a reference period, to which employees may be exposed by inhalation under any circumstances. Therefore, where there is exposure to a substance for which an OEL-CL is specified, the control of exposure shall, so far as inhalation of that substance is concerned, be treated as adequate only if the level of exposure is reduced so far as it is reasonably practicable and in any case below the OEL-CL.

An OEL-RL is the concentration of an airborne substance, averaged over a reference period, at which, according to current knowledge, there is no evidence that it is likely to be injurious to employees if they are exposed by inhalation, day after day, to that concentration. Therefore, where there is exposure to a substance for which an OEL-RL has been approved, the control of exposure shall, so far as the inhalation of that substance is concerned, be treated as adequate if (a) that OEL-RL is not exceeded, or (b) where that OEL-RL is exceeded, the employer identifies the reasons for the exceeding of the standard and takes appropriate action to remedy the situation as soon as it is reasonably practicable.

The OEL-CL for ethylene oxide set by the Regulations for Hazardous Chemical Substances is expressed as a long-term exposure limit (TWA OEL-CL) of 5 ppm (10 mg/m^3). There are no

short-term exposure limits set for ethylene oxide and in this case it is recommended that a figure of three times the long term limit be used as a guideline for controlling short term excursions in exposure.

In the USA, the National Institute for Occupational Safety and Health (NIOSH) recommends that the TWA8 be less than 0.1 ppm and that short-term peak exposure not exceed 5 ppm for more than 10 minutes per working day (Landrigan, 1992). The Occupational Safety and Health Administration's (OSHA) permissible exposure limit for ethylene oxide is an 8-hour TWA of 1 ppm with an excursion limit of 5 ppm for any 15-minute period (OSHA, 1985; US Department of Health and Human Services; CDC; NIOSH, 1988).

Elsewhere in the world, time-weighted average occupational exposure limits for ethylene oxide range from 1 ppm to 50 ppm. The short-term exposure limits range from 5 ppm to 75 ppm (IARC, 1994).

1.1.3.4. Engineering Control

The primary engineering controls used to reduce worker exposure to toxic substances are ventilation and process isolation or enclosure (Landrigan and Baker, 1991).

The following engineering control measures are recommended by the US Department of Health and Human Services, CDC and NIOSH to prevent health care workers' exposure to ethylene oxide (US Department of Health and Human Services, CDC and NIOSH, 1988):

- The steriliser should be enclosed either in a mechanical access room or a cabinet and the enclosure should be exhausted to a dedicated ventilation system[*];
- Sterilising operations should be centralized and access to steriliser rooms should be restricted;
- The steriliser should be checked with the infrared analyser at least once every 3 months in order to detect the presence of ethylene oxide in the surrounding air;
- Floor drains should have a cover with an anti-siphon air gap. The air gap, at the junction of the vacuum pump discharge line with the floor drain, should be enclosed. Dedicated exhaust ventilation should be provided for the enclosures;
- Local exhaust ventilation sufficient to effectively remove ethylene oxide should be as close as possible to the top of the steriliser door;

[*] A dedicated exhaust system is one that serves the steriliser area only and routes ethylene oxide directly to the outside of the building at a location where prevailing winds will not carry the exhaust into populated areas or into the air intakes of other buildings.

- The number of exhaust cycles recommended by the steriliser manufacturer should be completed before the door is opened; the door should remain only slightly open for at least 15 min;
- Supply cylinders should be located in a ventilated enclosure (either a ventilated cabinet or a hood that covers the point where the cylinder is connected to the steriliser supply line);
- Aerators and the overpressure relief valves (if present) should be vented to a dedicated exhaust system;
- Sensors should be provided to identify a ventilation failure and to detect ethylene oxide. Both audible and visual alarms should be activated by the sensors;
- Ventilation air from the sterilising room should not be re-circulated;
- Exhaust gases should preferably be vented directly to the outside of the building (away from intake vents); this procedure is strongly recommended for all sterilisers;
- Sterilised material and its packaging should be aerated in aeration cabinets, since approximately 5% of the ethylene oxide in the steriliser remains in these items. Materials that do not absorb ethylene oxide (metal and glass) need no aeration unless they are wrapped;
- Sterilisers that use glass ampoules in a plastic bag (flash bag) have a high potential for worker exposure to ethylene oxide. If they are used, all sterilisation procedures should be conducted in a ventilated enclosure.

In a survey conducted at 12 hospitals in the USA to characterize exposure to ethylene oxide during the utilisation of various types of engineering controls and work practices, Elliott *et al* (1988) showed that engineering controls or good work practices or both can effectively reduce exposure to ethylene oxide. Engineering controls are more effective than just good work practices in reducing exposure potential. The authors found that the following engineering controls collectively reduced ethylene oxide exposure in the hospitals included in the survey: local ventilation above the steriliser door, local ventilation at the steriliser drain, good general area ventilation of the steriliser room and additional vacuum purges.

1.1.3.5. Protective Equipment and Work Practices

Sterilisers should be operated only by personnel trained in sterilisation procedures and in the health and safety hazards of ethylene oxide. If local exhaust ventilation has been provided above the steriliser door, a worker should open the door slightly and step away for an established time period. The time period should be determined by monitoring and should be at

least 15 min. The door opening should be smaller than the capture distance of the hood (US Department of Health and Human Services; CDC; NIOSH, 1988).

A worker should use protective gloves and splash-proof goggles and/or a face shield when changing ethylene oxide supply cylinders. Protective gloves and long-sleeved garments should be worn when removing items from the steriliser or transferring them to the aerator (US Department of Health and Human Services; CDC; NIOSH, 1988).

To clean the steriliser, especially the back surfaces, a worker must often reach inside the chamber with the whole upper body. Ethylene oxide exposure during this cleaning can be controlled by (1) scheduling the cleaning activity as long as possible after processing a load, (2) leaving the steriliser door fully open for at least 30 min before cleaning and (3) wearing a respirator (US Department of Health and Human Services; CDC; NIOSH, 1988).

In the survey of the 12 USA hospitals for ethylene oxide exposure, Elliott *et al* (1988) found that proper work practices, even in the absence of good engineering controls, resulted in a considerable reduction in ethylene oxide exposure. Work practices that collectively aided in a reduction of ethylene oxide exposure included vacating the steriliser room area during the exhaust cycle, opening the door 2-5 cm at the end of the cycle and again vacating the area to allow ethylene oxide in the air to decrease and limiting the handling time of and close contact with the sterilised items.

1.1.3.6. Training

Each worker must be informed of the possible health effects of exposure to ethylene oxide. This information must include an explanation of the requirements of the standard on occupational exposure to ethylene oxide and must identify the areas and tasks in which there is potential exposure to ethylene oxide (Cardo and Sehulster, 1999).

Worker training is a crucial part of a comprehensive preventive approach to occupational health and safety and is required for anyone that works with ethylene oxide. “Training” requirements are distinguished from “information” requirements, which can be fulfilled simply by posting the required information on a bulletin board. Training must include ways to detect ethylene oxide, physical and health hazards, methods to protect employees from hazards (e.g., work practices, emergency procedures) and the details of the employer’s hazard communication program (LaMontagne *et al*, 1992).

LaMontagne *et al* (1992) developed an ethylene oxide health and safety training program for sterilisation workers. The training was provided at sterilising sites in 15 different hospitals in Massachusetts and its impact was evaluated thereafter. The authors found that the training

was followed by positive health and safety changes at most of the sites, including up to 50% reductions in ethylene oxide use and substantial expenditures on sterilising engineering controls and other safety equipment.

1.1.3.7. Medical Surveillance

Medical surveillance plays an important role that helps to detect significant morbidity, ranging from reversible acute to serious chronic effects.

Medical evaluation was recommended by Richmond *et al* (1985) as one of the procedures needed for protection of ethylene oxide-exposed workers. The authors recommended that medical evaluation include family history, reproductive history, past medical events, together with laboratory assessment of the possible documentable effects on health, including possible effects on the blood, liver, reproductive organs, respiratory tract and basic cellular structures.

According to the Standard on ethylene oxide passed by the Occupational Safety and Health Administration (OSHA), employers are required to institute a medical surveillance program for all employees (LaMontagne *et al*, 1993; LaMontagne[2] and Kelsey, 1998):

1. who are or may be exposed to ethylene oxide at or above the action level (0.5 ppm time-weighted average, TWA) for at least 30 days a year;
2. who have been exposed to ethylene oxide in an emergency situation;
3. who are showing ethylene oxide-associated signs and symptoms;
4. who desire medical advise concerning the effects of ethylene oxide on their ability to produce a healthy child;
5. upon job termination or transfer from an area where exposure was at or above the action limit for 30 or more days.

When provided, medical surveillance must include at least a medical and work history, a physical examination and a complete blood count (CBC) with leukocyte differential (LaMontagne[2] and Kelsey, 1998).

In a study assessing the utility of complete blood count in routine medical surveillance for ethylene oxide exposure, LaMontagne *et al* (1993) found that alteration in the CBC seem to occur only after ethylene oxide exposure at levels that also produce acutely toxic symptoms. In the authors' opinion, the finding of CBC abnormalities during medical surveillance would not help in the early detection of ethylene oxide health effects.

In a more recent study, Shaham *et al* (2000) analysed haematological changes in hospital workers chronically exposed to ethylene oxide (n=47) and compared them to a control group matched by age, sex and smoking habits (n=88). The measured levels of ethylene oxide

exposure ranged from below 0.01 ppm to 0.06 ppm. The researchers found an elevation in the absolute mean number of monocytes and eosinophils and a decrease in the absolute mean number of lymphocytes in the exposed group compared to the control group. Based on these findings, the authors suggested that changes in eosinophilia in workers exposed to ethylene oxide could represent a warning sign useful for routine medical surveillance.

In a survey conducted in 92 Massachusetts hospitals using ethylene oxide, LaMontagne[1] *et al* (1996) found that from 1985 to 1993, medical surveillance for ethylene oxide exposure was provided one or more times in 62% of ethylene oxide-using hospitals. Twenty-seven percent of medical surveillance providers reported detection of ethylene oxide-related symptoms or conditions, ranging from mucous membrane irritation to peripheral neuropathy. The authors also estimated that one or more workers at 19% of ethylene oxide-using Massachusetts hospitals have experienced ethylene oxide-related health effects.

In a third report referring to the survey conducted in Massachusetts hospitals using ethylene oxide, LaMontagne[2] *et al* (1996) investigated the determinants of the provision of medical surveillance for ethylene oxide exposure. Among the OSHA-specified triggers for provision of medical surveillance, only accidental worker exposures were related to provision of surveillance. The results of the study also showed a positive relationship between coverage of medical surveillance in training and information programmes and the provision of medical surveillance. A third important determinant for provision of medical surveillance for ethylene oxide was the medical surveillance policy coverage, which serves as an indicator of institutional awareness of the need for medical surveillance and the appropriate procedures to use.

1.2. HEALTH EFFECTS IN HUMANS

Ethylene oxide is absorbed through the skin and respiratory tract and may produce either local or generalised effects. The exposed individual's defensive reactions are hampered by the typical lag of several hours between exposure and the appearance of symptoms and by the fact that the gas is imperceptible to smell until quite high concentrations are present (700 ppm) (LaMontagne[1] and Kelsey, 1998).

Once inhaled, ethylene oxide passes through the pulmonary alveoli and enters into systemic circulation. The amount of ethylene oxide absorbed during an 8-hour period of exposure to 1 ppm is estimated to be 7 to 8 mg. Seventy five to eighty percent of an inhaled dose of

ethylene oxide is retained in humans. Ethylene oxide is very soluble in blood and it is rapidly distributed to various body tissues following absorption. During a work shift, the average blood concentration of ethylene oxide is approximately three times the environmental air ethylene oxide concentration, reflecting the high solubility of ethylene oxide in blood (Feldman, 1999).

Ethylene oxide is an alkylating agent that reacts directly and virtually irreversibly with organic molecules, including amino acids and nucleoproteins. Through binding to DNA it may cause cellular mutation (Gestal, 1987; Harrison, 1997; Sheikh, 1984; Vainio, 1982).

Ethylene oxide occurs endogenously in small amounts as a metabolite of ethylene. Two possible pathways responsible for metabolism of ethylene oxide in humans are: (a) hydrolysis by reaction with water and chloride ions to yield ethylene glycol and (b) conjugation with glutathione to yield thioethers. In the first of these pathways, ethylene glycol is the major metabolic product of ethylene oxide hydrolysis. Ethylene glycol can be excreted in urine or further metabolised by alcohol dehydrogenase to glycoaldehyde, which is further transformed to glycolic acid and glyoxal and then to glyoxylic acid, which is toxic and it will be detoxicated via several metabolic pathways. Ethylene oxide is also enzymatically deactivated via glutathione conjugation to form S-2-hydroxyethyl-glutathione, S-2-hydroxyethyl-cysteine and S-2-hydroxyethyl-mercapturic acid. Conjugation with glutathione prevents ethylene oxide from covalently binding to cellular proteins and nucleic acids. About two-thirds of the population posses the ability to enzymatically conjugate ethylene oxide with glutathione and the lack of this ability may increase an individual's susceptibility to the toxic effects of ethylene oxide. When glutathione levels are depleted by high or repeated exposure to ethylene oxide, ethylene oxide is eliminated more slowly. Ethylene oxide is excreted primarily through the urine. The half-life of absorbed ethylene oxide in humans has been estimated to be less than 1 hour, assuming steady-state conditions and first-order kinetics (Feldman, 1999; LaMontagne[1] and Kelsey, 1998).

Some of the ethylene oxide effects are dependent on the chemical's inherent propriety as an alkylator of proteins and nucleic acids (e.g., mutagenicity and sensitisation), whereas others may be attributable to its anaesthetic properties (e.g., acute neurotoxicity) (LaMontagne[1] and Kelsey, 1998).

Table 1 summarises the non-reproductive health effects of ethylene oxide in humans.

Table1: Non-reproductive health effects of ethylene oxide in humans

Health effect	**Clinical manifestations**
Dermatological	Mild irritation, oedema, vesiculation, frostbite burns, allergic sensitisation (LaMontagne[1] and Kelsey, 1998; Verraes and Michael, 1995)
Ocular	Conjunctivitis, cataract (Deschamps *et al*, 1990; IPCS, 1985; Sobaszek *et al*, 1999)
Respiratory	Upper respiratory tract irritation (hoarseness, cough), delay-onset pulmonary oedema, ethylene oxide-related occupational asthma (Deschamps *et al*, 1990; IPCS, 1985; LaMontagne[1] and Kelsey, 1998; Verraes and Michael, 1995)
Cardiovascular	Higher mortality from cerebrovascular diseases (Hogstedt[1] *et al*, 1979; Hogstedt *et al*, 1986)
Gastro-intestinal	Peculiar taste; high exposure leads to flu-like syndrome with nausea, recurrent vomiting, diarrhoea (LaMontagne[1] and Kelsey, 1998)
Neurological	Significant acute exposure leads to drowsiness, confusion, lethargy, weakness, delirium, dysarthria, ataxia; very high exposures lead to central nervous system depression with seizures, convulsions, loss of consciousness and death. Chronic exposure effects include personality dysfunction, cognitive impairment. Other manifestations: reversible peripheral neuropathy affecting both sensitive and motor neurons (numbness in the fingers and feet, muscular weakness in the lower limbs, incoordination, ataxia, distal muscle weakness). (Feldman, 1999; LaMontagne[1] and Kelsey, 1998)
Haematological	Lymphocytosis, leukaemia (Hogstedt *et al*, 1979; LaMontagne *et al*, 1993; Schulte et al, 1995)
Carcinogenic effects and effects on mortality	Leukaemia; Hodgkin's disease; non-Hodgkin lymphoma; stomach, pancreatic, bladder, brain, breast, kidney cancers. Excess mortality from neoplasms of lymphoid cells origin; cerebrovascular diseases. (Bisanti *et al*, 1993; Gardner *et al*, 1989; Hogstedt *et al*, 1979; Hogstedt[1] *et al*, 1979; Hogstedt *et al*, 1986; Hagmar *et al*, 1991; Hagmar *et al*, 1995; Morgan *et al*, 1981; Norman *et al*, 1995; Shore *et al*, 1993; Stayner *et al*, 1993; Steenland *et al*, 1991)

1.2.1. Dermatological Effects

Effects can range from mild irritation, erythema, oedema, vesiculation to frostbite burns. Repeated skin exposure to ethylene oxide can cause allergic sensitisation (LaMontagne[1] and Kelsey, 1998).

1.2.2. Ocular Effects

Accidental exposure to the vapour of ethylene oxide can lead to conjunctivitis (IPCS, 1985). Cataract has also been reported as an effect of chronic exposure to ethylene oxide. Deschamps *et al* (1990) investigated the effects of ethylene oxide on lens in 55 ethylene oxide-exposed workers employed in five Parisian hospitals. The prevalence of lens opacities and cataract in 21 of these exposed workers aged over 45 were compared to the prevalence of the same outcomes in 16 unexposed workers, matched for age and sex. The authors found no significant differences between the two groups (exposed and unexposed) with regard to the characteristics of lens opacities. However, cataract (defined as "association of lens opacities and of visual acuity less than or equal to 20/25, this loss not being imputable to another disease") was more common in exposed than in unexposed subjects. The cataract development following exposure to ethylene oxide could be explained by saturation of protective mechanisms against alkylating action of ethylene oxide.

In a more recent study investigating 16 health care workers assigned to ethylene oxide sterilisation in a French hospital, Sobaszek *et al* (1999) found 14 cases of lens opacities. The researchers attributed three of these cases to occupational exposure to ethylene oxide.

1.2.3. Respiratory Effects

Effects range from upper respiratory tract irritation (hoarseness, cough) to delayed-onset pulmonary oedema and ethylene oxide-related occupational asthma (IPCS, 1985; LaMontagne[1] and Kelsey, 1998).

Deschamps *et al* (1992) were the first to report a case of occupational asthma in a worker exposed to ethylene oxide at high concentration for a short period of time (the exposure was believed to be over 700 ppm and lasted for four days only). In the authors' opinion, in this case asthma occurred after the short accidental exposure to high concentrations of an alkylating gas, i.e. ethylene oxide.

Verraes and Michel (1995) also reported a case of occupational dermatitis followed by asthma in a trainee surgeon wearing latex rubber gloves sterilised with ethylene oxide. Based on

sensitivity testing, this case was considered the result of an IgE sensitisation to the ethylene oxide used to sterilise the gloves used by the surgeon.

1.2.4. Cardiovascular Effects

Hogstedt[1] *et al* (1979) carried out a cohort study of the mortality and the cancer incidence among full-time exposed workers in ethylene oxide production, a group of maintenance with intermittent exposure and a group of unexposed controls. For the exposed cohort, levels of ethylene oxide ranged from 0.6 to 27.8 ppm, with occasional peak exposures above 700 ppm. The authors found an excess of mortality from diseases of circulatory system (coronary heart disease and cerebrovascular disease) in the full-time exposed cohort, with 12 observed cases compared to 6.3 expected ($p<0.05$). The authors could not attribute the findings to any particular chemical in the production process, but they considered ethylene oxide and ethylene dichloride as "prime suspects"

In a follow-up of the previous study, Hogstedt *et al* (1986) found a significant excess mortality from cerebrovascular diseases in workers exposed to ethylene oxide, with five observed deaths compared with 1.5 expected ($p<0.05$).

1.2.5. Gastro-Intestinal Effects

Persons exposed to ethylene oxide usually complain of a peculiar taste. High exposure can lead to a flu-like syndrome including nausea, recurrent vomiting and diarrhoea (LaMontagne[1] and Kelsey, 1998).

1.2.6. Neurological Effects

Ethylene oxide is structurally similar to ethyl ether and was investigated historically as an anaesthetic before being abandoned because of its adverse side effects and after effects. Significant acute exposure leads to drowsiness, confusion, lethargy, weakness, delirium, dysarthria and ataxia. Very high exposures depress the central nervous system and can cause seizures or convulsions, loss of consciousness and death. Ethylene oxide-associated chronic exposure effects on central nervous system include personality dysfunction or cognitive impairment. Exposure to ethylene oxide has been also shown to cause peripheral neuropathy affecting both sensory and motor neurons. Symptoms are reversible and include numbness in the fingers and feet, muscular weakness in the lower limbs and in more severely affected cases, incoordination, ataxia, distal muscle weakness (Feldman, 1999; LaMontagne[1] and Kelsey, 1998).

1.2.7. Haematological Effects

Hogstedt *et al* (1979) reported three cases of leukaemia that had occurred between 1972 and 1977 in a relatively small group of Swedish workers exposed to ethylene oxide. The employees had been exposed to a mixture of 50% ethylene oxide and 50% methyl formate used to sterilise medical equipment. The levels of ethylene oxide ranged from 2 to 1500 ppm, with TWA=10-30 ppm. The number of cases of leukaemia was higher than the expected incidence for that period, which was calculated as 0.2 using the Swedish national statistics.

Currier *et al* (1984) conducted a cross-sectional study on employees potentially exposed to ethylene oxide to determine whether they had a higher prevalence of abnormalities of the haematopoietic, hepatic, or renal system than a control group. Amongst the exposed group, levels of ethylene oxide ranged from below 0.1 ppm to 5.7 ppm, with short term exposures of up to 235 ppm. The authors found no statistically significant difference in the laboratory findings between the exposed group and the control group, with the exception of an increased prevalence of proteinuria ($p=0.035$).

LaMontagne *et al* (1993) provided medical surveillance to the sterilisation department of a 300-bed Massachusetts hospital for 6 years. Medical surveillance included complete blood counts with differentials on the exposed group (workers employed at the sterilisation department) and on two control groups. The complete blood counts with differentials of the exposed group showed a relative lymphocytosis which persisted over 3-4 years after exposure to 0.07 ppm of ethylene oxide (TWA). Upon comparison to the unexposed groups, the findings were found not to be associated with ethylene oxide exposure or work in the sterilisation department. The authors also suggested that lymphocytosis may occur only in very highly overexposed workers who simultaneously show severe acute neurotoxic and other symptoms.

Schulte *et al* (1995) investigated the molecular, cytogenetical and haematological effects of exposure to ethylene oxide on women employed in nine hospitals in the United States and in one hospital in Mexico. The ethylene oxide exposure levels were in general below the OSHA TLV of 1 ppm. Workers were classified into three exposure categories, according to the estimated 4-month cumulative exposure (ppm-hours): none (0), low (0-32 ppm-hrs) and high (>32 ppm-hrs). The researchers found a statistically significant decrease in haematocrit and haemoglobin levels ($p=0.03$), an increase in lymphocyte percentages ($p=0.04$) and a relative decrease in neutrophil percentages ($p=0.03$) with exposure in US workers. The absolute number of lymphocytes decreased from low-exposure to high-exposure categories. The

authors considered that the relative lymphocytosis was due to a decrease in total granulocytes rather than to an increase in total lymphocytes, a pattern similar to the one described in chronic inflammation. They argued that chronic exposure to ethylene oxide could provoke such a reaction. In Mexican workers haematocrit decreased with exposure, but no statistically significant results were seen.

1.2.8. Carcinogenic Effects and Effects on Mortality

Hogstedt *et al* (1979) reported three cases of leukaemia that had occurred between 1972 and 1977 in a small group of Swedish workers exposed to ethylene oxide (see *1.2.7. Haematological Effects*).

Hogstedt[1] *et al* (1979) conducted a cohort study (1961-1977) of the mortality and the cancer incidence among workers from an ethylene oxide-producing Swedish company. They followed-up three groups of workers: full-time exposed workers, workers with intermittent exposure to ethylene oxide and unexposed controls. The researchers found an important excess mortality in the full-time exposed group, derived mainly from increased mortality due to tumours (stomach cancer and leukaemia): 9 observed cases compared to 3.4 expected ($p<0.01$). Given that the exposed workers had been occupationally exposed to several chemicals, the excess of malignancies could not be linked only to the ethylene oxide exposure. The authors considered ethylene oxide, ethylene dichloride, ethylene chlorohydrin and ethylene as possible causative agents for the malignancies found.

In a later follow-up of the two groups of workers exposed to ethylene oxide enrolled in the previous studies and of an additional third group of exposed workers, Hogstedt *et al* (1986) further investigated the role of ethylene oxide in the induction of malignancies. The findings of the study included eight cases of leukaemia occurring among 733 ethylene oxide-exposed workers (compared with 0.8 cases expected) and six cases of stomach cancer (compared with 0.65 cases expected).

Morgan *et al* (1981) undertook a mortality study of 767 male workers potentially exposed to ethylene oxide at a chemical plant in the USA producing ethylene oxide. The environmental sampling of ethylene oxide yielded values below 10 ppm with one exception: a level of 6000 ppm was detected from a leak that was quickly stopped. The results showed a reduced overall mortality for the cohort under study, with a standardised mortality ratio (SMR) of 58, 95%CI=42-77. The only excess mortalities were seen for a few cancers (pancreas, SMR=411, 95%CI=94-1149; bladder, SMR=353, 95%CI=15-1847; brain, SMR=313, 95%CI=45-1074; and Hodgkin's disease, SMR=626, 95%CI=90-2147). Apart from the deaths from Hodgkin's

disease, the study found no deaths from cancer of the lymphatic and haematopoietic system. Based on their findings, the authors did not rule out the possibility of a causal workplace exposure for pancreatic, bladder and brain cancer and for Hodgkin's disease.

Gardner *et al* (1989) followed up a cohort of 2876 workers occupationally exposed to ethylene oxide in the UK. Approximately half of the workers were employed at companies producing ethylene oxide and the other half at hospitals using ethylene oxide as a sterilant. Time-weighted average exposures were less than 5 ppm with occasional peak exposures up to several hundred ppms. Deaths from all causes combined were found to be less than expected (cause, sex and age specific death rates for appropriate five-year calendar periods in England and Wales), which the authors attributed to the healthy worker effect. The only excess of death was found for cancer, with a ratio of observed/expected of 1.14 (95%CI=0.85-1.49) for the companies' workers and 1.07 (95%CI=0.73-1.50) for the hospital workers. The authors found three deaths from leukaemia compared to 2.09 expected and also a slight excess of non-Hodgkin's lymphoma (4 versus 1.62).

Coggon *et al* (2004) extended the follow up of this cohort by 13 years (up to the year 2000) and they found no statistically significant increase in mortality from cancer overall, or from any specific category of tumour. The authors concluded that the risk of human cancer following exposure to ethylene oxide at levels of less than 5 ppm was low. They also suggested that this might reflect the capacity of human cells to repair DNA damage caused by ethylene oxide, which is a potent genotoxin and animal carcinogen.

Kiesselbach *et al* (1990) carried out a mortality study in 2658 male workers occupationally exposed to ethylene oxide in the Federal Republic of Germany. The overall standardised mortality ratio (SMR) was 0.87 (95%CI=0.77-0.98), which according to the authors may be attributed to the healthy worker effect. For all malignant neoplasms the SMR was 0.97 (95%CI=0.76-1.24); there was no excess mortality for leukaemia (SMR=0.85, 95%CI=0.10-3.07). The SMR for oesophageal cancer was 2.0 (95%CI= 0.41-5.85) and for stomach cancer was 1.38 (95%CI=0.75-2.31), but they were not statistically significant. In one of the plants, an internal control group was selected and compared to the exposed group. The total mortality and the mortality from malignant neoplasms were higher in the exposed group, but the differences were not statistically significant. There were no deaths from leukaemia in the exposed group and there was one in the control group.

Steenland *et al* (1991) conducted a study of mortality in a cohort of 18,254 workers exposed to ethylene oxide at 14 facilities in the United States. The population of the U.S. was used as the reference group. The standardised mortality ratios (SMRs) were 0.97 for leukaemia

(95%CI=0.52-1.67), 1.06 for haematopoietic cancers (95%CI=0.75-1.47) and 0.94 for stomach cancer (95%CI=0.45-1.70). The rate of death from haematopoietic cancer (especially non-Hodgkin lymphoma) was significantly increased among men (SMR=1.55), as it was mortality from leukaemia in recent years (1985 through 1987), with a SMR=3.45. The excess mortality from kidney cancer among the workers with the longest latency period was also significant (SMR=3.27).

In a second paper on this study (Stayner *et al*, 1993) the authors further investigated the relation between ethylene oxide exposure and cancer mortality in workers employed at 13 of the 14 facilities using ethylene oxide analysed previously. The outcomes of interest were haematopoietic cancer, cancers of the stomach, brain and pancreas. Rate ratios and 95 percent confidence intervals corresponding to a working lifetime (45 years) of exposure at the OSHA standard level for ethylene oxide (1 ppm) were estimated. The authors found a strong relation (rate ratio=1.22, 95% CI=1.09-1.35, p=0.004) between cumulative exposure to ethylene oxide and mortality from neoplasms of lymphoid cells origin. A weaker relation was observed between cumulative exposure to ethylene oxide and mortality from all haematopoietic cancers (rate ratio=1.20, 95% CI=1.05-1.38, p=0.03) and non-Hodgkin's lymphoma (rate ratio=1.24, 95% CI=1.04-1.47, p=0.046) when a 10-year lag period was assumed. A negative (inverse) exposure-response relation was observed between cumulative exposure to ethylene oxide and cancers of the stomach, pancreas, brain and kidney. The cumulative exposure to ethylene oxide was the only predictor for mortality due to all haematopoietic cancers or mortality from any of the other cancers examined in the study. The results suggested a positive exposure-response relation with cumulative ethylene oxide exposure and all haematopoietic neoplasms among males, but a negative relation for females.

Steenland *et al* (2004) continued the follow up of the initial cohort (see Steenland *et al*, 1991) from 1987 to 1998. In this 11 year update there was little evidence of cancer excess for the ethylene oxide exposed workers compared to the general population of the USA. The authors found positive exposure-response trends for males for lymphoid cancer mortality and for females for breast cancer mortality. They also found a significant bone cancer excess compared to the US population, but it did not show an increase with increasing exposure and it was based on a small number of deaths (6). Therefore, no conclusion was drawn from this excess of cancer mortality.

Wong and Trent (1993) analysed the same cohort of workers as did Steenland and Stayner (1993). They included a slightly higher number of workers (men and women) exposed to ethylene oxide in the United States (18,728 vs. 18,254 in the previous report). The ethylene

oxide exposure levels were estimated as between 2-16 ppm (eight hour time-weighted average, TWA8). The SMR for overall mortality was 73.3 (95%CI=69.5-77.2), showing a significant mortality deficit of 27% that was interpreted by the study's authors as due to the healthy worker effect. There was no significant increase in mortality from any site-specific cancers (SMR for overall cancer mortality was 90.3, 95%CI=81.7-99.6), particularly stomach (SMR=97.3, 95%CI=54.5-160.5), pancreas (SMR=96.7, 95%CI=59.1-149.3), brain (SMR=56.1, 95%CI=24.2-110.5) or leukaemia (SMR=86.6, 95%CI=47.3-145.3). The findings also included a statistically significant excess mortality from non-Hodgkin's disease in men (SMR=247.3, 95%CI=141.3-401.5), while deficit mortality from the same disease was observed in women (SMR=31.9, 95%CI=3.9-115.2). In the authors' view, the excess mortality from non-Hodgkin's disease in men could not be interpreted as related to ethylene oxide exposure due to the lack of a dose-response relationship and consistency.

Shore *et al* (1993) conducted a meta-analysis of eleven studies published between 1979 and 1993 assessing the mortality from cancer among workers occupationally exposed to ethylene oxide. The studies analysed included a total of about 29,800 exposed workers and 2,540 deaths. The meta-analysis was conducted for the following health outcomes: leukaemia, non-Hodgkin's lymphomas, stomach cancer, brain and nervous system cancer, pancreatic cancer, all cancers and deaths other than cancer. Despite earlier reports of possible ethylene oxide-related leukaemia, the standardised mortality ratio (SMR) for the combined studies was 1.06 (95%CI=0.73-1.48). There was a slight suggestion of a trend by duration of exposure and a suggested increase with longer latency, but the data analysis did not indicate a trend in leukaemia risk with respect to frequency and intensity of exposure. For non-Hodgkin's lymphoma there was a suggestive risk overall, with a SMR=1.35 (95%CI=0.93-1.90) and a trend in risk of non-Hodgkin's lymphomas with exposure with a 10-year exposure lag. For stomach cancer, the overall SMR was 1.28 (95%CI=0.98-1.65), but the authors considered that the weight of current evidence did not indicate that airborne ethylene oxide causes human stomach cancer. With a summary SMR of 0.89 and 0.98 for brain and pancreatic cancer, respectively, there was no evidence of association between ethylene oxide exposure and these types of cancer. When all cancers were considered as a possible health outcome, the SMR of 0.94 showed no evidence of an excess of total cancer among ethylene oxide exposed workers. The summary SMR of 0.73 for deaths other than cancer (e.g., circulatory disease) showed no material evidence for a risk of circulatory disease associated with ethylene oxide. Moreover, when all causes of death were examined, none of the eleven studies showed a significant

elevation in risk. This indicated that ethylene oxide has not had adverse effects upon overall mortality experience and life expectancy.

Bisanti *et al* (1993) examined the cancer mortality in 1971 male workers occupationally exposed to ethylene oxide in Italy. The workers were selected on the basis of a licence for handling ethylene oxide and/or other toxic gases, which was considered a surrogate of exposure to ethylene oxide. The standardised mortality ratio (SMR) for deaths of all cancers was 130 (95%CI=94-175). Excess mortality was statistically significant for lymphosarcoma and reticulosarcoma (SMR=682, 95%CI=186-1745). Focusing the analysis on the subcohort of workers licensed to handle ethylene oxide only, the excess of deaths for all lymphopoietic cancers was increased (SMR=700, 95%CI=227-1637), as it was the excess of deaths from lymphosarcoma and reticulosarcoma (SMR=1693, 95%CI=349-4953). The authors considered the findings of their study as supporting evidence for the association between occupational exposure to ethylene oxide and a higher risk of cancer.

Hagmar *et al* (1991; 1995) examined the association between exposure to ethylene oxide and risk of cancer, especially leukaemia, in a cohort of 2170 Swedish sterilant workers. The median value of the individual cumulative exposure was 0.13 ppm-years and the median follow up of the cohort was 11.8 years. The authors observed 6 lympho-haematopoietic tumours (standardised incidence ratio, SIR=1.78, 95%CI=0.65-3.88), 2 of which were leukaemias (SIR=2.44, 95%CI=0.3-8.81). When the workers with an individual cumulative exposure below the median (0.13 ppm-years) were excluded and a minimum induction latency period of 10 years was applied, the incidence ratio for leukaemia increased further (SIR=7.14, 95%CI=0.87-25.8). This was also true for brain cancer (SIR=3.80, 95%CI=0.78-11.1). These findings support the association between ethylene oxide exposure and cancer occurrence.

Norman *et al* (1995) examined the cancer incidence in a cohort of 1132 individuals exposed to ethylene oxide at a plant in New York State using ethylene oxide. A previous study (Stolley *et al*, 1984) had reported higher levels of sister chromatid exchange (SCE) in a sample of workers from the same plant than the unexposed people in the community. All the employees at the factory were considered potentially exposed to ethylene oxide and the proxy used for amount of potential exposure was the distinction between regular employees (considered more exposed) and temporary employees (considered less exposed). Among the regular female employees, the SMR for breast cancer ranged from 1.70 (95%CI=0.89-3.23, p=0.09) to 2.55 (95%CI=1.31-4.98, p=0.02), depending on calendar-year of follow up, assumptions about completeness of follow up and the reference rates used. No statistically

significant excess of breast cancer was noted for temporary female employees at any point during follow up. There was no statistically significant increase in observed number of cancers over those expected for any cancer site other than breast cancer for both male and female employees. The authors considered their findings inconclusive in respect to the association between occupational exposure to ethylene oxide and the incidence of breast cancer.

In an analysis of the study, Lucas and Teta (1996) offered as a possible explanation for the results an early detection bias occurring in this population. Based on some particularities of the population studied (individuals subjected to earlier investigation, placed under medical surveillance, possible awareness of chemically related health effects due to residential exposures, short latency period and diagnosis for most of the cases taking place closer to the end of the follow up), Lucas and Teta argued that the results may be attributable to early-case finding.

Olsen[1] *et al* (1997) investigated the risk of mortality from pancreatic and haematopoietic cancer in a cohort of 1361 male employees assigned to the Dow Chemical Company's ethylene and propylene chlorohydrine production processes. Workers who worked in the area of ethylene chlorohydrin production would have also worked in the area of ethylene oxide production through the chlorohydrin process. The standardised mortality ratio (SMR) for all malignant neoplasms was 94 (95%CI=74-118), SMR for mortality due to pancreatic cancer was 25 (95%CI=1-140) and SMR for mortality due to lymphopoietic and haematopoietic cancers was 129 (95%CI=62-238). The results showed that, to date, the cohort analysed in this study had not experienced significant increased risk for pancreatic cancer and lymphopoietic and haematopoietic cancer. The authors considered necessary that the cohort be followed up for an additional 5-10 years in order to ensure enough latency period for the outcomes studied and to be able to clarify any possible association with lymphopoietic and haematopoietic cancer.

In 1994, the Working Group of the International Agency for Research on Cancer (IARC) upgraded ethylene oxide from *group 2A*, i.e., probably carcinogenic to humans, to *group 1*, i.e., carcinogenic to humans (Axelson, 2004).

The Working Group evaluated the carcinogenicity of ethylene oxide and concluded the following: a) there is limited evidence in humans for its carcinogenicity and b) there is sufficient evidence in experimental animals for its carcinogenicity (IARC, 1994). In making

the overall evaluation, the Woking Group took into consideration the following supporting evidence. Ethylene oxide is a directly acting alkylating agent that:

i) induces a sensitive, persistent dose-related increase in the frequency of chromosomal aberrations and sister chromatid exchange in peripheral lymphocytes and micronuclei in bone-marrow cells of exposed workers;

ii) has been associated with malignancies of the lymphatic and haematopoietic system in both humans and experimental animals;

iii) induces a dose-related increase in the frequency of haemoglobin adducts in exposed humans and dose-related increases in the numbers of adducts in both DNA and haemoglobin in exposed rodents;

iv) induces gene mutations and heritable translocations in germ cells of exposed rodents; and

v) is a powerful mutagen and clastogen at all phylogenetic levels.

Following the overall evaluation ethylene oxide was classified as carcinogenic to humans, i.e., *group 1* (IARC, 1994).

1.3. ADVERSE REPRODUCTIVE OUTCOMES FOLLOWING OCCUPATIONAL EXPOSURE TO ETHYLENE OXIDE

The section will start with a brief presentation on the normal evolution of human pregnancy and on developmental toxicity. Then the following reproductive outcomes will be discussed: spontaneous abortion, stillbirth and low birth weight. The discussion will include definition, risk factors and data sources for each of them. For the outcomes whose association with exposure to ethylene oxide was already investigated, a summary of the papers published to date will also be provided.

The Occupational Safety and Health Administration (OSHA) of the USA have regulated ethylene oxide on the basis of its potential to cause reproductive dysfunction (OSHA, 2004; Rubenstein, 1992).

In general, the clinical response to a toxic agent depends on three categories of factors: 1) duration, frequency and route of exposure; 2) environmental factors; and 3) individual factors. When toxicants enter the human body via respiratory tract, they are readily absorbed in the general circulation due to the large alveolar area, high blood flow, and proximity of the blood to the alveolar air (Moeller, 1998; Rosenberg, 1997). In the pregnant woman, the respiratory

route of exposure to toxicants is of particular interest because of the physiological changes taking place during pregnancy: the increase of the minute ventilation and, to a lesser extent, of the oxygen consumption (Nuwayhid *et al*, 1998).

As ethylene oxide is absorbed through the respiratory tract, it becomes obvious that pregnant women exposed to this chemical are particularly at risk for a greater exposure. During an 8-hour exposure in the workplace at the same air concentration of ethylene oxide, the largest pulmonary dose of the chemical would be delivered to the pregnant woman. During an 8-hour exposure at the recommended exposure limit of 1.8 mg/m^3 (1 ppm), the pregnant woman would inhale 9 mg, compared to 5.2 mg inhaled by a non-pregnant woman and to 6.5 mg inhaled by a man in the same working conditions (Silvaggio and Mattison, 1993).

Ethylene oxide's size and solubility suggest that it readily crosses the foetal-placental barrier. In addition, its biochemical reactivity with nucleoproteins and amino acids gives ethylene oxide mutagenic and carcinogenic properties, which in turn indicate its potential for causing inheritable genetic damage and thus both male- and female-mediated reproductive problems. In experimental animals, ethylene oxide is toxic to reproductive function in both males and females. Although it may exert its reproductive toxicity through several mechanisms, the induction of dominant lethal mutations has been the mechanism most convincingly demonstrated (Baranski, 1993; Landrigan *et al*, 1984; LaMontagne[1] and Kelsey, 1998; Sheikh, 1984).

After fertilisation, the developing organism shows a very high level of cell division, migration and differentiation. Especially during these periods, DNA and other macromolecules are very susceptible to extraneous influences. It is very likely that after ethylene oxide has been absorbed by the body of the mother, it easily reaches the developing organism. If this results in DNA or other macromolecule alkylation, the developmental pathways may be disturbed. At an early embryonic stage this may lead to embryonic death which translates to a spontaneous abortion. Later on, structural and functional disturbances may be expected (Florack and Zielhuis, 1990).

Evidence now available on reproductive toxicity suggests that ethylene oxide exhibits a dose-rate[*] effect. Such an effect raises additional concerns about short-term exposures, even when they do not exceed the full-shift exposure limits (NIOSH, 1989).

[*] Dose rate is a synonym for dose-intensity, i.e., the rate of delivery of a substance to a target (Checkoway *et al*, 1989).

1.3.1. Fertilization, Implantation and Normal Evolution of Pregnancy

Fertilization, or conception, is the union of the male and female pronuclear elements. It usually takes place in the fallopian tube, after which the fertilized ovum continues to the uterus, where implantation occurs and development of the foetus continues.

The fertilized ovum reaches the endometrial cavity about 3 days after ovulation. This delayed transport of the fertilized ovum through the fallopian tube allows several stages of cell division to occur before the dividing ovum, now called a blastocyst with about 100 cells, enters the uterus. On reaching the uterine cavity, the embryo undergoes further development for 2 to 3 days before implanting. Implantation results from the action of trophoblast cells that develop over the surface of the blastocyst. These cells secrete proteolytic enzymes that digest and liquefy the adjacent cells of the uterine endometrium. Once implantation has taken place, the trophoblast cells and other adjacent cells (from the blastocyst and uterine endometrium) proliferate rapidly, forming the placenta and the various membranes of pregnancy (Guyton and Hall, 2000; Surrey *et al*, 1998).

Within 1 month after fertilization of the ovum, the gross characteristics of all the different organs of the foetus have already begun to develop and during the next 2 to 3 months, most of the details of the different organs are established. Beyond month 4, the organs of the foetus are grossly the same as those of the neonate. However, cellular development in each organ is usually far from complete and requires the full remaining 5 months of pregnancy for complete development (Guyton and Hall, 2000).

Term is reached at 40 weeks after the onset of the last menstrual period or at 38 weeks measured from the time of conception (ovulation). At this time, the foetus is fully developed, with the characteristic features of the newborn infant. The average crown-rump length of the foetus is about 36 cm and the weight is approximately 3400g (Bullock *et al*, 2001; Cunnigham *et al*, 1993).

1.3.2. Developmental Toxicology

In the pregnant woman, complex alterations in pulmonary, cardiovascular, renal, gastrointestinal and hepatic function occur. These physiological changes can alter toxicokinetic factors and affect the response of the mother and her foetus to certain chemicals. Foetal exposure to a foreign chemical is hence the result of a complex interaction among many factors: maternal, placental and embryonic factors (Kurzel, 1992; Silvaggio and Mattison, 1993).

- *Maternal factors*

Foetal exposure to toxic agents is closely linked to the mother's ability to detoxify and excrete foreign chemicals. Maternal distribution, detoxification and excretion of foreign chemicals serve to reduce foetal exposure. Once the developmental toxicant is absorbed by the mother, it is distributed within the maternal circulation and tissues. When the agent is sequestered in maternal tissues, circulating levels may be reduced. Maternal detoxification of foreign compounds further reduces foetal exposure, as does maternal excretion of foreign chemicals.

Any condition that alters these processes would tend to affect the environmental assault received by the foetus. Thus, the age of the mother and her nutritional status, general health and well being all affect her capacity to protect the developing embryo.

- *Placental factors*

Although the placenta has long been known to guard the foetus from environmental assault, there is ample evidence that many chemicals traverse the placenta. The critical question relative to foetal toxicity is the rate at which foreign chemicals do so. This rate is influenced by several factors, most important being the maternal plasma concentration of these chemicals.

Since the placenta is known to have all the common detoxification mechanisms, it is possible that the transplacental movement of foreign chemicals could be affected by such reactions. It appears, however, that the placental metabolism of xenobiotics is not sufficient to significantly affect their transfer across this organ.

- *Embryonic factors*

Very little data exist on the ability of young mammalian embryos to metabolise foreign compounds. It appears that the foetal capacity to detoxify foreign chemicals that have crossed the placenta is limited.

The foetal response to toxic agents depends on such factors as the agent, the dose, the duration of exposure and the timing of the insult relative to foetal development. The embryo is generally more sensitive to chemical exposures than the mother. The timing of toxic exposure relative to foetal development is most important. During the early pre-implantation period, when the blastocyst is free within the uterus, toxic exposures are most likely to be lethal, while toxic exposure occurring during organogenesis is most likely to produce malformations.

1.3.3. Loss of Conceptus

Cunningham *et al* (1993) define pregnancy as commencing at the time of implantation and they classify conceptus loss as follows:

1. *Early pre-implantation (preclinical) loss of conceptus* that takes place early after fertilization, just before implantation and therefore before the pregnancy occurred.
2. *Early post-implantation (preclinical) embryonic loss,* when the product of conception is sloughed before or at about the time of the next anticipated menstruation, with the pregnancy not being clinically recognised. These losses are also called *occult pregnancy* because they are not interpreted as a pregnancy episode. The bleeding that marks the loss begins around the time of expected menstrual period, an experience seldom recognized by the woman as a lost pregnancy. In this situation, pregnancy can be recognised only by detecting urinary levels of the pregnancy hormone hCG (human chorionic gonadotrophin), or detecting other pregnancy-specific proteins, such as early pregnancy factor (EPF) (Cunningham *et al*, 1993; Weinberg and Wilcox, 1998). Because of the methods used to detect a pregnancy at this stage, other authors call it *biochemical pregnancy* (Bennett, 1998; Howie, 1995).
3. *Spontaneous abortions,* also called *early clinical pregnancy wastage or spontaneous first-trimester abortion* that usually occur within the first trimester of pregnancy. Kmak and McNeeley (2000) argue that "spontaneous abortion" is an old terminology for pregnancy loss, suggesting the use of "early pregnancy loss/miscarriage" instead.
4. *Late pregnancy wastage (stillbirth, foetal death),* when foetal loss occurs after the time of expected viability.

1.3.4. Spontaneous Abortion

Definition: Failure of embryonic development, foetal death in utero and expulsion of all (complete) or any part (incomplete) of the products of conception before 20 weeks' gestation or of a foetus weighting less than 500 g or of a foetus less than 25 cm long (Dorfman, 1992; Lemasters, 1998).

Other definitions of spontaneous abortions are "termination of pregnancy before 24 weeks' gestation with no evidence of life" (UK) or "the delivery of a foetus-neonate that weighs less than 1000g" (some European countries) (Cunningham *et al*, 1993; Howie, 1995).

It is thought that about 10-15% of clinically manifested pregnancies end in spontaneous abortion (El Batawi *et al*, 1987; Weinberg and Wilcox, 1998).

Kmak and McNeeley (2000) consider that spontaneous abortions occur in 12 to 20% of all recognised clinical pregnancies. According to them, if clinically unrecognised pregnancies (biochemical pregnancies) are included, then 65% of all pregnancies result in spontaneous abortions.

In a study aimed at determining the proportion of pregnancies that end in legal termination of pregnancy in greater Soweto, Orange Farm and Lenasia, Buchmann *et al* (2002) also described the rates of spontaneous abortion found in these women. The study included only pregnancies managed in state-run health services in January–March 1999 and January-March 2001. Miscarriage rates were calculated for three groups of women: teenagers (aged less than 20 years), women aged 20-34 years and women aged 35 years and above and they were 4.9%, 5.9% and 9.7% respectively (2001 data).

Risk factors for spontaneous abortion

1. *Foetal factors*: the most common morphological finding in early spontaneous abortions is an abnormality of development of the zygote, embryo, early foetus, or at times placenta. In up to 60% of spontaneous abortions, the foetus is absent or grossly malformed and in 25 to 60%, it has chromosomal abnormalities incompatible with life. Thus, spontaneous abortions in more than 90% of cases may be a natural rejection of a maldeveloping foetus (Cunningham *et al*, 1993).
2. *Maternal factors*
    - *Infections*: herpes simplex, Mycoplasma hominis, ureaplasma urealiticum, acute maternal infections that generate a general toxic illness with high temperature (pyelitis or appendicitis) (Cunningham *et al*, 1993; Lemasters[1], 1998);
    - *Endocrine abnormalities*: uncontrolled diabetes mellitus, luteal phase defect (LPD – i.e., inadequate progesterone production by the corpus luteum and inadequate secretory changes of the endometrium), polycystic ovary syndrome, hypothyroidism (Baker, 1991; Bennett, 1998; Kmak and McNeeley, 2000);
    - *Severe malnutrition* (Cunningham *et al*, 1993; Lemasters[1], 1998);
    - *Recreational drugs and environmental factors*: tobacco smoking, alcohol consumption, coffee consumption (greater than four cups per day), contraceptives (intrauterine devices), ionising radiation, environmental toxins (anaesthetic gases, antineoplastic agents, arsenic, aniline, lead, formaldehyde, benzene, ethylene oxide), ergonomic factors (heavy lifting, bending and physical effort)

(Cunningham *et al*, 1993; Florack, 1993; Gold and Tomich, 1994; Goulet and Thériault, 1987; Xu *et al*, 1998);

- *Immunological factors*: autoimmune mechanisms (e.g., antiphospholipid antibodies, including lupus anticoagulant and anticardiopilin antibodies), alloimmune mechanisms (e.g., histocompatibility factors, failure to synthesise blocking antibodies that protect the foetus from the mother's own antibodies directed against paternal antigens shared by the foetus, absence of local (i.e., decidual) suppressor factors directed against immune response, failure in developing maternal antipaternal antileucocytotoxic antibodies) (Cunningham *et al*, 1993);
- *Aging gametes*: the age of both sperm and egg may influence the spontaneous abortion rate. Maternal age over 35 appears to be associated with an increased risk of abortion, but there is evidence that maternal age is an important risk factor in women under 35 years. Maternal age at conception is a strong and independent risk factor for spontaneous abortion, irrespective of a woman's reproductive history (Andersen *et al*, 2000; De la Rochebrochard and Thonneau, 2002; Jolly *et al*, 2000);
- *Trauma*: an intrauterine device which had failed to prevent pregnancy, abdominal surgery during early pregnancy, amniocentesis, chorion villus sampling (Howie, 1995);
- *Uterine defects*: congenital anomalies (e.g., unicornuate uterus, didelphic uterus, bicornuate uterus, septate uterus, incompetent cervix) or acquired anomalies (e.g., diethylstilbestrol exposure, intrauterine adhesions, uterine leiomyomas, incompetent cervix) (Kmak and McNeeley, 2000);
- *Reproductive history*: prolonged delay before achieving a recognised conception, history of subfertility, previous spontaneous abortions, birth of malformed babies (Baker, 1991).

3. *Paternal factors*: paternal age (over 40, particularly if maternal age is greater than 35) and exposure to anaesthetic gases, lead, mercury, selected solvents and some pesticides (De la Rochebrochard and Thonneau, 2002; Lemasters, 1992; Lemasters 1996; Schrader and Lemasters, 1998).

Data sources on pregnancy loss

Direct studies of early pregnancy loss are difficult to carry out. To do so, women should be recruited early in their attempt at conception and then followed-up to detect the occurrence of pregnancy. Pregnancies at this stage can only be confirmed using urinary assays for hCG (Lindbohm, 1999; Weinberg and Wilcox, 1998).

For clinically recognised spontaneous abortions two sources of data are available: interviews and medical records, both with advantages and disadvantages. Advantages of the interview technique include greater sensitivity in recording early spontaneous abortions, which would not always lead to medical contact and recording spontaneous abortions that could have happened outside the place of residence. On the other hand, as most spontaneous abortions are not always recognised clinically even by experienced observers, it is very likely that the ability of women to recognise their spontaneous abortions is highly variable. Therefore interview reporting may be rather sensitive to factors like social status, medical experience, concern about harmful environmental factors etc, which all may introduce severe bias (particularly if the social background is heterogeneous in the study population) unless data acquisition is very careful.

Medical records are more reliable, but hospital registration may be biased insofar as there are differential rates for hospitalisation on socio-economic or geographical grounds. Moreover, in case of migration records from local hospitals are incomplete sources of information (Axelsson and Rylander, 1984; Hemminki[1] *et al*, 1983).

The best approach is to combine the information gathered from the two data sources. Whenever objective data on spontaneous abortions are available, they should be used to control at least a sample of the information collected; this is particularly compelling if the involved study population is heterogeneous in factors that may influence subjective reporting (Hemminki[1] *et al*, 1983).

Animal research on spontaneous abortion in relation with exposure to ethylene oxide

Hardin studied the reproductive-toxicological effects of ethylene oxide, propylene oxide, butylenes oxide and styrene oxide on rats and rabbits (Hardin *et al*, 1983). Sexually mature female Sprague-Dawley sperm-positive rats and artificially inseminated New Zealand white rabbits were exposed to150 ppm (270mg/m^3) ethylene oxide for 7 hours per day before breeding and during different gestational stages. The animals were sacrificed on day 21 (rats) and day 30 (rabbits) of gestation and all the foetuses were analysed for external, visceral and skeletal defects. The assessment of ethylene oxide effects involved four groups of rats with

the following regimens of exposure: group 1 was exposed only to filtered air, group 2 was exposed to ethylene oxide at 7-16 days of gestation, group 3 was exposed to ethylene oxide at 1-16 days of gestation and group 4 was exposed to ethylene oxide three weeks before breeding and at 1-16 days of gestation. Embryo and foetal toxicity was evident in rat litters as a statistically significant increase in the incidence of the resorptions in group 4. The study also analysed the effects of ethylene oxide on three groups of rabbits with the following exposure regimens: group 1 was exposed only to filtered air, group 2 was exposed to ethylene oxide at 7-19 days of gestation and group 3 was exposed to ethylene oxide at 1-19 days of gestation. No evidence of embryo or foetal toxicity of developmental defects was detected in the ethylene oxide-exposed rabbits.

Human research on spontaneous abortion in relation with exposure to ethylene oxide

Table 2 summarises the studies published to date investigating the reproductive effects following exposure to ethylene oxide in pregnant women.

In a study from the USSR, Yakubova *et al* (1976, quoted in IPCS, 1985) followed the evolution and outcome of pregnancy in 57 operators, 38 laboratory workers and 65 administrative staff in an ethylene oxide-producing plant, the majority of the women being between 20 and 29 years of age. A group of 50 pregnant women working outside the plant served as additional controls.

It was estimated that the operators were exposed to ethylene oxide concentrations of 0.2-0.3 mg/m^3 (0.11–0.17 ppm) for 80% of the working time and 1.0 mg/m^3 (0.55 ppm) for the remaining 20%, while the laboratory workers were exposed only to the lower concentrations. Spontaneous abortion occurred in 6 out of 57 (10.5%) operators, 3 out of 38 (7.9%) laboratory workers and in 5 out of 65 (7.7%) administrative staff. The operators were subjected to the additional stress of high levels of noise and vibration and wide variations in atmospheric temperatures. Findings in this study did not indicate any unequivocal adverse effect of ethylene oxide exposure at these concentrations on the outcome of pregnancy.

Comments

As there was no access to the original paper, information on this study was obtained from other publications.

Hemminki *et al* (1982) analysed the incidence of spontaneous abortions in female sterilising staff working in all hospitals in Finland in 1980, occupationally exposed to ethylene oxide, glutaraldehyde and formaldehyde.

The study included two groups of female hospital employees: a group of women sterilising staff and a control group, represented by nursing auxiliaries in the same hospitals without previous exposure to sterilising agents, anaesthetic gases or X-rays.

Information on workers' exposure to any of the three chemicals of interest was obtained from the supervising nurses of the sterilising units enrolled in the study. The supervisors received a set of questionnaires enquiring about the chemical sterilants used and the names of female staff using a particular sterilising agent. The authors also mentioned in the paper that levels of ethylene oxide measured in several sterilising units in Finnish hospitals ranged from 0.1 to 0.5 ppm with peak levels of 250 ppm, but they did not offer details on when and how these measurements were performed.

Information on pregnancy outcome was gathered by means of a questionnaire sent to the two groups of women (exposed and control). This questionnaire collected social and demographic data (including social security number), details about all the pregnancies and their outcome, occupational history and life style (smoking, alcohol and coffee consumption). The participating women were not told about the purpose of the study, in order to avoid information bias. The response rate was 91.6% for the sterilising staff and 90.6% of the nursing auxiliaries, yielding a total number of pregnancies of 1443 and 1179, respectively. Information on the evolution and outcomes of the pregnancies of the study participants was also collected from the hospital discharge registers covering all Finland between 1973 and 1979, using social security number as means of identification.

The investigators calculated *the rate of spontaneous abortions* (number of spontaneous abortions divided by the sum of births, induced abortions and spontaneous abortions) and *the ratio of spontaneous abortions* (number of spontaneous abortions divided by the number of births). Data were analysed using both crude rates and adjusted rates controlled for age, parity, decade of reported pregnancy, smoking, alcohol intake and coffee consumption.

The overall *crude rates* of spontaneous abortions were 11.3% for sterilising staff and 10.6% for controls. Analysis was also performed inside the group of sterilising staff, comparing the outcome of pregnancies exposed to chemical sterilants to the outcome of pregnancies not exposed to chemical sterilants. The results showed a crude rate of spontaneous abortions of 16.7% for the exposed pregnancies vs. 6.0% for the unexposed ones.

The overall *adjusted rates* of spontaneous abortions were 9.7% for sterilising staff and 10.5% for controls. Inside the sterilising staff group, the adjusted rate of spontaneous abortions among exposed pregnancies was 15.1% and for the unexposed ones was 4.6%.

The authors also analysed the possible effect of each of the chemical sterilants used (ethylene oxide, glutaraldehyde and formaldehyde) on the frequency of spontaneous abortions occurring inside the sterilising staff group. The ethylene oxide exposure was classified into three categories: use of ethylene oxide with and without other agents, ethylene oxide with glutaraldehyde and ethylene oxide alone.

The *crude rates* of spontaneous abortions were as follows: for the category *ethylene oxide used with and without other agents* 9.9% for unexposed and 19.2% for exposed pregnancies ($p<0.001$); for the category *ethylene oxide used with glutaraldehyde* 7.1% for unexposed and 17.3% for exposed pregnancies ($p<0.001$); and for the category *ethylene oxide used alone* 10.3% for the unexposed and 20.7% for the exposed pregnancies ($p<0.01$).

The *adjusted rates* of spontaneous abortions were as follows: for the category *ethylene oxide used with and without other agents* 7.7% for unexposed and 12.7% for exposed pregnancies ($p<0.05$); for the category *ethylene oxide used with glutaraldehyde* 6.6% for unexposed and 11.3% for exposed pregnancies ($p<0.05$); and for the category *ethylene oxide used alone* 7.8% for the unexposed and 16.1% for the exposed pregnancies ($p<0.01$).

Rates and ratios of spontaneous abortions were also calculated using data collected from the hospital discharge registers for both the sterilising staff group and control group.

Amongst sterilising staff, the *rate of spontaneous abortions* for pregnancies exposed to *ethylene oxide* was 22.6% ($p<0.05$ when compared to controls), while the *rate of spontaneous abortions* for unexposed pregnancies was 9.9%. The controls had a *rate* of spontaneous abortions of 9.2%.

Amongst sterilising staff, the *ratio of spontaneous abortions* for pregnancies exposed to *ethylene oxide* was 33.3% ($p<0.05$ when compared to controls), while the *ratio of spontaneous abortions* for unexposed pregnancies was 12.6%. The controls had a *ratio* of spontaneous abortions of 11.8%.

Based on these findings, the authors concluded that "exposure to ethylene oxide in hospitals correlates with an increased frequency of spontaneous abortions".

Comments

Data on exposure are not straightforward in this study. The authors mentioned levels of exposure as assessed by the Finnish Institute of Occupational Health (TWA8 ranging from 0.1

ppm to 0.5 ppm and peak concentrations up to 250 ppm), without specifying whether the measurements were performed at the time of the study or over a different period of time and in which hospitals they were taken (all hospitals, a sample etc).

The information women enrolled in the study provided on occupation at the time of each pregnancy might have not been detailed enough to allow for an exposure classification (not all the workers sharing a working place are exposed to the same hazards and even so, they may have different levels of exposure).

Comparing overall pregnancy outcomes between sterilising staff and controls (using either crude or adjusted rates) is not appropriately used for the purpose of the study (i.e., finding an association between exposure to a particular chemical and a health outcome), since some of the pregnancies investigated might have occurred in very different occupational settings, with completely different exposures.

In the analysis the authors used two sets of data: the one obtained by the means of the questionnaire and the one collected from the hospital discharge registers. They did not specify whether the medical records were used to validate the information gathered by the means of questionnaire or they were just used for a second analysis.

A number of questions have been raised regarding the findings of this study (Austin, 1983; Gordon and Meinhardt, 1983).

- Ethylene oxide concentration measurements: the authors did not mention whether these measurements were representative of the sterilising units and women included in the study.
- Possible misclassification of exposure: the exposure status of all the pregnancies was assessed by the supervising nurse of each sterilising unit studied.
- The possibility of recall bias: as information was collected on all pregnancies experienced by the women investigated, some of them as far back as 1951, Gordon and Meinhardt questioned the ability of women to recall such distant events without bias.
- The possibility of differential reporting of adverse outcomes between controls and sterilising staff and between the sterilisers' exposed and unexposed pregnancies if women investigated were aware of the purpose of the study.
- The results showed that the unexposed pregnancies of the sterilising staff had lower rates of spontaneous abortions than the controls (also unexposed). A possible explanation for these findings could be: firstly, recent pregnancies were more likely to be exposed and reported more accurately, which would lead to a greater concentration of unexposed

pregnancies in the earliest years (1950s and 1960s), where recall was also poorest; secondly, unexposed pregnancies of the sterilising staff might have occurred when those women were largely unemployed, while the pregnancies of the controls might have occurred to women both unemployed and employed at that time.

- Inappropriate control of confounding factors: lack of sufficient information on adjustment for age and parity and classification of smoking habits, alcohol and coffee consumption (i.e., whether the duration and degree of these consumption patterns were accounted for at the time of each pregnancy or at the time the study was carried out); lack of control for employment status and history of previous spontaneous abortions.

Addressing these questions, Hemminki *et al* (1983) offered more details about their initial study:

- Ethylene oxide concentrations were not measured at the time of the study. The measurements mentioned in their paper had been carried out in Finnish hospitals since 1976 at the request of the hospitals. Therefore, the authors could not say how representative the measurements were of the hospitals included in the study, but they showed that there was exposure to ethylene oxide for the employees working with the steriliser (opening the sterilising chamber);
- Because interviewed women were not aware of the purpose of the study, the authors considered the possibility of biased exposure information unlikely;
- The pregnancies for which exposure status was not determined were included in a category of "exposure uncertain";
- The difference in the frequency of reported spontaneous abortion depended on how recent the pregnancy was: parity-adjusted frequency of reported spontaneous abortion was highest in the decade 1971-1981 for all three groups of interviewed women: exposed sterilising staff, controls and unexposed sterilising staff. The time of the pregnancy also correlated with employment, the most recent pregnancies being likely to be the exposed ones. The unexposed pregnancies usually dated far back in time and took place when the women were housewives. In the authors' opinion, this was why the rates of spontaneous abortion in the unexposed pregnancies were low.

The authors also provided a new analysis of the data from the original study, taking into account only pregnancies that started during hospital employment for both sterilising staff and controls. After age adjustment done in five-year age groups, the spontaneous abortions rates were as follows: for controls 11.3%; for the pregnancies exposed to ethylene oxide with or

without other agents, 18.6% (significantly different from controls' rate, $p<0.05$); for the pregnancies exposed to ethylene oxide or glutaraldehyde or both, 17.3% (significantly different from controls' rate, $p<0.01$); for the pregnancies exposed to ethylene oxide alone, 20.4% (significantly different from controls' rate, $p<0.05$).

Hemminki *et al* (1985) also investigated the occurrence of spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, sterilising agents (ethylene oxide, glutaraldehyde, formaldehyde), disinfectant soaps, cytostatic drugs and X-ray radiation during the first trimester of pregnancy. They conducted a case-control study; nurses who had been pregnant between 1973 and 1979 and who had worked in anaesthesia, surgery, intensive care, operating room or internal medicine department of a Finnish general hospital represented the study population. There were two types of cases: *a spontaneous abortion case*, defined as a woman from the study population that had been treated for spontaneous abortion between 1973 and 1979; and *a congenital malformation case*, defined as a woman that had given birth to a malformed child between 1973 and 1979. Only the part of the study concerned with spontaneous abortions will be discussed further.

To identify the spontaneous abortions cases (n=217) among the study population, the Hospital Discharge Register was linked to the Central Register of Health Care Personnel. Controls were selected among nurses who had given birth to a healthy child between 1973 and 1979 and who had not had a spontaneous abortion during the study period. Three controls were then selected for each case, matched for age and hospital of employment (n=571 meeting the matching criteria).

Exposure information during the first trimester of pregnancy was requested for all study participants (both cases and controls) from the head nurses of all general hospitals in Finland. Information was requested of exposure to: anaesthetic gases (nitrous oxide, halothane, other), sterilising agents (ethylene oxide, glutaraldehyde and formaldehyde), disinfectant soaps, cytostatic drugs and X-ray radiation.

Table 2: Summary of the studies investigating reproductive effects following exposure to ethylene oxide in pregnant women

Authors	Yakubova et al., 1976	Hemminki et al, 1982	Hemminki et al, 1983	Hemminiki et al, 1985	Rowland et al, 1996
Study	Course of pregnancy and birth followed in women working in an ETO production plant and controls	Spontaneous abortion in hospital staff engaged in sterilising instruments with chemical agents (ETO, FA, GA)	New analysis of the data from the previous study (see Hemminki et al, 1982)	Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs and other potential hazards in hospitals, based on registered information of outcome	Exposure to ETO and the risk of spontaneous abortion, preterm birth and postterm birth
Methods	• *Exposed group* = 57 operators and 38 laboratory workers • *Unexposed group* = 65 administrative staff and 50 pregnant women working outside the plant	• *Exposed* = female sterilising staff exposed to ETO while pregnant • *Unexposed* = female sterilising staff unexposed to ethylene oxide while pregnant • *Controls* = auxiliary nurses unexposed to ETO, X-ray, or anaesthetics	See Hemminki et al, 1982	• *Cases* = 217 nurses working in general hospitals in Finland who had had an spontaneous abortion and 46 who had had a malformed child between 1973-1979 • *Controls* = 571 for SAB cases and 128 for malformation cases	• *Exposed group*: 32 female dental assistants using ETO while pregnant • *Unexposed group* = 1,288 female dental assistants unexposed to ETO while pregnant

Authors	Yakubova et al., 1976	Hemminki et al, 1982	Hemminki et al, 1983	Hemminiki et al, 1985	Rowland et al, 1996
Exposure assessment	• *Operators:* 0.11-0.56 ppm ETO • *Laboratory staff:* 0.11-0.17 ppm ETO	Indirect assessment performed by the supervising nurse	Indirect assessment (see Hemminki et al, 1982) based on previous measurements of ETO levels: TWA8 exposure of 0.1-0.5 ppm and peak levels up to 250 ppm	Indirect assessment: exposures during the first trimester of nurse's pregnancy provided by the head nurses	Indirect assessment: self-reported data
Outcome assessment	No details	• Questionnaire administrated by the supervising nurses • Hospital discharge data for SAB	See Hemminki et al, 1982	Record linkage between: • The hospital discharge register (supplemented with policlinic cases of spontaneous abortions) • The central register of health care personnel	Mailed self-administrated questionnaire

Authors	Yakubova et al., 1976	Hemminki et al, 1982	Hemminki et al, 1983	Hemminiki et al, 1985	Rowland et al, 1996
Results	• SAB in 10.5% of operators and in 7.9% of laboratory workers • SAB in 7.7% of administrative staff	*Using questionnaire-collected data* • SAB rate[1] in exposed sterilising staff: 16.1% • SAB rate[1] in unexposed sterilising staff: 7.8% • SAB rate[1] in controls: 10.5% *Using data from the hospital discharge register* • SAB rate[1] in exposed sterilising staff: 22.6% • SAB rate[1] in unexposed sterilising staff: 9.9% • SAB rate[1] in controls: 9.2%	• SAB rate[2] in exposed sterilising staff: 20.4% • SAB rate[2] in controls: 11.3%	• Crude OR of SAB for ETO (unmatched series): OR=0.6 • OR of SAB for sterilising agents (matched series): 0.7 (95%CI=0.4-1.3)	• SAB frequency in exposed: 15.6% • SAB frequency in unexposed: 6.8% • PreTD frequency exposed: 9.4% • PreTD frequency in unexposed: 4.3% • PostTD frequency in exposed: 15.6% • PostTD frequency in unexposed: 10.9% • Relative risk[3] for either SAB or PreTD: RR=2.3 (95%CI=1.0-5.4) • Relative risk [4] for any of the three outcomes (SAB, PreTD, PostTD): RR=2.1 (95%CI=0.7-5.7)

Authors	Yakubova et al., 1976	Hemminki et al, 1982	Hemminki et al, 1983	Hemminiki et al, 1985	Rowland et al, 1996
Conclusions	No indication of any unequivocal adverse effect of ETO exposure at these concentrations on the outcome of pregnancy	Exposure to ETO in hospitals correlates with an increased frequency of spontaneous abortions		No information on the effects of ETO	Women exposed to ethylene oxide were 2.1 more likely to have any of the three adverse pregnancy outcomes
Comments	No direct information on the study methods	All pregnancies of the women enrolled in the study were analysed, including the ones occurring while the mother was not working in sterilisation or not working at all	Only pregnancies occurring while women working as sterilising staff were included in this analysis	No distinction was made between sterilising work and the use of instruments sterilised by ETO	

SAB = spontaneous abortions, ETO = Ethylene oxide, PreTD = preterm delivery, PostTD = postterm delivery.

[1]SAB rate = number of spontaneous abortions / number of pregnancies), adjusted for age, parity, decade of reported pregnancy, smoking, alcohol intake and coffee drinking.

[2] SAB rate = number of spontaneous abortions / number of pregnancies), adjusted for age.

[3]Relative risk (Poisson regression model) adjusted for age, unscavenged nitrous and high amalgam use.

[4]Relative risk (logistic model) adjusted for age, unscavenged nitrous oxide, high amalgam use and smoking.

Among the 164 cases dealing with sterilising agents or sterilised instruments, 7 had been exposed to ethylene oxide during the pregnancy studied (4.3%). Among the 467 controls dealing with sterilising agents or sterilised instruments, 30 had been exposed to ethylene oxide (6.4%). The crude odds ratio of spontaneous abortion (calculated for an unmatched series) for exposure to ethylene oxide was 0.6. The odds ratio of spontaneous abortion (calculated for matched series) for exposure to sterilising agents was 0.7 (95% CI=0.4-1.3). The authors mentioned that few nurses had been involved in sterilising instruments, most of them only using instruments that had been sterilised by ethylene oxide and had probably been exposed to negligible levels of this chemical. Therefore, the study failed to distinguish between sterilising work (where a daily ethylene oxide exposure to 1 ppm or less may take place) and the use of instruments sterilised by ethylene oxide (where very small exposures could be expected). Because only one nurse was identified as directly involved in sterilisation, the study provided no information on the effects of ethylene oxide on the evolution and outcome of the pregnancy.

Comments

The information on exposure was requested from the head nurse of all general hospitals enrolled in the study. This led to incomplete data on ethylene oxide exposure, which prevented the researchers from drawing any conclusions regarding the association between maternal exposure to ethylene oxide in early pregnancy and adverse reproductive health outcomes.

Rowland *et al* (1996) investigated the association between work-related exposure to ethylene oxide and adverse reproductive events (i.e., spontaneous abortions, preterm delivery and postterm delivery) in 1,320 female dental assistants aged 18-39 years, registered in California in 1987.

The authors randomly selected 7,000 female dental assistants aged 18-39 years from the dental-assistant registry of the California Department of Consumer affairs and mailed them a self-administered questionnaire. There were 4,856 respondents (69%), of whom 1,805 had been pregnant at least once. Rowland *et al* had also used this sample in two previous studies: a study that assessed the association between exposure to high levels of nitrous oxide and reduced fertility (Rowland *et al*, 1992) and a study that assessed the association between exposure to nitrous oxide and spontaneous abortion (Rowland *et al*, 1995).

The authors included in the analysis 1,320 dental assistants that met the following criteria: they provided information about the outcome and conception date of their most recent pregnancy, they did not have a twin pregnancy, they had been working full-time as a dental assistant at the time of conception (30 or more hours per week), they gave their age and they provided ethylene oxide exposure data.

The mailed self-administered questionnaire collected demographic data, information about the smoking status, work history as a dental assistant (including number of hours worked per week, details about exposure to ethylene oxide and possible exposure to nitrous oxide and mercury) and the history of the most recent pregnancy.

Exposure was not measured directly; instead, exposure status was assessed asking the women to list the methods used to sterilise instruments (with the following choices: chemiclave, dry-clave, autoclave, ethylene oxide, or cold solution). The women were considered exposed if they had listed ethylene oxide as one of the methods used during the job held at the last menstrual period date. The exposed group included 32 women that had reported exposure to ethylene oxide; the unexposed group included 1,288 women. Of these, 4 had missing data on smoking and 6 had missing data on nitrous oxide scavenging status, both covariates having been shown to be important risk factors for spontaneous abortion in other analyses using this data set.

The outcome (i.e., spontaneous abortions, preterm delivery and postterm delivery) was ascertained from a woman's report of her most recent pregnancy. Spontaneous abortion was defined as pregnancy loss occurring before 21 weeks, excluding induced abortions and molar pregnancies. Preterm delivery was defined as stillbirths or live births occurring between 21 and 36 weeks of gestation. Post-term delivery was defined as births with gestational age 42 weeks or longer.

Spontaneous abortions and preterm delivery data were analysed during a person-week model. Post-term births were analysed using standard logistic regression.

Ethylene oxide-exposed women reported a higher proportion of adverse pregnancy outcomes than unexposed women. The exposed women reported a frequency of spontaneous abortions of 15.6% vs. 6.8% reported by unexposed women, a frequency of preterm deliveries of 9.4% vs. 4.3% reported by the unexposed women and also a frequency of postterm deliveries 15.6% vs. 10.9% reported by unexposed women. In a life table, the exposed women had a higher risk of adverse events in almost every gestational week of pregnancy.

After adjusting for age, ethylene oxide was associated with 2.1 to 2.7-fold increases in spontaneous abortions, preterm births and postterm births. Using a Poisson regression model

that estimated the relative risk of either spontaneous abortions or preterm birth, ethylene oxide has an age-adjusted relative risk of 2.6 (95%CI=1.3–5.4). After additionally adjusting for unscavenged nitrous oxide and high amalgam use (mercury exposure), the relative risk was 2.3 (95%CI = 1.0-5.4). In a logistic regression model, ethylene oxide-exposed women were 2.7 times more likely to have any of the three adverse pregnancy outcomes (95%CI=1.2-6.1) after adjusting for age. This estimate became 2.5 (95%CI=1.0-6.1) after adjusting for age, unscavenged nitrous oxide and high amalgam use. After adjusting for smoking, the estimate became 2.1 (95%CI = 0.7-5.7).

To gauge the possible impact of missing smoking and nitrous oxide scavenging data on their conclusions, the authors performed a sensitivity analysis. When they assumed that everyone with missing smoking data was a heavy smoker and everyone with missing scavenging status was exposed to unscavenged nitrous oxide in the foetal loss/preterm birth analysis, the relative risk estimate changed from 2.6 (95%CI=1.3-5.4) to 2.4 (95%CI=1.2-5.1) in a model that adjusted for mercury, unscavenged nitrous oxide, age and smoking. When the authors reversed their assumption and supposed that everyone with missing data was not exposed to smoking or nitrous oxide, the point estimate became 2.5 (95%CI=1.2-5.2). The change in the point estimate was also negligible for the combined adverse outcome analysis. The authors used the sensitivity analysis to show that the missing smoking and scavenging data did not greatly bias their results.

The authors concluded that their results further implicated ethylene oxide as a possible reproductive toxicant in humans.

Comments

As the authors themselves admit, the definition of exposure was “crude”. The exposure assessment was based on the work history information, without measuring levels of ethylene oxide in the working place of the study subjects.

There was not any indication that possible paternal risk factors (age, occupational exposure, smoking etc) were taken into account in the analysis.

In a letter to the editor, Olsen[2] *et al* (1997) has brought into discussion some limitations of this study:

- The non-participation to the study was not random, which could bias the relative risk estimates away from the null. Out of the original sample of 7,000 women, 4,856 answered (69%) the mailed questionnaire. Amongst them, only 1,820 had ever been pregnant and

1,320 of these (73%) had usable data and were enrolled in the study. Therefore, the response rate has been calculated by Olsen[2] *et al* as 50% (0.69x0.73) of women in the original sample who had ever been pregnant;

- There was no validation of self-reported exposure and outcome responses among participants;
- Exposure status was determined asking the study participants to list the methods of sterilisation used during the job held at the time of the last menstrual period; if they listed ethylene oxide, they were considered exposed. Moreover, because sterilising process using ethylene oxide is commonly referred to as gas sterilisation and not ethylene oxide sterilisation, Olsen[2] *et al* were doubtful about accurately assessing ethylene oxide use;
- The self-reported gestation length (in weeks) and therefore the classification of pregnancies in preterm (34-37 weeks), full-term (37-41 weeks) and postterm (≥42 weeks) was also questioned;
- Ten of the 32 women that reported exposure to ethylene oxide did not provide complete information on two risk factors for spontaneous abortions: smoking and unscavenged nitrous oxide usage. Olsen[2] *et al* considered the sensitivity analysis conducted by the study's authors as a partial one, because the influence of data missing differentially by case status was not explored;
- The intervention of two possible biases occurring in retrospective studies of spontaneous abortions based on the outcome of the most recent pregnancy: first, the most recent pregnancy is less likely to be a spontaneous abortion for women who plan their pregnancies than for non-planners and second, the fact that pregnancies resulting in full-term births retain their most recent status longer than pregnancies ending in loss (length bias).

The authors of the study replied to these comments (Rowland *et al*, 1997):

- They disagreed with the calculation of the response rate of women in the original sample that had ever been pregnant; in their opinion one should not lump the non-responders with responders who are not eligible;
- Exposure data were admitted as being "crude", since studying the effect of ethylene oxide exposure on pregnancy was not the primary objective of the study;
- Despite the small number of exposed subjects (n=32), the authors considered "unusual" the fact that 13 (41%) of them had either a spontaneous abortion, a preterm delivery or a postterm delivery;

- The authors suggested that further epidemiologic studies should be conducted investigating the reproductive risks of ethylene oxide.

1.3.5. Stillbirth

Definition: Foetal death occurring after 20 weeks' gestation or spontaneous death of a foetus weighting more than 500 g, nonviable (Dorfman, 1992; LaMontagne[1] and Kelsey, 1998).

Vital statistics also use the following specific mortality rates (Moore, 1998; Whitfield, 1995):

- *Foetal death (intrauterine death)*: death of the foetus at any time before birth. Some authors consider the term synonymous with stillbirth;
- *Intrauterine foetal demise*: foetal death after 20 weeks' gestation but before the onset of labour;
- *Neonatal death*: the death of a baby within 28 days following birth, even if born when thought to be pre-viable at less than 28 weeks. *Early neonatal deaths* occur within 1 week of birth, while *late neonatal deaths* occur in the remaining 3 weeks of the neonatal period (of 28 days). The purpose of this convention (i.e., early and late neonatal deaths) is to separate neonatal deaths resulting from a complication of pregnancy, labour or delivery (early) from those that occur from a "new cause" (late) – usually an infection arising sometime after birth;
- *Perinatal deaths (mortality)*: the sum of stillbirths and early neonatal deaths, based on the fact that the latter usually result primarily from the same complications as do the stillbirths;
- *Post-neonatal death*: deaths occurring more than 28 days and up to 1 year following birth;
- *Infant death*: all deaths during the first year of life, i.e. both neonatal and postnatal deaths;

The foetal death rate is the number of foetal deaths with stated or presumed gestation of 20 weeks or more divided by the sum of live births plus foetal deaths, stated per 1,000 live births plus foetal deaths.

In 1998, the foetal death rate for all races in the USA was 6.7/1000 live births plus stillbirth (National Vital Statistics Report, 2001).

In 1990, the stillbirth rate in England and Wales was 4.6/1000 total births (i.e., live births and stillbirths) while in Scotland the stillbirth rate was 5.3/1000 total births (Whitfield, 1995).

Search for data on stillbirth in South Africa was not successful. Neither the database of the Department of Health (the South African Health Review 2002, Database of health indicators) nor the WHO database had any figures on stillbirth in South Africa.

Risk factors for stillbirth (foetal death)

1. *Foetal factors*
    - Congenital anomalies (e.g., multi-system anomalies, cardiovascular anomalies) (Hankins and Spong, 2001; Spong, 2000)
    - Erythroblastosis foetalis (Ogundipe and Hamilton, 1998)
    - Intrauterine infections (TORCH and *Listeria*) (Ogundipe and Hamilton, 1998)
2. *Maternal factors*
    - Placental and cord complications (Spong, 2000)
    - Pregnancy complications (preterm labour, premature rupture of membranes, pre-eclampsia/eclampsia, multiple gestation, antepartum haemorrhage, intrauterine growth retardation, autoimmune diseases) (Ogundipe and Hamilton, 1998; Spong, 2000; Walsh *et al*, 1993)
    - Maternal age (women over 35 are at increased risk because of higher incidence of medical problems in the mother and of higher risk of genetic abnormalities in the foetus) (Andersen *et al*, 2000; Jolly *et al*, 2000; Lindsey, 2001)
    - Maternal exposures to environmental and occupational factors: chemicals (e.g., pesticides), smoking, alcohol abuse (Walsh *et al*, 1993)
3. *Undetermined causes*: they represent 50% of the cases of foetal death (Ogundipe and Hamilton, 1998).

Data source on stillbirth

In some countries, stillbirth is reported in the birth registration system, where basic demographic information is collected on newborns and their parents.

Data can be collected from maternity wards or hospitals records, with the advantage of a high diagnostic confirmation rate or interviewing women that had a stillbirth.

Animal research on stillbirth in relation with exposure to ethylene oxide

Female mice were experimentally exposed to ethylene oxide gas (1200 ppm for 1.5 hours) at different exposure times (1, 6, 9, or 25 hours after mating). Exposure at 1 or 6 hours increased

the number of mid-gestational and late foetal deaths, but few such effects were seen after exposure at 9 hours and none after 25 hours (Generoso *et al*, 1987).

Human research on stillbirth in relation with exposure to ethylene oxide

The medical literature searched did not mention any study investigating the occurrence of stillbirth in women occupationally exposed to ethylene oxide.

1.3.6. Low Birth Weight

Definition: a *low birth weight* infant is defined as an infant weighing less than 2500 g at birth, a *very low birth weight* is defined as less than 1500 g and an *extremely low birth weight* is one that is less than 1000 g (Cunningham *et al*, 1993; Lemasters[1], 1998).

The average term infant at birth weighs about 3000 to 3600 g, depending upon race, parental economic status, size of the parents and parity of the mother, with boys about 100g heavier than girls. During the second half of pregnancy, the foetal weight increases in a linear manner with time until about the 37th week of gestation and then the rate slows variably. The principal determinants of foetal growth late in pregnancy are related, in large part, to the socio-economic status of the mother. In general, the greater the socio-economic deprivation, the slower the rate of late foetal growth (Cunningham *et al*, 1993).

A newborn's weight predicts its survival better than any other characteristic. Foetal growth retardation is an important predictor of perinatal morbidity and mortality, as well as a potential risk factor for cognitive disability later in childhood. Cognitive function is associated almost linearly with birth weight up to 4000 g (Olsen, 2000; Weinberg and Wilcox, 1998). A foetus or newborn infant whose weight is appreciably below normal is at increased risk of dying or, if he or she survives, at increased risk of physical and intellectual impairment. Two distinct mechanisms are responsible for these increased risks: altered gestational age and inappropriate foetal growth. In the low-birth weight neonate, gestational age may have been shortened or the foetus may have failed to maintain a normal growth rate (Cunningham *et al*, 1993). In developing countries, more than half of the cases of low birth weight probably result from intrauterine growth retardation, while in industrial countries most of such cases result from preterm delivery (Walsh *et al*, 1993).

In any pregnancy it is essential to have precise knowledge of the gestational age of the foetus; this knowledge is exceedingly important when the pregnancy is complicated. Gestational age must be known before any diagnosis of foetal growth retardation can be made. Without an

accurate gestational age, the appropriateness of foetal growth cannot be established (Cunningham *et al*, 1993).

In recent years, the term *small for gestational age* has been used widely, especially by neonatologists, to categorise an infant whose birth weight is clearly below average and usually below the 10th percentile for its gestational age. Obstetricians have more often used the terms *foetal growth retardation* or the less precise term *intrauterine growth retardation* (Cunningham *et al*, 1993).

It is also important to distinguish between asymmetrical and symmetrical growth retardation. Asymmetrical growth retardation, i.e., when the weight is affected more than the skeletal structure, is primarily associated with a risk factor operating during late pregnancy and it has also been observed in studies of environmental exposures. Symmetrical growth retardation may more likely be associated with an aetiology that operates over the entire length of gestation (Lemasters, 1996).

In considering the causal pathway, it is obvious that low birth weight in itself does not cause adverse outcomes but it serves as a biomarker for the truly causal factor. The question therefore is whether birth weight is a faithful correlate for some factor, such as impaired growth, that is on the causal pathway to disability and death (Olsen, 2000; Weinberg and Wilcox, 1998).

Low birth weight babies represent 3% to 8% of all births (Weinberg and Wilcox, 1998). According to the UNICEF database of nutrition indicators for 2002, in South Africa low birth weight babies represented 15% of all births (UNICEF, 2004).

Risk factors for low birth weight (small for gestational age)

1. Foetal factors

- Genetic: abnormalities of foetal chromosomes (Onyeije and Divon, 2000)
- Multifoetal pregnancy (foetal growth restriction resulting from abnormal placentation, foetal infection, physical restraints, shared foetal circulation) (Onyeije and Divon, 2000)
- Congenital malformations: dysplastic kidneys, cardiovascular malformations, significant neural tube defects (Baker, 1991; Onyeije and Divon, 2000)
- Foetal infections: rubella, cytomegalovirus infection, hepatitis A, hepatitis B, listeriosis, tuberculosis, syphilis, toxoplasmosis, congenital malaria (Beers and Berkow, 1999; Walsh *et al*, 1993)

- Sex: female sex is more likely to be associated with low birth weight (Weinberg and Wilcox, 1998)

2. Maternal and placental factors

- Maternal race (black) (Hobel, 1998)
- Maternal age: the frequency of birth of less than 2500g is greatest for mothers aged under 18 years, is least for mothers 25-29 years and rises again in mothers over 30 years (Lemasters, 1998)
- Constitutionally small mothers: mothers under 146 cm have around three times the risk of a low-birth-weight baby compared with mothers of 161 cm or more (Baker, 1991)
- Parity (none or more than four); birth weight increases with parity but the greatest increase takes place between parity 1 and 2 (Walsh *et al*, 1993)
- Poor reproductive history: adverse pregnancy history or foetal loss, preterm delivery, prior low birth weight infant, length of gestation, short interval between pregnancies (less than a year), multiple spontaneous abortions (Hobel, 1998; Lemasters, 1998; Lemasters[1], 1998)
- Poor maternal nutritional status at conception and poor maternal weight gain in pregnancy (Onyeije and Divon, 2000; Shetty, 2002)
- Maternal medical conditions (e.g., anaemia, cyanotic heart disease, viral pneumonia, respiratory insufficiency, liver disease, diabetes mellitus, chronic renal disease, anaemia, placental abnormalities, pre-eclampsia, eclampsia, chronic hypertension, hyperemesis, uterine constraints, antiphospholipid syndrome) (Baker, 1991)
- Social and environmental factors: malnutrition, low income/poor education, smoking, alcohol consumption, drug use, anticonvulsivants, high altitude (Baker, 1991; Lemasters, 1998; Onyeije and Divon, 2000)
- Occupational exposures: noise, occupational stress, shift work, physical effort, standing, organic solvents, polychlorinated biphenyls (PCBs), carbon monoxide, arsenic, mercury (Chen *et al*, 2000; Gold and Tomich, 1994; Ha *et al*, 2002; Osorio and Windham, 1997)

3. Paternal factors: paternal height, race (Walsh *et al*, 1993)

Data source on low birth weight

Birth weight is easily measured and routinely recorded for nearly every newborn and it can be found in vital statistics and hospital registries.

Data on birth weight can be also obtained interviewing the mother, using a questionnaire.

Animal research on low birth weight in relation with exposure to ethylene oxide

In the study assessing the reproductive-toxicological effects of ethylene oxide, propylene oxide, butylenes oxide and styrene oxide Hardin *et al* (1983) found reduced foetal body-weight and crown-rump length in all ethylene oxide-exposed rats (see *1.3.4. Spontaneous Abortion*).

Groups of 22 pregnant rats were exposed to 10, 33, or 100 ppm ethylene oxide for 6 hours per day on days 6-15 of gestation; two control groups were exposed to air only. Foetuses were delivered for examination on day 20. The only effect observed was a small but significant reduction in foetal weight at the highest dose (Snellings *et al*, 1982).

Human research on low birth weight in relation with exposure to ethylene oxide

In the reviewed medical literature no studies assessing the association between occupational exposure to ethylene oxide in pregnant women and low birth weight were found.

1.3.7. Summary

Experimental studies showed that exposure to ethylene oxide is associated with spontaneous abortion, foetal death and decreased foetal weight in animals.

Two human studies carried out to date also suggested an association between exposure to ethylene oxide during pregnancy and an increased frequency of spontaneous abortion. No human studies were conducted to investigate the relationship between exposure to ethylene oxide and stillbirth or low birth weight.

Therefore, the current scientific evidence concerning the association between exposure to ethylene oxide and adverse reproductive outcomes in humans is limited and further research is needed for a deeper understanding of the effects of ethylene oxide on human reproductive health.

1.4. ISSUES RELATED TO REPRODUCTIVE EPIDEMIOLOGY

Reproductive outcomes may act as sentinels for detecting occupational and environmental hazards (Osorio and Windham, 1997). Reproductive effects have a relatively short latency between exposure and clinical health event as compared to the long latency for cancer. If

workers or community residents are protected from exposures that are harmful to reproduction or the foetus, they may be protected from other health effects associated with these exposures as well. Although the extent to which workplace and environmental hazards affect reproductive function is unknown, these hazards are potentially preventable.

Reproductive failure associated with environmental exposures may result from exposure to toxicants either pre- or post-conception. The following markers can be used to monitor reproductive failure: sexual dysfunction (libido, potency); sperm abnormalities (number, motility, shape); decreased fertility; illness during pregnancy; abortions; stillbirths; infant deaths; birth defects (major, minor, mutations); chromosomal abnormalities; decreased birth weight; premature or postmature births; altered sex ratio; childhood morbidity and developmental disabilities; childhood malignancies; and age at menopause (Kurzel, 1992).

When carrying out epidemiologic studies investigating reproductive effects of environmental and occupational exposures, the researcher must take into account certain special considerations (Kurzel, 1992):

- Reproductive outcome is related to exposure of the father as well as the mother and therefore possible paternal risk factors need to be taken into account.
- Some reproductive impairments and losses are difficult to measure and record. For example, early spontaneous abortions may go unnoticed, and those occurring during the first trimester are not routinely recorded.
- The control of confounding variables: they are nearly all related to maternal exposure and characteristics, although male characteristics (e.g., paternal age, occupational exposures) need also to be taken into account.

1.4.1. Study Design in Reproductive Epidemiology

Cross-sectional, case-control and cohort studies can all be used in reproductive epidemiology. The discussion that follows will focus only on particularities of these study designs when they are used to assess the association between exposure and reproductive outcomes.

- *Cross-sectional studies*: retrospective questionnaire-based surveys of occupationally defined populations have an important place in reproductive epidemiology (Joffe, 1989). A retrospective survey design enables a large population to be studied quickly: the majority of workers (or their partners) have had at least one pregnancy or attempt at conception, and frequently two or more. Another advantage of this study design is that it is based on a defined population, comparable to a cohort study as long as all ex-workers for the study period are included. This population is truncated, in the sense that younger

workers have yet to complete their reproductive experience, but for older workers it is a total enumeration. However, if ex-workers are not included, selection effects may be present, especially in the case of female workers. In this situation, the potential selection bias is due to the fact that the population existing in the workplace at the time of the study may not be representative of the workforce during the time of previous exposure. For example, women who have a live birth may leave the workforce to care for their children, whereas women experiencing spontaneous abortions may continue to work and are at greater risk for subsequent abortions. On the other hand, women who experience adverse outcomes that they associate with a workplace exposure may change jobs and therefore they will not be enrolled in a study that involves women working in that particular working place at the time of the study (Joffe, 1989; Osorio and Windham, 1997).

- *Case-control studies*: they are most appropriate to evaluate relatively rare diseases in large populations (e.g., birth defects or childhood cancers). Because the outcome of interest is specified at the onset, the continuum of reproductive effects that may result from a given exposure cannot be evaluated (Osorio and Windham, 1997). In case-control studies used in reproductive epidemiology there are also issues related to definition of a case and to choice of a sampling strategy (Weinberg and Wilcox, 1998). For example, if one compares hospitalised cases of spontaneous abortion with hospitalised live births, there is a potential for case self-selection, because many women with spontaneous abortion are not hospitalised, whereas most women delivering an infant are. The biological entity used in a case-control study is a second issue: if the unit of the study is the pregnancy, then usual methods can be applied to evaluate the influence of particular exposures experienced early in that pregnancy. If the unit of the study is the woman (or the couple), then case definition should take the whole reproductive history into account, rather than the outcome of a single pregnancy. Third, there is a possible bias related to the gestational age at which pregnancy is recognised. For example, women with a history of spontaneous abortion or who have been trying to conceive for some time tend to recognise their pregnancy earlier and they will appear to have a higher rate of spontaneous abortion than the other women.
- *Cohort studies*: this is the most preferred study design for most reproductive outcomes (Osorio and Windham, 1997). A prospective cohort study allows specific measures of an exposure and potential confounders to be ascertained at the etiologically relevant time periods and also allows exposures to be evaluated before the outcome, thus avoiding potential for differential recall. The major limitation of this study design is the time and

resources required for completion of the study and attrition of the exposed group that is being followed. An alternative retrospective type of study may be used that ascertains the outcome of the most recent pregnancy and relates this outcome to exposures at that time. This is a particularly convenient approach for studies of a rare exposure or a small fixed population, such as an occupational cohort, where few women will be currently pregnant and available for a prospective cohort study. A problem with this design arises because a wanted pregnancy ending in loss is likely to be replaced by other pregnancies until a viable one is conceived. This replacement phenomenon produces a lowered overall rate of spontaneous abortion among most recent pregnancies even with perfect recall (Kurzel, 1992; Weinberg and Wilcox, 1998).

1.4.2. Assessing Exposure in Reproductive Epidemiology

1.4.2.1. Collecting Information on Exposure

As most of the studies on reproductive health in relation to occupational exposures have a retrospective design, exposure is seldom measured directly at the time the research is carried out. Exposure information is usually gathered at the time of the study, using one of the following methods:

- Estimation of past exposure (exposure at the time of the reproductive outcome of interest) is made on the basis of existing exposure data. Statistical models have been developed for this purpose (Greife *et al*, 1988; Hornung *et al*, 1994), but their main limitation is that the researcher needs a large quantity of data on exposure levels for the period between the time the reproductive outcome took place and the time the research is carried out.
- Levels of exposure are assigned to the workers included in the research by the study investigators, based on their subjective judgements concerning levels in past years and the probable effect of changes in operating conditions (Hornung *et al*, 1994).
- Past exposure is estimated by industrial hygienists familiar with current and past exposure at the study site (Hornung *et al*, 1994).
- Biological monitoring data are used: when effects of ethylene oxide on human health are studied, the biological markers that can be used are the level of ethylene oxide-haemoglobin adducts and cytogenetical changes (chromosome aberrations, SCE, micronuclei). The limitation of the former marker category is that the life span of erythrocytes (and hence of the ethylene oxide-haemoglobin adducts) is 120 days and therefore it can only be used to asses recent exposure (4 months). The latter marker category can be used to assess exposure to ethylene oxide for longer periods of time

(years). The main advantage of using biological markers to assess exposure is that they can provide quantitative data on an internal or biological effective dose of toxicants and they are independent of workers' own reporting of exposure (Lindbohm, 1999).

- Information on exposure at the time of the pregnancy (or of the reproductive outcome) studied is gathered from the women at the time of the study using questionnaires or interviews. This was the technique used by Rowland *et al* (1996) when they investigated the association between exposure to ethylene oxide and spontaneous abortion, pre-term and postterm birth in dental nurses in the USA.
- Information on exposure at the time of the pregnancy (or the reproductive outcome) studied is gathered from the workplace supervisor or from industrial hygiene records. Hemminki *et al* (1982) used supervisor-provided data on exposure when they carried out the study on the relationship between exposure to ethylene oxide and spontaneous abortion in female sterilising staff in Finland.

1.4.2.2. Validity of Questionnaire-Collected Exposure Information

Information bias due to inaccurate assessment of exposure or outcome is one of the most common weaknesses of many reproductive study designs (Hemminki *et al*, 1995; Lindbohm, 1999).

When interviews or self-administered questionnaires are used to collect exposure data, they have the advantage of providing more detailed information on occupation, specific work tasks and exposures than registers or company records. Usually, workers give valid information about their jobs, but they may often be unaware of specific exposures (Hemminki *et al*, 1995). On the other hand, under-reporting of exposure is a common problem in studies using self-reported data. Reporting varies by agent: specific chemical agents are more likely to be under-reported than more general agents. It has been observed that under-reporting of exposure is even a greater problem than over-reporting in studies on reproductive heath. Under-reporting leads to exposure misclassification (when exposed persons are classified as unexposed or vice versa), resulting in biased association (Hemminki *et al*, 1995; Lindbohm, 1999).

When the diseased and non-diseased persons are equally likely to be misclassified according to exposure, then the misclassification is said to be non-differential. Non-differential misclassification will bias the effect estimate of the study towards the null value, masking a possible effect of the exposure on the outcome. When the likelihood of misclassification of exposure is different amongst diseased and non-diseased persons, the misclassification is said

to be differential. Differential misclassification can bias the effect estimate either towards or away from the null value (Checkoway, 1989).

Misclassification of exposure also affects the power of a study: at the same prevalence of exposure and under the assumption that sensitivity and specificity of the method used for exposure classification are equal, the power decreases when the proportion of non-differential misclassification increases. This effect is greater when the prevalence of exposure is lower (Hemminki *et al*, 1995).

In a study assessing the validity of exposure data collected by the means of a questionnaire, Joffe (1992) compared this information with data derived from the management in five factories in England. The subjects enrolled in the study (men and women) were asked to report exposure to eight chemical agents used at their workplace. Values of sensitivity above 50% were obtained for chemicals that had a description of a more general nature; the higher the level of generality, the higher the sensitivity (fewer false negative reports, i.e., less under-reporting of exposure) and the lower the specificity (more false positives, i.e., more over-reporting of exposure). The author also investigated whether respondents who had reported a miscarriage or a phase of subfertility tended to over-report exposure in an attempt to explain their reproductive mishap or as a part of a general tendency to report more adverse experiences. The findings of the study showed that false positive reports (over-reporting of exposure) were no more common in these groups than in the population as a whole.

1.4.3. Assessing Outcome in Reproductive Epidemiology

1.4.3.1. Collecting Information on Outcome

Unless a prospective study is carried out that allows the information on the reproductive outcome to be collected at the time the event of interest occurs, data on reproductive outcomes are gathered using one of the following approaches:

- Information on the reproductive outcome is gathered from the women at the time of the study using questionnaires or interviews. This method is open to information bias: recall bias (a woman who had a spontaneous abortion might be more likely to remember exposure to ethylene oxide while pregnant than a woman whose pregnancy ended with a normal birth) and reporting bias (a woman who had a pregnancy loss might be reluctant to admit drinking or smoking heavily during her pregnancy, at the same time trying to emphasise the role of occupational exposure to ethylene oxide in order to show that the adverse reproductive outcome is not her fault, but rather the effect of the workplace-related exposure) (Gordis, 2000; Last, 2001).

- Information on the reproductive outcome is gathered from the existing medical records (individual medical records or nationwide registries, e.g., hospital discharge registries, medical birth registries). The advantage of medical records is that the information is recorded at the time of the event and it is usually based on medical diagnosis (Hemminki *et al*, 1995). The main limitation of this approach is that medical records are not complete. For example, if spontaneous abortions are investigated medical records will provide information only on those spontaneous abortions occurring to women who sought medical care (not all women experiencing a miscarriage seek medical care; this depends on the woman's level of education, her economic status and her accessibility to a medical facility).

1.4.3.2. Validity of Questionnaire-Collected Outcome Information

In epidemiological research, information obtained by interviewing and questioning is often referred to as "soft" data, as opposed to the presumably "hard" data derived from observations and documentary sources. Factors that influence accuracy of the these questionnaire-reported data are the importance of the event to the individual, the way the trait of interest is defined, the time frame in which the event occurs, the way a question is asked (i.e., a list, open-ended, or a closed question) and the individual's knowledge about the exposure. This is why the subjects' responses to an interview should be validated by comparison with other records (Abramson and Abramson, 1999; Lilienfeld and Stoley, 1994; Olson *et al*, 1997).

According to Abramson and Abramson (1999), the best and most obvious way to appraise validity of a measurement is using *criterion validity*, that is, to find a criterion (a reference standard or, in epidemiological jargon, "a gold standard") that is known or believed to be close to the truth and to compare the results of the measure with this criterion. The ideal criterion is the "true value" of the attribute that is being measured. As this is seldom possible, the best criterion is a measure that has higher face validity than the measure being tested, or that has been tested previously and found to be of high criterion validity.

The criterion validity of a measure can be expressed depending on the scale the variable outcome is measured (Abramson and Abramson, 2001; Bradford Hill and Hill, 1991; Kirkwood and Sterne, 2003; Lumey *et al*, 1994; MacLure and Willett, 1987; Streiner and Norman, 1995):

1) When a scale with a *dichotomous outcome* (e.g., "yes" or "no") is being evaluated, indices of sensitivity, specificity and predictive value of the "test" (method of measurement) can be used. Sensitivity and specificity are characteristics of the test and their values do not depend

on the prevalence of the disease in the population. The values of the positive and negative predictive values depend on the prevalence of the disease in the population, as well as on the sensitivity and specificity of the procedure used.

Using sensitivity, specificity, positive and negative predictive values to evaluate a method of measurement on a dichotomous scale

- If the goal is to report the validity of a method that is hoped to be widely generalizable and have utility in diverse populations among whom the prevalence of the phenomenon varies markedly, then sensitivity and specificity have the advantage of being invariant with changes in prevalence.
- If the prevalence is relatively invariant among the populations in which the investigators hope the method will have utility, then the predictive values positive and negative obtained from the validation study are fairly generalizable. The predictive values are more intelligible and practically usable measures of validity than sensitivity and specificity because by incorporating information on both accuracy and prevalence, they describe the performance of the method in an actual population.

2) When a scale with *ordinal or continuous outcome* (e.g., birth weight) is being evaluated, the following methods can be used:

- The correlation between the observed and true measurements (expressed as the correlation coefficient). When the two variables being compared are normally distributed, the product-moment correlation coefficient is used. When the two variables are not normally distributed, rank correlation coefficients are used (Spearman and Kendal).
- The size of the discrepancies between the observed and true values: discordant birth weight (defined as a difference of at least 250 grams between mother-reported birth weight, i.e., the observed value and the recorded birth weight, i.e., the true value) and the difference between the mean values (assessed using either *t* tests or distribution-free tests, e.g., Wilcoxon signed rank tests, Wilcoxon rank sum test).

Using correlation, discrepancy between the observed and true values and the difference between means to evaluate a method of measurement on a continuous scale

- If the goal is to report the validity of an exposure measurement method in the hope that it will be widely used amongst populations with diverse exposure distributions,

then it is useful to report the mean and the standard deviation of the difference between the surrogate measurement being assessed and the valid reference value. These measurements are independent of the exposure distribution in the study population (if the measurement error is constant across the range of true values);

- If the goal is to report the accuracy of an exposure assessment method as concisely and intelligibly as possible, the correlation coefficient is preferable.

1.4.3.3. Assessing the Validity of Questionnaire-Collected Information on Reproductive Outcomes: Research Review

Wilcox and Horney (1984) used prospectively collected records on spontaneous abortions to validate reproductive histories obtained up to 40 years later. Prospective data were spontaneous abortions recorded by 348 women enrolled in the Menstrual and Reproductive Health Study, conducted in the USA between 1935 and 1980. All the women enrolled in this study were given at the beginning at each year a calendar card on which they were asked to make note of their menses as they occurred. They were instructed to record on the back of the card any reasons for disruption of menses, including pregnancy. The 348 women had recorded a total of 507 spontaneous abortions. Retrospective data were obtained using questionnaires administered to these 348 women at the time of the present study. The women were asked to recall their pregnancy history. They recalled a total of 382 spontaneous abortions. Overall, the women participating in the study recalled 75% of their recorded spontaneous abortions (382 out of 507). The major determinant of spontaneous abortions recall was the length of pregnancy at the time of abortion. Completeness of recall was also affected by the length of time that had elapsed since the abortion. Completeness of recall was unrelated to the total number of pregnancies, total number of spontaneous abortions, pregnancy order of the abortion, or mother's age at the time of abortion. The date of abortion was recalled with a modest degree of accuracy: a recalled date within one year of the correct date was given for nearly three fourths of these abortions. Gestational age was accurately recorded in 83% of the correctly recalled abortions. Since one quarter of the total number of recorded spontaneous abortions was not recalled, the authors suggested that the presence of "recall bias" could be a problem in retrospective studies of environmental hazards and abortion risk. In the authors' opinion, women who are concerned about exposure to possible toxins may be prompted to recall a larger proportion of their originally recognised abortions. This type of bias should be considered as a possible influence in situations in which women are alarmed about tier exposure to possible reproductive toxins.

Tilley *et al* (1985) assessed the completeness of medical records and the precision of subject recall using data from the Diethylstilbestrol-Adenosis Project, a study of daughters exposed in utero to diethylstilbestrol (DES) and a comparison group. The researchers gave mothers of the study participant's questionnaires regarding their obstetric histories and also obtained their prenatal records. This permitted the investigators to assess the limitations of a mother's recall and of the medical record characterising a woman's pregnancy history and to determine if the differences in recall were associated with exposure status. The authors also compared data from mothers of DES-exposed daughters who initiated their own enrolment in the study (walk-in and referrals). To obtain prenatal records from these women, physicians were contacted. They would usually supply drug exposure data but not the other obstetric history requested. The analysis of agreement between questionnaire-collected information and medical records was restricted to women with complete information from their records and questionnaires. Analysis of agreement employed kappa statistics, which adjusts for chance agreement. The authors could not evaluate sensitivity and specificity because it was not possible to determine whether the record or the questionnaire was correct. The authors could not interpret the results for agreement on threatened miscarriage, hospitalisation, trunk x-rays and hormones in terms of validity. If one of these events was reported in the record, it was assumed that the record was correct. Thus, a comparison of a mother's negative response with a positive response in the record was meaningful. However, a mother's positive response could not necessarily be classified as incorrect when there was lack of documentation in the record because it is possible that events may not always be recorded. In general, there was good to excellent agreement when mothers' recall of personal history (past miscarriage, past pregnancy) was compared with their medical records. The kappas ranged from 0.67 to 0.89 and differed between the walk-in, referral and unexposed groups, indicating that agreement was better in the unexposed group than in the walk-in and referral group. However, for medical intervention such as drugs and x-rays, agreement was poor, with kappa values ranging from 0.11 to 0.54 (for trunk x-rays) and from 0.08 to 0.51 (for drug exposure). Statistically significant differences in agreement were rarely detected between the exposed and unexposed group. Generally, when differences in agreement did exist, they were usually between the mothers of the walk-in and referral group and the mothers of unexposed record reviews, the two groups most different in their motivation to participate in the study. Although overall agreement for birth weight was good to excellent, there was a tendency for mothers to report a larger number of high and low birth weight babies than recorded in the chart. This finding suggests the need for documentation of birth weight beyond mothers'

recall when carrying out studies in which the determination of high and low birth weight is essential.

Harlow and Linet (1989) conducted a literature review to identify studies comparing data obtained from questionnaires with information derived from medical records. The majority of studies identified examined one of three types of "exposure": chronic illness or medical care-related events, medication use and female reproductive history. Of the six papers evaluating recall of reproductive events, two address menstrual history, three address pregnancy history and two address childbirth. Pregnancy history, including number of children, pregnancies and miscarriages was recalled with great accuracy over extended periods of time.

In a letter to the editor referring to this review, Velema and Blettner (1990) discussed studies performed among working women in Scandinavian countries where the occurrence of spontaneous abortions was treated as an "outcome" and not as an "exposure". These studies found both a higher and a lower number of reported spontaneous abortions amongst exposed women compared to the unexposed ones. In their letter, Velema and Blettner raise the possibility of a differential recall of reproductive outcomes between subjects selected according to their exposure status. In their view, this selective recall might be due either to the difference in educational level between exposed and unexposed subjects or to the fact that the exposed women are aware of their exposure or they are concerned about the possibility of exposure or any health hazard that might ensue. The authors considered this differential recall of outcomes similar to the differential recall of exposure in case-control studies.

In a response to the review described by Harlow and Linet and to the comments of Velema and Blettner, Hertz-Piciotto (1991) pointed out differences in the medical records themselves as another possible explanation for the differential recall of reproductive outcomes. First, underreporting of spontaneous abortions in the medical records is substantial, since not all spontaneous abortions are diagnosed medically. Second, the underreporting of pregnancy loss in medical records is likely to be differential because 1) the probability of diagnosis is related to how early in pregnancy a woman seeks prenatal care and 2) the seeking of medical care is highly variable and differs by cultural, social, economic and demographic factors. In the author's opinion, while failure to report a medically confirmed spontaneous abortion is clear evidence of inaccurate recall, the lack of medical confirmation for a reported spontaneous abortion is not meaningful. The authors concluded that "medical records are a biased 'standard' and provide a misleading picture of the accuracy of self-reports of spontaneous abortion".

Olson *et al* (1997) assessed the validity and reliability of maternally reported pregnancy and birth characteristics, influence of varying recall periods and differential misclassification using data from a case-control study on infant leukaemia. Cases were gathered from the Children's Cancer Group, a cooperative clinical trials group with members in USA and Canada that sees approximately 53% of all childhood cancers in the USA diagnosed at less than 5 years of age. Controls were randomly selected and individually matched with the cases on date of birth and telephone area code and exchange. Pregnancy-related data were collected from the mothers (302 cases and 558 controls) using telephone interviews. Medical records were obtained for 287 cases and 467 controls. Time between delivery and interview ranged from 0 to 8 years. The medical records served as the "gold standard" for the following variables: birth weight, gestational age, delivery variables, certain pregnancy complications and medical care variables. These variables were tested for both reliability and validity. For other variables, such as reproductive history, the medical records could not serve as "gold standard", but only as another source of information. This is why in this case only reliability was assessed. High correlations were found between maternally reported information and the information recorded in the medical chart for birth weight ($r = 0.98$) and gestational age ($r = 0.84$). Problems after delivery and pregnancy complications had low reliability and validity. Time between delivery and interview did not influence validity and reliability of variable such as birth weight, gestational age, reproductive history, medial procedures and post-delivery complications. However, time did affect recall of some pregnancy-related conditions such as anaemia, high blood pressure and toxaemia.

Tomeo *et al* (1999) assessed the reproducibility and validity of a questionnaire that asked mothers to recall pregnancy-related events from thirty or more years ago. The reproducibility study enrolled 146 women that completed the same questionnaire twice, 2 years apart. They were on average 78 years old at the time of the second questionnaire and they had to recall events that had occurred on average 53 years in the past. Mothers' responses were highly reproducible for the following factors: pre-pregnancy height and weight, history of miscarriage and abortion, pregnancy complications, cigarette and alcohol use, coffee intake, preterm delivery, birth weight and breastfeeding history. The validity study enrolled 154 women whose answers to the questionnaire were compared to NCPP (the National Collaborative Prenatal Project) data. They were on average 57 years old at the time of the questionnaire completion and they had to recall events that had occurred on average 32 years in the past. Maternal reports were highly correlated with NCPP records for the following factors: height, pre-pregnancy weight, smoking during pregnancy and birth weight. Maternal

recall and NCPP data were discordant for pregnancy complications and initiation of prenatal care. The study found that mothers were consistent, if not always accurate, in their long-term recall of pregnancy-related events. Mothers may recall most accurately information that they previously reported or have been at least told (e.g., mothers most likely provided information on birth weight if they received a birth certificate on which birth weight was recorded). It was also suggested that maternal recall does not deteriorate substantially over time. Pregnancy appeared to be a salient enough event that maternal recall of many, but not all, factors was both reproducible and reasonably accurate thirty or more years after delivery.

Ellison *et al* (2000) assessed the reliability and validity of self-reported reproductive history and obstetric morbidity amongst mothers who delivered children at Baragwanath Hospital (now Chris Hani-Baragwanath Hospital) in Soweto. The authors compared self-reported obstetric morbidity, collected during interviews with the mothers of children enrolled in a longitudinal birth cohort study (Birth to Ten study), with self-reported data and clinical measurements recorded in their obstetric notes. The Birth to Ten study enrolled all the singleton births to mothers resident in Soweto-Johannesburg metropolis during a 7-week period in 1990. The authors identified obstetric notes for 517 Birth to Ten mothers, which comprised the sample subsequently analysed. Antenatal record charts were identified for 313 mothers out of 517 and neonatal record charts were identified for 200 mothers out of 517. Antenatal record charts provided self-reports of: maternal age, gravidity and previous obstetric problems including miscarriages, terminations, premature- and stillbirths. They also provided clinical measurements of blood pressure, proteinuria and glycosuria. Seropositivity for sexually-transmitted disease (STD) was recorded in the neonatal record charts of any child that had been admitted for postnatal care. These clinical data provided objective measures of five clinical disorders (i.e., pre-existing (essential) hypertension, pregnancy-induced hypertension, pre-eclampsia, glycosuria and seropositivity for sexually-transmitted infections) and the putative "gold standard" against which the validity of self-reported obstetric morbidity could be assessed. Self-reports of maternal age, reproductive history and obstetric morbidity were also obtained at enrolment into the Birth to Ten study, using detailed interviews conducted during antenatal care or shortly after delivery. Only 255 out of the 517 women examined in the current study were interviewed about their obstetric morbidity. Reliability of self-reported reproductive histories was assessed comparing the self-reports of maternal age, gravidity and prior reproductive outcomes recorded in the antenatal record cards to those collected during the Birth to Ten interviews. The reliability of self-reported age and gravidity was high (reliability coefficient R=0.810-0.993), while self-reports of previous

miscarriages, terminations, premature- and still-births were only fairly reliable (Kappa=0.48-0.50). Validity of self-reported obstetric morbidity was assessed comparing hospital-recorded diagnoses for each of the five clinical conditions to the self-reports of obstetric morbidity collected during the Birth to Ten interviews. Self-reported diabetes and high blood pressure had specificities of more than 95% for glycosuria, hypertension and pre-eclampsia. The specificity of self-reported oedema for hypertensive disorders and the specificity of self-reported urinary tract infection for STD seropositivity were only around 65%. The authors concluded that "the modest reliability and limited validity of self-reported obstetric morbidity undermines the clinical utility of this information". At the same time, they suggested that validity of self-reported obstetric morbidity could be improved through better communication between antenatal clinicians and the mothers in their care and by ensuring greater access to education and health information for women (e.g., providing mothers with their own medical records).

In epidemiological studies on reproductive outcomes in relation to exposure, researchers often employ questionnaire-collected information both on exposure and the outcome of interest. While this represents a convenient approach for gathering information in a reproductive study, one should bear in mind the following problems that may arise with this method:

- The possibility of recall bias: women might recall differently exposures during pregnancy if the outcome of their pregnancy was a failure (pregnancy loss) or if she was concerned about toxic exposures at the workplace.
- The possibility of "faulty recall" (Kurzel, 1992): as the period of time between the moment the study is carried out and the pregnancy investigated increases, a woman may not recall all the details of her pregnancy.
- Birth weight: when studies in which the determination of high and low birth weight is essential are carried out, there is a need for documentation of birth weight beyond mothers' recall.
- The "gold standard" for the outcome diagnosis: despite being usually employed as the "gold standard" in assessing the validity of questionnaire-collected information, medical records are not always such a 'standard'. For example, there is an under-recording of pregnancy loss in the medical records and this under-recording is likely to be influenced by two factors: 1) the diagnosis is related to how early in pregnancy a woman seeks prenatal care and 2) the seeking of prenatal care is variable and differs by socio-economic, cultural and demographic factors.

- Communication between the pregnant woman and her health care provider: if there is a good communication between the pregnant woman and her health care provider (e.g., if the woman is given the details on her new born) she is more likely to remember accurately this information, even several years after her pregnancy.

1.4.4. Missing Data in Reproductive Epidemiology

When some subject records in a database are incomplete, the following methods can be used in analysis (de Vaus, 1993; Greenland and Rothman, 1998):

- Deleting records: the incomplete records are deleted from any analysis that involves variables for which the records have missing values. This is a common approach to handle missing data and it is called complete-subject analysis or listwise deletion of missing data.
- Deleting variables: if a particular variable is responsible for a large number of missing data, the variable can be dropped from the analysis.
- Predicting (imputing) and filling in the missing values based on the missing data pattern (the pattern of missing values seen among all records). Examples of such methods are: sample mean approach, when the missing variable is replaced with the value of the mean for the sample; group mean approach, when the sample is first divided into groups on a background variable (e.g., ethnicity, gender, education) and then the mean for the missing data is obtained within each category of the selected background variable; and random assignment within groups, when the sample is first divided into groups on a background variable (e.g., ethnicity, gender, education) and then the missing value is replaced with the value of the same variable of the nearest preceding case.
- Using methods that directly analyse only the complete records but assign special weights to those records based on the missing-data patterns.

1.4.5. Confounding in Reproductive Epidemiology

The strength of confounding in reproductive epidemiology seems to be greater than in any other areas of epidemiology (Hemminki[1] *et al*, 1983). Confounding variables that need to be controlled for in reproductive epidemiology include both maternal and paternal characteristics (Kurzel, 1992):

- Maternal characteristics: maternal age, parity and previous successful pregnancies, exposure to drugs (smoking, alcohol consumption, pharmaceuticals), contraceptive use, surgical procedures (tubal ligation), geographic and social factors, genetic factors,

infectious disease (herpes simplex, rubella, cytomegalic virus, syphilis, toxoplasmosis) and concurrent illness, metabolic disease (diabetes mellitus), nutritional deficiency.

- Paternal characteristics: paternal age, surgical procedures (vasectomy), occupational exposures.

When studying pregnancy loss as an outcome, the approach to the history of spontaneous abortion as a potential confounder deserves a special discussion (Weinberg, 1993). History of spontaneous abortion is known to be related to risk, even amongst unexposed, and, under the study hypothesis, could also be related to exposure, if the exposure tends to have been long term. At the same time, history of spontaneous abortion is almost certainly not on the causal pathway between exposure and loss of the current pregnancy. Such a history is rather viewed as a "marker for elevated risk", and this elevated risk may be due in part to effects of the same exposure on past pregnancies. History of spontaneous abortion does satisfy the criteria for "confounding": it presumably serves as a marker for causally related factors, and it may also be associated (under the study hypothesis) with the exposure under study. But history of pregnancy loss is in fact a "correlated factor", which is caused in part by the exposure and it is also correlated to the outcome under study and it should not be adjusted for in the estimation of the exposure-associated risk. Adjusting for such a "correlated factor" during analysis can bias the effect estimate of the study.

In reproductive epidemiology, confounders are selected using the same statistical approaches employed in any epidemiological study:

- Measuring the overall association (using either odds ratios or relative risks) between exposure and disease and the change in this value after control for a variable. To select confounders the analyst must choose a cut-off for what constitutes an important change in the estimate. The exact cut-off for importance is somewhat arbitrary but limited in range by the subject matter and the most important point is that one should report the criterion used to select confounders for adjustment (Checkoway *et al*, 1989; Greenland and Rothman, 1998; Lilienfeld and Stolley, 1994).
- Using statistical tests of confounder-disease association or of the difference between the unadjusted and adjusted estimates (collapsibility testing). These testing approaches will perform adequately if the tests have high enough power to detect any important confounder effects. One way of insuring adequate power is to raise the alpha-level for

rejecting the null (of no confounding) to 0.20 or more, instead of using the traditional 0.05 level (Greenland and Rothman, 1998).

- When more than one potential confounder must be examined, two strategies have been proposed: backward deletion and forward selection (Greenland and Rothman, 1998). In backward deletion, one adjust for all potential confounders and then one deletes the confounders from adjustment one by one in a step-wise fashion, at each step deleting that confounder that makes the smallest change in the exposure effect estimate upon deletion. One stops deleting confounders when the total change in the estimate and confidence limits accrued from the start of the process would exceed the chosen limit of importance. In forward selection, one starts with the exposure effect estimate from the simplest acceptable stratification, then stratifies by the confounder that makes the most difference in the estimate, then adds confounders one-by-one to the stratification, at each step adding the confounder that makes the most difference amongst those not yet added. The process stops when addition of variables ceases to make an "important" difference.

One then controls for the selected confounders stratifying the available data by the levels of confounders and calculating the Mantel-Haenszel estimate of the summary odds ratio for the exposure-outcome association (Kirkwood and Sterne, 2003).

1.4.6. Effect Modification in Reproductive Epidemiology

The effect of an exposure on reproductive health might be increased or decreased in the presence of a modifying factor. For example, an excess of abortions might be found to have occurred among the exposed smokers, even when the exposed and unexposed groups have identical smoking frequencies. Then this is an inherent biological phenomenon that might be important to consider in preventive efforts, and decrease of smoking would then be adjuvant to decreasing the specific hazardous exposure (Hemminki[1] *et al*, 1983).

Testing for effect modification can be done applying the χ^2 test for heterogeneity (Kirkwood and Sterne, 2003). Under the null hypothesis of no effect modification, all the individual stratum odds ratios would equal the overall summary odds ratio (Mantel-Haenszel). The greater the differences between the stratum-specific odds ratios and OR (MH), the larger will be heterogeneity statistic and the smaller the p-value. The test however has low power and it is unlikely to yield evidence for effect modification unless there are large differences between strata. A large p-value does not therefore establish the absence of effect modification. In fact, as true odds ratios are never likely to be exactly the same in each stratum, effect modification

is always present to some degree. Most researchers would accept, however, that minor effect modification should be ignored in order to simplify the presentation of data (Kirkwood and Sterne, 2003).

1.4.7. Summary

While general epidemiological issues also apply to reproductive epidemiology, there are a few topics that are more specific to this field.

Most studies on reproductive health use a retrospective approach, collecting data on both exposure and outcome by the means of a self-administered questionnaire or an interview. With this approach, the possibility of information bias (recall bias, both for exposure and outcome), which in turn can lead to misclassification of exposure or outcome, is an important point to take into account. Faulty recall, depending on the length of time between either the reproductive outcome studied or the pregnancy and the time the study is carried out, is also important, particularly when low birth weight is assessed.

This is why, when possible, it is important to attempt to validate the classification of exposure and outcome by comparing interview results with other data sources (e.g., employer records or medical records, respectively), in order to assess the potential magnitude of bias due to misclassification of exposure or outcome. As a certain amount of information bias is unavoidable in epidemiological research, it is usually desirable to ensure that is non-differential, as the effect estimate is then biased in a known direction, i.e., towards the null value (Checkoway, 1989).

Methods of assessing and dealing with confounders and effect modifiers in reproductive epidemiology are the same as in any epidemiological field, but there is one noteworthy issue: as reproductive failure depends on both partners and not only on the characteristics of the mother, both maternal and paternal risk factors have to be taken into account.

1.5. RATIONALE FOR RESEARCH

Current available data describe ethylene oxide as a chemical used on a large scale, despite a long list of adverse health effects documented in workers occupationally exposed to it. Although recognised as a reproductive hazard, only two studies published so far suggested that it might be associated with adverse reproductive outcomes in pregnant women exposed to it (spontaneous abortion, pre-term and postterm delivery). No assessment of ethylene oxide

use and its associated health effects has been yet done in South Africa, and it seems, no assessment of occupation-related adverse reproductive effects in working women has been yet the subject of any research in this country.

Therefore the decision to carry out a study that would investigate the association between exposure to ethylene oxide during pregnancy and the occurrence of adverse reproductive effects in women occupationally exposed to this chemical.

The study had the following goal and objectives:

Study Goal

The goal of this study was to assess the association between adverse reproductive outcome and occupational exposure to ethylene oxide during pregnancy in women sterilising staff working in sterilising units using ethylene oxide in Gauteng province, South Africa.

Study Objectives

1. To describe the extent and nature of ethylene oxide use in sterilising units operational in medical facilities in Gauteng;
2. To assess the current exposure to ethylene oxide in sterilising units in Gauteng;
3. To collect information on the last recognised pregnancy using a questionnaire;
4. To assess the validity of the information on the evolution and outcome of the last recognised pregnancy collected by the means of the questionnaire;
5. To assess the association between occupational exposure to ethylene oxide during pregnancy and adverse reproductive outcomes.

2. METHODS

In this chapter the methods used for each of the five objectives of the research will be described.

2.1. DESCRIBING THE EXTENT AND NATURE OF ETHYLENE OXIDE USE IN STERILISING UNITS OPERATIONAL IN MEDICAL FACILITIES IN GAUTENG

A list of public and private medical facilities in the province was obtained from the Gauteng Department of Health. The list contained all the contact details for each of these medical facilities: name, address, telephone and fax numbers and manager's name.

- *Public Medical Facilities*

Together with the Gauteng Department of Health all the public hospitals in the province that had a sterilising unit were identified. A letter was then sent to all these hospitals, informing them about the research and requesting their participation in the study if they were using ethylene oxide to sterilise medical equipment.

- *Private Medical Facilities*

Letters were also sent to the three major private health care providers in South Africa (Medi-Clinic, Netcare and Afrox Healthcare) and to all the independent private medical facilities in the province. The letters explained the goal and objectives of the research and requested the participation in the study of the medical facilities using ethylene oxide to sterilise medical equipment.

Private medical facilities that had not responded to the first letter were sent a reminder letter. Those that had not answered to the second letter were sent a third and last request for participation in the research.

At the end of this step there were still non-respondent medical facilities and they were contacted telephonically to find out whether they were using ethylene oxide or not.

After all participating hospitals had answered, preliminary field visits were organised. Public hospitals were visited in the first six months of the year 2002 and private medical facilities during the period October 2002 and May 2003 (they were visited as they answered the request to participate in the study).

The team of investigators included the project supervisor, the project leader, three occupational hygienists and one laboratory technician.

A checklist (see Appendix 1) was designed to be used for field visits in order to gather the following information:

- Number of sterilising units in each medical facility
- Type of ethylene oxide steriliser used and number of ethylene oxide sterilisers of each type in use
- Total number of staff employed in the sterilising unit and total number of female workers in the sterilising unit
- Jobs description for the sterilising staff and daily tasks for each of these jobs
- Number of working shifts per 24 hours in the sterilising unit and number of loads sterilised during each working shift
- Type of protection measures in use
- Recorded levels of ethylene oxide in the sterilising unit
- Changes in sterilising equipment and/or technology in the last 10 years and dates when these changes were made

2.1.1. Sterilising Units Using Ethylene Oxide

During the field visits information was obtained on the total number of sterilising units using ethylene oxide in each medical facility visited.

2.1.2. Ethylene Oxide Sterilisers in Use

There are three types of ethylene oxide sterilisers that can be used in a sterilising unit: general-purpose steriliser using single-dose ethylene oxide cartridge, general-purpose steriliser using compressed-gas cylinder and steriliser using glass ampoule.

In each of the sterilising units visited information was collected on the type and the number of ethylene oxide sterilisers used and on the place were they were kept (e.g., in an enclosed area dedicated to ethylene oxide sterilisation or in the general sterilising area).

2.1.3. Staff Characterisation

The following information about the staff employed in these sterilising units was gathered: total number (men and women), percentage of women employees, jobs held and daily tasks performed during a working shift.

2.1.4. Work Practices

Information was collected on the number of working shifts in each sterilising unit participating in the study (day shift and/or night shift) and on the number of loads sterilised using ethylene oxide.

2.1.5. Protection Measures

Information was collected on protective measures (e.g., gloves, masks) used by the employees working with the ethylene oxide steriliser and also on the way the freshly-sterilised loads offloaded from the steriliser were transported to the storing place (e.g. use of trolleys).

2.1.6. Ethylene Oxide Monitoring

Information was gathered on a possible ethylene oxide monitoring programme. Where the sterilising unit had such a programme, details on the levels detected by the measurements were requested.

2.1.7. Changes in Sterilising Equipment and/or Technology

Information was requested on changes in equipment and/or technology that were made in the last 10 years. This question included type of change and date the change was made.

2.2. ASSESSING THE CURRENT EXPOSURE TO ETHYLENE OXIDE IN STERILISING UNITS IN GAUTENG

2.2.1. Sample Collection

Between May and August 2003, specialists from the Occupational Hygiene Unit of the National Institute for Occupational Health (NIOH) performed personal and static measurements of the ethylene oxide levels in the sterilising units of all public medical facilities participating in the study.

The samples were collected over a period of 5 days for each hospital using hydrobromic acid-coated petroleum charcoal tubes as sampling media. The charcoal tubes were connected to calibrated Gilian pumps through which a known volume of air containing ethylene oxide was drawn. The hydrobromic acid-coated petroleum charcoal tubes collected ethylene oxide as its 2-bromoethanol reaction product. This reaction is fast and complete and produces a stable product which has a high affinity for charcoal. The sampling media were then sent to the

NIOH laboratory for analysis, which resulted in a calculated level of exposure to ethylene oxide (NIOSH, 1989; NIOSH, 1994).

Personal sampling involved all the employees in the sterilising units operating the ethylene oxide steriliser (the maximum risk employees) and was carried out during loading and unloading the ethylene oxide steriliser. The sampling used Gilian pumps that were drawing air at a flow rate of 0.15litres/min. The pumps were worn by the employees while performing their daily tasks in the sterilising unit.

Static sampling was carried out during the entire work process using Gilian pumps that were drawing air at a flow rate of 0.05litres/min. The pumps were placed in the following three locations: on top of the ethylene oxide steriliser, at the door of the ethylene oxide sterilisation room and in the middle of the general sterilisation room.

Blank samples were also collected (they were hydrobromic acid-coated charcoal tubes identical to the sampling tubes, but no air was drawn through them).

Pump calibration

The pumps were calibrated using a calibrating machine. One end of the hydrobromic acid-coated petroleum charcoal tubes was attached to the calibrating machine and the other one to the sampling pump. The calibrating machine was sending a known flow of air into the pump, which was therefore adjusted according to the required flow rate for the sampling method used (e.g., for the NIOSH method 1614 the required flow rate was between 0.05-0.15 litres/minute).

2.2.2. Sample Analysis

The samples collected were analysed by the Analytical Services of the National Institute for Occupational Health using the gas chromatography technique (NIOSH method 1614). This method has been shown in laboratory and field studies to provide a reliable, convenient and accurate means of measuring ethylene oxide exposures in workplaces (NIOSH, 1994).

Ethylene oxide was recovered from the sampling media using desorption with dimethylformamide (DMF). An aliquot of the sample was then derivatized with heptafluorobutyrylimidazole (HFBI) in isooctane to 2-bromoethylheptafluorobutyrate. Following hydrolysis of the excess reagent with water, the ester product (heptafluorobutyrate ester of 2-bromoethanol) was analysed by gas chromatography with electron capture detection. The resulting air concentration of ethylene oxide was reported in ppm (NIOSH, 1994).

2.2.3. Quality control

Quality control was carried out using the following methods:

- Approved Inspection Authority (AIA) verification: this was performed by COSEN Safety, Health and Environment, CC and included the following: instrumentation used and its performance; calibration procedures; methodology; conformance to methodology; measurement procedures; reports and documentation; representativeness of samples; accuracy and reproducibility of results; record keeping; identification of samples.
- Duplicate samples: a total of 40 duplicate samples were taken to determine the sensitivity of the NIOH analytical procedure in terms of detection of ethylene oxide. Half of the samples were analysed by OSHA and the other half by NIOH. The purpose of this exercise was to provide qualitative information on the presence or absence of ethylene oxide in the sample. Seventy-five percent of the samples were in concordance in terms of detecting ethylene oxide (ethylene oxide was either detected or not detected by both laboratories) and 25% were not in concordance (one of the laboratories detected ethylene oxide in the sample, while the other did not detect ethylene oxide in the sample).
- Blank samples: a total of 100 blank samples were taken and analysed. These samples were not exposed to the ethylene oxide-contaminated atmosphere and were expected not to yield detectable levels of ethylene oxide. Ethylene oxide could not be detected in any of the blank samples.

2.3. COLLECTING INFORMATION ON THE LAST RECOGNISED PREGNANCY USING A QUESTIONNAIRE

2.3.1. Study Population

The study population included singleton pregnancies meeting the following criteria:

- They occurred in the women currently working in sterilising units using ethylene oxide in Gauteng who agreed to participate in the study,
- They represented the last recognised pregnancies occurring in these women after the 1st of January 1992 and
- They occurred while the mother was employed.

The following multi-step approach was used to select the study population:

a) All the sterilising units in Gauteng using ethylene oxide were identified.
b) The sterilising units that agreed to participate in the study were enrolled in the research.
c) The total number of women working in these sterilising units was assessed.
d) The manager of the sterilising unit helped in identifying all the women who had had a pregnancy after the 1st January 1992. The manager asked the women working under her supervision about the time their last recognised pregnancy had occurred and selected the ones whose last recognised pregnancy had occurred after the 1st January 1992. These women were then informed about the research and they were asked to participate in the study.
e) Detailed information on the last recognised pregnancy and a complete reproductive history was obtained from the women who agreed to participate in the research, using the questionnaire described below.
f) After data collection, the following pregnancies were excluded from the study population: multiple pregnancies, pregnancies whose calculated conception date was before the cut-off point (1st January 1992) and pregnancies conceived while the mother was not employed.

2.3.2. Designing the Consent Forms and the Questionnaire

The Consent Forms

Two consent forms were designed (see Appendices 3 and 4): one for "women working with ethylene oxide" at the time of the study and one for "women not working with ethylene oxide" at the time of the study. Because not all the women employed in a sterilising unit were working with the ethylene oxide steriliser, the women not working with the steriliser had to be explained why their contribution to the study was needed. This was the reason for which two consent forms were used instead of one; it had nothing to do with the job these women were performing at the time of her last recognised pregnancy.

The two consent forms (one for "women working with ethylene oxide" and the other one for "women not working with ethylene oxide") were very similar. The only difference was that the consent form for women currently not working with ethylene oxide contained an explanation about the nature and use of ethylene oxide and also about the necessity to enrol in the study women not exposed to ethylene oxide.

The consent forms introduced the study investigators and summarised the scientific evidence of the association between occupational exposure to ethylene oxide in pregnant women and possible adverse reproductive outcomes.

The consent forms briefly presented the questionnaire, describing the type of questions that the women would have to answer. They also emphasised the need to assess the validity of the questionnaire-collected information.

It was made clear that the participation in the study was completely voluntary; there were no consequences on women's employment and the women had the right of withdrawal at any point during the research.

Women were also told that no personal direct benefits were to be expected from the participation in the study and that only indirect benefits would result from a better understanding of the effects of ethylene oxide on reproductive health.

The consent forms promised confidentiality for all study participants emphasising the responsibility of the research team in this regard.

Contact numbers and address of the research team were offered, if any of the study participants needed more information about the study.

The written consent was required two times from the women to be enrolled in the study:

- With the first signature, the woman accepted to participate in the study.
- With the second signature, the woman allowed the team of researchers to access the medical records on her last recognised pregnancy so they could assess the validity of the questionnaire-collected information.

Obtaining the second signature on the consent form (the one giving access to the medical records concerned with the pregnancy on which the woman provided information) was a very important step in the research. Without this signature the medical records containing details on this pregnancy could not be accessed and therefore the validity of the information provided by the mother could not be assessed. Nevertheless, the women that did not agree to have their medical records checked were still included in the study and the pregnancies on which they provided information were included in the analysis (but not in the validity assessment).

The Questionnaire

To record the data needed for this research, a questionnaire was designed (see Appendix 5). The questionnaire collected demographic data, reproductive history, medical data, other risk factors for the adverse reproductive outcome (environmental exposures, occupational exposures and lifestyle) and data regarding the evolution and outcome of the last recognised

pregnancy. The questionnaire also collected information on the job held at the time of the last recognised pregnancy. If a woman was working in a sterilising unit using ethylene oxide, a detailed description of the daily tasks involved was also required.

The questionnaire was based on the bibliography quoted in the introduction of this thesis (see *1.3. Adverse Reproductive Outcomes Following Occupational Exposure to Ethylene Oxide*).

The risk factors listed for the reproductive outcomes of interest were considered potential confounders or effect modifiers. Therefore, questions about possible exposure to these factors were introduced in the questionnaire in order to understand whether the results were due to exposure to ethylene oxide or rather to confounding and to understand whether any of them modified the possible effect of ethylene oxide on the reproductive outcome of interest.

In formulating the questions, the following requirements were taken into account (Abramson and Abramson, 1999):

- Questions must have face validity (i.e., the question has to supply the information needed).
- Respondents can be expected to know the answer to the questions.
- Questions must be clear and unambiguous.
- Questions must be "user-friendly".
- Questions must not be offensive or embarrassing.
- Questions must be fair.

Both open-ended and closed questions were used in the questionnaire. Open-ended questions were employed whenever a complete description of a situation was needed (e.g., working place, job description, type of a medical condition, treatment administered etc). Closed questions were used for situations in which the answer could be expressed either as "yes/no" or as one of several categories (e.g., race, weight, height, educational background, alcohol and cigarettes consumption etc).

In order to facilitate computer entry of the data gathered, the answers to the questions have been coded. Codes were only assigned to the variables measured on a categorical scale (e.g., race, educational background, occupational exposures, smoking, alcohol consumption, presence of certain conditions etc). The variables measured on a metric scale (e.g., maternal age, number of previous pregnancies, interval from the previous pregnancy, birth weight) were entered as such. Some of the metric variables were collapsed later in the analysis into dichotomous variables (e.g., maternal age below or over 35 years, paternal age below or over 40 years, birth weight below or over 2500 g).

Before starting the information collection, the questionnaire used two questions to exclude from the study the women who had never been pregnant and those who had not been pregnant after the 1st January 1992. This method was used in order to ensure that information was collected only on those pregnancies meeting the study criteria.

The questionnaire was divided in three sections: A. Personal information, B. Reproductive history and C. History of the last (most recent) pregnancy.

A. Personal information

This section contained questions that collected the following information:

- demographical data (race, height, weight);
- information on educational background (the highest level of education that the respondent had finished);
- data on current employment status (current working place, job title and starting date).

B. Reproductive history

This section gathered information on the overall reproductive experience of the respondent women. It contained questions that collected information on all the pregnancies ever occurring in the woman, including the total number of pregnancies, their outcome, the number of live births, stillbirths, spontaneous abortions and induced abortions.

- for pregnancies that had ended with the delivery of a child, the questionnaire gathered the following details: date of delivery, newborn's vital status and birth weight;
- for pregnancies that had ended with a spontaneous or induced abortion, the questionnaire collected the following details: date of abortion (either spontaneous or induced) and if known, its cause.

C. History of the last (most recent) pregnancy

This section contained questions that collected detailed information on the evolution and outcome of last recognised pregnancy of the respondent woman:

- the rank of the pregnancy and the interval between this pregnancy and the one before (the second last);
- the weight of the mother at the beginning at the pregnancy, at delivery and weight gain during pregnancy;
- height and age of the partner (the man who fathered the last recognised pregnancy);
- working place during the last recognised pregnancy, number of hours worked per week, working in shifts and when maternity leave started (month of pregnancy);

- if occupational exposure to ethylene oxide took place during the last recognised pregnancy, details about this exposure were requested: working place, section, work title, description of the daily tasks requested by the job;
- other possible maternal occupational exposures and time of exposure during the last recognised pregnancy: mercury, lead, arsenic, cadmium, organic solvents, carbon disulfide, carbon monoxide, pesticides, polychlorinated biphenyls (PCBs), anaesthetic agents, antineoplastic agents, ionising radiation, microwave radiation, video display terminals (VDTs), hypobaric environment, excessive physical activity, noise and excessive psychological stress;
- possible occupational paternal exposures before the last recognised pregnancy: lead, mercury, organic solvents, pesticides, anaesthetic gases;
- possible maternal household exposures: insecticides, carbon monoxide other than environmental tobacco smoking (heating system) and environmental tobacco smoking (paternal smoking);
- possible maternal exposure to recreational drugs: maternal smoking and drinking;
- use of contraceptive methods at the time pregnancy occurred;
- health problems during or before the last recognised pregnancy: high blood pressure, diabetes mellitus, uterine conditions, hormonal disorders, or any other serious sickness. For all these health problems the date of diagnosis and the kind of treatment, if any, were required;
- presence of infections during the last recognised pregnancy and time they occurred (if applicable): rubella, mumps, herpes simplex infection, varicella, zoster, HIV infection, toxoplasmosis, genital mycoplasma infection, syphilis, uterine infection, or high fever (over 41°C);
- whether antenatal care was received during last recognised pregnancy and if yes, when it started (month of pregnancy);
- the total gestation length in months;
- if the last recognised pregnancy ended as a miscarriage, date, confirmed diagnosis and cause are required;
- if the last recognised pregnancy ended with the birth of a child, details about delivery were requested: place of delivery, date of delivery (expressed as day/month/year), gestation length, newborn's gender, newborn's vital status, birth weight, whether there was a multiple gestation and whether there were any malformations present at birth.

The last page of the questionnaire contained the personal details of the respondent woman:

- full name
- date of birth
- ID number
- contact address
- medical aid details: name of the medical aid scheme and membership number.

This information was used to identify the corresponding medical records of the pregnancies when the validity of the information provided by the mother was to be assessed. For confidentiality purposes, after completion this page was detached and kept apart from the rest of the questionnaire.

Due of the ethnic diversity of the South African population, the questionnaire and the consent forms were written in English and then translated in Afrikaans, Zulu and Sotho. The translation was done by scientists from the National Institute for Occupational Health. They were experienced in questionnaire translation and their first language was the one in which they translated the questionnaire and the consent forms.

2.3.3. Questionnaire Testing – Methods and Results

This section contains both the methods used to perform the questionnaire testing as well as the results, as these would help deciding the final version of the study questionnaire.

Methods

Before being administered to the study population, the questionnaire was tested on a sample of working women similar to the women participating in the study. The purpose of this testing was to find out to what extent the questions were understandable and whether they were easy to answer or not.

A sample of working women similar to the ones participating in the study was needed in order to perform the testing. Hilbrow Community Centre was chosen as the place for questionnaire testing because it was a medical institution with a sufficient number of women employees and also close to the National Institute for Occupational Health. The management of this institution was contacted; they were informed about the research and explained why testing the questionnaire was important for the study. After having obtained the management's permission, women nurses and auxiliary personnel were gathered with the help of the head nurse and they were requested to participate in the questionnaire testing.

Consent forms were distributed to the women who agreed to participate in the testing. After they had signed the consent forms, questionnaires were administered. The questionnaire was

administered by trained interviewers from the National Institute from Occupational Health in four languages: English, Afrikaans, Zulu and Sotho. The interviewers distributed the questionnaires and the women filled them in by themselves. The women were told to ask for assistance at any time when there was a need for clarifications, if there were misinterpretations of questions etc.

Because the number of women working at Hilbrow Community Centre who had accepted to participate in the questionnaire testing was low, the questionnaire was also administered to a small group of women not working at this institution (they were friends and colleagues of the interviewers).

After questionnaire administration, the interviewers were requested to fill in a form (see Appendix 2) recording the following information (Abramson and Abramson, 1999; De Vaus, 1993):

- the name of the interviewer;
- the number of women who completed the questionnaire;
- the number of women who refused to answer the questionnaire;
- questions that were difficult to understand;
- questions that needed to be repeated;
- questions that were misinterpreted;
- questions where the respondent would have liked to say more then the choices offered by the questionnaire;
- questions that made the respondent uncomfortable.

Results

Twenty women working at Hilbrow Community Centre agreed to participate in the questionnaire testing (8 English speakers, 5 Zulu speakers, 1 Afrikaans speaker and 6 Sotho speakers).

The number of women who completed the questionnaire was 16: 8 English speakers, 4 Zulu speakers, 1 Afrikaans speaker and 3 Sotho speakers (1 Zulu-speaker woman had never been pregnant and 3 Sotho-speaker women did not complete their questionnaires).

Five more women not working at Hilbrow Community Centre completed the Afrikaans version of the questionnaire. They were friends and colleagues of the interviewers and they were high school or college graduates.

Thus, the total number of respondents that took part in the questionnaire testing was 21, which is considered sufficient for a small-scale test of the questionnaire (Abramson and Abramson, 1999).

After questionnaire testing the following conclusions were drawn:

- The most important finding was that the questionnaires should be administrated by the interviewers themselves (i.e., each question should be read by them and explained to the respondents if necessary). Although explained in the questionnaire, some of the terminology used was not always clear to the respondents (e.g., "partner" meaning the man who fathered the last recognised pregnancy and not the partner at the time of the study, the difference between a "miscarriage" and an "induced abortion");
- Some issues had to be explained to the respondents before they started answering the questions (e.g., signing the consent form was essential for participation in the study);
- Some of the questions needed to be adjusted in order to be rendered more "respondent-friendly". Height and weight were changed from a variable on a metric scale (centimetres and kilograms, respectively), as they had initially been, to categories (short, medium or tall and slim, medium or fat respectively); the gestation length was changed from weeks to months and the birth weight from grams to kilograms.

After making the necessary changes in the questionnaire, there were still some questions left that could not be modified (e.g., most of the questions enquiring about potential occupational exposures and possible medical conditions during pregnancy). These questions remained to be explained during the questionnaire administration if necessary.

2.3.4. Questionnaire Administration

The questionnaire was administered between November 2002 and August 2003 in the preferred language of the subject.

The women who had accepted to participate in the research were first distributed consent forms and they were asked to read and sign them.

All the questionnaires were administered in English, with one exception (one questionnaire was administered in Sotho).

The questionnaire was administered using the interview technique: questionnaires were distributed to the study participants from the same working place (the size of such a group ranged from 1 to 6 persons); the interviewer then read the questions to the participants and they filled in the answers.

Questions were asked exactly as they had been written. If absolutely necessary (e.g., misunderstanding, misinterpretation of the question), they were reformulated and sometimes supplemented by explanations. The questionnaire administration took on average 45 minutes.

2.4. ASSESSING THE VALIDITY OF THE INFORMATION ON THE EVOLUTION AND OUTCOME OF THE LAST RECOGNISED PREGNANCY COLLECTED BY THE MEANS OF THE QUESTIONNAIRE

The criterion validity of the questionnaire-collected information on the evolution and outcome of the pregnancies included in the study was assessed by comparing this information to the existing medical data (i.e., data recorded in labour ward registers or medical files). The medical records were used as the "gold standard" for the diagnosis of the reproductive outcome of interest.

To achieve this objective the following multi-step approach was used:

1. Permission to check the medical records concerned with the last recognised pregnancy was requested from the women enrolled in the study.
2. Permission to check the medical records concerned with the last recognised pregnancy was requested from the medical facilities where the outcome of the pregnancy had been recorded (e.g., spontaneous abortion or delivery).
3. Information on the pregnancy studied was collected from the medical records to which access was given.
4. The information on the last recognised pregnancy provided by the mother was compared to the one existing in the medical records.

2.4.1. Requesting Permission from Mothers

This step has been described in the previous section (see *2.3. Collecting Information on the Last Recognised Pregnancy Using a Questionnaire).*

2.4.2. Requesting Permission from Medical Facilities

Having obtained the permission to check the pregnancy records, the next step was to identify the medical facilities where these pregnancies were registered.

Letters of request were sent to these medical facilities explaining the validity assessment process and the need to get access to the medical records concerned with the pregnancies under study. Medical facilities that had not answered after the first letter were sent a reminder

letter. Those that had not answer to the second letter were sent a third and last request to participate in the validity assessment.

2.4.3. Collecting Information on the Pregnancies under Study from Medical Records

A validation checklist was designed (see Appendix 6) that verified the following questionnaire-collected information on the pregnancy outcome against the existing medical data:

- The name of the mother – used only for identification purposes
- The medical facility where the event was recorded
- Date of spontaneous abortion or delivery, as appropriate
- Gestation length
- Vital status of the newborn
- Number of foetuses
- Gender
- Birth weight
- Malformations at birth
- Complications of delivery
- Diseases/medical problems during pregnancy
- Treatment during pregnancy

All the medical facilities that had granted access to their medical records were visited and the information existing in the medical records concerned with the pregnancies under study was collected.

2.4.4. Data Management and Analysis

All the information collected during the previous step was then compared to the information provided by the mother during questionnaire administration. The two sets of data (mother-reported and recorded data) were compared as follows:

- *Variables measured on a categorical scale*

The following variables measured on a categorical scale were assessed: the medical facility where the event was recorded; gestation length; vital status of the newborn; number of foetuses; gender; malformations at birth; complications of delivery; diseases/medical problems during pregnancy; and treatment during pregnancy.

Specificity, sensitivity, positive predictive value, negative predictive value or percentage of accurately reported data on the variables of interest were used to assess the validity of the information provided by the mother on these variables.

- *Variables measured on a metric scale*

The following variables measured on a metric scale were assessed: the date of the outcome (spontaneous abortion or delivery, as appropriate) and the birth weight of the new-born.

To assess the validity of the information provided by the mother on date of the outcome, the percentage of accurate reporting of this variable was calculated.

To assess the validity of the information provided by the mother on the birth weight, the following approach was used:

1. As the questionnaire collected data on the birth weight of the last born in two different questions (question 9, where information was asked on all the children ever born to the interviewed woman and question 60, where detailed information was requested on the last child), during data processing the following situations were encountered: 1) the answers to the two questions provided the same birth weight and this was the figure used in the analysis; 2) the answers to the two questions provided different birth weights and in this case the figure provided by the answer to question 60 was used (as this was the question collecting detailed information on the last born); 3) birth weight was provided only in one of the two answers (either to question 9 or question 60) and this was the figure used in the analysis; 4) no birth weight was provided by the respondent mother and these pregnancies were not included in the analysis.
2. The mother-reported birth weight was transformed from kilograms into grams (e.g., if the mother had reported the birth weight of her last born as 1.25 kilograms, that was transformed into 1250 grams). As the women participating in the study reported the birth weight of their last born in kilograms and the medical records registered birth weight in grams, this transformation was made in order to have a common unit of measurement and thus to be able to compare the two sets of data.
3. The frequency distribution of the mother-reported birth weight, recorded birth weight and of the difference between the two sets of data was assessed.
4. According to this frequency distribution, the decision was taken whether to use parametric or non-parametric tests: if the frequency distribution showed normally distributed data, then parametric methods would be used (i.e., product-moment correlation coefficient, *t*

test); if the frequency distribution was different from a normal curve, then non-parametric tests would be used (rank correlation coefficients, Wilcoxon rank tests).

2.5. ASSESSING THE ASSOCIATION BETWEEN OCCUPATIONAL EXPOSURE TO ETHYLENE OXIDE DURING PREGNANCY AND ADVERSE REPRODUCTIVE OUTCOME

2.5.1. Classification of Exposure

The exposure information used in this study was gathered indirectly, using three sources: walk-through survey, measurements of the current levels of ethylene oxide in the sterilising units of all public hospitals participating in the research and questionnaire-collected data.

1. Following the walk-through survey (field visit), a classification of the jobs encountered in the sterilising units visited was established (see *Table 8: Job description and main tasks involved for the women participating in the study*). Based on this classification and on the description of the tasks involved by each job, the jobs where exposure to ethylene oxide was most likely to occur were identified.
2. Measurements of the current levels of ethylene oxide in the sterilising units of all the 10 public hospitals using this chemical were performed at the time the study was conducted. The results of these measurements were combined with the information collected during the previous step in order to identify the jobs most exposed to ethylene oxide.
3. The questionnaire gathered information on the job description of the woman at the time of her last recognised pregnancy. If she was working in a sterilising unit using ethylene oxide at that time, the questionnaire also collected details about the daily tasks required by her job.

The findings of the three steps were combined in order to identify the women who had been exposed to ethylene oxide during their last recognised pregnancy. Based on this, the pregnancies under study were classified in exposed and unexposed (as it is fully presented in *3. Results*).

- *Exposed pregnancy* = a pregnancy meeting the study criteria that occurred while the woman was occupationally exposed to ethylene oxide.
- *Unexposed pregnancy* = a pregnancy meeting the study criteria that occurred while the woman was not occupationally exposed to ethylene oxide.

2.5.2. Classification of Outcome

Information on the evolution and outcome of all the pregnancies included in the study was collected using the questionnaire described previously (see *2.3. Collecting Information on the Last Recognised Pregnancy Using a Questionnaire* and Appendix 5).

The following operational definitions for the adverse reproductive outcomes under study were used:

- *Spontaneous abortion (SAB)* = a recognised pregnancy that terminated before 20 weeks of gestation. Induced abortions and ectopic pregnancies were excluded from this category.
- *Stillbirth (SB)* = a recognised pregnancy that advanced beyond 20 weeks of gestation but whose outcome was a dead child.
- *Pregnancy loss (PL)* = the occurrence of either spontaneous abortion or stillbirth.
- *Low birth weight (LBW)* = an infant weighing less than 2500 g at birth.
- *Combined adverse reproductive outcome (CARO)* = the occurrence of spontaneous abortion, stillbirth or low birth weight.

Based on the questionnaire-collected data, the relative frequencies of these outcomes were calculated as follows:

- *Relative frequency of spontaneous abortion* = number of spontaneous abortions divided by the total number of pregnancies under study;
- *Relative frequency of stillbirth* = number of stillbirths divided by the total number of pregnancies under study;
- *Relative frequency of pregnancy loss* = number of spontaneous abortion and stillbirths divided by the total number of pregnancies under study;
- *Relative frequency of low birth weight* = number of low birth weight infants divided by the total number of live births;
- *Relative frequency of combined adverse reproductive outcome* = number of spontaneous abortions and stillbirths plus the number of low birth weight infants divided by the total number of pregnancies under study.

2.5.3. Data Management and Analysis

2.5.3.1. Missing Data

A complete-subject analysis was used, including only records with complete data on the variables of interest and excluding the records with incomplete data.

2.5.3.2. Variables

During questionnaire administration, information on possible risk factors associated with the adverse reproductive outcomes under study was collected (see *1.3. Adverse Reproductive Outcomes Following Occupational Exposure to Ethylene Oxide*).

During data management and analysis the distribution of these variables in the study population was evaluated, calculating their prevalence amongst the pregnancies under study. Only the variables that had a prevalence of at least 3% were analysed further (as it was presented in a similar study - Xu et al, 1998).

New variables were also derived, either modifying existing variables (e.g., transforming a variable measured on a categorical scale with more than two categories into a variable measured on a categorical scale with only two categories, i.e., dichotomous) or deriving a new variable from an existing one. The reason for creating new variables was data stratification needed to identify and control confounding and to identify and assess effect modification. Stratification would be easier to perform with dichotomous variables than with variables measured on a categorical scale with more than two categories or with variables measured on a metric scale.

2.5.3.3. Measuring Associations

To assess the association between exposure to ethylene oxide and each of the five adverse reproductive outcomes, risk ratios were calculated (the ratio between the frequency of outcome in exposed and the frequency of outcome in unexposed).

First, crude risk ratios were calculated. If confounders were identified for an association between exposure and outcome, stratified analysis by the levels of those confounders was performed and the risk ratios adjusted for the confounders were calculated.

All calculations have been done using the statistical program Epi Info 2003.

2.5.3.4. Identifying Confounders

To select the confounders that would be controlled for during the analysis the following procedure was used:

First, the variables associated with exposure were identified. Second, the variables associated with the outcome were identified. Third, the results were combined, thus selecting the variables associated both with the exposure and the outcome (Greenland and Rothman, 1998).

All the calculations were done using the statistical program Epi Info 2003.

1. Identifying the variables associated with the exposure to ethylene oxide

Statistical tests (chi-square or Fischer exact tests) were applied to identify possible associations between the variables considered potential confounders and exposure to ethylene oxide at a level of statistical significance p=0.20. This level of statistical significance was chosen instead of the traditional 0.05 as a way to insure adequate power for the statistical tests to detect any important confounder effects (Greenland and Rothman, 1998).

2. Identifying the variables associated with the five outcomes

Next, statistical testing (chi-square or Fischer exact tests) was performed to identify possible associations between the variables found to be associated with exposure to ethylene oxide in the previous step and the adverse reproductive outcomes studied. The level of statistical significance was also p=0.20.

3. Selecting the variables associated both with the exposure and outcome as confounders of the relation exposure-outcome

The results of the first two steps were combined, therefore identifying the confounders to control for in the analysis. They were represented by the variables associated both with exposure to ethylene oxide (at a level of statistical significance p=0.20) and with the adverse reproductive outcome studied (at a level of statistical significance p=0.20).

2.5.3.5. Identifying Effect Modifiers

To detect any effect modification in this study stratified analysis was performed using the statistical program Epi Info 2003.

The association between exposure to ethylene oxide and adverse reproductive outcome was performed stratifying the available data by the variables that could have potentially been an effect modifier. The pattern of odds ratios in different strata (in the presence and in the absence of the variable) was then examined and the chi-square test for heterogeneity (chi-square for differing odds ratios between strata) was applied, obtaining the corresponding p-value. A low p-value suggested interaction between the exposure studied and the variable by which stratification was done.

The variables for which the level of p-value was 0.20 or lower were considered effect modifiers: if the p-value≤0.05, this was considered a c*lear evidence* of effect modification; if 0.05<p-value≤0.20, this was considered a m*oderate evidence* of effect modification.

3. RESULTS

This chapter will present the results of each of the five objectives of the study.

3.1. DESCRIBING THE EXTENT AND NATURE OF ETHYLENE OXIDE USE IN STERILISING UNITS OPERATIONAL IN MEDICAL FACILITIES IN GAUTENG

The following sterilising units operational in medical facilities in Gauteng using ethylene oxide to sterilise medical equipment were identified:

- *Public Medical Facilities*

There were 28 public hospitals in the province and 22 of them had a sterilising unit (using ethylene oxide or other type of sterilisation, i.e. heat sterilisation, hydrogen peroxide sterilisation etc). All 22 hospitals answered the request; 10 of them reported using ethylene oxide to sterilise medical equipment. All ten agreed to participate in the research (the participation rate amongst ethylene oxide users in the public sector was 100%).

- *Private Medical Facilities*

According to the data provided by the Gauteng Department of Health there were 132 private medical facilities (hospitals and day clinics) in the province. Of these, 58 belonged to the three major medical care providing companies (4 Medi-Clinic, 30 Netcare and 24 Afrox Healthcare). The other 74 were independent private medical facilities.

Table 3: Response rate for medical facilities asked to participate in the study

Type of medical facility		***Number of medical facilities contacted***	***Number of respondent medical facilities***	***Response rate (%)***
Public medical facilities		22	22	100
Private medical facilities	Medi-Clinic	4	3	75.0
	Netcare	30	17	56.7
	Afrox Healthcare	24	8	33.3
	Independent	74	11	14.9
	Total	132	39	29.5
Total medical facilities		**154**	**61**	**39.6**

The response rate for the private medical facilities was as follows: 75% (3 out of 4) for Medi-Clinic; 56.7% (17 out of 30) for Netcare; 33.3% for Afrox Healthcare (8 out of 24); and

14.9% (11 out of 74) for private independent medical facilities. The response rate for all private medical facilities was 29.5% (39 out of 132 private medical facilities in Gauteng - see Table 3).
The overall response rate for the medical facilities contacted (public and private) was 39.6% (61 medical facilities out of a total of 154).

Amongst the 39 respondent private medical facilities 12 were using ethylene oxide: 3 Medi-Clinic, 5 Netcare, 1 Afrox Healthcare and 3 independent private medical facilities and all twelve agreed to participate in the study.
When the 93 non-respondent private medical facilities were contacted telephonically, 10 additional medical facilities were found to use ethylene oxide to sterilise medical equipment. This brought the total number of private medical facilities using ethylene oxide to 22 (12 respondents and 10 non-respondents). Thus, the participation rate amongst ethylene oxide users in the private sector was 54.5% (12 out of 22 - see Table 4).

The overall participation rate for the medical facilities using ethylene oxide (public and private) was 68.8% (22 ethylene oxide users out of 32 - see Table 4).

Table 4: Ethylene oxide users amongst medical facilities in Gauteng and percentage participating in the study

Type of medical facility	***Number of ethylene oxide users***	***Number of ethylene oxide users participating in the study***	***Participation rate (%)***
Public	10	10	100
Private	22	12	54.5
Total	***32***	***22***	***68.8***

3.1.1. Sterilising Units Using Ethylene Oxide

Twenty-two medical facilities were visited, with a total number of 26 sterilising units. Twenty-one medical facilities had just one sterilising unit using ethylene oxide, but one medical facility had 5 sterilising units using ethylene oxide at the time of the study. This gave a total of 26 sterilising units (21+5).

3.1.2. Ethylene Oxide Sterilisers in Use

The findings of the field visits concerned with the type and number of ethylene oxide sterilisers are summarised in Table 5.

Table 5: Type and number of ethylene oxide sterilisers used in the medical facilities investigated

Total number of sterilisers	***General-purpose steriliser using single-dose cartridge***	***General-purpose steriliser using compressed-gas cylinder***	***Steriliser using glass ampoule***
31	30	1	0

The medical facilities visited were using only two types of ethylene oxide sterilisers: general-purpose steriliser using single-dose ethylene oxide cartridges; and general-purpose steriliser using compressed-gas cylinders. None of the medical facilities had an ethylene oxide steriliser using glass ampoules.

A total of 31 sterilisers were found in the medical facilities investigated, the majority (30) being general-purpose sterilisers using a single-dose ethylene oxide cartridges. Only one general-purpose steriliser was using compressed-gas cylinders.

Table 6 summarises the distribution of ethylene oxide sterilisers by medical facility investigated.

Table 6: Number of ethylene oxide sterilisers by medical facility investigated

Number of ethylene oxide sterilisers per medical facility	***Number of medical facilities (out of a total of 22)***	***Percentage of medical facilities (%)***
1	18	81.8
2	1	4.5
3	2	9.1
5	1	4.5

The number of ethylene oxide sterilisers used per medical facility participating in the study ranged from 1 to 5. Eighteen out of twenty-two (81.8%) were using one ethylene oxide steriliser each. One medical facility (4.5%) was using two ethylene oxide sterilisers, two

medical facilities (9.1%) were using three ethylene oxide sterilisers each and one medical facility (4.5%) was using five ethylene oxide sterilisers.

The ethylene oxide sterilising area (EOSA) was defined as the area where the ethylene oxide steriliser was kept. The way this area was organised was also investigated.

- 20 EOSA were closed areas (small rooms), included in the general sterilising area. Their door was designated to be closed during the ethylene oxide sterilising cycle;
- 4 EOSA were closed areas, with a different location to the general sterilising area. Of these, two were also used as store rooms for supplies used in the general sterilising area or in the theatre;
- 2 EOSA were completely open, situated in the general sterilising area.

3.1.3. Staff Characterisation

Table 7 summarises the number of employees found in the sterilising units participating in the study (the information was provided by the managers of the sterilising units).
A total of 522 employees were working in the sterilising units visited (men and women), 504 of whom (96.6%) were women.

Table 7: Number of employees working in the sterilising units participating in the study and percentage of women employees

Total number of employees in sterilising unit (males and females)	***Number of female employees in the sterilising unit***	***Percentage of female employees in the sterilising unit (%)***
522	*504*	*96.6*

The unit manager also provided information on the jobs titles in the sterilising unit and on the main tasks performed during working shifts (see Table 8).

- *The technician* (operator) was the person in charge of the operation of the ethylene oxide steriliser. This employee was responsible for loading the steriliser, installing the ethylene oxide cartridge and turning on the machine. When the sterilising cycle was over, the operator offloaded the freshly sterilised load and either transferred it to the aerator cabinet or took it to the storeroom of the sterilising unit, ready to be used. The technician usually

loaded the steriliser in the afternoon (towards the end of the day shift) and offloaded it the following morning (at the beginning of the day shift).

- *The instrument packer* was the person in charge of cleaning the used medical instruments and packing them, so that they were ready to be sterilised using ethylene oxide sterilisation or other sterilising methods available (e.g., heat sterilisation).
- *The cleaner* was the person in charge of cleaning up the working site (the sterilising unit). This job description also included cleaning inside the ethylene oxide steriliser.

Table 8: Job description and main tasks involved in a sterilising unit

Job description	***Main tasks involved***
Technician (operator)	• Loading the ethylene oxide steriliser • Offloading the ethylene oxide steriliser • Moving the freshly sterilised loads to the aerator cabinet and/or to the store room
Instrument packer	• Scrubbing the used medical instruments • Arranging these instruments into packs that will be loaded into the ethylene oxide steriliser/heat steriliser
Cleaner	• Cleaning the sterilising unit, including the room where the ethylene oxide steriliser was kept (EOSA) • Cleaning the inside of the ethylene oxide steriliser when the sterilising cycle was over

3.1.4. Work Practices

Work practices varied among sterilising units: in some of them only the technician (operator) was allowed to offload the ethylene oxide steriliser, while in others an employee-rotation system was used. This meant that any employee available at the moment the steriliser was to be offloaded could perform this task.

Sometimes this staff rotation did not include only employees working in the sterilising unit, but it extended to the employees working in the theatre. That is, every 2-3 months a rotation took place and the theatre employees went to work at the sterilising unit, while the sterilising unit employees went to work at the theatre.

All sterilising units visited were working in two shifts (day and night). The number of loads sterilised in a week per sterilising unit ranged from 3 to 126, with an average of 14 loads. Most of the sterilising units (17 out of 26, or 65.4%) were sterilising 7 loads per week (one load every day).

3.1.5. Protection Measures

Only four out of 26 sterilising units visited (15.4%) reported the use of protective clothing (e.g., masks and gloves) by the employees working with the ethylene oxide steriliser.

All sterilising units reported using baskets and trolleys to transfer the sterilised loads to the storing places.

3.1.6. Ethylene Oxide Monitoring

All the sterilising units visited reported monitoring the levels of ethylene oxide on a regular basis. In all cases monitoring was performed by the same company. This was also the supplier of ethylene oxide sterilisers, with one exception (in one sterilising unit the supplier of the ethylene oxide steriliser and the company performing the monitoring were different).

Data on the latest measurements of ethylene oxide levels were available only in 12 out of 26 sterilising units (46.2%). All the recorded levels found were below 0.25 ppm.

3.1.7. Changes in Sterilising Equipment and/or Technology

In 11 out of 26 sterilising units (42.3%) there had been changes in equipment and/or technology in the past 10 years. Example of these changes were: installing an ethylene oxide steriliser, replacing the old ethylene oxide steriliser, moving the ethylene oxide steriliser to a different location, closing the ethylene oxide sterilising area or improving the extraction and ventilation system.

3.1.8. Summary

The vast majority of the employees working in the sterilising units using ethylene oxide were women (96.6%). There were three categories of job titles in a sterilising unit, and only one (technician or operator) involved working directly with the ethylene oxide steriliser.

Amongst measures aimed at preventing exposure to ethylene oxide, monitoring the ethylene oxide concentration was mentioned by almost half of the sterilising units visited (46.2% provided measured levels of ethylene oxide), followed by engineering control measures

(42.3%). Personal protective measures (protective clothing) was found only in only 15.4% of the cases.

The findings of this objective showed that the employee most exposed to ethylene oxide in a sterilising was the technician (operator) and that the exposure was likely to have remained the same or to have diminished in the past ten years (it did not appear to have increased).

3.2. ASSESSING THE CURRENT EXPOSURE TO ETHYLENE OXIDE IN STERILISING UNITS IN GAUTENG

A total of 418 samples (97 personal samples, 221 static samples and 100 blank samples) were collected in the sterilising units of all 10 public hospitals using ethylene oxide.

3.2.1. Personal Sampling

Table 9 summarises the levels of ethylene oxide detected by personal sampling (97 samples taken for all the employees operating the ethylene oxide sterilisers, i.e., the maximum risk employees) in all 10 public hospitals in Gauteng using ethylene oxide.

The number of personal samples taken in each hospital ranged from 9 to 11, with a mean of 10 samples. In four out of 10 hospitals (40.0%) personal sampling did not detect ethylene oxide in the air sampled. In the remaining 6 hospitals (60.0%) personal sampling detected levels of ethylene oxide between 0.78 ppm and 38.22 ppm.

Table 9: Levels of ethylene oxide (ppm) detected by personal sampling by hospital

Hospital	**Number of samples**	**Ethylene oxide levels (ppm)**		
		Range	**Mean**	**Median**
Hospital 1	9	ND*	ND	ND
Hospital 2	9	ND-10.16	2.79	0.95
Hospital 3	10	ND -38.22	5.48	1.77
Hospital 4	9	ND -2.78	0.56	ND
Hospital 5	10	ND -4.79	0.94	ND
Hospital 6	11	ND -2.57	0.42	ND
Hospital 7	10	ND	ND	ND
Hospital 8	10	ND -0.78	0.08	ND
Hospital 9	10	ND	ND	ND
Hospital 10	9	ND	ND	ND

*ND = not detected

Table 10 summarises the overall results of the 97 personal samples collected during the study.

Table 10: Levels of ethylene oxide detected by personal sampling of the technician (ppm) and sampling duration (minutes)

ETO levels (ppm)	Personal samples (n=97)	Sampling duration in minutes
ND	80	1-20 (mean=10.5)
0.78	1	8
0.95	1	10
1.01	1	7
2.10	1	9
2.28	1	9
2.57	1	9
2.78	1	14
3.54	1	2
3.65	1	15
3.87	1	10
4.60	1	4
4.79	1	10
5.54	1	10
5.61	1	8
7.39	1	8
10.16	1	9
38.22	1	11

*ND = not detected

The levels of ethylene oxide detected by personal sampling ranged from not detected (ND) to 38.22 ppm, with a mean of 1.03 ppm and a median of not detected (ND). Most of the personal samples (80 or 82.5%) did not detect the presence of ethylene oxide in the air sampled. The remaining 17 personal samples (17.5%) detected levels of ethylene oxide between 0.78-38.22 ppm.

The personal samples were collected over a period of time ranging from 1 to 20 minutes, with an average of 10.2 minutes and a median of 10 minutes.

Due to the specific tasks performed by the personnel working with the ethylene oxide steriliser (i.e., loading and offloading the steriliser), exposure to this chemical is likely to occur for a brief period of time. Exposure occurs mostly at the end of the sterilisation cycle, when the ethylene oxide steriliser is opened, offloaded and when freshly sterilised loads are handled. Therefore, the levels of ethylene oxide detected by personal sampling were very likely to represent short-term exposures to the chemical. When these results were compared to the short-term exposure limit (15 ppm) set by the South African Regulations (Regulations for Hazardous Chemical Substances, 1995), only 1 measurement (1.0%) was above this limit. When they were compared to the internationally accepted short-term exposure limit (5 ppm)

set by the Occupational Safety and Health Administration (US Department of Health and Human Services; CDC; NIOSH, 1988), 5 measurements (5.2%) were above this limit.

3.2.2. Static Sampling

Table 11 summarises the levels of ethylene oxide detected by static sampling (221 samples) in all 10 public hospitals in Gauteng using ethylene oxide.

The number of static samples taken in each hospital ranged from 17 to 31, with a mean of 22 samples. In one of the 10 hospitals (10.0%) static sampling did not detect ethylene oxide in the air sampled. In the remaining 9 hospitals (90.0%) static sampling detected levels of ethylene oxide between 0.01-3.16 ppm.

Table 11: Levels of ethylene oxide (ppm) detected by static sampling by hospital

Hospital	**Number of samples**	**Ethylene oxide levels (ppm)**		
		Range	**Mean**	**Median**
Hospital 1	30	ND*	ND	ND
Hospital 2	18	ND -1.41	0.28	ND
Hospital 3	26	ND -0.61	0.11	0.08
Hospital 4	31	ND -1.67	0.10	ND
Hospital 5	18	ND -0.37	0.06	ND
Hospital 6	19	ND -3.16	0.31	ND
Hospital 7	17	ND -2.69	0.82	0.26
Hospital 8	22	ND -0.04	0.02	0.03
Hospital 9	23	ND -0.28	0.04	ND
Hospital 10	17	ND -0.18	0.02	ND

*ND = not detected

Table 12 summarises the results of the 221 static samples collected during the study.

Table 12: Levels of ethylene oxide detected by static sampling (ppm) and sampling duration (minutes)

ETO Level (ppm)	Ethylene oxide steriliser (n=103)	Door of the ethylene oxide sterilising area (n=66)	General sterilising room (n=52)	Total static samples (n=221)	Sampling duration in minutes (mean)
ND	34	52	49	135	13-1073 (406)
0.01	0	1	1	2	965-972 (968.5)
0.02	2	1	1	4	386-967 (688.3)
0.03	6	6	0	12	195-803 (454.9)
0.04	5	0	1	6	415-731 (491.5)
0.05	5	0	0	5	234-690 (390.2)
0.06	2	0	0	2	232-668 (450)
0.07	1	1	0	2	124-276 (200)
0.08	4	0	0	4	253-291 (272.8)
0.10	2	0	0	2	136-672 (404)
0.13	2	0	0	2	157-181 (169)
0.14	0	1	0	1	960
0.15	4	0	0	4	167-516 (267)
0.16	2	0	0	2	167-190 (178.5)
0.18	4	1	0	5	181-524 (355)
0.19	1	0	0	1	275
0.20	1	0	0	1	516
0.21	2	0	0	2	237-297 (267)
0.23	1	0	0	1	668
0.24	1	0	0	1	165
0.26	0	1	0	1	1067
0.28	2	1	0	3	234-964 (668)
0.29	1	0	0	1	515
0.32	1	0	0	1	291
0.36	1	0	0	1	380
0.37	1	0	0	1	226

ETO Level (ppm)	Ethylene oxide steriliser (n=103)	Door of the ethylene oxide sterilising area (n=66)	General sterilising room (n=52)	Total static samples (n=221)	Sampling duration in minutes (mean)
0.53	1	0	0	1	181
0.56	1	0	0	1	356
0.59	1	0	0	1	119
0.61	1	0	0	1	156
0.62	0	1	0	1	316
0.63	1	0	0	1	224
0.87	1	0	0	1	353
0.95	1	0	0	1	235
1.34	1	0	0	1	263
1.41	1	0	0	1	224
1.42	1	0	0	1	1065
1.60	1	0	0	1	961
1.66	1	0	0	1	994
1.67	1	0	0	1	300
1.83	1	0	0	1	1065
1.97	1	0	0	1	1075
1.98	1	0	0	1	995
2.69	1	0	0	1	968
3.16	1	0	0	1	335

*ND = not detected

The levels of ethylene oxide detected by static sampling ranged from not detected (ND) to 3.16 ppm, with a mean of 0.15 ppm and a median of not detected (ND).

The sampling period ranged from 13 to 1075 minutes (approximately between one quarter of an hour and 18 hours), with an average of 427 minutes (approximately 7 hours) and a median of 356 minutes (approximately 6 hours).

Overall, 86 out of 221 static samples (38.9%) yielded detectable levels of ethylene oxide that ranged from 0.01 to 3.16 ppm. The remaining 135 static samples (61.1%) did not detect ethylene oxide in the air sampled.

Static samples taken on top of the ethylene oxide steriliser

These were the samples that detected most frequently ethylene oxide in the air sampled: 69 out of 103 samples (67.0%) yielded levels of ethylene oxide ranging between 0.02-3.16 ppm. They represented 80.2% of the total number of static samples detecting ethylene oxide (69 out of 86).

Static samples taken at the door of the ethylene oxide sterilising area

Fourteen out of 66 (21.2%) static samples taken at the door of the ethylene oxide sterilising area detected levels of ethylene oxide ranging from 0.01 to 0.62 ppm. They represented 16.3% of the total number of static samples that detected ethylene oxide (14 out of 86). The static sample that detected the highest level of ethylene oxide at the door of the ethylene oxide sterilising room (0.62 ppm) was collected during an accidental leakage of the ethylene oxide steriliser, followed by a failure of the ventilation system.

Static samples taken in the general sterilising area

Only 3 out of 52 (5.8%) static samples taken in the general sterilising area yielded detectable levels of ethylene oxide that ranged from 0.01 to 0.04 ppm. They represented 3.5% of the total number of static samples that detected ethylene oxide (3 out of 86).

When the results of the static sampling were compared to the occupational exposure limits, 11 static samples out of 221 (5.0%) detected levels of ethylene oxide above the Occupational Safety and Health Administration (OSHA) long-term exposure limit for ethylene oxide (1 ppm). All these samples were taken on top of the ethylene oxide steriliser.

None of the static samples detected levels of ethylene oxide above the South African long-term exposure limit (5 ppm).

3.2.3. Summary

Personal and static samples were taken over a 5-day period for each public hospital using ethylene oxide.

Personal sampling involved all employees working directly with the ethylene oxide steriliser (the maximum risk employees) during the sampling period (5 days) and it was carried out during loading and offloading the steriliser. The levels of ethylene oxide detected by personal sampling reflected the exposure to this chemical of the employees working directly with the ethylene oxide steriliser (technicians or operators). Personal sampling detected ethylene oxide in 17.5% of the cases (17 out of 97 samples). The highest concentration of ethylene oxide detected by personal sampling of the technicians (operators) was 38.22 ppm.

Static sampling was performed during the entire work shift and it was carried out in the following areas: on top of the ethylene oxide steriliser, at the door of the ethylene oxide sterilising room and in the middle of the general sterilising room. The levels of ethylene oxide detected by static sampling reflected the exposure to this chemical of the following categories of employees: technicians only (for samples taken on top of the ethylene oxide steriliser) and technicians, cleaners or instrument packers (for samples taken at the door of the ethylene oxide sterilising room and in the middle of the general sterilising room). Static sampling detected ethylene oxide in 38.9% of the cases (86 out of 221 samples). Most of the static samples that detected ethylene oxide were samples taken on top of the ethylene steriliser (69 out of 86, or 80.2%). Only 14 out of 86 (16.3%) were static samples taken at the door of the ethylene oxide sterilising room and 3 out of 86 (3.5%) were static samples taken in the middle of the general sterilising area. The highest level of ethylene oxide detected by static sampling performed at the door of the ethylene oxide sterilising room was 0.62 ppm and this sample was collected during an accidental leakage of the steriliser followed by a failure of the ventilation system. The highest level of ethylene oxide detected by static sampling performed in the general sterilising area was 0.04 ppm (well bellow the occupational exposure limits for ethylene oxide, both in South Africa and in the USA – 5 ppm and 1 ppm, respectively).

Based on these findings, the technician (operator, i.e. the person working directly with the ethylene oxide steriliser) was identified as the job category most exposed to ethylene oxide in a sterilising unit.

3.3. COLLECTING INFORMATION ON THE LAST RECOGNISED PREGNANCY USING A QUESTIONNAIRE

3.3.1. Study Population

The results of *steps a)*: identifying all the sterilising units in Gauteng using ethylene oxide, *step b)*: enrolling the sterilising units that agreed to participate in the study and *step c)*: identifying the total number of women working in these sterilising units were presented in *3.1: Describing the Extent and Nature of Ethylene Oxide Use in Sterilising Units Operational in Medical Facilities in Gauteng*.

Step d): With the help of the sterilising unit manager, 113 female employees who had been pregnant after the 1st January 1992 were identified. They represented 22.4% of the total number of women (504) working in the sterilising units participating in the study.

When the 113 women were asked to take part in the research, 109 agreed (response rate 96.5%) and four (3.5%) refused for personal reasons (in one case, her last born had died soon after birth and the woman did not want to talk about it and in the other three cases no explanation was given).

The results of *step e)*: obtaining detailed information on the last recognised pregnancy and a complete reproductive history from the women who agreed to participate in the research, using a questionnaire and *f)*: excluding from the study population multiple pregnancies, pregnancies whose calculated conception date was before the 1st January 1992 and pregnancies conceived while the mother was not working will be described in section *3.3.2. Questionnaire Administration*.

3.3.2. Questionnaire Administration

In the previous steps, 113 women who had had a recognised pregnancy after the 1st January 1992 were identified working in the sterilising units participating in the study. Four of them (3.5%) refused to be enrolled in the study for personal reasons.

The remaining 109 women agreed to participate in the research, which yielded a response rate of 96.5%.

All 109 women signed the first part of the consent form, but only 94 of them signed the second part. The questionnaire was administered to all 109 women, which included the ones refusing access to their medical records.

Using the questionnaire, data was collected on the evolution and outcome of 109 recognised pregnancies (see Figure 1). Two (1.8%) of the 109 pregnancies on which information was gathered were multiple pregnancies, 5 (4.6%) were conceived while the mother was not employed and 4 (3.7%) were conceived before the 1st January 1992. All these pregnancies (11 out of 109, or 10.1%) were excluded from the study population.

The study population consisted of the remaining 98 singleton pregnancies.

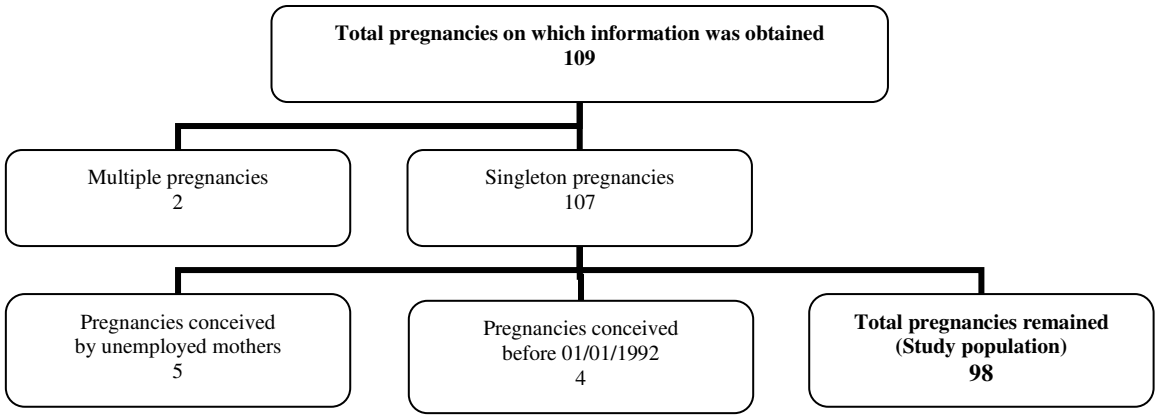

Figure 1: Selection of the study population

3.4. ASSESSING THE VALIDITY OF THE INFORMATION ON THE EVOLUTION AND OUTCOME OF THE LAST RECOGNISED PREGNANCY COLLECTED BY THE MEANS OF THE QUESTIONNAIRE

3.4.1. Requesting Permission from Mothers

Permission to check the medical records of the last recognised pregnancy was obtained for 94 (86.2%) out of 109 pregnancies on which information had been collected. For 15 pregnancies (13.8%) access to medical records was refused.

Table 13 summarises the situation of the pregnancies for which mothers had allowed access to the medical records.

Out of the 94 pregnancies for which mothers had allowed access to the medical records, 2 (2.1%) were not registered at a medical facility (according to the mother, they were spontaneous abortions that occurred at home and were not followed by medical care).

The records of thirteen pregnancies reportedly registered at a medical facility (13.8%) could not be accessed for the following reasons: 3 were registered at medical facilities so far away from Gauteng that accessing them was impractical, 2 were registered at a medical facility in Gauteng that was closed down at the time of the research and 8 were registered at medical facilities that could not be identified.

The remaining 79 pregnancies (84.1%) were recorded at 29 medical facilities in Gauteng and these medical facilities could be contacted.

Table 13: Situation of pregnancies for which mothers allowed access to medical records

Situation of pregnancy		***Number (%)***
Pregnancies not registered at a medical facility		2 (2.1)
Pregnancies reportedly registered at a medical facility	**t**hat could not be contacted	13 (13.8)
	that could be contacted	79 (84.1)
Total number of pregnancies		**94 (100%)**

3.4.2. Requesting Permission from Medical Facilities

Between May and August 2003 letters were sent to these 29 medical facilities informing them about this objective of the research (assessing the validity of mother-provided information) and the need to get access to the medical records concerned with the pregnancies enrolled in the study. Medical facilities that had not answered after the first letter were sent a reminder letter. Those that had not answered to the second letter were sent a third and last request.

In the end, 19 medical facilities out of 29 replied. Eighteen of them granted access to the medical records concerned with the pregnancies analysed in the study and one denied access to their obstetrical medical records.

Amongst the 10 non-respondent medical facilities just one (10.0%) was a public hospital, the remaining 9 (90%) being private medical facilities. The number of pregnancies recorded at the non-respondent medial facilities was 11.

Amongst the 19 respondent medical facilities there were 10 public hospitals (52.6%) and 9 private medical facilities (47.4%). The number of pregnancies recorded at the respondent medical facilities was 68. The hospital that answered, but denied access to their medical records, had 4 pregnancies recorded in its files. This brought the number of pregnancies whose records could be checked down to 64.

3.4.3. Collecting Information on the Pregnancy from Medical Records

There were 64 pregnancies recorded at the 18 medical facilities that had granted access to their obstetrical records.

According to the mothers, 44 of these 64 pregnancies were recorded at public hospitals and 20 at private hospitals. Amongst the 44 pregnancies registered at public hospitals, 37 (84.1%) could be found; for the remaining 7 pregnancies no records were found at the indicated medical facilities. Amongst the 20 pregnancies registered at private hospitals, 18 (90.0%) could be found; for the remaining 2 pregnancies, no records were found at the indicated medical facilities.

In the end, these 55 pregnancies (37+18) were the pregnancies whose records could be checked. They represented 69.6% (55 out of 79) of the pregnancies whose records could be traced and 58.5% (55 out of 94) of the pregnancies for which initially mothers had allowed access to the medical records.

The 64 pregnancies recorded at the medical facilities that granted access to their files had occurred between 1992 and 2003 (6x1992, 4x1993, 10x1994, 5x1995, 4x1996, 6x1997, 5x1998, 5x1999, 4x2000, 5x2001, 9x2002 and 1x2003). The seven pregnancies that could not be identified in the existing medical records of public hospitals had occurred between 1992 and 2002 (1x1992, 2x1993, 2x1994, 1x1997 and 1x2002). The two pregnancies that could not be found in the medical records of private hospitals had occurred in 1992 and 1995. In the former case the private hospital did not have any records older than 10 years at the time of the study (2003). In the latter case the medical record was missing due to recent administrative changes taking place at the medical facility.

Medical records checked included medical files ("bed letter") and labour ward registers.

- Medical files contained detailed information on the pregnancy, including reproductive history, data on pregnancy evolution (problems during pregnancy, treatment received) and pregnancy outcome (date and time of delivery, duration of labour, method of delivery,

complications at delivery, details on the newborn: sex, birth weight, Apgar score, vital status).

- Labour ward registers were just a "summary" of the pregnancy outcome, recording only the name and age of the mother, address, parity, date and time of delivery, duration of labour, method of delivery (normal vaginal delivery or caesarean section and reasons for that), sex of the newborn, birth weight, Apgar score and status of the mother and child after delivery.

During the field visits it was learnt that public hospitals kept bed letters for an average of 3-4 years, after which they destroyed them, due to the lack of storing space. Labour ward registers were usually available for a longer period of time, averaging 7-8 years (one public hospital had labour ward registers dating back from the 1980's). For some reason, one public hospital visited had sent most of the labour ward registers (up to the year 2000) to the territorial home affairs office and there they were found.

In private hospitals detailed medical files were usually available for the last 9-10 years. They were not kept on the premises, but they were available on request (they could be ordered through the medical facility's management from a central archive where they were stored).

Medical files could be found for 18 out of the 55 pregnancies checked. For the remaining 37 pregnancies, recorded information on the outcome was gathered from the corresponding labour ward registers.

3.4.4. Mother-Reported versus Recorded Information on Pregnancy Evolution and Outcome

This step started with the assumption that medical records were the "gold standard" for all the variables that had to be evaluated during this objective.

Mothers providing information on their last recognised pregnancy had to recall events that had taken place between 4 months and 10 years prior to questionnaire administration (mean=5.4 years, median=5 years).

Medical facility

Data checking at the medical facilities indicated by the respondent mothers led to one of the following situations:

- For 55 pregnancies: the medical records from the corresponding period of time were found and the 55 pregnancies were registered in these records;
- For 2 pregnancies: the medical records from the corresponding period of time were found, but the 2 pregnancies were not registered in these records;
- For 7 pregnancies: no medical records from the corresponding period of time were found. As it was not known whether or not the 7 pregnancies were registered in the missing medical records, they were excluded from the analysis.

There were 2 pregnancies that could not be found in the medical records from the corresponding period of time. Under the initial assumption "medical records=gold standard", the 2 pregnancies were not found in the medical records at the indicated medical facility because this was not where the mother had received medical care for her pregnancy. In other words, the missing entries in the labour ward register were due to inaccurate reporting of the medical facility by the mother and not to faulty medical recording.

In this case the proportion of mothers accurately reporting the medical facility where their last recognised pregnancy was registered was 96.5% (55 out of 57).

Date of the pregnancy outcome (spontaneous abortion, stillbirth or live birth)

Date of the pregnancy outcome was recorded in the questionnaire as day/month/year and this was how it was checked against medical records.

Out of the 55 pregnancies that were found to be registered at the indicated medical facilities, 3 had a different outcome date from the one reported by the mother.

Therefore, the proportion of mothers accurately reporting the date of the outcome of their last recognised pregnancy was 94.5% (52 out of 55).

Gestation length

Gestation length was assessed in the questionnaire by asking the mother to chose one of the following options: "the child was born: premature, at term and after the confinement date" (question 60). The questionnaire did not ask for gestation length in weeks, as the questionnaire testing had showed that women were not familiar with it. The following definitions were used instead: "premature" = a child born before 36 weeks of gestation; "at term" = a child born between 36 and 42 weeks; and "after confinement date" = a child born after 42 weeks of gestation (Kalousek *et al*, 1992).

In five out of the 55 pregnancies the gestation length indicated by the mother did not coincide with the one registered in the medical records.

Thus, the proportion of mothers who accurately reported the length of their last recognised pregnancy was 90.9% (50 out of 55).

Vital status of the newborn

For all the 55 pregnancies checked, the mothers have indicated the right live status of their offspring.

This was equivalent to a 100% accurate reporting on this parameter.

Number of foetuses

All the 55 pregnancies whose records could be checked were singleton pregnancies and the mothers reported them as such.

This was equivalent to a 100% accurate reporting on this parameter.

Gender

One out of the 55 pregnancies checked ended as spontaneous abortion and hence it was not included in the analysis.

For the remaining 54 pregnancies, one of the mothers reported the wrong gender for her offspring.

The proportion of mothers accurately reporting the gender of their last offspring was therefore 98.1% (53 out of 54).

Birth weight

Table 14 summarises the mother-reported against recorded birth weights (grams) for the 55 pregnancy records that could be checked.

Of 55 pregnancy records that could be checked, 3 were records of spontaneous abortions and stillbirth and they were not included in this analysis, 3 did not mention any birth weight for the new born and 7 corresponded to pregnancies for which the mother had not reported the birth weight. Hence, the validity of the information on birth weight provided by the respondent mother could be assessed for 42 pregnancies.

Table 14: Mother-reported against recorded birth weights (grams) for the 55 pregnancy records checked

Questionnaire code	Mother-reported birth weight (grams)	Recorded birth weight (grams)
1	1250	2250
56	1500	1500
51	1650	1700
98	2000	2860
54	2300	2800
19	2500	2550
45	2600	2350
101	2680	1870
34	2700	2900
46	2800	2900
21	2800	2920
100	2850	2935
73	2900	2760
85	2900	2960
43	2950	3100
88	2970	2970
50	3000	3750
106	3200	3200
41	3050	3050
68	3050	3240
105	3080	3380
8	3080	3540
93	3100	3126
102	3200	2710
62	3200	3085
24	3200	3300
44	3400	3180
10	3400	3225
42	3400	3410
52	3400	3850
67	3500	2420
70	3500	2865
66	3500	3200
20	3500	3450
3	3530	3290
84	3625	3625
48	3750	3705
69	3800	3065
13	4000	3700
61	4000	4000
53	4100	2700
2	4500	3350
83	missing	
27	missing	
31	missing	
39	missing	
40	missing	
71	missing	
96	missing	

Questionnaire code	Mother-reported birth weight (grams)	Recorded birth weight (grams)
9	N/A*	
12	N/A*	
36	N/A*	
5		missing
30		missing
65		missing

*These pregnancies ended as spontaneous abortion or stillbirth.

Using the approach described under *2.4.4. Data Management and Analysis*, the following frequency distributions were assessed first: the frequency distribution of the mother-reported birth weight, of the recorded birth weight and of the difference between mother-reported and recorded birth weight.

Figure 2 shows the frequency distribution of the mother-reported birth weight, with the number of reported birth weights within each 100-gram interval.

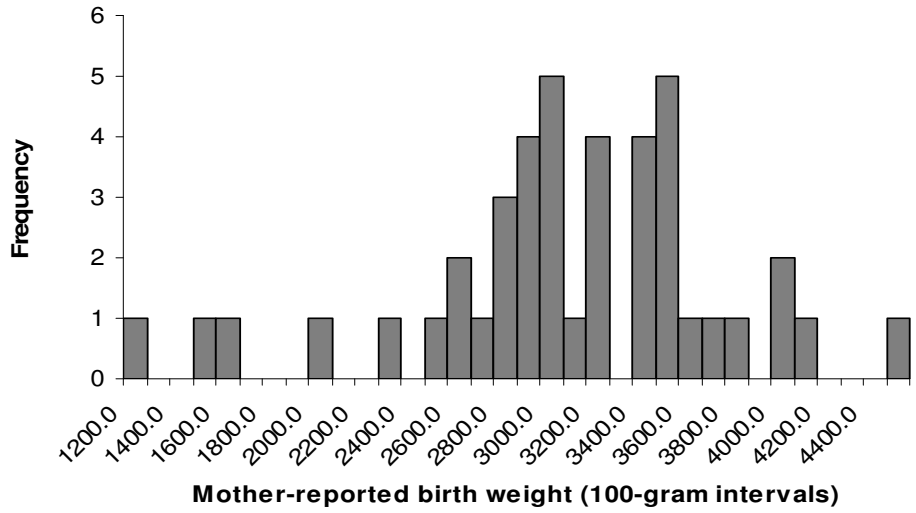

Figure 2: Frequency distribution of the mother-reported birth weight (grams)

Figure 3 shows the frequency distribution of the recorded birth weight, with the number of recorded birth weights within each 100-gram interval.

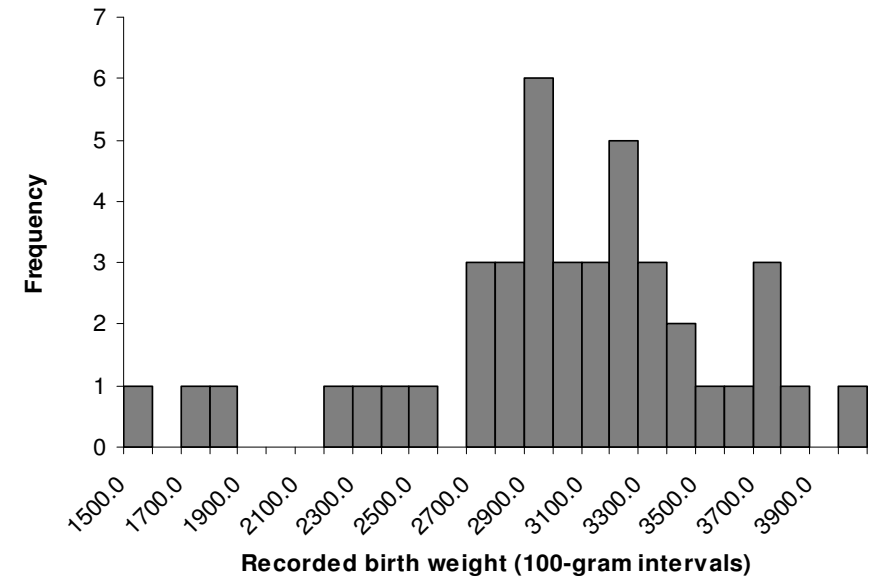

Figure 3: Frequency distribution of the recorded birth weight (grams)

Figure 4 shows the frequency distribution of the difference between mother-reported and recorded birth weight, with the number of differences between mother-reported and recorded birth weights within each 100-gram interval.

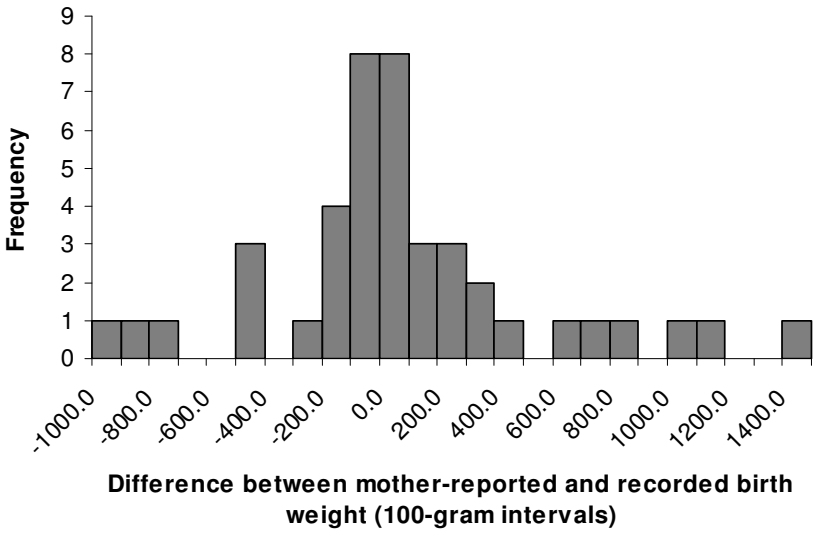

Figure 4: Frequency distribution of the difference between mother-reported and recorded birth weight (grams)

Given that these frequency distributions approximated a normal curve, parametric methods were used to compare the mother-reported and the recorded birth weight for the 42 pregnancies on which data were available.

To compare the two data sets the following methods were used: the product-moment correlation coefficient (r), discordant birth weight and the *t* test.

Product-moment correlation coefficient (r)

The two sets of data (mother-reported birth weight and recorded birth weight) were plotted against each other and a linear relationship was obtained (see Figure 5).

The calculated product moment correlation coefficient was r = 0.69 ($p<0.01$), which showed a positive, linear relationship and a good correlation between the two data sets.

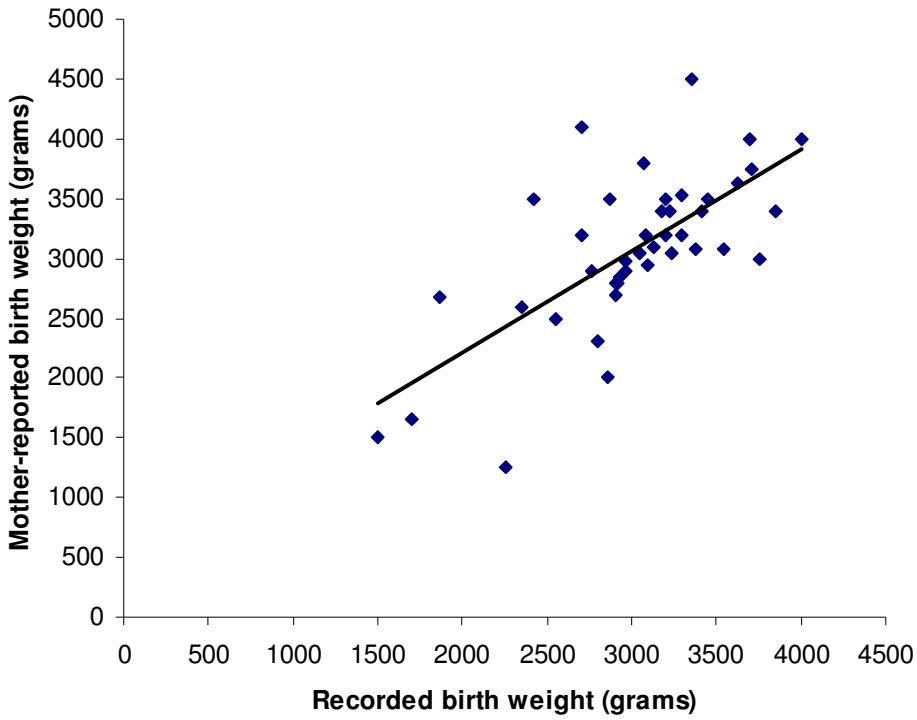

Figure 5: Scatter plot of mother-reported birth weight (grams) against recorded birth weight (grams)

The 95% confidence interval for the correlation coefficient was also calculated using the z transformation (Bradford Hill and Hill, 1991):

$z=1/2\ln(1+r)/(1-r)$, where ln is the natural logarithm

Hence z=1/2ln5.45, z=0.848

z is normally distributed with a standard error 1/√n-3, where n=42

Hence the standard error of z is 0.16 and the 95% confidence limits for z are 0.848±1.96x0.16, which are 0.534 to 1.162 respectively.

To get back from z to r, the following formula was used:

$r= (e^{2z}-1)/(e^{2z}+1)$, where e=the base of natural logarithms (e=2.71828…)

The calculated 95% confidence limits for the correlation coefficient were 95% CI=0.49-0.82.

Discordant birth weight

When the mother-reported birth weights were checked against the recorded birth weights, a positive discordance was found for 10 out of 42 mother-reported birth weights (23.8%) and a negative discordance for 7 out of 42 mother-reported birth weights (16.7%).

Overall, 40.5% (17 out of 42) of the mother-reported birth weights in this study were discordant, i.e., they differed with 250 g or more from the recorded values.

Detecting a bias in the mother-reported birth weight (Bland, 1995; Bradford Hill and Hill, 1991)

The mother-reported and the recorded birth weights were considered as two sets of measurements taken on the same subject under two conditions. A possible bias in the mother-reported birth weight was represented by the mean difference between mother-reported and recorded birth weight.

The one sample (paired) *t* test was used to test the null hypothesis that the mean difference between the two sets of measurements was zero and that there was no bias in the mother-reported birth weight.

If the null hypothesis were true and the differences between the two sets of measurements followed a normal distribution, the test statistic mean/standard error would be from a *t* distribution with n-1 degrees of freedom (n=sample size).

The mean of the difference between mother-reported and recorded birth weights was 63.67 g and the standard deviation was 487.42 g.

The standard error of the mean difference between the two sets of measurements was then

s.e. = s/√n, where s is standard deviation of the mean difference and n is the sample size (n = 42), s.e. = 487.42/√42 or 75.22 g

t = mean difference/standard error of the mean difference, therefore

t = 63.67/75.22, which was 0.85.

The probability of getting such a *t* value with 41 degrees of freedom arising was between 0.20 and 0.50 (from the table of two tailed probability points of the *t* distribution). The data were therefore consistent with the null hypothesis; the mean difference between mother-reported and recorded birth weight was zero and the difference found (63.67 grams) was likely to be due to chance.

In conclusion, this method demonstrated no bias in the mother-reported birth weight.

Estimating the true mean difference between mother-reported and recorded birth weight using the *t* distribution

The true mean difference between mother-reported and recorded birth weight for all pregnancies that could have been included in such a study (i.e., the population mean difference) was estimated using its 95% confidence interval. The *t* distribution was used to find this 95% confidence interval (Bland, 1995).

95% CI (population mean difference) = sample mean difference ± *t* x standard error of the sample mean difference

The sample mean difference was 63.67 g and its standard error was s.e.=75.22g (calculated as above).

The *t* value with n-1 degrees of freedom (i.e., 41) corresponding to a probability of p=0.05 is *t*=2.02 (from the table of two tailed probability points of the *t* distribution).

Hence the 95% confidence interval for the true mean difference between mother-reported and recorded birth weight in the population from which the sample was drawn was

95%CI=63.67 ± 2.02x75.22, which was between -88.27 and +215.61 g

95%CI=(-88g; +216 g)

These data showed that the mothers could tend to under-report the birth weight of their new born by as much as 88 g or to over-report it by as much as 216 g.

Malformations at birth

None of the 55 mothers providing information on the outcome of the pregnancies checked mentioned any malformation present at birth for their newborns. These data coincided with the information found in the medical records (no recorded malformations at birth), which showed 100% accurate reporting for this parameter.

Complications of delivery

This variable included the occurrence of maternal complications during delivery (e.g., uterine rupture followed by heavy blood loss and hysterectomy) and/or foetal complications (e.g., foetal distress). It excluded elective caesarean section or caesarean section performed because of previous medical conditions (e.g., previous caesarean section, pregnancy-related high blood pressure, heart disease).

The questionnaire did not ask directly for these "complications at delivery". The question closest to this was question 48, enquiring about any serious sickness before or during the last pregnancy and the answer to this question was also considered as answer for "complications at delivery".

Two out of the 55 pregnancies ended as spontaneous abortion and they were excluded from this analysis. For the remaining 53 pregnancies, there were 7 with recorded delivery-related complications and 46 without recorded delivery-related complications. None of the complications was reported by the respondent mothers in this study (0% accuracy in reporting on this parameter).

Diseases / medical problems during pregnancy

Out of the 55 pregnancy records checked, only 18 were detailed enough to provide information on medical conditions during pregnancy and these were included in the current analysis. The remaining 37 pregnancies for which no data on this parameter were gathered from the medical records were excluded from the current analysis. There were 12 pregnancies with recorded medical conditions and the questionnaire correctly identified 10 of them. There were 6 pregnancies with no recorded medical condition and the questionnaire correctly identified all of them.

The calculated sensitivity, specificity and predictive values were as follows:

Sensitivity = 83.3%

Specificity = 100%

Predictive Value Positive = 100%

Predictive Value Negative = 75%

Treatment during pregnancy

Only 18 medical records were detailed enough to provide information on medical conditions and treatment during pregnancy and these were included in the current analysis. The remaining 37 pregnancies for which no data on this parameter were gathered from the medical

records were excluded from the analysis. There were 8 pregnancies with recorded treatment and the questionnaire correctly identified 7 of them. There were 10 pregnancies with no recorded treatment and the questionnaire correctly identified all of them.

The calculated sensitivity, specificity, predictive values were as follows:

Sensitivity = 87.5%

Specificity = 100%

Predictive Value Positive = 100%

Predictive Value Negative = 90.9%

3.5. ASSESSING THE ASSOCIATION BETWEEN OCCUPATIONAL EXPOSURE TO ETHYLENE OXIDE DURING PREGNANCY AND ADVERSE REPRODUCTIVE OUTCOME

3.5.1. Classification of Exposure

Exposure to ethylene oxide was assessed indirectly, combining three sources: walk-through survey, measurements of ethylene oxide levels performed at the time of the study and questionnaire-collected information on the job held at the time of the last recognised pregnancy.

During the walk through survey, the technician (operator) was identified as the employee most exposed to ethylene oxide because this job involved working directly with the ethylene oxide steriliser. The tasks performed by the technician were loading and offloading the ethylene oxide steriliser, followed by transporting the freshly sterilised packs to the storing place.

Measurements of ethylene oxide levels performed at the time of the study showed that exposure did occur in the majority of the medical facilities sampled (9 out of 10, or 90.0%). Exposure affected the employees in charge of the steriliser (technicians or operators). The highest levels of ethylene oxide were detected by personal samples (involving all employees working with the ethylene oxide steriliser and performed while these employees were loading and offloading the steriliser) and by static samples taken on top of the steriliser (static samples placed at the door of the ethylene oxide sterilising room or in the general sterilising area detected lower levels of ethylene oxide than the ones placed on top of the steriliser). These results supported the findings of the walk through survey, showing that the employee most exposed to ethylene oxide in a sterilising unit was the technician (operator).

During questionnaire administration, information was collected on the job held at the time of the last recognised pregnancy, with details on the daily tasks involved. Based on the findings of the previous two steps, all the women who during their last pregnancy were working directly with the ethylene oxide steriliser, performing specific tasks like loading and offloading the steriliser were considered exposed to ethylene oxide.

The results of the three sources of exposure assessment lead to the classification of the pregnancies according to their exposure, as follows:

- *Exposed pregnancy*: a pregnancy occurring in a woman who was directly involved in ethylene oxide sterilisation, i.e. her main work tasks involved loading and offloading the ethylene oxide steriliser. Nineteen out of 98 pregnancies (19.4%) were classified as exposed.
- *Unexposed pregnancy*: a pregnancy occurring in a woman who was not directly involved in ethylene oxide sterilisation. Women performing the following work tasks were not considered exposed to ethylene oxide: cleaning the floor of the ethylene oxide sterilising room, cleaning the ethylene oxide steriliser, fetching sterilised packs from the storing places inside the sterilising units, getting inside the ethylene oxide sterilising room or passing in front of the ethylene oxide steriliser (if this was situated in the general sterilising area). Seventy-nine out of 98 pregnancies (80.6%) were classified as unexposed.

3.5.2. Classification of Outcome

The questionnaire collected information on a total of 109 pregnancies.
Two of them (1.8%) were multiple pregnancies, 5 (4.6%) had occurred while the mother was not employed and 4 (3.7%) were conceived before the 1st January 1992. After all these 11 pregnancies (10.1%) were excluded, 98 singleton pregnancies were left to be analysed.

The overall distribution of pregnancy outcome amongst the pregnancies included in the analysis is shown in Figure 6 and Table 15.

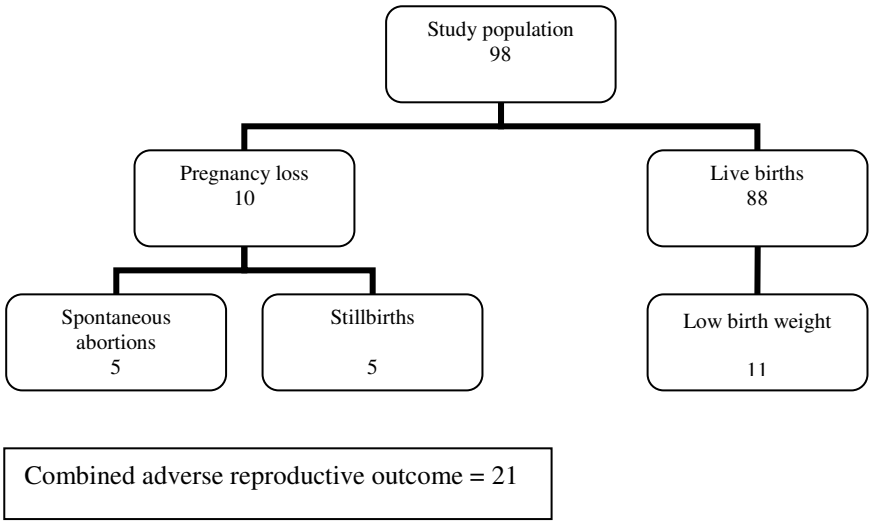

Figure 6: Distribution of outcomes amongst the study population

Five pregnancies ended as spontaneous abortion, 5 pregnancies ended as stillbirths and 88 pregnancies ended in live births.

- *Relative frequency of spontaneous abortion (SAB)* = 5.1% (5 out of the 98 pregnancies under study);
- *Relative frequency of stillbirth (SB)* = 5.1% (5 out of the 98 pregnancies under study);
- *Relative frequency of live birth* = 89.8% (88 out of the 98 pregnancies under study);
- Relative *frequency of pregnancy loss (PL)* = 10.2% (10 out of the 98 pregnancies under study);
- *Relative frequency of low birth weight (LBW)* = 12.5% (11 out of the 88 pregnancies that ended in live births);
- *Relative frequency of combined adverse reproductive outcome (CARO)* = 21.4% (21 out of the 98 pregnancies under study).

Table 15: The overall distribution of pregnancy outcome for the pregnancies included in the analysis (n=98)

Pregnancy outcome	***Number***	***%***
Spontaneous abortion		
Yes	5	5.1
No	93	94.9
Stillbirth		
Yes	5	5.1
No	93	94.9
Pregnancy loss		
Yes	10	10.2
No	88	89.8
Live birth		
Yes	88	89.8
No	10	10.2
Low birth weight*		
Yes	11	12.5
No	67	76.1
Unknown**	10	11.4
Combined adverse reproductive outcome		
Yes	21	21.4
No	67	68.4
Unknown**	10	10.2

* Low birth weight applied only to live births (n=88). Percentages were calculated using as a denominator n=88.

** Due to lack of information on birth weight for 10 pregnancies, the outcome of these pregnancies (as low birth weight or combined adverse reproductive outcome) could not be established.

3.5.3. Data Management and Analysis

3.5.3.1. Missing Data

Because of the missing data on birth weight for 10 pregnancies, the analysis was performed using the following number of subjects:

- For the association between exposure to ethylene oxide and spontaneous abortion: 98 subjects;
- For the association between exposure to ethylene oxide and stillbirths: 93 subjects;
- For the association between exposure to ethylene oxide and pregnancy loss: 98 subjects;
- For the association between exposure to ethylene oxide and low birth weight: 78 subjects;
- For the association between exposure to ethylene oxide and combined adverse reproductive outcome: 88 subjects.

3.5.3.2. Variables

Table 16 lists the main characteristics for the 98 singleton pregnancies included in the analysis.

The following characteristics were created during analysis: maternal race, maternal education, mother's age at conception, maternal age, father's age at conception, paternal age, maternal height, paternal height, parity, time between pregnancies and pre-term delivery.

Table 16: Characteristics of the total number of pregnancies included in the analysis (n=98)

Pregnancy characteristics	***Number***	***%***
Mother's age at conception		
<35 years	65	66.3
≥35 years	33	33.7
Maternal age		
Yes	33	33.7
No	65	66.3
Mother's race		
Black	87	88.8
White	7	7.1
Coloured	4	4.1
Maternal race		
Yes	91	92.9
No	7	7.1
Mother's education		
No formal education	1	1.0
Primary school	2	2.0
High school	84	85.7
Higher education (college)	11	11.3
Maternal education		
Yes	3	3.1
No	95	96.9
Mother's height		
Short	24	24.5
Medium	48	49.0
Tall	26	26.5
Maternal height		
Yes	24	24.5
No	74	75.5
Father's age at conception		
<40 years	68	69.4
≥40 years	30	30.6
Paternal age		
Yes	30	30.6
No	68	69.4

Pregnancy characteristics	***Number***	***%***
Father's height		
Short	12	12.2
Medium	49	50.0
Tall	37	37.8
Paternal height		
Yes	12	12.2
No	86	87.8
Pregnancy rank		
1	10	10.2
2-4	72	73.5
≥5	16	16.3
Parity		
Yes	26	26.5
No	72	73.5
Time since the previous pregnancy*		
1 year	8	9.1
> 1 year	80	90.9
Time between pregnancies*		
Yes	8	9.1
No	80	90.9
Antenatal care**		
Yes	89	95.7
No	4	4.3
Gestation length (assessed by time of delivery)**		
Pre-term delivery	11	11.8
Term delivery	77	82.8
Post-term delivery	5	5.4
Pre-term delivery		
Yes	11	11.8
No	82	88.2
Diabetes mellitus		
Yes	4	4.1
No	94	95.9
Other medical conditions		
Yes	8	8.2
No	90	91.8
Treatment		
Yes	24	24.5
No	74	75.5
Maternal smoking		
Yes	1	1.0
No	97	99.0
Passive smoking		
Yes	34	34.7
No	64	65.3

Pregnancy characteristics	*Number*	*%*
Maternal exposure to carbon monoxide		
Yes	23	23.5
No	75	76.5
Maternal drinking		
Yes	4	4.1
No	94	95.9
Maternal high blood pressure		
Yes	19	19.4
No	79	80.6
History of pregnancy loss		
Yes	27	27.6
No	71	72.4
Maternal occupational exposure to ionising radiation		
Yes	8	8.2
No	90	91.8
Maternal occupational exposure to anaesthetics		
Yes	16	16.3
No	82	83.7
Maternal occupational exposure to excessive physical activity		
Yes	77	78.6
No	21	21.4
Shift work during pregnancy		
Yes	46	46.9
No	52	53.1
Maternal exposure to emotional stress		
Yes	18	18.4
No	80	81.6
Maternal exposure to noise		
Yes	11	11.2
No	87	88.8

*Time since the previous pregnancy and time between pregnancies applied only to pregnancies with rank 2 or above (n=88). Relative frequencies were calculated using as denominator n=88.

**The five pregnancies that ended in spontaneous abortion were excluded from this calculation. Percentages were calculated using as denominator n=93.

- *Mother's age at conception*: variable measured on a metric scale and computed from the mother's date of birth and the date of the reproductive event (date of spontaneous abortion or date of child birth): first, date of conception was calculated subtracting the estimated gestation length (in days) from the date of the reproductive event of interest. Gestation length was considered 140 days (20 weeks) for pregnancies ending in spontaneous

abortion and 266 days (length of gestation from conception) for pregnancies that had advanced beyond 20 weeks of gestation (Beers and Berkow, 1999). Then mother's age at conception was calculated subtracting the mother's date of birth from the calculated date of conception and dividing it by 365.25 days (the average length of a year). The 98 pregnancies occurred in women aged between 19 and 42 years, with a mean age=32.5 years (median=33). Sixty-five mothers (66.3%) were younger than 35 years of age, while 33 of them (33.7 %) were aged 35 and older.

- *Maternal age*: variable measured on a dichotomous scale (Yes = mother 35 years or more at the time of conception; No = mother younger than 35 years at the time of conception). Thirty-three out of 98 pregnancies (33.7%) were classified as "Yes" and the remaining 66 (66.3%) were classified as "No".
- *Mother's race*: variable measured on a categorical scale with the following categories: black, white, coloured (mixed race), or Asian. Eighty-seven of the 98 mothers (88.8%) were black, 7 (7.1%) were white and 4 (4.1%) were coloured. There were no Asian women amongst the study participants.
- *Maternal race*: variable measured on a dichotomous scale (Yes = black or coloured mother; No = white or Asian mother). Ninety-one out of 98 pregnancies (92.9%) were classified as "Yes" and the remaining 7 were classified as "No" (7.1%).
- *Mother's education*: variable measured on a categorical scale with the following categories: no school, primary school, high school without matric, high school with matric, or college/university. The majority of mothers (84 out of 98, or 85.7%) were high school graduates (with or without matric) and 11 mothers (11.3%) were college graduates. Two mothers (2.0%) had only graduated primary school and one mother (1.0%) did not have any schooling.
- *Maternal education*: variable measured on a dichotomous scale (Yes = mother with no schooling or mother who had graduated only primary school; No = mother who had graduated high school or college). Three out of 98 pregnancies (3.1%) were classified as "Yes" and the remaining 95 (96.9%) were classified as "No".
- *Mother's height*: variable measured on a categorical scale with the following categories: short, medium or tall (self-reported). Twenty-four of the 98 women (24.5%) considered themselves short, 48 (49.0%) reported a medium height and 26 (26.5%) reported being tall.

- *Maternal height*: variable measured on a dichotomous scale (Yes = short mother; No = mother with medium height or tall). Twenty-four out of 98 pregnancies (24.5%) were classified as "Yes" and the remaining 74 (75.5%) were classified as "No".
- *Father's age at conception*: variable measured on a metric scale and computed from the father's age at the time of the study and the calculated date of conception: first the interval between the time of the study and the date of conception was calculated and this figure was then subtracted from the father's age at the time of the study. The men who fathered the 98 pregnancies were aged between 27 and 64 years, with a mean age=42 years (median=41). Sixty-eight men (69.4%) were younger than 40, while thirty (30.6%) were 40 years of age or older.
- *Paternal age*: variable measured on a dichotomous scale (Yes = father 40 years or more at the time of conception; No = father younger than 40 years at the time of conception. Thirty out of 98 pregnancies (30.6%) were classified as "Yes" and the remaining 68 (69.4%) were classified as "No".
- *Father's height*: variable measured on a categorical scale with the following categories: short, medium or tall (as reported by the woman). Twelve out of 98 men (12.2%) were short, 49 (50.0%) had medium height and 37 (37.8%) were considered tall by their partners.
- *Paternal height*: variable measured on a dichotomous scale (Yes = short father; No = father with medium height or tall). Twelve out of 98 pregnancies (12.2%) were classified as "Yes" and the remaining 86 (87.8%) were classified as "No".
- *Pregnancy rank*: variable measured on a metric scale (the women was asked to state the number of pregnancies she had had before the last recognised pregnancy; then one was added to the figure reported by the woman to obtain the rank of the last recognised pregnancy). Pregnancy rank ranged from 1 to 7, with a mean rank=3 (median=3). Ten pregnancies (10.2%) had rank 1, i.e., they were the first pregnancy occurring to the woman. There were 72 pregnancies (73.5%) with the rank between 2 and 4 and 16 pregnancies (16.3) with rank 5 or more.
- *Parity*: variable measured on a dichotomous scale (Yes = pregnancy rank 1 or above 4; No = pregnancy rank 2, 3 or 4. Twenty-six out of 98 pregnancies (26.5%) were classified as "Yes" and the remaining 72 (73.5%) were classified as "No".
- *Time since the previous pregnancy*: variable measured on a metric scale and defined as the number of years between the pregnancy under study and the previous pregnancy). It applied only to the 88 pregnancies with rank 2 or above and it ranged from 1 to 19 years,

with a mean=5.8 years (median=5 years). There were 8 pregnancies out of 88 (9.1%) that had occurred 1 year after the previous pregnancy. The remaining 80 pregnancies (90.9%) had occurred at more than 1 year after the previous pregnancy.

- *Time between pregnancies*: variable measured on a dichotomous scale (Yes = last recognised pregnancy occurred in approximately one year after the previous one; No = last recognised pregnancy occurred more than one year after the previous one. Eight out of 88 pregnancies (9.1%) were classified as "Yes" and the remaining 80 were classified as "No".
- *Mother's weight at the beginning of pregnancy and/or weight gain during pregnancy*: variables measured on a metric scale. Most of the respondent women did not answer these questions and therefore these variables were not included in the analysis.
- *Antenatal care*: variable measured on a dichotomous scale (Yes or No). The 5 pregnancies that ended as spontaneous abortion were excluded from this calculation (they ended before 20 weeks of gestation and therefore before the time the antenatal care would have started). Eighty-nine out of the remaining 93 pregnancies (95.7%) received antenatal care compared to 4 pregnancies (4.3%) that did not received antenatal care. For the 89 pregnancies receiving antenatal care, this started at various times during gestation, ranging from 1 to 9 months. The mean gestational age at the beginning of the antenatal care programme was 3.7 months (median=3 months).
- *Gestation length*: variable measured on a metric scale (in months) and on a categorical scale with following categories: pre-term delivery, term delivery, or postterm delivery. Gestation length ranged from 1 to 10.5 months, with a mean length of 8.5 months (median=9 months). When the 93 pregnancies advancing beyond 20 weeks were classified according to the time of delivery, the distribution was as follows: 11 pre-term deliveries (11.8%), 77 term deliveries (82.8%) and 5 postterm deliveries (5.4%).
- *Pre-term delivery*: variable measured on a dichotomous scale (Yes = delivery before term; No = delivery at term or after the confinement date). It applied only to the 93 pregnancies advancing beyond 20 weeks of gestation and it was reported in 11 out of the 93 pregnancies (11.8%). The remaining 82 pregnancies (88.2%) were delivered either at term or after the confinement date.
- *Diabetes mellitus*: variable measured on a dichotomous scale (Yes or No) and defined as presence of the disease at any time during the last recognised pregnancy. Diabetes mellitus was reported in 4 out of 98 pregnancies (4.1%).

- *Other medical conditions during pregnancy*: variable measured on a dichotomous scale (Yes or No) and defined as any medical condition present at any time during the last recognised pregnancy. They were reported in 8 out of 98 pregnancies (8.2%) and they were represented by depression, epilepsy, respiratory disorders, cardiac disorders, hepatitis A and vaginal infection.
- *Treatment during pregnancy*: variable measured on a dichotomous scale (Yes or No) and defined as any treatment received at any time during the last recognised pregnancy. It was reported in 24 of 98 pregnancies (24.5%) and it consisted of antihypertensive drugs, pain killers, antidepressant drugs, antiepileptic drugs, oxygen and topical antibiotics.
- *Maternal smoking*: variable measured on a dichotomous scale (Yes or No) and defined as mother smoking at any time during the last recognised pregnancy, regardless of the number of cigarettes a day. It was reported in only one of the 98 pregnancies analysed (1.0%).
- *Passive smoking*: variable measured on a dichotomous scale (Yes or No) and defined as partner smoking at home while the woman was pregnant, regardless of the number of cigarettes a day. It was reported in 34 of the 98 pregnancies (34.7%).
- *Maternal exposure to carbon monoxide other than due to smoking*: variable measured on a dichotomous scale (Yes or No) and defined as mother living in a house where gas, wood, charcoal or paraffin heating was used during the last recognised pregnancy. It was reported in 23 out of 98 pregnancies (23.5%).
- *Maternal drinking*: variable measured on a dichotomous scale (Yes or No) and defined as maternal alcohol consumption at any time during the last recognised pregnancy, no matter the quantity. It was reported in 4 out of 98 pregnancies (4.1%).
- *Maternal high blood pressure*: variable measured on dichotomous scale (Yes or No) and defined as presence of maternal high blood pressure at any time during the last recognised pregnancy. It was reported in 19 out of 98 pregnancies (19.4%).
- *History of pregnancy loss*: variable measured on a dichotomous scale (Yes or No) and defined as history of spontaneous abortion, ectopic pregnancy or stillbirth. It was positive in 27 of the 98 pregnancies analysed (27.6%).
- *Maternal exposure to ionising radiation*: variable measured on a dichotomous scale (Yes or No) and defined as maternal occupational exposure to ionising radiation at any time during the last recognised pregnancy. It was positive in 8 out of 98 pregnancies (8.2%). Women reporting occupational exposure to ionising radiation during the last recognised pregnancy also reported using protective equipment (lead aprons).

- *Maternal exposure to anaesthetics*: variable measured on a dichotomous scale (Yes or No) and defined as maternal occupational exposure to anaesthetics at any time during the last recognised pregnancy. It was positive in 16 out of 98 pregnancies (16.3%).
- *Maternal exposure to excessive physical activity*: variable measured on a dichotomous scale (Yes or No) and defined as mother being exposed to one of the following: bending for more than one hour a day, standing for more than six hours a day or heavy lifting during the last recognised pregnancy. It was positive for 77 out of 98 pregnancies (78.6%).
- *Shift work during pregnancy*: variable measured on a dichotomous scale (Yes or No) and defined as mother working either day and night shifts or only night shifts during the last recognised pregnancy. It was positive for 46 out of 98 pregnancies (46.9%).
- *Maternal exposure to emotional stress*: variable measured on a dichotomous scale (Yes or No) and defined qualitatively, as perceived by the mother. It was reported in 18 out of 98 pregnancies (18.4%).
- *Maternal exposure to noise*: variable measured on a dichotomous scale (Yes or No) and defined qualitatively, as perceived by the mother. It was reported in 11 out of 98 pregnancies (11.2%).

3.5.3.3. Measuring Associations

Table 17 lists the outcome of the analysed pregnancies according to their ethylene oxide exposure status.

Table 17: Outcome of exposed versus unexposed pregnancies and measure of association (risk ratio, RR) between exposure to ethylene oxide and adverse reproductive outcomes

Outcome	***Pregnancies exposed to ethylene oxide (n=19)***		***Pregnancies unexposed to ethylene oxide (n=79)***		***Risk ratio (RR)***
	Number	***%***	***Number***	***%***	
Spontaneous abortion	4/19	21.1	1/79	1.3	16.63
Stillbirth*	2/15	13.3	3/78	3.8	3.47
Pregnancy loss	6/19	31.6	4/79	5.1	6.24
Low birth weight**	1/11	9.1	10/67	14.9	0.61
Combined adverse reproductive outcome***	7/17	41.2	14/71	19.7	2.09

*Relative frequencies of stillbirths and live births were calculated using as denominator the total number of births (stillbirths plus live births), n= 93 (15 exposed and 78 unexposed).

**Relative frequency of low birth weight was calculated only for the 78 pregnancies that ended in live births on which there were complete birth weight data (11 exposed and 67 unexposed).

*** Relative frequency of combined adverse reproductive outcome was calculated only for the 88 pregnancies for which there were complete birth weight data (17 exposed and 71 unexposed).

- *Exposure to ethylene oxide and spontaneous abortion*

Overall, 5 out of the 98 pregnancies under study (5.1%) ended with spontaneous abortion.

The relative frequency of spontaneous abortions was higher amongst the exposed pregnancies compared to unexposed pregnancies (see Table 18): 21.1% of the pregnancies exposed to ethylene oxide ended as spontaneous abortion compared to only 1.3% of the pregnancies unexposed to ethylene oxide.

The crude risk ratio was RR=16.63 (95%CI=1.97-140.42) and the association was statistically significant (Fischer exact p=0.004).

Table 18: Spontaneous abortion by exposure to ethylene oxide

	Spontaneous abortion Yes	Spontaneous abortion No	Total
Ethylene oxide exposure Yes (%)	4 (21.1)	15 (78.9)	19 (100.0)
Ethylene oxide exposure No (%)	1 (1.3)	78 (98.7)	79 (100.0)
Total	5	93	98

- *Exposure to ethylene oxide and stillbirth*

Overall, 5 out of the 98 pregnancies under study (5.1%) ended with stillbirth.

The frequency of stillbirth was higher in the exposed pregnancies than in the unexposed (see Table 19): 13.3% of the pregnancies exposed to ethylene oxide ended as stillbirth compared to 3.8% of the pregnancies unexposed to ethylene oxide.

The crude risk ratio was RR=3.47 (95%CI=0.63-19.01) and the association was not statistically significant (Fischer exact p=0.18).

Table 19: Stillbirth by exposure to ethylene oxide

	Stillbirth Yes	Stillbirth No	Total
Ethylene oxide exposure Yes (%)	2 (13.3)	13 (86.7)	15 (100.0)
Ethylene oxide exposure No (%)	3 (3.8)	75 (96.2)	78 (100.0)
Total	5	88	93

- *Exposure to ethylene oxide and pregnancy loss*

Overall, 10 out of 98 pregnancies under study (10.2%) ended as pregnancy loss.

There was an increased relative frequency of pregnancy loss amongst the exposed pregnancies compared to the unexposed (see Table 20): 31.6% of the pregnancies exposed to ethylene oxide ended as pregnancy loss compared to only 5.1% of the pregnancies unexposed to ethylene oxide.

The crude risk ratio was RR=6.24 (95%CI=1.95-19.93) and the association was statistically significant (Fischer exact p=0.003).

Table 20: Pregnancy loss by exposure to ethylene oxide

	Pregnancy loss Yes	Pregnancy loss No	Total
Ethylene oxide exposure Yes (%)	6 (31.6)	13 (68.4)	19 (100.0)
Ethylene oxide exposure No (%)	4 (5.1)	75 (94.9)	79 (100.0)
Total	10	88	98

- *Exposure to ethylene oxide and low birth weight*

Overall, 11 out of the 88 live births under study (12.5%) were low birth weight newborns.

The relative frequency of low birth weight amongst exposed pregnancies was lower than amongst unexposed (see Table 21): 9.1% of the pregnancies exposed to ethylene oxide ended with a low birth weight child compared to 14.9% of the pregnancies unexposed to ethylene oxide.

The crude risk ratio was RR=0.61 (95%CI=0.09-4.30) and the association was not statistically significant (Fischer exact p=0.51).

Table 21: Low birth weight by exposure to ethylene oxide

	Low birth weight Yes	Low birth weight No	Total
Ethylene oxide exposure Yes (%)	1 (9.1)	10 (90.9)	11 (100.0)
Ethylene oxide exposure No (%)	10 (14.9)	57 (85.1)	67 (100.0)
Total	11	67	78

- *Exposure to ethylene oxide and combined adverse reproductive outcome*

Overall, 21 out of the 98 pregnancies under study (21.4%) ended with a combined adverse reproductive outcome.

The relative frequency of combined adverse reproductive outcome amongst exposed was higher than amongst unexposed pregnancies (see Table 22): 41.2% of the pregnancies exposed to ethylene oxide ended with a combined adverse reproductive outcome compared to 19.7% of the pregnancies unexposed to ethylene oxide.

The crude risk ratio was RR=2.09 (95%CI=1.00-4.36) and the association was not statistically significant (Fischer exact p=0.06).

Table 22: Combined adverse reproductive outcome by exposure to ethylene oxide

	Combined adverse reproductive outcome Yes	Combined adverse reproductive outcome No	Total
Ethylene oxide exposure Yes (%)	7 (41.2)	10 (58.8)	17 (100.0)
Ethylene oxide exposure No (%)	14 (19.7)	57 (80.3)	71 (100.0)
Total	21	67	88

3.5.3.4. Identifying Confounders

1. Identifying the variables associated with the exposure to ethylene oxide

Possible associations between all the pregnancy characteristics described above and exposure to ethylene oxide were assessed. In order to select the variables associated with exposure to ethylene oxide, a level of statistical significance of p=0.20 was used.

Tables 22-46 show the ethylene oxide exposure status by the pregnancies characteristics included in the analysis. Variables (pregnancy characteristics) that show a positive association with exposure to ethylene oxide at this level of statistical significance (e.g., there is a greater frequency of pregnancies exposed to ethylene oxide in the group of pregnancies having the characteristic) could artificially induce an association between exposure to ethylene oxide and adverse reproductive outcome, should these variables be also associated with the outcome. Variables that show a negative association with exposure to ethylene oxide at this level of statistical significance (e.g., there is a lower frequency of pregnancies exposed to ethylene oxide in the group of pregnancies having the characteristic) could mask an existing association between exposure to ethylene oxide and adverse reproductive outcome, should these variables be also associated with the outcome.

The distribution of exposure to ethylene oxide was slightly different between the two groups of maternal age (see Table 23): 21.2% of the pregnancies occurring in women aged 35 years or older were exposed to ethylene oxide, compared to 18.5% of the pregnancies occurring in women younger than 35 years, but the difference was not statistically significant (chi-square Mantel-Haenszel p=0.74).

Table 23: Exposure to ethylene oxide by maternal age

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal age Yes (%)	7 (21.2)	26 (78.8)	33 (100.0)
Maternal age No (%)	12 (18.5)	53 (81.5)	65 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal race (see Table 24): 18.7% of the pregnancies occurring in black or coloured women

were exposed to ethylene oxide, compared to 28.6% of the pregnancies occurring in white women, but the difference was not statistically significant (Fischer exact p=0.40).

Table 24: Exposure to ethylene oxide by maternal race

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal race Yes (%)	17 (18.7)	74 (81.3)	91 (100.0)
Maternal race No (%)	2 (28.6)	5 (71.4)	7 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal education (see Table 25): none (0.0%) of the pregnancies occurring in mothers without schooling or with primary school only was exposed to ethylene oxide compared to 20.0% of the pregnancies occurring in mothers with higher education, but the difference was not statistically significant (Fischer exact p=0.51).

Table 25: Exposure to ethylene oxide by maternal education

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal education Yes (%)	0 (0.0)	3 (100.0)	3 (100.0)
Maternal education No (%)	19 (20.0)	76 (80.0)	95 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal height (see Table 26): 4.2% of the pregnancies occurring in short mothers were exposed to ethylene oxide, compared to 24.3% of the pregnancies occurring in mothers with medium height or tall and the difference was statistically significant (Fischer exact p=0.02). The lower frequency of pregnancies exposed to ethylene oxide in the group of pregnancies occurring in short mothers might mask an association between exposure to ethylene oxide and

adverse reproductive outcome, should this variable (i.e., maternal height) be also associated with the outcome.

Table 26: Exposure to ethylene oxide by maternal height

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal height Yes (%)	1 (4.2)	23 (95.8)	24 (100.0)
Maternal height No (%)	18 (24.3)	56 (75.7)	74 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of paternal age (see Table 27): 26.7% of the pregnancies fathered by men 40 years of age or older were exposed to ethylene oxide, compared to 16.2% for the pregnancies fathered by men younger than 40, but the difference was not statistically significant (chi-square Mantel-Haenszel p=0.22).

Table 27: Exposure to ethylene oxide by paternal age

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Paternal age Yes (%)	8 (26.7)	22 (73.3)	30 (100.0)
Paternal age No (%)	11 (16.2)	57 (83.8)	68 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of paternal height (see Table 28): 25.0% of the pregnancies fathered by short men were exposed to ethylene oxide compared to 18.6% of the pregnancies fathered by men with medium height or tall, but the difference was not statistically significant (Fischer exact p=0.42).

Table 28: Exposure to ethylene oxide by paternal height

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Paternal height Yes (%)	3 (25.0)	9 (75.0)	12 (100.0)
Paternal height No (%)	16 (18.6)	70 (81.4)	86 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was similar between the two groups of parity (see Table 29): 19.2% of the pregnancies with rank 1 or >4 were exposed to ethylene oxide compared to 19.4% of the pregnancies with rank 2, 3 or 4 (chi-square Mantel-Haenszel p=0.98).

Table 29: Exposure to ethylene oxide by parity

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Parity Yes (%)	5 (19.2)	21 (80.8)	26 (100.0)
Parity No (%)	14 (19.4)	58 (80.6)	72 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of time between pregnancies (see Table 30): 12.5% of the pregnancies occurring one year after the previous pregnancy were exposed to ethylene oxide compared to 22.5% of the pregnancies occurring more than 1 year after the previous pregnancy, but the association was not statistically significant (Fischer exact p=0.44).

*Table 30: Exposure to ethylene oxide by time between pregnancies**

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Time between pregnancies Yes (%)	1 (12.5)	7 (87.5)	8 (100.0)
Time between pregnancies No (%)	18 (22.5)	62 (77.5)	80 (100.0)
Total	19	69	88

*This variable applies only to the 88 pregnancies with rank ≥2.

The distribution of exposure to ethylene oxide was different between the two groups of antenatal care (see Table 31): only 14.6% of the pregnancies that had received antenatal care were exposed to ethylene oxide compared to 50.0% of the pregnancies that had not received antenatal care and the difference was statistically significant (Fischer exact p=0.12). The higher frequency of pregnancies exposed to ethylene oxide in the group of pregnancies that had not received antenatal care might artificially induce an association between exposure to ethylene oxide and adverse reproductive outcome, should this variable (i.e., antenatal care or rather the lack of it) be also associated with the outcome.

Table 31: Exposure to ethylene oxide by antenatal care

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Antenatal care Yes (%)	13 (14.6)	76 (85.4)	89 (100.0)
Antenatal care No (%)	2 (50.0)	2 (50.0)	4 (100.0)
Total	15	78	93

The distribution of exposure to ethylene oxide was slightly different between the two groups of pre-term delivery (see Table 32): 18.2% of the pregnancies that had ended with a pre-term delivery were exposed to ethylene oxide compared to 15.9% of the pregnancies that had been

delivered at term or after the confinement date, but the difference was not statistically significant (Fischer exact p=0.56).

Table 32: Exposure to ethylene oxide by pre-term delivery

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Pre-term deliveries Yes (%)	2 (18.2)	9 (81.8)	11 (100.0)
Pre-term deliveries No (%)	13 (15.9)	69 (84.1)	82 (100.0)
Total	15	78	93

The distribution of exposure to ethylene oxide was different between the two groups of diabetes mellitus (see Table 33): none (0.0%) of the pregnancies whose mothers had reported diabetes mellitus were exposed to ethylene oxide compared to 20.2% of the pregnancies whose mothers had not reported diabetes mellitus, but the difference was not statistically significant (Fischer exact p=0.41).

Table 33: Exposure to ethylene oxide by diabetes mellitus during pregnancy

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Diabetes mellitus Yes (%)	0 (0.0)	4 (100.0)	4 (100.0)
Diabetes mellitus No (%)	19 (20.2)	75 (79.8)	94 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of other medical conditions during pregnancy (see Table 34): 12.5% of the pregnancies whose mothers had reported other medical conditions during pregnancy were exposed to ethylene oxide compared to 20.0% of the pregnancies whose mothers had not reported other medical conditions during pregnancy, but the difference was not statistically significant (Fischer exact p=0.51).

Table 34: Exposure to ethylene oxide by other medical conditions during pregnancy

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Other medical conditions Yes (%)	1 (12.5)	7 (87.5	8 (100.0)
Other medical conditions No (%)	18 (20.0)	72 (80.0)	90 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was slightly different between the two groups of treatment received during pregnancy (see Table 35): 16.7% of the pregnancies whose mothers had reported receiving treatment during pregnancy were exposed to ethylene oxide compared to 20.3% of the pregnancies mothers had not reported receiving treatment during pregnancy, but the difference was not statistically significant (Fischer exact p=0.47).

Table 35: Exposure to ethylene oxide by treatment received during pregnancy

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Treatment received Yes (%)	4 (16.7)	20 (83.3)	24 (100.0)
Treatment received No (%)	15 (20.3)	59 (79.7)	74 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was slightly different between the two groups of passive smoking (see Table 36): 20.6% of the pregnancies exposed to passive smoking were exposed to ethylene oxide compared to 18.8% of the pregnancies not exposed to passive smoking, but the difference was not statistically significant (chi-square Mantel-Haenszel p=0.82).

Table 36: Exposure to ethylene oxide by passive smoking

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Passive smoking Yes (%)	7 (20.6)	27 (79.4)	34 (100.0)
Passive smoking No (%)	12 (18.8)	52 (81.2)	64 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of exposure to carbon monoxide other than due to smoking (see Table 37): 26.1% of the pregnancies that had been exposed to carbon monoxide were exposed to ethylene oxide compared to 17.3% of the pregnancies not exposed to carbon monoxide, but the difference was not statistically significant (Fischer exact p=0.25).

Table 37: Exposure to ethylene oxide by exposure to carbon monoxide

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Exposure to carbon monoxide Yes (%)	6 (26.1)	17 (73.9)	23 (100.0)
Exposure to carbon monoxide No (%)	13 (17.3)	62 (82.7)	75 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal drinking (see Table 38): none (0.0%) of the pregnancies whose mothers had reported drinking during pregnancy were exposed to ethylene oxide compared to 20.2 of the pregnancies whose mothers had not reported drinking during pregnancy, but the difference was not statistically significant (Fischer exact p=0.41).

Table 38: Exposure to ethylene oxide by maternal drinking

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal drinking Yes (%)	0 (0.0)	4 (100.0)	4 (100.0)
Maternal drinking No (%)	19 (20.2)	75 (79.8)	94 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal high blood pressure (see Table 39): 15.8% of the pregnancies whose mothers had reported high blood pressure during pregnancy were exposed to ethylene oxide compared to 20.3% of the pregnancies whose mothers had not reported high blood pressure during pregnancy, but the difference was not statistically significant (Fischer exact p=0.46).

Table 39: Exposure to ethylene oxide by maternal high blood pressure (HBP)

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal HBP Yes (%)	3 (15.8)	16 (84.2)	19 (100.0)
Maternal HBP No (%)	16 (20.3)	63 (79.7)	79 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of history of pregnancy loss (see Table 40): 14.8% of the pregnancies that had been preceded by a pregnancy loss were exposed to ethylene oxide compared to 21.1% of the pregnancies that had not been preceded by pregnancy loss, but the difference was not statistically significant (chi-square Mantel-Haenszel p=0.48).

Table 40: Exposure to ethylene oxide by history of pregnancy loss

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
History of pregnancy loss Yes (%)	4 (14.8)	23 (85.2)	27 (100.0)
History of pregnancy loss No (%)	15 (21.1)	56 (78.9)	71 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal exposure to ionising radiation (see Table 41): 25.0% of the pregnancies that had been exposed to ionising radiation were exposed to ethylene oxide compared to 18.9% of the pregnancies that had not been exposed to ionising radiation, but the difference was not statistically significant (Fischer exact p=0.48).

Table 41: Exposure to ethylene oxide by maternal exposure to ionising radiation (X-rays)

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal exposure to X-rays Yes (%)	2 (25.0)	6 (75.0)	8 (100.0)
Maternal exposure to X-rays No (%)	17 (18.9)	73 (81.1)	90 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal exposure to anaesthetics (see Table 42): 25.0% of the pregnancies that had been exposed to anaesthetics were exposed to ethylene oxide compared to 18.3% of the pregnancies that had not been exposed to anaesthetics, but the difference was not statistically significant (Fischer exact p=0.37).

Table 42: Exposure to ethylene oxide by maternal exposure to anaesthetics

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal exposure to anaesthetics Yes (%)	4 (25.0)	12 (75.0)	16 (100.0)
Maternal exposure to anaesthetics No (%)	15 (18.3)	67 (81.7)	82 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was similar between the two groups of maternal exposure to excessive physical activity (see Table 43): 19.5% of the pregnancies whose mothers had reported excessive physical activity during pregnancy were exposed to ethylene oxide compared to 19.0% of the pregnancies whose mothers had not reported excessive physical activity during pregnancy (Fischer exact p=0.61).

Table 43: Exposure to ethylene oxide by maternal exposure to excessive physical activity

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal exposure to excessive physical activity Yes (%)	15 (19.5)	62 (80.5)	77 (100.0)
Maternal exposure to excessive physical activity No (%)	4 (19.0)	17 (81.0)	21(100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was similar between the two groups of shift work (see Table 44): 19.6% of the pregnancies whose mothers had reported shift work during pregnancy were exposed to ethylene oxide compared to 19.2% of the pregnancies whose mothers had not reported shift work during pregnancy (chi-square Mantel-Haenszel p=0.96).

Table 44: Exposure to ethylene oxide by shift work

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Shift work during pregnancy Yes (%)	9 (19.6)	37 (80.4)	46 (100.0)
Shift work during pregnancy No (%)	10 (19.2)	42 (80.8)	52 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was different between the two groups of maternal exposure to emotional stress (see Table 45): 33.3% of the pregnancies whose mothers reported exposure to emotional stress were exposed to ethylene oxide compared to 16.3% of the pregnancies whose mothers had not reported exposure to emotional stress and the difference was statistically significant (Fischer exact =0.09). The higher frequency of pregnancies exposed to ethylene oxide in the group of pregnancies occurring in mothers exposed to emotional stress might artificially induce an association between exposure to ethylene oxide and adverse reproductive outcome, should this variable (i.e., maternal exposure to emotional stress) be also associated with the outcome.

Table 45: Exposure to ethylene oxide by maternal exposure to emotional stress

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal exposure to emotional stress Yes (%)	6 (33.3)	12 (66.7)	18 (100.0)
Maternal exposure to emotional stress No (%)	13 (16.3)	67 (83.7)	80 (100.0)
Total	19	79	98

The distribution of exposure to ethylene oxide was similar between the two groups of maternal exposure to noise (see Table 46): 18.2% of the pregnancies whose mothers had reported exposure to noise during pregnancy were exposed to ethylene oxide compared to 18.5% of the pregnancies whose mothers had not reported exposure to noise during pregnancy (Fischer exact p=0.63).

Table 46: Exposure to ethylene oxide by maternal exposure to noise

	Ethylene oxide exposure Yes	Ethylene oxide exposure No	Total
Maternal exposure to noise Yes (%)	2 (18.2)	9 (81.8)	11 (100.0)
Maternal exposure to noise No (%)	17 (18.5)	70 (80.5)	87(100.0)
Total	19	79	98

Table 47 summarises the results of the statistical testing (expressed as p-values) for the association between variables and exposure to ethylene oxide.

Table 47: Results of the statistical testing of the association between variables and exposure to ethylene oxide (expressed as p-*value)*

Variable	**p-*value***
Maternal race	0.40*
Maternal education	0.51*
Maternal age	0.74**
Paternal age	0.22**
Maternal height	0.02*
Paternal height	0.42*
Parity	0.98**
Time between pregnancies	0.44*
Antenatal care	0.12*
Pre-term delivery	0.56*

Diabetes mellitus	0.41*
Other medical conditions	0.51*
Treatment	0.47*
Passive smoking	0.82**
Maternal exposure to carbon monoxide	0.25*
Maternal drinking	0.41*
Maternal high blood pressure	0.46*
History of pregnancy loss	0.48**
Maternal occupational exposure to ionising radiation	0.40*
Maternal occupational exposure to anaesthetics	0.37*
Maternal occupational exposure to excessive physical activity	0.61*
Shift work	0.96**
Maternal exposure to emotional stress	0.09*
Maternal exposure to noise	0.63*

*Fischer exact p-value; **chi-square Mantel-Haenszel p-value.

Using these results the following variables were found to be associated with exposure (at a level of statistical significance of p=0.20): maternal height (p=0.02); antenatal care (p=0.12); and maternal exposure to emotional stress (p=0.09).

2. Identifying the variables associated with the five adverse reproductive outcomes

Next, statistical testing of the association between the variables shown to be associated with exposure (maternal height, antenatal care and maternal exposure to emotional stress) and the five outcomes studied was performed (Tables 47-59). The level of statistical significance was also set at p=0.20.

The distribution of spontaneous abortion was different between the two groups of maternal height (see Table 48): none (0.0%) of the pregnancies occurring in short women ended with spontaneous abortion compared to 6.8% of the pregnancies occurring in women with medium height or tall, but the difference was not statistically significant (Fischer exact p=0.23).

Table 48: Spontaneous abortion by maternal height

	Spontaneous abortion Yes	Spontaneous abortion No	Total
Maternal height Yes (%)	0 (0.0)	24 (10.0)	24 (100.0)
Maternal height No (%)	5 (6.8)	69 (93.2)	74 (100.0)
Total	5	93	98

The distribution of stillbirth was slightly different between the two groups of maternal height (see Table 49): 8.3% of the pregnancies occurring in short women ended with stillbirth compared to 4.3% of the pregnancies occurring in women with medium height or tall, but the difference was not statistically significant (Fischer exact p=0.38).

*Table 49: Stillbirth by maternal height**

	Stillbirth Yes	Stillbirth No	Total
Maternal height Yes (%)	2 (8.3)	22 (91.7)	24 (100.0)
Maternal height No (%)	3 (4.3)	66 (95.7)	69(100.0)
Total	5	88	93

*The five pregnancies that ended as spontaneous abortion were excluded from this analysis.

The distribution of pregnancy loss was slightly different between the two groups of maternal height (see Table 50): 8.3% of the pregnancies occurring in short women ended with pregnancy loss compared to 10.8% of the pregnancies occurring in women with medium height or tall, but the difference was not statistically significant (Fischer exact p=0.53).

Table 50: Pregnancy loss by maternal height

	Pregnancy loss Yes	Pregnancy loss No	Total
Maternal height Yes (%)	2 (8.3)	22 (91.7)	24 (100.0)
Maternal height No (%)	8 (10.8)	66 (89.2)	74 (100.0)
Total	10	88	98

The distribution of low birth weight was different between the two groups of maternal height (see Table 51): 9.5% of the pregnancies occurring in short women ended with a low birth weight baby compared to 15.8% of the pregnancies occurring in women with medium height or tall, but the difference was not statistically significant (Fischer exact p=0.38).

*Table 51: Low birth weight by maternal height**

	Low birth weight Yes (%)	Low birth weight No	Total
Maternal height Yes (%)	2 (9.5)	19 (90.5)	21(100.0)
Maternal height No (%)	9 (15.8)	48 (84.2)	57 (100.0)
Total	11	67	78

*This analysis applied only to pregnancies that ended in live birth (n=88). The ten pregnancies whose outcome was a live born with unknown birth weight have been excluded from the analysis.

The distribution of combined adverse reproductive outcome was different between the two groups of maternal height (see Table 52): 17.4% of the pregnancies occurring in short women ended with a combined adverse reproductive outcome compared to 26.2% of the pregnancies occurring in women with medium height or tall, but the difference was not statistically significant (chi-square Mantel-Haenszel p=0.39).

*Table 52: Combined adverse reproductive outcome by maternal height**

	Combined adverse reproductive outcome Yes	Combined adverse reproductive outcome No	Total
Maternal height Yes (%)	4 (17.4)	19 (82.6)	23 (100.0)
Maternal height No (%)	17 (26.2)	48 (73.8)	65 (100.0)
Total	21	67	88

*The ten pregnancies whose outcome was a live born with unknown birth weight were excluded from this analysis.

The distribution of stillbirth was slightly different between the two groups of antenatal care (see Table 53): 5.6% of the pregnancies that had received antenatal care ended with stillbirth compared to 0.0% of the pregnancies that had not received antenatal care, but the difference was not statistically significant (Fischer exact p=0.79).

Table 53: Stillbirth by antenatal care

	Stillbirth Yes	Stillbirth No	Total
Antenatal care Yes (%)	5 (5.6)	84 (94.4)	89 (100.0)
Antenatal care No (%)	0 (0.0)	4 (100.0)	4 (100.0)
Total	5	88	93

The distribution of low birth weight was different between the two groups of antenatal care (see Table 54): 14.9% of the pregnancies that had received antenatal care ended with a low birth weight baby compared to 0.0% of the pregnancies that had not received antenatal care, but the difference was not statistically significant (Fischer exact p=0.53).

*Table 54: Low birth weight by antenatal care**

	Low birth weight Yes	Low birth weight No	Total
Antenatal care Yes (%)	11 (14.9)	63 (85.1)	74 (100.0)
Antenatal care No (%)	0 (0.0)	4 (100.0)	4 (100.0)
Total	11	67	78

*This analysis applied only to pregnancies that ended in live birth (n=88). The ten pregnancies whose outcome was a live born with unknown birth weight were excluded from the analysis.

As the pregnancies that ended in spontaneous abortion were excluded from any calculation involving antenatal care, the other adverse reproductive outcomes whose definition was based on spontaneous abortion (pregnancy loss and combined adverse reproductive outcome) were also excluded from this calculation.

The distribution of spontaneous abortion was similar between the two groups of maternal exposure to emotional stress (see Table 55): 5.6% of the pregnancies occurring in women who had been exposed to emotional stress ended with a spontaneous abortion compared to 5.0% of the pregnancies occurring in women who had not been exposed to emotional stress (Fischer exact p=0.64).

Table 55: Spontaneous abortion by maternal exposure to emotional stress

	Spontaneous abortion Yes	Spontaneous abortion No	Total
Maternal exposure to emotional stress Yes (%)	1 (5.6)	17 (94.4)	18 (100.0)
Maternal exposure to emotional stress No (%)	4 (5.0)	76 (95.0)	80 (100.0)
Total	5	93	98

The distribution of stillbirth was similar between the two groups of maternal exposure to emotional stress (see Table 56): 5.9% of the pregnancies occurring in women who had been exposed to emotional stress ended with stillbirth compared to 5.3% of the pregnancies occurring in women who had not been exposed to emotional stress (Fischer exact p=0.64).

Table 56: Stillbirth by maternal exposure to emotional stress

	Stillbirth Yes (%)	Stillbirth No (%)	Total
Maternal exposure to emotional stress Yes	1 (5.9)	16 (94.1)	17
Maternal exposure to emotional stress No	4 (5.3)	72 (94.7)	76
Total	5	88 (100.0)	93

The distribution of pregnancy loss was similar between the two groups of maternal exposure to emotional stress (see Table 57): 11.1% of the pregnancies occurring in women who had been exposed to emotional stress ended in pregnancy loss compared to 10.0% of pregnancies occurring in women who had not been exposed to emotional stress (Fischer exact p=0.58).

Table 57: Pregnancy loss by maternal exposure to emotional stress

	Pregnancy loss Yes	Pregnancy loss No	Total
Maternal exposure to emotional stress Yes (%)	2 (11.1)	16 (88.9)	18 (100.0)
Maternal exposure to emotional stress No (%)	8 (10.0)	72 (90.0)	80 (100.0)
Total	10	88	98

The distribution of low birth weight was different between the two groups of maternal exposure to emotional stress (see Table 58): 21.4% of the pregnancies occurring in women who had been exposed to emotional stress ended with a low birth weight baby compared to 12.5% of the pregnancies occurring in women who had not been exposed to emotional stress, but the difference was not statistically significant (Fischer exact p=0.30).

*Table 58: Low birth weight by maternal exposure to emotional stress**

	Low birth weight Yes	Low birth weight No	Total
Maternal exposure to emotional stress Yes (%)	3 (21.4)	11 (78.6)	14 (100.0)
Maternal exposure to emotional stress No (%)	8 (12.5)	56 (87.5)	64 (100.0)
Total	11	67	78

*This analysis applied only to pregnancies that ended in live birth (n=88). The ten pregnancies whose outcome was a live born with unknown birth weight were excluded from the analysis.

The distribution of combined adverse reproductive outcome was different between the two groups of maternal exposure to emotional stress (see Table 59): 31.3% of the pregnancies occurring in women who had been exposed to emotional stress ended with a combined adverse reproductive outcome compared to 22.2% of the pregnancies occurring in women who had not been exposed to emotional stress, but the difference was not statistically significant (Fischer exact p=0.31).

*Table 59: Combined adverse reproductive outcome by maternal exposure to emotional stress**

	Combined adverse reproductive outcome Yes	Combined adverse reproductive outcome No	Total
Maternal exposure to emotional stress Yes (%)	5 (31.3)	11 (68.7)	16 (100.0)
Maternal exposure to emotional stress No (%)	16 (22.2)	56 (77.8)	72 (100.0)
Total	21	67	88

*The ten pregnancies whose outcome was a live born with unknown birth weight were excluded from this analysis.

Table 60 shows the results of the statistical testing of the association between the variables shown to be associated with exposure (maternal height, antenatal care and maternal exposure to emotional stress) and the five adverse reproductive outcomes. The associations are expressed as p-values corresponding to chi-square Mantel-Haenszel or Fischer exact tests.

Table 60: Association between the variables statistically associated with exposure and the five adverse reproductive outcomes

***Variable (*p-*value**)**	**p-*values***				
	SAB	***SB***	***PL***	***LBW***	***CARO***
Maternal height (0.02)	0.23**	0.38**	0.53**	0.38**	0.39***
Antenatal care (0.12)	N/A****	0.79**	N/A	0.53**	N/A
Maternal exposure to emotional stress (0.09)	0.64**	0.64**	0.58**	0.30**	0.31**

*p-value showing association between the variable and exposure to ethylene oxide.

**Fischer exact p-value.

***chi-square Mantel-Haenszel p-value.

****N/A: not applicable.

SAB=spontaneous abortion, SB=stillbirth, PL=pregnancy loss, LBW=low birth weight, CARO=combined adverse reproductive outcome.

3. Selecting the variables associated both with the exposure and outcome

When the results of the first two steps were combined, no confounders were identified (none of the variables studied was associated both with exposure to ethylene oxide and one of the adverse reproductive outcomes). Therefore there were no confounders to control for in the analysis.

3.5.3.5. Identifying Effect Modifiers

The available data was stratified by the levels of each variable and the association between exposure to ethylene oxide and the five adverse reproductive outcomes was assessed in each stratum.

Chi-square test was applied and the corresponding p-values were used in order to detect possible effect modification.

Table 61 shows the corresponding p-values for the chi-square test (χ^2) for heterogeneity (chi-square for differing odds ratios between strata) applied during stratified analysis.

Table 61: The corresponding p-values for the χ^2 test for heterogeneity applied during stratified analysis (ETO=ethylene oxide, SAB=spontaneous abortion, SB=stillbirth, PL=pregnancy loss, LBW=low birth weight, CARO=combined adverse reproductive outcome)

Variables used for stratification	**p- *values***				
	ETO -SAB	***ETO - SB***	***ETO - PL***	***ETO -LBW***	***ETO –CARO***
Maternal race	No data*	No data	No data	No data	No data
Maternal education	No data	No data	No data	No data	No data
Maternal age	No data	No data	0.44	No data	0.06
Paternal age	No data	0.80	0.27	No data	0.05
Maternal height	No data	No data	No data	No data	No data
Paternal height	No data	No data	No data	No data	No data
Parity	No data	No data	0.67	No data	0.94
Time between pregnancies	No data	No data	No data	No data	No data
Antenatal care	N/A**	No data	N/A	No data	N/A
Pre-term delivery	N/A	No data	N/A	No data	N/A
Diabetes mellitus	No data	No data	No data	No data	No data
Other medical conditions	No data	No data	No data	No data	No data
Treatment	No data	0.74	0.34	No data	0.33

Variables used for stratification	**p-** ***values***				
	ETO -SAB	***ETO - SB***	***ETO - PL***	***ETO -LBW***	***ETO –CARO***
Passive smoking	No data	0.66	0.41	No data	0.05
Maternal exposure to carbon monoxide	No data	No data	No data	No data	No data
Maternal drinking	No data	No data	No data	No data	No data
Maternal high blood pressure	No data	0.40	0.40	No data	0.94
History of pregnancy loss	No data	No data	No data	No data	0.57
Maternal exposure to ionising radiation	No data	No data	No data	No data	No data
Maternal exposure to anaesthetics	No data	No data	No data	No data	0.34
Maternal exposure to excessive physical activity	No data	0.76	0.72	No data	0.70
Shift work	No data	0.73	0.98	No data	0.97
Maternal exposure to emotional stress	No data	No data	No data	No data	0.65
Maternal exposure to noise	No data	No data	No data	No data	No data

*Not enough data to allow stratification by the levels of this variable; **Not applicable.

Using stratified analysis and applying the chi-square test for differing odds ratios between strata lead to the following:

a) Clear evidence of effect modification ($p \leq 0.05$) of the association between exposure to ethylene oxide and combined adverse reproductive outcome by two variables: paternal age ($p=0.05$) and passive smoking ($p=0.05$).

- When stratifying by *paternal age*, the following stratum-specific measures of association between exposure to ethylene oxide and combined adverse reproductive outcome were found: in the stratum of paternal age=No (father's age at conception less than 40 years) OR=0.57 (95%CI=0.06-5.14), while in the stratum of paternal age=Yes (father's age at conception 40 years or more) OR=9.60 (95%CI=1.45-63.50). The results showed that the effect of exposure to ethylene oxide during pregnancy on the occurrence of combined adverse reproductive outcome was increased by older paternal age.
- When stratifying by *passive smoking*, the following stratum-specific measures of association between exposure to ethylene oxide and combined adverse reproductive outcome were found: in the stratum of passive smoking=No, OR=1.21 (95%CI=0.27-5.46), while in the stratum of passive smoking=Yes, OR=15.33 (95%CI=1.91-122.80).

The results showed that the effect of exposure to ethylene oxide during pregnancy on the occurrence of combined adverse reproductive outcome was increased by passive smoking.

b) Moderate evidence of effect modification ($0.05<p\leq0.20$) of the association between exposure to ethylene oxide and combined adverse reproductive outcome by maternal age ($p=0.06$).

- When stratifying by *maternal age*, the following stratum-specific measures of association between exposure to ethylene oxide and combined adverse reproductive outcome were found: in the stratum of maternal age=No (mother's age at conception less than 35 years) OR=0.98 (95%CI=0.18-5.33), while in the stratum of maternal age=Yes (mother's age at conception 35 years or more) OR=11.25 (95%CI=1.58-80.30). The results showed that the effect of exposure to ethylene oxide during pregnancy on the occurrence of combined adverse reproductive outcome was increased by older maternal age.

4. DISCUSSION

This study, the first in South Africa on ethylene oxide exposure and adverse reproductive outcomes, confirmed the widespread use of ethylene oxide, exposure to this agent in public sector hospitals and associations between exposure to ethylene oxide and spontaneous abortion and between exposure to ethylene oxide and pregnancy loss (either spontaneous abortion or stillbirth).

Moreover, the study provided data on reproductive outcomes in employed women (on which scant data are available in South Africa) and added information on the validity of self-reported pregnancy data relative to medical records.

Effect modification of the association between exposure to ethylene oxide and combined adverse reproductive outcome (spontaneous abortion, stillbirth or low birth weight) by paternal age, passive smoking and maternal age is a potentially important finding that requires further investigation.

The study detected an increased risk of spontaneous abortion amongst pregnancies exposed to ethylene oxide (crude RR=16.63, 95%CI=1.97-140.42, p=0.004) and an increased risk of pregnancy loss amongst pregnancies exposed to ethylene oxide (crude RR=6.24, 95%CI=1.95-19.93, p=0.003).

The frequency of spontaneous abortion found amongst exposed pregnancies was high (21.1%). This frequency is similar to the findings of the two above mentioned studies: in the study carried out by Hemminki *et al* (1982), the rate of spontaneous abortion (adjusted for age) amongst exposed pregnancies was 20.4%; in the study conducted by Rowland *et al* (1996), the frequency of spontaneous abortion amongst exposed pregnancies was 15.6%.

The confidence intervals of the risk ratios were wide due to the small number of subjects (pregnancies) in the outcome category, but they did not include 1. The associations detected were statistically significant (p=0.004 and p=0.003 respectively) making unlikely that the associations were due to chance. As no confounders of the two associations were identified, the findings could not be attributed to confounding either.

Moreover, the results of this research are consistent with the findings of two of the three earlier studies published in this field: Hemminki *et al* (1982) found that exposure to ethylene oxide in hospitals correlates with an increased frequency of spontaneous abortion; Rowland *et al* (1996) found that women exposed to ethylene oxide were 2.1 more likely to have any of the following adverse reproductive outcomes: spontaneous abortion, pre-term delivery or postterm delivery.

The association between exposure to ethylene oxide and spontaneous abortion and pregnancy loss are convincing in this study and add to the concern that ethylene oxide is a reproductive hazard for women. Preventive strategies are warranted.

However, this study had some limitations that are addressed below.

Study design

The study included only the last recognised pregnancy of the participating women.

- *Advantages of the study design*

Ascertaining only the outcome of the last recognised pregnancy and relating this outcome to exposures at the time is a retrospective type of study particularly convenient for rare exposures or small populations such as an occupational cohort, where few women will be currently pregnant and available for a prospective cohort study (Weinberg and Wilcox, 1998). Another advantage of this approach is that studying the last recognised pregnancy minimises the possibility of poor recall. As this pregnancy represents the most recent reproductive event in a woman's life, it is very likely that it is well remembered even several years afterwards (Olsen and Skov, 1993).

- *Disadvantages of the study design*

Such a study design never detects all the abortions experienced by the women included in the research, as a high number of pregnancies are not clinically recognised (they are lost either before implantation or before the next period). This is why the study included the last clinically recognised pregnancy and not simply the last pregnancy occurring in the women participating in this study.

Another problem with this study design arises because a wanted pregnancy ending in loss is likely to be replaced by other pregnancies until a viable one is conceived. This replacement phenomenon produces a lowered overall rate of spontaneous abortions amongst most recent pregnancies even with perfect recall (Weinberg and Wilcox, 1998).

This might be the explanation for the low frequency of spontaneous abortion found in this study: overall, the relative frequency of spontaneous abortion was 5.1%, which is much lower than the internationally reported data (10-15%). Another South African study (Buchmann *et al*, 2002) found low rates of spontaneous abortion amongst the population studied (4.9% for teenagers, 5.9% for women aged between 20-34 years and 9.7% for women 35 years or older), but these rates were based on the number of spontaneous abortions recorded at medical facilities and therefore they could be due to under-recording of spontaneous abortion at

medical facilities. In the study on ethylene oxide, the relative frequency of spontaneous abortion was based on the mother-reported number of spontaneous abortion, so the problem of under-recording this reproductive outcome disappears. To check whether the study findings could have been due to the "replacement phenomenon", the relative frequency of spontaneous abortion amongst the pregnancies that had preceded the pregnancies under study (i.e., the second last pregnancy of the participating women) was calculated and it was compared to the frequency of spontaneous abortion found amongst the last recognised pregnancies. The women enrolled in the study had reported 88 second last pregnancies, 15 of which (17.0%) had ended in spontaneous abortion. When this frequency was compared to the frequency of spontaneous abortion found amongst the last recognised pregnancies (5.1% or 5 out of 98 pregnancies), there was a statistically significant difference (chi-square Mantel-Haenszel p=0.008). This result supports the hypothesis that the low frequency of spontaneous abortion found amongst the last recognised pregnancies studied was likely to be due to the "replacement effect".

Sample size

Despite a high participation rate amongst the women employees working in sterilising units (96.6%), the number of subjects in the study was small (98 pregnancies). Two reasons were identified for this: first, the participation rate amongst the medical facilities in Gauteng using ethylene oxide was reasonable, but not complete (68.8%) and second, the proportion of the women in their reproductive age employed in the sterilising units was low (only 22.4% of these women had a pregnancy in the last 10 years).

Due to the small number of subjects it was not always possible to stratify for all the variables considered potential effect modifiers; as a consequence, not all possible effect modification could be identified during analysis.

Selection bias

- *Response rate*

The necessary information about the evolution and outcome of the pregnancies studied was gathered from mothers. As most of the women contacted participated in the study (participation rate 96.6%), the selection bias in this study was rather minimal.

A greater selection bias might have intervened during the selection of the sterilising units included in the study, since 31.2% of the medical facilities in the province using ethylene oxide did not participate in the research (particularly surprising was the refusal of Afrox

Healthcare management to take part in this study, knowing that the company is part of one of the major producers of ethylene oxide in South Africa). It might be that these non-participating sterilising units had already had problems with ethylene oxide exposure in their women staff and this was the reason for which they refused to participate in a research investigating these problems. In this situation, their non-participation might have biased the effect estimate towards the null value, showing little or no association between exposure and outcome.

- *Survivor effect*

Studies that include only current workers in an industry to examine the relation of past exposures to past pregnancy outcomes can suffer from a type of selection bias called "survivor effect". These studies enrol only "survivors", or women who stayed in the industry until the time the study is carried out. The problem that such an approach creates is that women who have had spontaneous abortions may be more likely to continue employment while those who have given birth may be more likely not to return to work or to lose their jobs if parental leave is not provided (Gold and Tomich, 1994).

The women participating in this study were employed in a sector that is well organised and where maternity leave is provided (health care sector, both public and private). Moreover, the income of these women is important for their family and it is unlikely that they can afford to leave their job and stay home to look after their new-born child. The most likely situation is that they get maternity leave following delivery and then they get back to work. This is what makes the presence of the survivor bias in this study rather unlikely.

A particular type of survivor effect that could have intervened is represented by women leaving the job in a sterilising unit because they had already experienced adverse reproductive outcomes (spontaneous abortion either recognised as such or perceived as failure to conceive). In this case, their adverse reproductive outcomes were not included in the study population and this again might have biased the effect estimate towards the null value, showing little or no association between exposure and outcome.

Information bias

Data on exposure and outcome in the study were obtained retrospectively from the mothers and usually this approach makes information bias likely. To reduce the possibility of information bias, the analysis was restricted only to pregnancies that had occurred after the 1st January 1992 (it had been previously shown that women recall events related to their

reproductive life reasonably well after 10 years [Axxelson and Rylander, 1984; Lemasters, 1998)].

However, as a certain degree of information bias is unavoidable in any epidemiological study, the two categories of information bias that might have intervened in this study (i.e., misclassification of exposure and misclassification of outcome) will be discussed further.

- *Misclassification of exposure*

Exposure data from the time the pregnancies included in the study occurred was not available and thus an indirect approach was used to assess the exposure to ethylene oxide at the time of the last recognised pregnancy.

Indirect assessment of exposure to ethylene oxide was also used in two of the three previous studies that investigated the effects of this chemical on reproduction: Hemminki *et al* (1982) used supervising nurse-reported data (the supervising nurse classified the pregnant woman as exposed or unexposed according to her job) and Rowland *et al* (1996) used self-reported data (questionnaire-collected data about exposure to several sterilising procedures, including ethylene oxide, during the most recent pregnancy).

Although indirect, the approach used in this study was more thorough, combining exposure data from three sources:

1. The walk-through survey (field visit) helped in establishing a classification of the jobs encountered in the sterilising units visited. Based on this classification and on the tasks performed for each job, technician (operator) was identified as the employee most exposed to ethylene oxide.

2. Measurements of the current levels of ethylene oxide in the sterilising units of all the 10 public hospitals using this chemical were performed at the time of the study. The results of these measurements (personal and static sampling) supported the findings of the previous step that had suggested that the employees working directly with the ethylene oxide steriliser (technician or operator) were the ones most exposed to ethylene oxide.

3. The questionnaire gathered information on the job description of the woman at the time of her last recognised pregnancy. If she was working in a sterilising unit using ethylene oxide at that time, the job description included details about the daily tasks she was performing. These results were combined with the findings of the previous two steps and used to identify the women who had been exposed to ethylene oxide during their last recognised pregnancy (i.e. the women working directly with the ethylene oxide steriliser). Subsequently, the pregnancies under study were classified as exposed or unexposed to ethylene oxide according to the mother's exposure at the time of her last recognised pregnancy.

There is no reason to believe that the job category currently assessed as exposed (technician or operator) was not exposed in the past. The walk-through survey showed that exposure to ethylene oxide in sterilising units either diminished in the last 10 years or remained the same (the findings did not suggest an increased of exposure over time). There is a possibility that employees currently assessed as unexposed (e.g., cleaners) might have been exposed in the past, e.g., at the time of the last recognised pregnancy. Had this happened, the resulting misclassification of exposure would have only diluted the effect estimate of the study (reducing the apparent effect of exposure to ethylene oxide on the occurrence of spontaneous abortion or pregnancy loss).

- *Misclassification of outcome*

One of the objectives of this study was to asses the validity of the mother-reported information on the pregnancy evolution and outcome, in order to 1) understand whether the information collected using a questionnaire could be used instead of medical records in a study investigating possible associations between exposure and reproductive outcomes; 2) detect a possible misclassification of outcome.

Women providing information on the pregnancies included in the research had to recall events taking place between 4 months and 10 years prior to the collection of information (mean=5.4 years, median=5 years).

The mother-reported data on the pregnancy evolution and outcome were compared with the existing medical records concerned with these pregnancies, which were considered to be the "gold standard".

This process was limited by the lack of a standard policy concerning medical records in medical facilities across Gauteng province (which medical records have to be kept, for how long and where).

Overall, it was easier to get these records from private medical facilities than from public medical facilities. As much as 90.0% of the records concerned with the pregnancies under study were found at private medical facilities and 84.1% of the records at public medical facilities.

The more difficult task of finding records at public medical facilities had to do with different policies on old medical records. During the field visits, more time was spent searching various rooms, storing places and lockers than compiling the information from the record once this was found. Some medical facilities were using separate labour ward registers for normal deliveries, caesarean sections and spontaneous or induced abortions and the registers were not kept in the same place. The situation was not better when the central computerised database at

the admission room was used to gather basic information. This database was either very recent and it did not contain the old information searched for, or it was being updated exactly at the very moment the information was needed and again it was not of any help.

Another problem encountered was missing information from the medical records. In 3 out of 55 medical records checked (5.5%) the birth weight of the newborn was not mentioned. The existence of incomplete medical records casts doubt over the essential assumption one makes when assessing validity of mother-reported data: medical records are the "gold standard" for the reproductive information. If they are not so, then the only alternative left is not to assess the validity of the questionnaire-collected information, but to assess the reliability of this information. In this situation, one can only compare the two data sources (questionnaire-collected information and information from the medical records) and assess the extent to which they agree.

There was also missing information amongst the mother-reported birth weight: in 7 out of 55 cases (12.7%) the mother did not report the birth weight of her last child. The lack of mother-reported birth weight might have been poor maternal recall but it might have also been lack of communication between the mother and the medical personnel that assisted her during delivery. It seems obvious that if mothers are not given basic information on their new born at delivery they cannot recall this information, try as they might.

When the mother-provided information was compared to the medical records concerned with the pregnancies under study, the mothers' recall was found to be accurate for the following variables: medical facility where the pregnancy was recorded, date of the reproductive event (spontaneous abortion, stillbirth or live birth), gestation length, live status of the newborn, number of foetuses, child gender, disease / medical problems during pregnancy and treatment during pregnancy.

The questionnaire failed to identify any of the complications related to the delivery of their last child that the women enrolled in this study had experienced. It is very likely that this failure was due to the fact that the questionnaire used a proxy measure for this parameter, instead of a direct measure. That is, the questionnaire used an indirect question enquiring about medical conditions before or during the last recognised pregnancy, instead of formulating a direct question addressing specific events around the time of delivery.

The validity of mother-reported birth weight was assessed in several ways. The correlation coefficient ($r=0.69$) showed a good correlation between mother-reported and recorded birth weight, although discordant birth weight (mother-reported birth weight that differed 250 g or more from the recorded birth weight) was found in 40.5% of the pregnancies investigated.

The one sample *t* test was used to test the null hypothesis that the mean difference between mother-reported and recorded birth weight was zero (that there was no bias in mother-reported information). The result of this testing was consistent with the null hypothesis and therefore no bias was detected in the mother-reported birth weight. The true mean difference between the questionnaire-collected and the recorded birth weight for all pregnancies that could have been included in such a study was estimated using its 95% confidence interval and it ranged from -88 g to +216 g. This showed an error in mothers' reporting of the birth weight of their babies: mothers could tend to under-report the birth weight of their newborns by as much as 88 g and to over-report it by as much as 216 g.

Under- or over-reporting birth weight might introduce a misclassification of outcome (low birth weight can be misclassified as normal birth weight or normal birth weight can be classified as low birth weight). It is important to know whether misclassification of outcome is differential or non-differential (as it would influence the effect estimate in a different way). To check whether the mothers reported low birth weight differentially, according to their exposure status, a variable called "different LBW" was created, measured on a dichotomous scale (with two categories, Yes or No) and defined as follows: "Yes" when the mother-reported birth weight was below 2500 g but it was not confirmed by the medical records or when the mother-reported birth weight was 2500 g or above but it was not confirmed by the medical records and "No" when both the mother-reported and recorded birth weight were either low birth weight or normal birth weight.

Next the distribution of the new variable by exposure to ethylene oxide was studied. There was a higher percentage of unexposed mothers who had reported a "different LBW" compared to exposed mothers (see Table 62): 13.5% of the unexposed mothers had reported "different LBW" compared to 0.0% of the exposed mothers, but the difference was not statistically significant (Fischer exact p=0.51).

Table 62: "Different low birth weight" by mother's exposure to ethylene oxide

	Different LBW Yes	Different LBW No	Total
Ethylene oxide exposure Yes (%)	0 (0.0)	5 (100.0)	5 (100.0)
Ethylene oxide exposure No (%)	5 (13.5)	32 (86.5)	37 (100.0)
Total	5	37	42

The result of this analysis showed that reporting a "different LBW" did not depend on the mother's exposure status. This means that the possible misclassification of outcome resulting from under- or over-reporting of low birth weight by the mother was non-differential and therefore it could only bias the effect estimate towards the null value or could produce no bias in the risk ratio (Checkoway, 1989; Kenneth and Greenland, 1998).

A possible association between the "different LBW" and the recall period (the period of time between the last recognised pregnancy and the time the information was collected) was also investigated. The mean recall period for the mothers reporting "different LBW" was 7 years, with a standard deviation of 1.9 years. The mean recall period for the mothers not reporting "different LBW" was 5.8 years, with a standard deviation of 3.3 years. Using the unpaired *t* test the two means were compared (PEPI statistical software, 1993-2001) and the difference between them was not statistically significant (p=0.43). This result showed that reporting a "different LBW" was not likely to be associated with the length of the recall period.

The assessment of the validity of the questionnaire-collected information on pregnancy outcome led to the following conclusions:

1. The questionnaire used in this study can be used in reproductive studies instead of medical records provided that birth weight is the outcome and not the exposure under study (Lumey *et al*, 1994). As the possible misclassification of outcome resulting from an error in the mother-reported birth weight was non-differential, it could change the effect estimate towards the null value or it could not change it al all.
2. As the effect estimate can be biased towards the null value in the presence of a non-differential misclassification of outcome, this issue becomes more important in studies showing little or no association between the exposure and outcome (Kenneth and Greenland, 1998).

The study conducted investigated two outcomes whose definition included low birth weight: low birth weight as such and combined adverse reproductive outcome. The effect estimates for the association between exposure to ethylene oxide and these variables were crude RR=0.61 (95%CI=0.09-4.30) and crude RR=2.09 (95%CI=1.00-4.36), respectively. The fact that no association was detected between exposure to ethylene oxide and low birth weight and between exposure to ethylene oxide and combined adverse reproductive outcome might be due to the presence of the non-differential misclassification of outcome mentioned earlier. This can hold true particularly when the association between exposure to ethylene oxide and combined adverse reproductive outcome is concerned: the crude risk ratio (RR=2.09) shows a possible association between the exposure and outcome and the probability that an effect

estimate this size or higher (crude RR=2.09) occurs by chance if there is no association between exposure and outcome is p=0.06, which is just above the customarily used p=0.05.

Pregnancies occurring in employed vs. unemployed women

As reproductive health should not be compared directly between working and non-working women (Savitz *et al*, 1990), pregnancies occurring in unemployed women were excluded from the analysis. The pregnancies occurring in women who were studying (student nurses) at the time of their last recognised pregnancy were included in the analysis, because these women reported working at medical facilities as part of their school curriculum.

Confounders

During analysis no confounders were identified for any of the five associations investigated. The sample studied was very homogeneous, with the variables analysed being evenly distributed amongst the pregnancies under study.

Due to the homogeneity of the study sample all the effects estimates (risk ratios, RR) calculated were crude, unadjusted for confounders.

Effect modifiers

The following variables were found to modify the effect of exposure to ethylene oxide on the occurrence of combined adverse reproductive outcome: paternal age (father's age at conception 40 years or more), passive smoking and maternal age (mother's age at conception 35 years or more). These variables might interfere with the exposure to ethylene oxide on the pathway leading to the occurrence of the combined adverse reproductive outcome.

The problem that arises here is that the combined adverse reproductive effect is a combination of three independent reproductive outcomes (spontaneous abortion, stillbirth and low birth weight) and it is therefore difficult to answer the following question: do these effect modifiers change the effect of ethylene oxide on the occurrence of only one of them (and if so, on which one), or on the occurrence of more than one? Unfortunately, due to the small sample size it was not possible to detect an effect modification produced by these risk factors (paternal age 40 or more, maternal age 35 or more and passive smoking) on any of the three independent adverse reproductive outcomes (spontaneous abortion, stillbirth and low birth weight) and at this point the question cannot be answered. This issue requires to be further investigated.

5. CONCLUSIONS AND RECOMMENDATIONS

The study investigated the association between exposure to ethylene oxide during pregnancy in women sterilising staff working in sterilising units in Gauteng and the occurrence of the following adverse reproductive outcomes: spontaneous abortion, stillbirth, pregnancy loss, low birth weight and combined adverse reproductive outcome. The objectives of this study were: 1) to describe the extent and nature of ethylene oxide use in sterilising units operational in medical facilities in Gauteng; 2) to assess the current exposure to ethylene oxide in sterilising units in Gauteng; 3) to collect information on the last recognised pregnancy using a questionnaire; 4) to assess the validity of the information on the evolution and outcome of the last recognised pregnancy collected by the means of the questionnaire; 5) to assess the association between occupational exposure to ethylene oxide during pregnancy and adverse reproductive outcome.

The study population was represented by 98 singleton pregnancies meeting the following criteria: 1) they occurred in the women currently working in sterilising units using ethylene oxide in Gauteng who agreed to participate in the study; 2) they represented the last recognised pregnancy occurring in these women after the 1st January 1992; 3) they occurred while the mother was employed.

The study enrolled 68.8% (22 out of 32) of the medical facilities in Gauteng using ethylene oxide to sterilise medical equipment. These included all public hospitals (10 out of 10) and 54.5% (12 out of 22) of the private medical facilities using ethylene oxide. The total number of sterilising units operational at these medical facilities was 26.

The vast majority of the employees working in the sterilising units included in the study were women (96.6%) and they were employed in one of the following jobs: technician (operator), instrument packer or cleaner. The employee most exposed to ethylene oxide was the technician (operator).

The majority of the sterilising units had one steriliser using ethylene oxide cartridges (100% ethylene oxide) and they were using it every day. In only 15.4% of the sterilizing units the employees in charge of the ethylene oxide steriliser were using protective clothing (gloves, masks) when working with the steriliser.

Despite the fact that all sterilising units reported monitoring the levels of ethylene oxide on a regular basis, only 46.2% of them provided recent measurements; all of them were below 0.25 ppm.

Measurements of the current levels of ethylene oxide were performed by the Occupational Hygiene Unit of the National Institute for Occupational Health using hydrobromic acid-coated petroleum charcoal tubes connected to calibrated Gilian pumps through which air containing ethylene oxide was drawn. The samples were analysed by the Analytical Services of the National Institute for Occupational Health using the gas chromatography technique (NIOSH method 1614). The total number of samples taken was 418 (100 blank, 97 personal and 221 static samples). Quality control was ensured using the following methods: 1) verification by an Approved Inspection Authority; 2) collection of duplicate samples; 3) collection of blank samples.

Personal sampling detected levels of ethylene oxide between 0.0-38.22 ppm. These levels of exposure were considered short-term exposure and they were compared to the short-term exposure limits set by the South African Regulations (15 ppm) and by the Occupational Safety and Health Administration, USA (5 ppm). One measurement (1.0%) exceeded the South African limit and 5 measurements (5.2%) exceeded the American limit.

Static sampling detected levels of ethylene oxide between 0.0-3.16 ppm. These levels of exposure were compared to the long-term exposure limits set by the South African regulations (5 ppm) and by the Occupational Safety and Health Administration (1 ppm). Eleven measurements (5.0%) exceeded the American limit (all of them detected by samples placed on top of the ethylene oxide steriliser) and none exceeded the South African limit.

The results of these measurements showed that exposure to ethylene oxide still occurs in sterilising units, particularly in the employees working with the ethylene oxide steriliser (technician). Ethylene oxide was detected in 9 out of the 10 public hospitals sampled and the highest levels were detected by personal samples or by static samples taken on top of the ethylene oxide steriliser. These findings also showed that the employee most exposed to ethylene oxide in a sterilising unit was the technician (operator).

There were 113 women who had been pregnant after the 1st January 1992 employed in the sterilising units participating in the study. One hundred and nine of them agreed to participate in the research (response rate of 96.5%).

Information on the evolution and outcome of the 109 pregnancies was gathered administering a questionnaire to the women enrolled in the study. The questionnaire collected demographic data, reproductive history, medical data, other risk factors for the adverse reproductive outcomes of interest (environmental and occupational exposures, lifestyle), and data regarding the evolution and outcome of the last recognised pregnancy. The questionnaire also collected detailed information on the job held by the woman at the time of her last recognised

pregnancy (if she was working with ethylene oxide, she was asked for a complete description of her daily tasks). Prior to administration the questionnaire was tested on a small sample of working women.

Amongst the 109 pregnancies on which the questionnaire collected information, 2 were multiple pregnancies (1.8%), 5 (4.6%) where conceived while the mother was not employed and 4 (3.7%) were conceived before the 1st January 1992. These 11 pregnancies were excluded from the study population. The study population included the remaining 98 singleton pregnancies.

The pregnancies included in the study had occurred from 1992 to 2003 and therefore the mothers had to recall past events that had taken place between 4 months and 10 years prior to the information collection.

To asses the validity of the mother-reported information on the pregnancy evolution and outcome this information was compared against medical records, which were considered the "gold standard". The assessment of the validity of mother-reported information on pregnancy evolution and outcome showed that the mothers' recall was accurate for the following variables: medical facility where the pregnancy was recorded, date of the reproductive event (spontaneous abortion, stillbirth or live birth), gestation length, vital status of the newborn, number of foetuses, child gender, disease/medical problems during pregnancy and treatment received during pregnancy.

The questionnaire failed to identify any of the delivery-related complications that the women enrolled in this study had experienced with their last child. This was due to the lack of a direct question enquiring about delivery-related complications in the questionnaire.

There was an error in the mothers' reporting of the birth weight of their babies: it was shown that the true mean difference between the mother-reported and recorded birth weight for all pregnancies that could have been be included in such a study could range from -88 g to 216 g. A possible misclassification of outcome due to this error was shown to be non-differential (the proportion of subjects misclassified with regard to the outcome did not depend on exposure) and therefore, it could either bias the effect estimate towards the null value (masking or minimising an existing association) or it could not produce any bias at all.

In conclusion, the validity assessment showed that mother-reported information on the pregnancy outcome can be used instead of the medical records, provided that birth weight is used as an outcome and not an exposure in the study and that findings are interpreted carefully if no association between exposure and outcome is found.

The analysis to detect possible association between exposure to ethylene oxide and adverse reproductive outcome included 98 of the initial 109 pregnancies on which information was gathered by the means of the questionnaire.

The findings of the study suggested that there is an increased risk for spontaneous abortion and pregnancy loss in pregnancies exposed to ethylene oxide compared to unexposed pregnancies.

For the association between exposure to ethylene oxide and spontaneous abortion the crude risk ratio was RR=16.63 (95%CI=1.97-140.42) and the association was statistically significant (p=0.004).

For the association exposure to ethylene oxide and pregnancy loss the crude risk ratio was RR=6.24 (95%CI=1.95-19.93) and the association was statistically significant (p=0.003).

These results support the findings of two previous studies (Hemminki *et al*, 1982; Rowland *et al*, 1996) that suggested that exposure to ethylene oxide during pregnancy could lead to the occurrence of adverse reproductive outcomes.

No statistically significant association was found between exposure to ethylene oxide and stillbirth (crude risk ratio was RR=3.47, 95%CI=0.63–19.01, p=0.18) and between exposure to ethylene oxide and combined adverse reproductive outcome (crude risk ratio was RR=2.09, 95%CI=1.00-4.36, p=0.06). No association was found between exposure to ethylene oxide and low birth weight (crude risk ratio was RR=0.61, 95%CI=0.09–4.30, p=0.51). For the lack of association between exposure to ethylene oxide and low birth weight and between exposure to ethylene oxide and combined adverse reproductive outcome, the explanation might be the misclassification of outcome resulting from the biased information on birth weight provided by the mother.

RECCOMMENDATIONS

Given the positive and statistically significant association found between exposure to ethylene oxide during pregnancy and spontaneous abortion and pregnancy loss and taking into account that women represent the majority of employees working in sterilising units using ethylene oxide, the following recommendations are to be made:

1. A study involving a greater number of pregnancies should be carried out (possible a multi-centre study at national level) in order to establish whether exposure to ethylene oxide during pregnancy could lead to other adverse reproductive effects (low birth weight, stillbirth).
2. When possible, information on both exposure and pregnancy outcome should be collected prospectively.
3. Elimination or reduction of reproductive risk in the workplace should be accomplished by non-discriminatory means; the reproductive health of both male and female employees should be protected (Kaczmarczyk and Paul, 1996). “Foetal protection” policies (policies that restrict fertile women from hazardous jobs on the basis of potential foetal risk) should not be used as means of protecting the reproductive health of working women.
4. Ethylene oxide sterilising units should be established in closed areas and the enclosure should be exhausted to a dedicated ventilation system (US Department of Health and Human Services, CDC and NIOSH, 1988).
5. Sensors should be provided to identify a ventilation failure and to detect ethylene oxide. Both audible and visual alarms should be activated by the sensors (US Department of Health and Human Services, CDC and NIOSH, 1988).
6. Ethylene oxide sterilising units should not be used for any other purpose apart from their designated function (e.g., they should not be used as storage facilities).
7. Only personnel trained in sterilisation procedures and in the health and safety hazards of ethylene oxide should be allowed to enter in the enclosed ethylene oxide sterilising area.
8. No rotation system should be allowed, as it exposes employees more randomly, without the possibility of an appropriate exposure control.
9. Personnel working with ethylene oxide should wear protective equipment (masks) when opening the steriliser to offload the sterilised packs.
10. Engineering control measures should be taken in order to minimise the exposure to ethylene oxide of all staff working in sterilising units.

11. Levels of ethylene oxide in the sterilising units should be monitored according to the provisions of the Regulations for Hazardous Chemical Substances (1995).
12. The monitoring programme should not be carried out by the same company that supplies the ethylene oxide sterilisers to the sterilising units.

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Appendix 1

HOSPITALS' CHECK LIST

1. Date	
2. Interviewer	
3. Health care facility	
4. Sterilising unit number (if there are more than one sterilising unit per health care facility)	
5. Sterilisation with ethylene oxide is used	Yes No
6. If sterilisation with ethylene oxide is used, which type of steriliser is used	General steriliser Ampoule steriliser Other
7. How many sterilisers of each type	General Ampoule Other
8. Total number of staff in this sterilising unit	
9. Number of female staff in this sterilising unit	
10. How many working shifts per 24 hours	1 2 3
11. How many loads are sterilised per shift	1st shift 2nd shift 3rd shift
12. Job descriptions for the sterilising staff	1. 2. 3. 4.
13. Daily tasks for each job described above	1. 2. 3. 4.

14. Protection measures in use	1. 2. 3. 4. 5.
15. Current levels of exposure to ethylene oxide	Peak exposure (highest exposure level) 8-hour TWA (time-weighted average)
16. What changes in sterilising equipment and/or technology did your unit make in the last 10 years?	1. 2. 3. 4.
17. Please specify when (dates) your unit made these changes	1. 2. 3. 4.

Appendix 2

SUMMARY OF THE QUESTIONNAIRE TESTING

TESTING THE QUESTIONNAIRE

Name of the investigator _____

Date / /

Medical facility _____

Number of women that completed the questionnaires

Number of women that refused to participate

Number of women that were not available at the time of the interview (e.g., because there were off, on leave, or working on a different shift)

Please fill in the following table with the appropriate answer for each of the 60 questions in the questionnaire that you administered:

Question number (as it is in the questionnaire)	This question was difficult to understand (Yes/No)	This question had to be repeated (Yes/No)	This question appeared to be misinterpreted (Yes/No)	Here the respondent wanted to say more (Yes/No)	This question made the respondent uncomfortable (Yes/No)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					

Question number (as it is in the questionnaire)	This question was difficult to understand (Yes/No)	This question had to be repeated (Yes/No)	This question appeared to be misinterpreted (Yes/No)	Here the respondent wanted to say more (Yes/No)	This question made the respondent uncomfortable (Yes/No)
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
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36					
37					
38					
39					
40					
41					
42					
43					
44					
45					
46					
47					
48					
49					
50					

Question number (as it is in the questionnaire)	This question was difficult to understand (Yes/No)	This question had to be repeated (Yes/No)	This question appeared to be misinterpreted (Yes/No)	Here the respondent wanted to say more (Yes/No)	This question made the respondent uncomfortable (Yes/No)
51					
52					
53					
54					
55					
56					
57					
58					
59					
60					

Thank you!

Appendix 3

CONSENT FORM FOR ETHYLENE OXIDE STUDY FOR WOMEN WORKING WITH ETHYLENE OXIDE (English version)

Hello,

My name is Daniela Gresie-Brusin, I am a medical doctor and I am doing my postgraduate degree (PhD) with the University of Witwatersrand and the National Institute for Occupational Health.

Together we are studying the health effects of exposure to ethylene oxide in women sterilising staff working in health care facilities in Gauteng.

Previous medical research has suggested that exposure to ethylene oxide during pregnancy might affect the evolution of the pregnancy and the health of the baby. Because not all the scientists agree on this, we have decided to carry out a study that will investigate the health effects of work exposure to ethylene oxide in pregnant women. We hope that at the end of the research we will be able to say with more certainty whether the ethylene oxide has adverse health effects in pregnant women exposed to it or not.

To do the study we need to ask you some questions about yourself, your habits, illnesses and your pregnancies, particularly about your last pregnancy. It will take approximately 30 minutes to answer all the questions in the questionnaire and trained personnel will help you to understand and answer the questions.

Because we need to get complete information about the evolution and outcome of your last pregnancy, we will need to check the medical records of this pregnancy only.

Your participation to the study is important to us and we would be grateful if you agree to participate. However, the participation to the study is completely voluntary and whether you agree or decline to participate will have no negative consequences on your employment. You can withdraw from the study at any point in time, without any negative consequences.

There are no direct benefits from participating to this study, but at the end of the research we will be able to come up with a complete presentation of the situation and with recommendations that will help you work in a safer place.

All the information you will provide will be kept strictly confidential and no personal data will be disclosed. In order to ensure confidentiality, your personal details (e.g., name, date of birth, ID number, address) will be recorded on a separate page, so we can keep them apart

from the rest of the questionnaire. Therefore, only the principal investigators of this research will know your personal details and it is their responsibility to keep this information secret.
At the end of the research, the results will be published as collective data, without mentioning any personal details of the study participants. You will also be informed about the results of the study.
If you want more information or if you need to talk to somebody about it, please do not hesitate to contact me (tel: 082-680-9559), or Dr Danuta Kielkowski (tel: 011-712-6436). You can also contact us at the following address: National Institute for Occupational Health, 25 Hospital Road, PO Box 4788, Johannesburg 2000.

If you agree to participate to this study, please sign the form and fill in the questionnaire, as advised.
Thank you for your co-operation!

Yes, I agree to participate to the study
Name
Signature

Yes, I agree to have the medical records on my last pregnancy checked by the study investigators,
Name
Signature

Date (dd/mm/yy) / /

Appendix 4

CONSENT FORM FOR ETHYLENE OXIDE STUDY FOR WOMEN NOT WORKING WITH ETHYLENE OXIDE (English version)

Hello,

My name is Daniela Gresie-Brusin, I am a medical doctor and I am doing my postgraduate degree (PhD) with the University of Witwatersrand and the National Institute for Occupational Health.

Together we are studying the health effects of exposure to ethylene oxide in women sterilising staff working in health care facilities in Gauteng.

Ethylene oxide is a chemical used in hospitals to sterilise things that can be damaged by heat.

Previous medical research has suggested that exposure to ethylene oxide during pregnancy might affect the evolution of the pregnancy and the health of the baby. Because not all the scientists agree on this, we have decided to carry out a study that will investigate the health effects of work exposure to ethylene oxide in pregnant women. We hope that at the end of the research we will be able to say with more certainty whether the ethylene oxide has adverse health effects in pregnant women exposed to it or not.

We know that you are not working with ethylene oxide, but for the purpose of this study we need a control group as well, in order to compare data obtained from women exposed to ethylene oxide to data obtained from women not exposed to this chemical.

To do the study we need to ask you some questions about yourself, your habits, illnesses and your pregnancies, particularly about your last pregnancy. It will take approximately 30 minutes to answer all the questions in the questionnaire and trained personnel will help you to understand and answer the questions.

Because we need to get complete information about the evolution and outcome of your last pregnancy, we will need to check the medical records of this pregnancy only.

Your participation to the study is important to us and we would be grateful if you agree to participate. However, participation to the study is completely voluntary and whether you agree or decline to participate will have no negative consequences on your employment. You can withdraw from the study at any point in time, without any negative consequences.

There are no direct benefits from participating to this study, but at the end of the research we will be able to come up with a complete presentation of the situation and with recommendations that will help you and your colleagues work in a safer place.

All the information you will provide will be kept strictly confidential and no personal data will be disclosed. In order to ensure confidentiality, your personal details (e.g., name, date of birth, ID number, address) will be recorded on a separate page, so we can keep them apart from the rest of the questionnaire. Therefore, only the principal investigators of this research will know your personal details and it is their responsibility to keep this information secret.

At the end of the research, the results will be published as collective data, without mentioning any personal details of the study participants. You will also be informed about the results of the study.

If you want more information or if you need to talk to somebody about it, please do not hesitate to contact me (tel: 082-680-9559), or Dr Danuta Kielkowski (tel: 011-712-6436). You can also contact us at the following address: National Institute for Occupational Health, 25 Hospital Road, PO Box 4788, Johannesburg 2000.

If you agree to participate to this study, please sign the form and fill in the questionnaire, as advised.

Thank you for your co-operation!

Yes, I agree to participate to the study

Name

Signature

Yes, I agree to have the medical records on my last pregnancy checked by the study investigators,

Name

Signature

Date (dd/mm/yy) / /

Appendix 5

QUESTIONNAIRE FOR INVESTIGATING OCCUPATIONAL EXPOSURE TO ETHYLENE OXIDE AND ADVERSE REPRODUCTIVE OUTCOMES IN WOMEN STERILISING STAFF WORKING IN GAUTENG (English version)

In completing the questionnaire, please tick the appropriate option or fill in the date, as required.

Please note that throughout the questionnaire, when we talk about "your spouse/partner" we mean the father of your last child (or the partner that you had last time when you became pregnant).

Before answering the questionnaire, please answer the following questions (please tick the appropriate option):

- Have you ever been pregnant? Yes No

- *IF THE ANSWER IS **NO**, **PLEASE STOP HERE** AND DO NOT FORGET TO HAND IN YOUR FORM. THANK YOU!*

- *If the answer is Yes, did your last pregnancy occur after the 1st of January 1992?*

  Yes No

- *IF THE ANSWER IS **NO**, **PLEASE STOP HERE** AND DO NOT FORGET TO HAND IN YOUR FORM. THANK YOU!*

A. Personal information

1. Personal code (given by the study investigators)

2. Race (for statistical purposes only) Black White Coloured Asian

3. Compared to other women you are short medium tall

4. Compared to other women you are slim medium fat

5. What was the highest level of education that you finished?

No school Primary school High school without matric

High school with matric University

6. Your current working place _____

7. Job title _____

8. When did you start doing your current job (mm/yy) /

B. Reproductive history

In this section we ask you questions about all your pregnancies and their evolution**. Please note that a pregnancy does not always end up with the birth of a child (e.g., some pregnancies may finish with a spontaneous or an induced abortion).**

Please tick the appropriate option or fill in the date, as required:

9. Please specify the total number of children you gave birth to, the date of birth for each of them and their birth weight.

Note:

a) please include all live born children, even the ones who died shortly after birth

b) please include also the stillbirths (dead newborns)

c) please count the multiple births (e.g. twins etc) as one child

	Date of birth (dd/mm/yy)	**The child was born alive or dead?**	**Child's weight at birth (kg)**
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

10. Have you ever had a miscarriage (spontaneous abortion, not a termination of pregnancy)?

Yes No

11. If yes, please specify how many:

12. Please specify when you had the miscarriages and what their cause was:

	Date (mm/yy)	**Cause**
1		
2		
3		
4		
5		

13. Have you ever had a termination of pregnancy (induced abortion)? Yes No

14. If yes, please specify how many:

15. Please specify when you had a termination of pregnancy and why:

	Date (mm/yy)	**Cause**
1		
2		
3		
4		
5		

C. History of your last (most recent) pregnancy

The next set of questions is about the last time you were pregnant, about the evolution of this last pregnancy and the way the pregnancy finished (e.g., with a spontaneous abortion, an induced abortion or with the birth of a child?).

If you are pregnant now, please tell us about the last pregnancy before this one.

Please do not forget that when we talk about "your spouse/partner" we mean the father of your last child (or the partner that you had last time when you became pregnant).

Please tick the appropriate option or fill in the date, as required.

16. How many times had you been pregnant before the last pregnancy?

none 1 2 3 4 5 6 7 8 9 10

17. What was the interval between your last pregnancy and the one before that (years)?

18. What was your weight at the beginning of the last pregnancy (kg)?

19. What was your weight at the time of delivery (kg)?

20. How much weight did you gain during the last pregnancy (kg)?

21. How tall is your spouse/partner? short medium tall

22. How old is your spouse/partner?

23. What was your working place during the last pregnancy?

Place _____

Job description _____

From / / to / /

24. If you were working with ethylene oxide during your last pregnancy, please specify:

Place of work _____

Work section _____

Work title _____

Description of your daily tasks _____

_____

For how many hours per day were you doing that job?

For how many months did you do that job during your last pregnancy?

25. For how many hours per week did you work on average during the last pregnancy?

26. Did you work in shifts during your last pregnancy? Yes No

27. When did you stop working during your last pregnancy (month of pregnancy)?

28. The next table lists various conditions that one may encounter in working places; if you have been exposed to any of them during your last pregnancy, please tick the appropriate box and specify the duration of exposure (from mm/yy to mm/yy).

Exposure	Exposed (yes or no)	From (mm/yy)	To (mm/yy)
Mercury			
Lead			
Arsenic			
Cadmium			
Organic solvents			
Carbon disulfide			
Carbon monoxide			
Pesticides			
Polychlorinated biphenyls (PCBs)			
Ethylene oxide			
Anaesthetic agents			
Antineoplastic agents			
Ionising radiation			
Microwave radiation (not microwave ovens)			
Video display terminals (VDTs)			
Hypobaric environment (high altitude)			
Excessive physical activity • Bending for more than 1 hour/day • Standing for more than 6 hours a day • Heavy lifting			
Noise			
Excessive psychological stress			

29. The next table lists various conditions that one may encounter in working places; if your spouse/partner has been exposed to any of them before your last pregnancy, please tick the appropriate box and specify the duration of exposure (from mm/yy to mm/yy).

Exposure	Exposed (yes or no)	From (mm/yy)	To (mm/yy)
Lead			
Mercury			
Organic solvents			
Pesticides			
Anaesthetic gases			

30. Did you use insecticides or herbicides (bug or weed killers, flea and tick sprays, collars, powders or shampoos) in your household during the last pregnancy? Yes No

31. If yes, please specify:

What kind of chemicals _____

How often (how many times a month)

For how many months

32. Which of the following did you have in your home during your last pregnancy?

gas stove

coal stove

paraffin stove

central heating using gas

central heating using oil

fireplace

wood stove

33. Did you smoke during the last pregnancy? Yes No

34. If yes, how many cigarettes a day? more than 20 11-20 1-10

35. Did your spouse/partner smoke at home during your last pregnancy? Yes No

36. If yes, how many cigarettes a day? more than 20 11-20 1-10

37. For each of the following drinks, how much did you drink per week during the last pregnancy?

Drink	**Number of drinks per week**
Beer (bottle)	
Wine (glass)	
Spirits: whisky, brandy (tot)	

38. Were you using any contraceptive measure last time when you became pregnant?

Yes No

39. If yes, please specify which one of the following:

intra-uterine device (coil) oral contraceptive (pill) jelly, cream or foam other

40. Have you ever been diagnosed with high blood pressure? Yes No

41. If yes, please specify:
When (mm/yy) / /
Treatment received _____

42. Have you ever been diagnosed with sugar diabetes? Yes No

43. If yes, please specify:
When (mm/yy) / /
Which type: Insulin dependent Insulin independent
Treatment received injections tablets

44. Have you ever been diagnosed with any disorders of the womb (uterus)? Yes No

45. If yes, please specify
When (mm/yy) / /
Which disorder _____

46. Have you ever been diagnosed with any disorders of hormones? Yes No
47. If yes, please specify
When (mm/yy) / /
Which disorder _____
Which treatment did you receive for it? _____

48. Have you ever been diagnosed with any serious sickness during or before the last pregnancy? Yes No

49. If yes, please specify
When (mm/yy) /
Which disease _____

50. Did you receive any medical treatment during your last pregnancy? Yes No

51. If yes, pleases specify

What kind of medication _____

Date: from / / to / /

52. The next table lists various infections that may occur during pregnancy. If you had any of them during your last pregnancy, please tick the appropriate box and specify the date of diagnosis (mm/yy) and the month of pregnancy.

Infection	**Yes/No**	**Date of diagnosis**	**Month of pregnancy**
Rubella (German measles)			
Mumps			
Herpes simplex infection			
Varicella (chickenpox)			
Zoster (shingles)			
HIV infection			
Toxoplasmosis (toxoplasma gondii infection)			
Genital Mycoplasma infections			
Syphilis			
Any uterine infection			
High fever (over 41°C)			

53. Did you receive any antenatal care during your last pregnancy? Yes No

54. If yes, in which month of your last pregnancy did the antenatal care start?

55. How many months have you been pregnant for?

56. Was the outcome of your last pregnancy a miscarriage (spontaneous abortion, not a termination of pregnancy)? Yes No

57. If yes, please specify the date of miscarriage (mm/yy) / /

58. Did a doctor examine you when you had the miscarriage? Yes No

59. If yes, what was the diagnosis (the reason for the miscarriage) _____

60. If a child was born at the end of your last pregnancy, please specify

Place of birth (which medical facility) _____

Date of birth (dd/mm/yy) / /

Gender Male Female

The child was born premature at term after the confinement date

Born alive Yes No

Stillborn (dead newborn) Yes No

Birth weight (kilograms) .

Multiple birth (e.g., twins, triplets etc) Yes No

Malformations present at birth Yes No

Thank you for completing this questionnaire!

PERSONAL DETAILS

The questionnaire does not ask for your name, date of birth, ID number, address and your medical aid details.
We do need this information and we want to keep it strictly confidential. This is why we are asking you to answer the following questions that will be kept separately from the rest of the questionnaire. Therefore, only the principal investigators of this research will know your personal details and it is their responsibility to keep this information secret.

Personal code (given by the study investigators)

Name and surname _____

Date of birth (dd/mm/yy) / /

ID number

Contact address
Street _____

House number

Flat number

Town _____

Postal code

If you have a medical aid, please specify

- Which medical aid are you on _____
- What is your medical aid number?

Appendix 6

VALIDATION CHECKLIST

Date (dd/mm/yy)	
Questionnaire code	
Mother's name	
Medical facility	
Date of spontaneous abortion (if applicable)	
Child's date of birth (dd/mm/yy)	
Gestation length	
Live born / Stillborn	
Singleton / Multiple birth	
Gender	
Birth weight	
Malformations at birth	
Complications of delivery	
Diseases/ medical problems during pregnancy	
Treatment during pregnancy	