CHROMOSOME DAMAGE IN ASBESTOS-EXPOSED WORKERS, MEASURED BY SISTER CHROMATID EXCHANGE

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ABSTRACT

Objective: To determine the relationship between asbestos exposure and chromosome damage, as depicted by sister chromatid exchange frequency.

Design: Descriptive, cross-sectional study.

Setting: Asbestos-products factory

Subjects: 31 asbestos-exposed subjects and 21 unexposed subjects

Main outcome measure: Mean sister chromatid exchange (SCE) frequency per metaphase cell.

Results: The control group had a slightly higher mean SCE frequency per cell than the exposed group (3.4%) but this difference was not statistically significant ($p = 0.5935$). Smoking contributed significantly to SCE frequencies in both the exposed and unexposed groups. The mean SCE frequencies per cell in the exposed group were 10.49 for smokers and 8.59 for non-smokers ($p = 0.0078$). The frequencies for smokers and non-smokers in the unexposed group were 10.83 and 8.58, respectively ($p = 0.0257$).

Conclusions: The failure to observe an increase in SCE frequency does not rule out asbestos exposure as a genotoxic agent. Rather, it may help to resolve the limitations of this method for detecting genetic damage. Alternatively, the fibre levels to which this group was exposed may have been too low to cause chromosome damage.

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science (Medicine) in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

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For my parents

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CHAPTER 1: INTRODUCTION

1.1 Asbestos: uses and health effects

Asbestos is a generic name for six naturally occurring fibrous minerals from the amphibole and serpentine group of rock-forming minerals. South Africa is the third largest producer of asbestos, after the former USSR and Canada. Most of the fibre is exported and in 1988 asbestos ranked seventh among South Africa's non-gold mineral exports (Hart 1988).

More than three quarters of all the asbestos mined is used in the manufacture of asbestos-cement products, such as prefabricated walls, corrugated roofing and tiles, water pipes and tanks, and garden furniture and flower pots. Asbestos is an important component of friction products such as clutch plates and brake pads for cars. It is woven into material that is used for heat and fire resistant clothing such as that worn by foundry workers and firemen. It is also used for pipe and electrical insulation. It increases the structural strength of the plastics used in battery cases, and can also be found in heaters, ovens, toasters, hair dryers and polyvinyl chloride (PVA) floor tiles (Hart 1988). Because the asbestos industry is so large, many people are exposed to the fibre during the course of their work.

A number of diseases have been associated with asbestos exposure, including many cancers. The strong association between exposure to crocidolite, or

blue asbestos, and the development of mesothelioma (a cancer which affects the pleura of the lung, in most cases) was first demonstrated by Wagner *et al.* (1960) in South Africa. Other cancers have also been associated with asbestos exposure, although the evidence for the association is weaker than that for mesothelioma. These cancers include lung cancer, gastrointestinal tract cancers (Mossman and Gee 1989), and cancers of the bladder (Puntoni *et al.* 1979) and kidney (Selikoff *et al.* 1979, Enterline *et al.* 1987). Despite the health risks associated with asbestos, the fibre is still widely used.

1.2 Detection of DNA damage

There is growing concern about possible mutagenic and carcinogenic effects of genotoxic agents in human populations exposed to these agents occupationally, environmentally or by lifestyle (Carrano and Natarajan 1988). For many years efforts have been made to protect populations from the harmful effects of chemical substances. In Norway especially, special attention has been paid to genotoxins and carcinogens with a view to establishing a general labelling of all carcinogens. The Norwegian authorities agreed to have a warning printed on all products containing 0,1% by weight or more of one of several carcinogens on the "cancer list". By June 1981, 106 substances were classified in the Norwegian cancer list. The criteria for classification are based primarily on human epidemiology and long-term experiments in animals (Brogger 1982a).

Unfortunately, there are relatively few direct methods to measure mutations (unrepaired DNA damage that develops into a permanent heritable alteration when the damaged molecule is replicated) or other forms of induced damage in humans exposed to potential mutagens or carcinogens. One of the few direct methods which does exist measures gross changes occurring in the deoxyribonucleic acid (DNA) that can be visualised by looking at chromosomes through the light microscope. These alterations include overt breakage and rearrangement of the chromosomes within the cell (structural chromosome aberrations) and more subtle changes involving the switching of parts of a single chromosome (sister chromatid exchange). The technique chosen for this study, to quantify chromosome damage in a group of asbestos workers, was sister chromatid exchange (SCE).

1.3 Sister chromatid exchange and genetic damage

A prerequisite for the successful prevention of neoplastic disease is the identification of persons at risk either of having offspring with hereditary defects or of developing disease from mutation in somatic cells (Brogger 1982b). Persons may be at risk either because of an inherited genetic defect or because of exposure to environmental genotoxins, i.e. agents that cause a change in the quality or quantity of the DNA in any of the cells of an individual. In the latter case, prevention requires identification of the genotoxin and termination of exposure.

Carcinogens are thought to act by causing mutations which can be divided into two categories, viz. microlesions which are visible at only the molecular level, and macrolesions (such as those depicted by SCEs) which are visible at the chromosomal level (Hemminki *et al.* 1979).

The identification of a human carcinogen is a long process. The disease manifests itself many years after onset of exposure and it may take 15 to 20 years before reliable epidemiological evidence of an association is available (Brogger 1982c). To avoid such a delay, animal experimentation is often used to identify carcinogens. However, this experimentation is time-consuming and expensive, and the implications for human health are uncertain. The SCE test, using cultured human cells, is a quick and efficient method for detecting chromosome damage.

SCE is a symmetrical exchange of genetic material between the two chromatids of a metaphase chromosome before the separation of the replicated material into two daughter chromosomes (Brogger 1982d). It involves breaks in the DNA sequences of each chromatid, followed by the interchange of DNA chains, and the subsequent repair of breaks with no alteration of the overall morphology (Perry and Evans 1975). The finding that these exchanges arise during replication on damaged templates (Wolff *et al.* 1974, Kato 1980) led to the possibility of using the SCE technique to identify potential DNA-damaging agents (Perry and Evans 1975).

SCE is believed to reflect damage to the DNA material which, if the exchange involves errors, results in possible mutations. If a mutation occurs in a germ cell (oocyte or spermatocyte), the effect is hereditary, i.e. the offspring will be affected with the possibility of congenital malformations. A change in a somatic cell (any cell of the body, other than a germ cell) is transmissible only horizontally to daughter cells, an event significant in the aetiology of cancer (Brogger 1982b, Hemminki *et al.* 1979, Albertini *et al.* 1993). Thus, SCE is not only a good indicator of mutagenic alterations in DNA but also a fairly reliable signal of alterations which lead to carcinogenesis (Carrano *et al.* 1978, Abe and Sasaki 1982, Popescu and DiPaolo 1982).

Even if the exchange is error-free, and not harmful in itself, it still indicates exposure to an agent that may cause damage. In either case, exposure to a hazardous agent is indicated and therefore SCE can be used in occupational monitoring as an indirect measure of possible mutation and an indicator of potential health risks (Husum 1987).

So far there is no clear observational evidence for any hereditary effect in man from exposure to mutagens (Brogger 1982b). Negative findings, nonetheless, have not deterred efforts to protect human populations from environmental mutagens (Brogger 1982b).

The first observations that SCEs could be readily induced by alkylating agents (Abe and Sasaki 1977, Kato 1973, Latt 1974a, Perry and Evans 1975, Solomon and Bobrow 1975) led to a large number of studies, but not all (Latt and Schreck 1980, Latt *et al.* 1981, Perry 1980, Wolff 1977) supported the idea that an agent's ability to induce SCEs correlated well with its effectiveness as a mutagenic carcinogen. However, controversies surrounding the ability of some agents (e.g. tumour promoters) to induce SCEs (Fujiwara *et al.* 1980, Kinsella and Radman 1978, Loveday and Latt 1979, Nagasawa and Little 1979, Popescu *et al.* 1982, Schwartz *et al.* 1982, Thompson *et al.* 1980) may have been resolved, to some extent, by demonstrations that these agents can have indirect effects; for example, induction of free radical production by certain cell types, with the radicals then producing the DNA damage (Emerit and Cerutti 1981, Emerit *et al.* 1982, Weinberg 1982, Emerit 1984).

Some typical cancer-promoting agents do induce SCEs *in vitro*, e.g. phorbol esters (Kinsella and Radman 1978). However, in members of families with high cancer incidence, SCE frequency has not been found to be higher than normal (Cheng *et al.* 1979). Although there is no definite correlation between chromosome damage and the development of cancer, a number of carcinogenic substances have been proven to cause chromosome damage, e.g. benzene (Fredga *et al.* 1982), ethylene oxide (Yager *et al.* 1983, Tates *et al.* 1991), and vinyl chloride (Hansteen *et al.* 1978).

The SCE technique has been used to estimate individual cancer risk. For example, Hopkins and Evans (1980) found that the SCE frequency in response to smoke condensate treatment was significantly higher in smokers with lung cancer than in healthy smokers or non-smokers. This suggests that smokers with lung cancer develop DNA lesions, measured by SCEs, more readily than those smokers without cancer (Vainio *et al.* 1981).

Some investigators have postulated that chromosome instability, as reflected by quantitative changes in SCE, may signal changes associated with the pathogenesis of malignancy (Livingston *et al.* 1983). This is supported by studies showing elevated SCE levels in malignant lymphoma (Kurvink *et al.* 1978a), acute lymphoblastic leukaemia (Otter *et al.* 1979) and Bloom's Syndrome (Chaganti *et al.* 1974).

1.4 History of sister chromatid exchange

The possibility of exchanges between sister chromatids was first suggested in 1938 by McClintock, as an explanation for the behaviour of dicentric ring chromosomes in somatic maize cells (McClintock 1938). It was not until the 1950s, however, that SCEs were cytogenetically demonstrated, by Taylor and co-workers, using autoradiographic techniques (Taylor *et al.* 1957, Taylor 1958). They demonstrated the formation of SCEs in plant root-tip cells following the incorporation of tritiated (^{3}H) thymidine into replicating DNA. SCEs were seen as switches in the radioactive label between chromatids at

the second mitosis after ^{3}H-thymidine labelling at the S phase of the first cycle of mitosis (Taylor 1958). In 1960, Taylor published a report on SCEs in human cells in culture.

In 1972 Zakharov and Egolina described a new method for detecting SCEs, incorporating 5-Bromodeoxyuridine (BrdU) into the sister chromatids instead of a radioactive label (see section 3.3 for further discussion). This vastly improved technique resulted in a much higher resolution of SCEs and has been used for their detection ever since.

Implicit in the early work of Taylor (1958) and later substantiated by others, was the ability of at least one exogenous agent to induce SCEs. This was extended to include ultraviolet light or alkylating agents, using either autoradiography (Kato 1973) or the BrdU method (Kato 1974a, Latt 1974b, Perry and Evans 1975, Solomon and Bobrow 1975) to detect the SCEs. Consequently, it was suggested that SCE could be used as a possible test for certain types of DNA damage (Latt 1974b).

1.5 Mechanism of sister chromatid exchange

It has been suggested that SCE is a means by which a cell copes with DNA damage (Brogger 1982d). In some way the replicating machinery bypasses unrepaired DNA damage by producing an SCE (Evans 1977, Shafer 1982). Thus, increased DNA damage leads to increased SCE. Alternatively, SCE

might be a slightly unreliable process in which unequal exchange is taking place; too small to be recognised cytologically but large enough at the molecular level to lead to duplication-deficiency chromatids (Brogger 1982d).

It is unclear just how SCE induction can generate genetic damage if, as is apparent at a cytological level, it reflects a precise reciprocal exchange of genetic material. Perhaps because of errors in DNA replication enhanced during bypass of DNA damage, which may be involved in the generation of SCEs (Latt 1982, Shafer 1977, Tatsumi and Strauss 1978), the exchange might not be absolutely perfect. Alternatively, SCE formation may serve as a 'signature' of some other process which, like SCE formation, accounts for only a fraction of the total DNA damage, but which, unlike SCE formation, can be directly linked to some mutagenic or carcinogenic process (Latt *et al.* 1984a).

The exact mechanism of SCE formation is unknown but it is thought to reflect changes in DNA resulting from adduct formation or changes in conformational structure after exposure to exogenous agents (Yager *et al.* 1983). The many examples linking DNA sequence rearrangement to neoplastic changes (Cairns 1981, Klein 1981) suggest that DNA interchange is a possible mechanism linking the biological consequences and cytological manifestations of cellular responses to DNA damage that induces SCEs (Latt *et al.* 1984b).

SCEs are double-strand exchanges between polynucleotide chains of corresponding polarity. The DNA strand breakage (which interrupts the continuity of the strands) and reunion occur during the S phase, independent of the time in the cell-cycle at which the responsible DNA alteration occurred (Speit *et al.* 1984a). The general assumption of a connection between certain types of DNA damage and SCEs is due to the fact that agents responsible for characteristic types of DNA damage can also induce SCEs. However, none of the well-known DNA damages has been proven to be a necessary requirement for SCEs (Wolff 1977, Cassel and Latt 1980, Duncan and Evans 1982).

For example, Speit *et al.* (1984b) studied the involvement of single strand breaks (a form of DNA damage) in the formation of SCEs and chromosome aberrations. They showed that various chemicals and physical agents can cause single strand breaks in DNA and can also induce SCEs and chromosome aberrations. This connection has also been demonstrated by other investigators (Bradley *et al.* 1979, Perry and Evans 1975, Gebhart and Kappauf 1978, Kato 1974b, Ikushima 1977, Moan *et al.* 1980, Speit *et al.* 1982). It was unclear, however, whether there was a causal connection between the induction of single strand breaks and SCEs.

As SCEs involve the breaking and subsequent double strand exchange of two identical DNA molecules, a break in one polynucleotide chain could represent

a favourable starting point for the development of an SCE (Speit *et al.* 1984c). One of the early models of SCE proposed by Kato (1974b) was based on this consideration. However, DNA single strand breaks do not seem to be a necessary requirement for SCEs (Bradley *et al.* 1979) and involvement of single strand breaks has not been directly demonstrated. Furthermore, since X-rays and other DNA-breaking agents are not very efficient in the induction of SCEs, it has been assumed that single strand breaks do not represent a major pathway for the production of SCEs (Wolff 1978).

The more recent models of SCE formation assume that the required breaks in the DNA strands are enzymatically induced (Ishii and Bender 1980, Painter 1980). In the model by Ishii and Bender, breaks are induced at the replication fork as the result of damage. Painter postulates that the breaks frequently occur at the connections between neighbouring replicon clusters during their replication, and that SCEs are initiated whenever daughter strands of a replicating cluster recombine with daughter strands of a partially replicated cluster. Such situations particularly occur when the fork displacement rate is reduced, and this is why agents blocking DNA-fork displacement are supposed to lead to increased SCE formation (Painter 1980).

The general replication bypass model suggests that SCEs result from a temporal encounter between the arrival of replication at the site of DNA damage and the simultaneous occurrence of a particular lesion which

impedes the normal advance of replication (Shafer 1984a). This implies that, in most cases, the lesion itself does not induce the SCE. Rather, an SCE is more likely due to some intermediate stage of lesion repair involving an open strand incision of the DNA. SCEs thus result from switching of incised parental strands at, or adjacent to, a lesion site in order for replication to continue past that site (Shafer 1984a).

Central to this general replication bypass principle is the concept that an SCE will only be induced under specific temporal interactions, i.e. not when lesion repair is completed before replication occurs at that site, or when lesions are not repaired but are of a type which does not inhibit replication (Shafer 1984b). Thus, few potential SCE-inducing lesions will actually result in an SCE, and SCEs, like mutations, are a relatively rare outcome of many DNA lesions which may have been present, whether endogenous or induced.

In addition, it was noted that many of the agents which induce SCEs, whether directly or indirectly, result in a few common repair intermediate stages including endonuclease binding to DNA, strand incision, DNA resynthesis, and ligation (Shafer 1984b). It was suggested that this might be a basis for hypothesising a few primary SCE mechanisms to handle the wide variety of agents or conditions that impede the successful completion of DNA replication. The evolution of multiple SCE mechanisms would thus be analogous to the evolution of multiple repair mechanisms, i.e. a small class

of general SCE processes designed to handle a wide variety of discrete lesion conditions (Shafer 1984b).

Independent of whether DNA lesions lead to SCEs directly or indirectly (for instance, via replication delay), and whether there is one or several mechanisms leading to SCE formation, one must assume that SCE formation is a complex enzymatic process which includes both the breaking and rejoining of DNA strands. The breaks must obviously occur in a defined manner so that an orderly reunion becomes possible (Speit *et al.* 1984d).

Whatever the mechanism of SCE formation, the predictability of a genetic hazard by SCE induction is of value, at least to the extent that it warrants subsequent examination by more complicated tests (Latt *et al.* 1984c).

1.6 Advantages of the sister chromatid exchange test

Cytogenetic analyses yield qualitative information and rough quantitative estimates of the degree of exposure to mutagens (Vainio and Sorsa 1983). SCE analysis is a more sensitive method than conventional metaphase analysis of chromosome aberrations, especially directly following the exposure in question (Vainio and Sorsa 1983). Large increases in the number of SCEs occur with doses of mutagenic agents well below the level needed to cause chromosome aberrations, thus the analysis of SCEs is a very sensitive method of assessing DNA damage. If cells are exposed to chemicals

at concentrations as low as one hundredth that necessary to produce chromosome aberrations, large numbers of SCEs can be seen (Latt 1974b, Solomon and Bobrow 1975, Perry and Evans 1975). It is also likely that SCEs are more representative of events compatible with cell survival (such as mutagenesis) than are aberrations (Wolff 1977).

In conclusion, because of its sensitivity and ease of scoring, the SCE test has been used increasingly as a short term test for the detection of mutagenic carcinogens; an excellent correspondence has been found between chemicals that induce SCEs and those that induce mutations, as determined by a battery of other short-term tests (Wolff 1983).

1.7 Interpreting sister chromatid exchanges

The most important health effects of induced chromosome damage are, perhaps, the changes which functionally alter the cell but leave it capable of proliferation. Changes at the chromosomal level may play an important role in carcinogenesis, e.g. in the promotion of the malignant potential of the transformed cell through an expression of the initiated oncogenic factors of the genome (Vainio and Sorsa 1981).

Visible chromosome damage in a sample of somatic cells taken from an exposed individual provides a direct indication of exposure to clastogenic agents (agents which break chromosomes). Even though the unrepaired

damage in the indicator cells may be lethal to the cell, and thus of no significance as such (especially in the case of non-dividing T lymphocytes), the observation of chromosome damage suggests that alterations of genetic material may also have occurred in cells of other tissues (Vainio and Sorsa 1981). Visible damage also indicates that invisible lesions may have occurred. Such microlesions may have an even higher probability of being transmitted in cell proliferation and thus, for being manifested (Vainio and Sorsa 1981).

The significance of chromosome mutations inherited in the germinal cell line has been clearly documented in relation to spontaneous abortions and live births with chromosome anomalies (Vogel 1979). However, as yet, there is no clear evidence that a population exposed to chromosome-breaking agents would show an increased incidence of constitutional chromosomal or genetic abnormalities among their offspring (Vainio and Sorsa 1981).

On an individual basis, increased somatic chromosome damage that is clearly above the level of the average of the group may point to an inherited susceptibility of the individual to, for example, develop cancer. The role of chromosomes in human cancer proneness is supported by correlations of high chromosome instability (both 'spontaneous' and induced) and an increased risk of malignancies in such human hereditary disorders as xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, and Fanconi's

anaemia (Knudson 1977). The quantitative estimate of risk cannot, however, be deduced from the chromosomal data of a single individual (Vainio and Sorsa 1981).

Although an individual prognosis cannot be derived on the basis of chromosome data, we may suspect that an individual with a prolonged increased level of somatic chromosome damage has been exposed to an agent at concentrations high enough to cause ill health effects (Vainio and Sorsa 1981).

In terms of potential genetic hazard to a population, the production of a low level of damage in a large number of individuals is of greater consequence to the population than the induction of large amounts of damage in a small number of individuals (O'Riordan and Evans 1974). On the group level, the demonstration of chromosome damage can be considered proof of genotoxic risks requiring actions to decrease exposure (Vainio *et al.* 1981).

One may conclude that a group of exposed persons showing increased levels of chromosome damage has a higher risk of developing cancer than a group showing no such increase.

1.8 Factors affecting sister chromatid exchange frequency in individuals

SCEs arise spontaneously, and in fairly large numbers per cell, on average, in the absence of genotoxin exposure (Bender *et al.* 1992). Nevertheless, there is considerable variation in the SCE frequency in lymphocytes of healthy human subjects (Lambert *et al.* 1976, Lambert *et al.* 1978, Morgan and Crossen 1977, Crossen *et al.* 1977, Pedersen *et al.* 1979). Different studies have reported mean baseline SCE frequencies per cell from as low as 4.41 to as high as 14.26 (Table 1).

TABLE 1. Baseline sister chromatid exchange frequencies in normal populations

Reference	Country	SCE/cell range	SCE/cell mean	SCE/cell SD
Galloway and Evans 1975	Britain	12.19 - 19.96	14.26	ns
Latt *et al.* 1975	USA	9.50 - 14.60	12.50	ns
Hatcher *et al.* 1976	USA	7.20 - 10.60	8.70	2.73
Morgan and Crossen 1977	New Zealand	1.00 - 12.00	7.90	1.36
Hollander *et al.* 1978	USA	9.80 - 15.00	11.90	1.77
Lambert *et al.* 1978	Sweden	7.80 - 20.00	13.20	2.90
Cervenka *et al.* 1979	Nigeria	4.80 - 6.33	5.20	ns
Meiying *et al.* 1982	China	3.70 - 5.75	4.41	0.72
Hirsch *et al.* 1984	USA	6.63 - 9.61	8.26	0.79
Soper *et al.* 1984	USA	5.00 - 17.50	9.90	2.00
Bender *et al.* 1992	USA	< 4.40 - 13.40	8.12	ns
Park *et al.* 1992	Korea	3.49 - 16.96	8.78	0.24
Anderson *et al.* 1993	UK	ns	6.69	ns

ns = not stated

The higher baseline frequencies of SCE in populations of developed countries (compared to those in developing countries) may be due to higher environmental contamination (Meiying *et al.* 1982).

In most investigations, groups for study are selected from a "normal" human population. It is therefore important to know which factors may influence the baseline SCE variation. Genetic differences are one possible explanation; SCE has been found to be relatively consistent in repeated cultures for the same donor (Morgan and Crossen 1981, Morgan and Crossen 1977, Crossen *et al.* 1977). Pedersen *et al.* (1979) studied mono- and dizygotic twins and found the same variation within pairs as between pairs.

Studies demonstrating the effect of age on SCE frequency have shown conflicting results. Some authors have reported no differences across wide age ranges (Morgan and Crossen 1977, Galloway and Evans 1975, Schneider *et al.* 1979). Other studies have demonstrated differences between children and adults (Funes-Cravioto *et al.* 1977, Ardito *et al.* 1980, Schuler *et al.* 1979, Murthy *et al.* 1980). Zanzoni *et al.* (1979) reported a significant difference between 20 to 40 year olds, and 60 to 80 year olds. Goh (1981) showed a higher SCE frequency in 66 to 88 year olds than in 24 to 65 year olds. On the other hand, Wen and Liew (1983) found a significant correlation between age and SCE frequency in non-smokers between 13 and 84 years of age. Other reports have also described increases with age (Waksvik *et al.* 1981a, Sarto *et al.* 1985).

When genetic factors are ruled out, differences in environmental exposure levels must be considered as a cause for variation.

Tobacco smoke is the most widespread exposure in the human population (Hansteen 1982a). Many studies have demonstrated an increase in SCE frequency among cigarette smokers (Murthy 1979, Reuterwall 1990, Lambert *et al.* 1978, Degrassi *et al.* 1993). The amount of smoking, inhalation, and even the time between smoking and collection of the blood sample may influence the SCE frequency (Lambert *et al.* 1982a). Smoking has been shown to increase the frequency of SCEs by between five and 23% (Nordic study group 1990, Bender *et al.* 1988, Reidy *et al.* 1988).

Other factors affecting SCE frequency include anticancer drugs (Jacobson-Kram *et al.* 1993), organic solvents (Funes-Cravioto *et al.* 1977), ethylene oxide (Stolley *et al.* 1984), oral contraceptives (Lambert *et al.* 1982b, Murthy and Prema 1979), caffeine (Reidy *et al.* 1988, Shim 1989) severe protein malnutrition (Murthy *et al.* 1980), infection of the urinary tract (Kowalczyk 1980), vaccinations (Lambert *et al.* 1979), and hepatitis, herpes simplex virus and uncharacterised cold or influenza virus infections (Kurvink *et al.* 1978b).

Increased SCE frequency has been reported in females (Hedner *et al.* 1982, Bender *et al.* 1988), and single XX cells have been shown to have a higher SCE frequency than XY cells (Wulf and Niebuhr 1985). This is believed, by some authors (Margolin and Shelby 1985, Bender *et al.* 1988), to be due to the longer second X chromosome in females, in relation to the Y chromosome in males. Wulf (1990), however, states that this difference is too large to be

solely due to the different genomic lengths of the X and Y chromosomes. Whatever the reason, sex must be considered as a confounding factor in SCE studies.

Studies of *in vivo* exposed persons are also complicated by variation in the dose of individual exposure which is often difficult to determine in retrospective studies (Hansteen 1982a). Distribution of the test chemical in the body, the metabolic activation of the compound, and the rate of inactivation of the metabolites are unknown for most chemicals. Metabolic activation, inactivation of metabolites, and DNA repair processes may also be subject to genetically determined individual variation. Therefore, it is also difficult to make dose estimates in prospective studies.

All of the aforementioned factors can influence SCE and consequently mask the effect of the particular agent being tested. A precise characterisation of the subjects under study is therefore crucial in an investigation which is designed to evaluate the effect of a particular industrial or environmental toxin. The exposed subjects and their referents should ideally be a homogenous group with regard to all exposures except the one under scrutiny (Hansteen 1982b).

The possible environmental exposures that may influence the baseline SCE frequency underline the extreme importance of collecting detailed

information on age, smoking habits, drugs, alcohol, recent viral infections, hobbies, etc., for all subjects under investigation (Hansteen 1982a).

1.9 The distribution of sister chromatid exchanges within chromosomes

Although SCEs seem to occur randomly in that they are distributed among the chromosomes according to Poisson expectations, there are several indications that their location within chromosomes might not be completely random. A large proportion of SCEs occur in the interband regions of G-banded chromosomes (Wolff 1977).

1.10 Sister chromatid exchange and occupational exposures

Once an increased incidence of cancer in an industry becomes evident, the opportunity for prevention of the disease is lost. Latency time between primary damage and expression may be many years. It is therefore important to detect potential ill health effects before they occur. SCEs can be used in occupational monitoring as an indirect measure of possible mutation and an indicator of potential health risks (Husum 1987, Gebhart *et al.* 1993). Visible chromosome damage in a sample of somatic cells taken from an exposed individual provides a direct indication of possible exposure to clastogenic agent(s). Cytogenetic monitoring of suitable occupational groups is a feasible method to identify individuals and populations with potential genotoxic risk (Sorsa 1985).

SCE frequencies may be affected by individual differences in metabolism and susceptibility to chemicals (Degrassi *et al.* 1993). Other problems with *in vivo* studies lie in identifying a specific factor in an occupational setting, where simultaneous exposure to a number of organic and inorganic chemicals is a common phenomenon (Vainio and Sorsa 1981). The interaction between different agents may affect the properties of one or the other, for example, toluene modifies the metabolism and toxicity of hexane (Takeuchi *et al.* 1993). The use of the SCE technique in occupational settings is also hindered by limited information on the distribution of SCE frequencies in non-exposed persons (Soper *et al.* 1984).

TABLE 2. Increased sister chromatid exchange frequencies observed in some occupational settings

Agent	Reference
Anaesthetic gases	Karelova *et al.* 1992
Chromium	Stella *et al.* 1982
Coke oven emissions	Miner *et al.* 1983
Cytostatic drugs	Norppa *et al.* 1980
Ethylene oxide	Stolley *et al.* 1984
Lead	Maki-Paakkanen *et al.* 1981
Organic solvents	Funes-Cravioto *et al.* 1977
Organophosphate (insecticide)	Larripa *et al.* 1983
Pentachlorophenol	Bauchinger *et al.* 1982
Petroleum	Edwards and Priestly 1993
Pesticides	De Ferrari *et al.* 1991
Stone dust (silica)	Sobti and Bhardwaj 1991
Styrene	Andersson *et al.* 1980
Toluene	Bauchinger *et al.* 1983
Trichloroethylene	Gu *et al.* 1981
Vinyl chloride	Kucerova *et al.* 1979

Many studies on different occupational exposures have demonstrated increased SCE frequencies in workers (Table 2). Not a single case, however, has been observed in which it is evident that increased SCE in lymphocyte

chromosomes led to a hereditary defect or a cancer (Brogger 1982e). Nevertheless, workers exposed to vinyl chloride monomer have developed angiosarcoma of the liver (Creech and Johnson 1974) and exposures of similar levels have been large enough to induce both SCEs and chromosome aberrations (Hansteen *et al.* 1978).

Large prospective studies are perhaps the only way of obtaining reliable, quantitative estimates of the risks indicated by observed SCE increases (Brogger 1982f). In occupational health, exposures leading to increased SCE and chromosome aberration call for immediate efforts to diminish the unwanted exposure (Brogger 1982f).

1.11 Sister chromatid exchange and asbestos

Many researchers have investigated the relationship between *in vitro* exposure of different types of cells to asbestos and SCE frequency but results have been inconclusive (Table 3).

Very few studies have been done on the *in vivo* effects of asbestos. Bajerska *et al.* (1988) found an increase in SCE frequency in mouse bone marrow cells after *in vivo* exposure to asbestos. Only three reports on human exposure to asbestos and SCE have been published (Rom *et al.* 1983, Fatma *et al.* 1991, Kelsey 1986a), with contradictory findings (see chapter 5).

TABLE 3. Sister chromatid exchange studies on *in vitro* exposure to asbestos

Reference	Cell Type	Increase in SCE
Livingston *et al.* 1980	CHO fibroblasts	Yes
Kaplan *et al.* 1980	RPM cells	No
Price-Jones *et al.* 1980	V79-4 CH cell line	No
Babu *et al.* 1981	CHO cells	Yes
Casey 1983	CHO, human fibroblasts and lymphoblastoid lines	No
Hei *et al.* 1985	mouse embryo fibroblasts	No
Kelsey *et al.* 1986b	CHO cells	No
Archard *et al.* 1987	RPM cells	Yes

CHO - Chinese hamster ovary
RPM - rat pleural mesothelial

1.12 Objective of the study

The objective of the study reported here was to determine the relationship between asbestos exposure and chromosome damage, as depicted by sister chromosome exchange frequency.

CHAPTER 2: METHODS

2.1 Study population and sampling

The study population consisted of two groups of black males, viz. a group exposed to chrysotile asbestos and an unexposed reference group. The exposed group originated from two neighbouring asbestos products factories (factories A and B) which manufactured brake linings, asbestos heaters, etc. The reference group comprised men such as cleaners and clerks, employed in a Government institute and not exposed to asbestos at work. The two groups were of similar socio-economic status.

A total exposed sample of 122 workers was selected from the two factories. All workers (61) were selected from factory A and a further 61 were randomly chosen (using random numbers generated by the computer package, Epistat) from the 115 workers in factory B. Sixty one of the 63 men in the reference group were selected. The study sample thus comprised 122 exposed and 61 unexposed subjects (a ratio of 2:1).

For the purpose of analysis, the workers from factory A and those sampled from factory B were combined into a single asbestos-exposed group.

2.2 Implementation

Eighteen cases were randomly selected each week for a period of 10 weeks (six from each of the two factories and six from the reference group). Each study subject was given an explanation about the research being conducted and an informed consent form was signed if he agreed to participate (appendix 1).

In one day two questionnaires were administered by two trained interviewers and a 10ml sample of blood was drawn, in a heparinised vacutainer tube, from each person. The first questionnaire (appendix 2) covered basic demographic information, family history (with regard to birth abnormalities), smoking history, health status, and other information about exposure to factors known to increase the frequency of SCE. The second questionnaire (appendix 3) dealt with previous and present exposure to asbestos, and occupational histories.

2.3 Culturing of cells for sister chromatid exchange analysis

One day later, T lymphocytes were cultured from each of the blood samples, according to standard techniques (described below), in a "batch". In other words, all the blood samples were prepared at the same time in a uniform manner to overcome problems relating to culturing cells under different conditions (see 3.4 - factors influencing SCE in culture). At the end of the study there were 10 such batches of cells.

Double cultures (two cultures per study subject) were initiated in sterile 15ml plastic screw top centrifuge tubes by adding 0.5ml heparinised blood to 4ml Ham's F-10 medium with L-glutamine and antibiotics, 0.5ml foetal bovine serum, and 0.05ml phytohaemagglutinin. Lastly, 0.3ml BrdU (concentration 10ug/ml) was added. The tubes were then wrapped in aluminium foil to protect the cells from light. The cultures were incubated at 37°C, at an angle of approximately 30°, for 72 hours. Thereafter, 0.1ml of colchicine (concentration 0.05ug/ml culture medium) was added to each 5ml culture. Thirty minutes later the cultures were terminated by centrifuging at 1 600 rpm for 10 minutes, removing the supernatant, and adding 5ml of 0.075M KCl at 37°C. The cultures were incubated at 37°C for a further 20 minutes, after which the tubes were again centrifuged and the supernatant removed. The cells were "fixed" by adding 5ml fixative (three parts methanol to one part glacial acetic acid) to each culture, centrifuging, and removing the supernatant. This process was repeated until the supernatant was clear. Usually, three fixative changes were sufficient.

2.4 Microscope slide preparation

Two microscope slides were prepared from the lymphocyte cultures of each study subject. Pre-cleaned slides were soaked in absolute alcohol for at least 24 hours at room temperature; they were then dried and polished with facial tissue. Approximately 0.5ml fixative was added to the cell pellet immediately before preparing the slides and the cells were resuspended,

using a narrow calibre glass pipette. The slides were flooded with cold fixative and the excess was tipped off. Three to four drops of the cell suspension were dropped onto each slide from a height of approximately one metre. The slides were again gently flooded with fixative as they began to dry. Once they had air dried completely, the slides were stored for four days at room temperature before being stained.

2.5 Slide staining

The staining procedure followed was a modification by Crossen (1982a) of the fluorescence plus Giemsa (FPG) technique developed by Perry and Wolff (1974). The slides were stained for 15 minutes in a 100ug/ml 33258 Hoechst stain (a bis-benzimidazole fluorochrome), rinsed in distilled water, dried, and mounted in 0.6M Na_2HPO_4 (anhydrous). After being exposed to natural light for 24 hours, the coverslips were removed and the slides were incubated for 20 minutes in 0.06M phosphate buffer (0.06M Na_2HPO_4/KH_2PO_4; pH 6.8). They were then stained in a 5% Giemsa solution (Gurr R66) for six minutes.

2.6 Microscopic analysis of sister chromatid exchange

Each slide was given an identification number to ensure that the counting of SCEs was a blind procedure, i.e. the reader was unaware of the exposure status of the individual when counting the SCEs, and observer bias was prevented. All the slides were examined by a single reader.

The cells were examined under 1 000 X magnification (oil immersion), using a Nikon Alphaphot YS microscope, and a drawing tube. Each change in colour along the length of a chromatid was counted as an SCE (see Fig. 1).

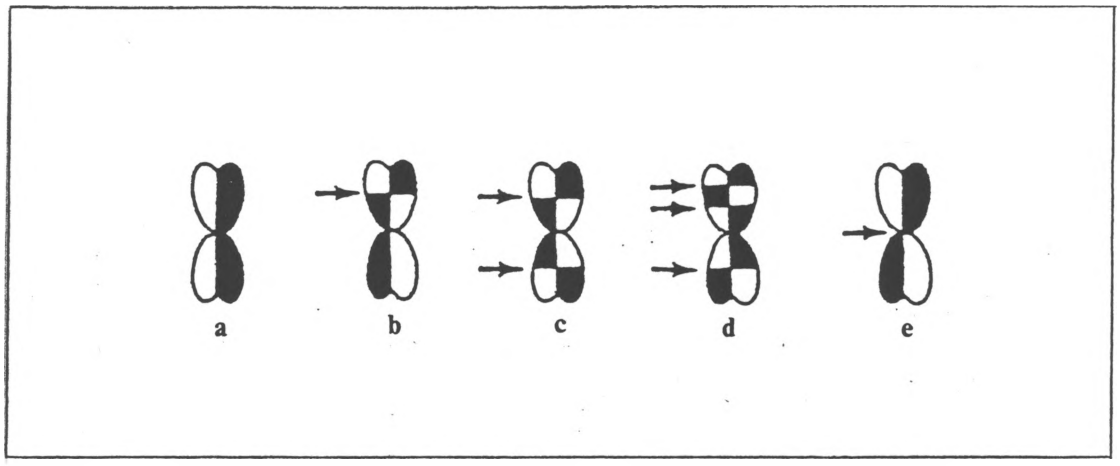


Figure 1. Schematic representation of various differential staining patterns, showing method used to score sister chromatid exchanges. (a) No SCEs are present. (b) A single SCE. The arrow indicates the point of reciprocal exchange of the chromatids. (c) Two SCEs. (d) Three SCEs. (e) An SCE occurring at the centromere. (Adapted from Carrano and Natarajan 1988).

The chromosomes of each metaphase cell were drawn onto paper and were colour coded by chromosome group (A to G). Each SCE was indicated by a black dot (Appendix 4). The SCEs in each cell were added and the numbers were transcribed onto a data capture sheet (appendix 5). The mean number of SCEs per cell and per chromosome were then calculated and transcribed onto a second data capture sheet (appendix 6) from which the data were computerised into a dBase IV file.

SCEs were counted in all metaphase cells where there were less than four overlapping chromosomes. At least 44 of the 46 chromosomes had to be

present, and there had to be visible differential staining of the chromatids. Up to 50 metaphase cells were analysed per subject. In cases where 50 cells did not fulfil the criteria, the maximum number of suitable cells was analysed.

2.7 Preparation of data for analysis

A successful case was defined as follows. Firstly, lymphocytes had to have successfully undergone mitotic division, with metaphase cells visible under the light microscope. Secondly, at least 15 cells had to fulfil the criteria for the clear scoring of SCEs.

The computer packages used for data management and statistical analysis were dBase IV and Epiinfo.

Data were first analysed to test whether the successful cases were representative of the sample that had been selected from the total exposed and unexposed populations. This was done by comparing:

1. All successful unexposed cases (21) with all unsuccessful unexposed cases (33).
2. All successful exposed cases (31) with all unsuccessful (80) exposed cases.

These two sets of analyses were done to test for any statistical differences in those variables which have previously been shown to affect the frequency of SCE, and which were included in the questionnaire. These variables were:

1. Viral diseases during the previous month (influenza, diarrhoea, chicken pox).
2. Hepatitis or glandular fever during the previous six months.
3. Immunisations during the previous six months.
4. Medications during the previous three months (including those administered by a traditional healer).
5. Current smoking status.
6. Caffeine consumption (tea, coffee and cold drinks).
7. Alcohol consumption.
8. Use of hair dyes during the previous three months.
9. Use of artificial sweeteners during the previous three months.

Frequency distributions were calculated for differences between the successful exposed and unexposed cases with regard to demographic data and factors known to affect SCE frequency.

Indices were calculated for smoking habits, and for caffeine and alcohol consumption. The smoking index was calculated by adding the number of commercial and hand rolled cigarettes currently smoked per day. Pipe

smoking was ignored as only four of the 165 cases smoked a pipe (only one of which was a successful case). The alcohol index was calculated on the basis of the percentage of alcohol contained in a standard glass of wine (15% in 150ml, equivalent to 22.5g of alcohol), a tot of liquor (43% in 25ml, equivalent to 10.75g of alcohol), a can of beer (5% in 340ml, equivalent to 17g of alcohol), and carton of sorghum beer (2% in 1 000ml, equivalent to 20g of alcohol). The calculated daily amount of caffeine consumed was based on the caffeine content of an average cup of coffee (85mg), average cup of tea (50mg), and 340ml can of cola (50mg) (Iserson 1990). Means were calculated using only those cases who smoked, drank alcohol, or drank caffeine, i.e. zero values were excluded from the calculations.

2.8 Statistical methods

Chi-square analyses were used to test differences between absolute numbers. Analysis of variance (ANOVA) was the method applied to test differences between means. When the variances in the samples did not differ, p values equivalent to the two sample, two-sided Student's t test were quoted. When the variances did differ, the non-parametric Kruskal-Wallis test was used to calculate the p value.

Multiple linear regression analysis was performed to determine which of the following variables were predictors of increased SCE frequency:

1. Conventional medication in the last three months
2. Traditional medication in the last six months
3. Vaccines in the last six months
4. Use of hair dye
5. Use of artificial sweeteners
6. Any viral infection within the last six months, viz. influenza, diarrhoea, chicken pox, hepatitis, or glandular fever
7. "Environmental" exposure to asbestos - taken as positive if the person had lived in an asbestos area, lived with someone who worked in the asbestos industry, cut asbestos while working on a hobby, etc.
8. Age - this was divided into 20 year categories for reasons discussed in chapter 1, section 1.8
9. Number of cigarettes currently smoked - divided into non, light, moderate, heavy and very heavy smokers
10. Amount of alcohol consumed - divided into low, moderate and high alcohol intake
11. Amount of caffeine consumed - divided into equivalent cups of coffee
12. Occupational exposure to asbestos - exposed or unexposed cases

The dependent variable was the mean SCE frequency per cell.

CHAPTER 3: DISCUSSION OF METHODS

3.1 Study subjects

The ratio of exposed to unexposed subjects in the study was 2:1 (122 exposed and 61 unexposed men). It would have been preferable to have equal numbers of exposed and unexposed subjects but the size of the reference group was limited. A decision was made to include as many of the asbestos workers as possible in the study. This same ratio was used by Sobti and Bhardwaj (1991) in a study on cytogenetic damage after exposure to stone dust.

3.2 The use of lymphocytes in cytogenetic analysis

In 1960 Nowell discovered that phytohaemagglutinin (PHA) stimulated T lymphocytes to divide in culture. Since then, these mitogen-stimulated blood lymphocytes have become the most frequently used cells for cytogenetic analysis of short-term cultures of peripheral blood taken from subjects by venipuncture (Vainio and Sorsa 1981).

Although real in vivo detection of damage can be obtained only from direct samples of dividing human cells, e.g. bone marrow or testicular tissue, the use of these cells has obvious disadvantages. SCE is therefore applied *in vitro* to lymphocytes exposed to certain agents in vivo (Vainio and Sorsa 1981). Circulating T lymphocytes are easy to obtain and they grow well in culture so that the chromosomes are easily visible at mitosis (Crossen 1982b).

Most of the T lymphocytes belong to the redistributional pool, i.e. they leave the blood, pass through the different organs, carrying nutrients and metabolites, and reenter the peripheral blood (Carrano and Natarajan 1988). Thus, lymphocytes exposed to a mutagen anywhere in the body can eventually occur in peripheral blood and can therefore be used as indicators of exposure (Carrano 1986). In other words, the lymphocyte is a surrogate cell that may be an indicator of an effect produced in other cells of the body, including germ cells.

3.3 The role of 5-Bromodeoxyuridine in the production of sister chromatid exchange

All techniques for SCE visualisation rely on the fact that DNA synthesis in eukaryotic cells is semi-conservative and, after two rounds of DNA synthesis in the presence of either labelled or analogue forms of thymidine, there will be SCEs in the same chromosome which are different in their thymidine residue composition (Du Frain 1984).

Almost all methods for the *in vitro* detection of SCE use the incorporation of 5-Bromodeoxyuridine (BrdU), an halogenated thymidine analogue, for two cell cycles, to enable the visualisation of the exchanges under a light microscope (fig. 2). When the cells are stimulated to divide by PHA, BrdU is incorporated into the DNA instead of thymidine. If cells undergo two consecutive rounds of replication in the presence of BrdU, the second

generation metaphase chromosomes will contain one chromatid with similar DNA strands (either both substituted or both unsubstituted) while the other chromatid has a hybrid DNA strand with one chain substituted and one chain unsubstituted (Schvartzman and Tice 1982).

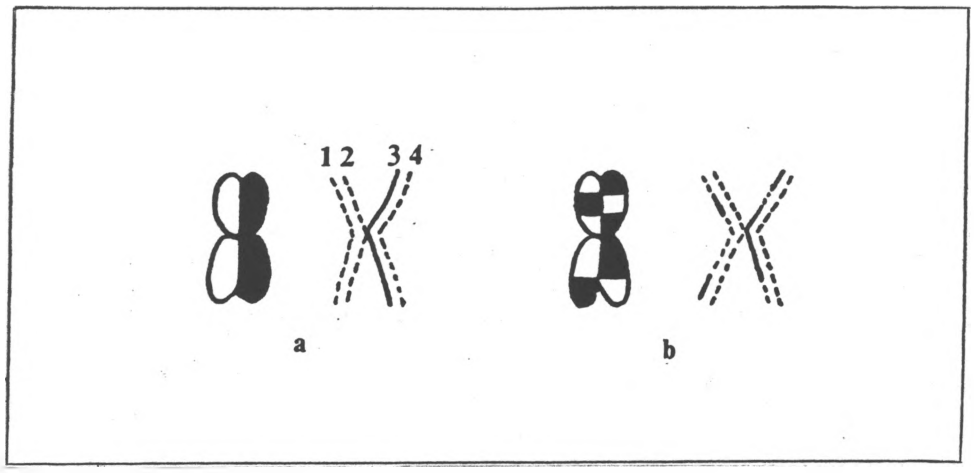


Figure 2. Diagrammatic representation of sister chromatids with bromodeoxyuridine substitution. DNA strands are labelled 1 to 4. (a) A chromosome that has replicated twice in BrdU without forming an SCE. Strands 1,2 and 4 have BrdU incorporated in them. The chromatid containing strands 3 and 4 (one strand without BrdU and one strand with BrdU) stains dark, relative to the sister chromatid (BrdU present in both strands). (b) A chromosome with three SCEs. Segments have switched between strands 1 and 3, and also between strands 2 and 4, to maintain DNA polarity. (Adapted from Carrano 1986).

The lesser substituted chromatid can be made to stain (or fluoresce) either more or less intensely than the more substituted one, resulting in chromosomes with one chromatid staining darker (or fluorescing more brightly) than the other. Thus, exchanges appear as sharp demarcations in the intensity of staining along a chromatid with the opposite staining pattern on the other chromatid.

Latt (1973), using autoradiography to assess the incorporation of tritiated BrdU and Hoechst 33258 to achieve sister chromatid differentiation, concluded that the dull-fluorescing chromatids had incorporated nearly twice as much BrdU as the brightly fluorescing ones. Soon after, other fluorochromes, such as acridine orange (Kato 1974c), were also shown to differentiate sister chromatids. Staining techniques based on the use of Giemsa (Perry and Wolff 1974, Korenberg and Freedlender 1974, Goto *et al.* 1978) have largely replaced fluorescent techniques as the former allow the preparation of permanent microscope slides and fluorescent microscopy is not needed. Unifilarly BrdU substituted chromatids stain darkly with Giemsa, whereas bifilarly substituted chromatids stain lightly.

3.4 Factors influencing sister chromatid exchange in culture

Reproducibility of individual results, at least between laboratories, tends to be poor, probably due to the different conditions under which cells are cultured, and the different SCE techniques used.

A number of technical problems arise during the SCE procedure. Cells must survive until the second division to be counted or the response will be biased due to the non-survival of severely damaged cells. In addition, since the number of SCEs increases with increasing concentration of BrdU in culture (Kato 1974c, Davidson *et al.* 1980), it is important to control baseline conditions and to keep the BrdU at a concentration low enough to maintain

a low baseline exchange rate, but high enough to be able to distinguish between chromatids.

Some authors believe that a high cell density in the culture may result in a lower SCE frequency because a smaller amount of BrdU will be available (Stetka and Carrano 1977, Mazrimas and Stetka 1978, Carrano *et al.* 1980). However, Davidson *et al.* (1980) found that the ratio of exogenous BrdU molecules to cell number had no significant effect on the frequency of SCEs.

After addition of BrdU to a cell culture, the culture must be protected from visible light as this can increase the frequency of SCE (Kato 1974b, Ikushima and Wolf 1974, Schvartzman *et al.* 1979). Exposure of cultures to other than red or yellow light causes photolysis of the BrdU-substituted DNA (Carrano and Natarajan 1988).

Morgan and Crossen (1981) found that the culture medium used can influence the number of SCEs. The effect could, to some extent, be dependent upon the amount of thymidine in the medium. Bianchi *et al.* (1979), however, found no difference between Ham's F10 which contains thymidine, and RPMI 1640 and Dulbecco's MEM, which do not.

Morgan and Crossen (1981) tested two different sera; neither caused an increase in SCE. Kato and Sandberg (1977) and McFee and Sherrill (1981), on

the other hand, have demonstrated variations in SCE frequency with different batches of serum. This may indicate that some batches of serum contain factors that increase SCE.

Other factors that affect SCE frequency are rises in temperature during incubation of cell cultures (Livingston and Dethlefsen 1979, Abdel-Fadil *et al.* 1982), and differing incubation times of cell cultures (Santesson *et al.* 1979). The effect of time is prevented, as much as possible, by incubating cultures for a standard period of 72 hours for SCE analysis.

Because of these factors, it is essential that standardised culture conditions be applied, and that control cultures be processed simultaneously.

Taken together, the culture and biological factors (as discussed in chapter 1) that potentially confound the baseline SCE frequency in humans, are numerous. It is therefore not surprising that considerable variation exists among individuals.

3.5 The scoring of sister chromatid exchange

An SCE may be defined as a reciprocal exchange between sister chromatids of the same chromosome, shown by differences in staining intensity (Block 1982a). Each exchange of stain (a darkly stained region on one chromatid, accompanied by a reciprocal lightly stained region on the other chromatid)

is counted as an SCE (Figure 1). Exchanges of stain at the centromere are counted as an SCE unless there is an obvious twist of the chromatids (Carrano and Natarajan 1988).

Multiple closely spaced SCEs or very small SCEs may be difficult to detect and accurately count; their detection is dependent upon the staining quality of the cells (Morgan and Crossen 1981). It is often difficult to judge whether or not an SCE has occurred at the centromeric region. Variations in SCE frequencies in otherwise identical experiments may therefore be due to some extent to variations in staining technique and scoring procedures among observers. One should, ideally, have a single scorer during the entire study to avoid observer bias.

Kato (1977) suggested that SCEs be counted per cell and not per chromosome because baseline SCE is directly related to DNA content and not to chromosome number. Swierenga *et al.* (1991), however, state that the number of SCEs per cell tends to be a more accurate parameter since it does not change with the number of chromosomes which may vary from cell to cell. For this reason, and because data are not consistently reported in one way or the other in different studies, the data in this study are presented as both SCE per chromosome and SCE per cell.

3.6 Statistical methods

The recommended number of cells to examine to detect small differences in SCE frequency with statistical confidence, varies from between 10 and 20 per subject, for a liberal criterion of accuracy (Hirsch *et al.* 1984, Block 1982b), to 50 cells, for a strict criterion of accuracy (Hirsch *et al.* 1984).

CHAPTER 4: RESULTS

4.1 Response and success rates

The total number of subjects in the study was 183; 122 were exposed to asbestos and 61 were not exposed (Table 4). The response rate of both groups was high, viz. 91.0% for the exposed group and 88.5% for the unexposed group (90.2% overall). Only 18 people did not have blood taken for lymphocyte culturing. Reasons for this included refusal, absenteeism, and difficulty in taking a blood sample.

TABLE 4. Sample sizes and success rates

	Unexposed	Factory A	Factory B	Total
Population	63	61	115	239
Sample	61 (96.8%)	61 (100.0%)	61 (53.0%)	183 (76.6%)
Refusal	4 (6.6%)	2 (3.3%)	1 (1.6%)	7 (3.8%)
Absent	3 (4.9%)	2 (3.3%)	1 (1.6%)	6 (3.3%)
Unable to draw blood	0	2 (3.3%)	3 (4.9%)	5 (2.7%)
No. cultures	54 (88.5%)	55 (90.2%)	56 (91.8%)	165 (90.2%)
No. successful cases	21 (38.9%)	11 (20.0%)	20 (35.7%)	52 (31.5%)

Table 4 also shows the success rates of the blood cultures, as defined in chapter 2, section 2.7. These were disappointing in both the exposed and the unexposed groups (27.9% and 38.9%, respectively). The overall success rate was 31.5%.

4.2 Testing for representativeness of successful cases

To test whether the successful cases were representative of the samples of exposed and unexposed cases, two comparisons were made. All successful unexposed cases (21) were compared with all unsuccessful unexposed cases (33), and all successful exposed cases (31) were compared with all unsuccessful (80) exposed cases. The variables tested are discussed in chapter 2, section 2.7.

More detailed analyses (see chapter 2 for methodology) were then carried out on the successful exposed cases (31) and the successful unexposed reference group (21). Statistically significant differences are indicated in all tables by bold p values.

4.2.1 Comparison between successful and unsuccessful unexposed cases

Table 5a shows the differences between the successful and unsuccessful unexposed cases for those variables known to affect sister chromatid exchange frequency. There were no statistically significant differences although more of the successful cases were taking some sort of conventional medication (47.6% as opposed to 24.2% of the unsuccessful cases), and more of the successful cases smoked. Most of these unexposed cases had had an X ray in the past year, and most of them drank tea and/or coffee on a daily basis.

TABLE 5a. Comparison between successful and unsuccessful unexposed cases

	Successful n = 21	Unsuccessful n = 33	p value*
Medical:			
Influenza	7 (33.3%)	10 (30.3%)	0.8749
Diarrhoea	6 (28.6%)	10 (30.3%)	0.8370
Chicken pox	1 (4.8%)	1 (3.0%)	1.0000
Hepatitis	0	2 (6.1%)	0.5157
Glandular fever	0	0	
Any viral disease	12 (57.1%)	17 (51.5%)	0.6887
Exposures:			
X rays	20 (95.2%)	31 (93.9%)	1.0000
Medication (non traditional)	10 (47.6%)	8 (24.2%)	0.0784
Medication (traditional)	4 (19.1%)	9 (27.3%)	0.4947
Immunizations	0	1 (3.0%)	1.0000
Hair dye	2 (9.5%)	1 (3.0%)	0.5530
Artificial sweeteners	1 (4.8%)	0	0.3889
Environmental asbestos	3 (14.3%)	2 (6.1%)	0.3660
Smoking:			
Cigarettes (commercial)	16 (76.2%)	17 (51.5%)	0.0724
Cigarettes (hand rolled)	4 (19.1%)	2 (6.1%)	0.1933
Pipe	0	0	
Marijuana	1 (4.8%)	4 (12.1%)	0.6377
Caffeine:			
Tea or coffee	18 (85.7%)	30 (90.9%)	0.6673
Cold drinks	15 (71.4%)	23 (69.7%)	0.8929
Alcohol	11 (52.4%)	19 (57.6%)	0.7106

* Mantel-Haenszel Chi-square test or Fisher exact test

Table 5b shows the differences in the mean ages, number of cigarettes smoked, and amount of caffeine and alcohol consumed. The only statistically significant difference between the successful and unsuccessful unexposed cases was the mean age ($p = 0.0016$). This difference is, however, probably not meaningful with regard to the frequency of sister chromatid exchange as baseline SCE frequency appears to be independent of age (Carrano and Moore 1982).

TABLE 5b. Comparison between successful and unsuccessful unexposed cases (cont.)

	Successful n = 21			Unsuccessful n = 33			p value*
	mean	SD	range	mean	SD	range	
Age (yrs)	35.9	12.5	18 - 64	47.2	11.5	27 - 72	0.0016
Cigarettes (no./day)	9.4	5.9	1 - 20	12.2	6.8	3 - 20	0.2516
Caffeine (mg/day)	186.5	139.6	14.3 - 490.7	190.5	110.5	7.1 - 557.1	0.9066
Alcohol (g/day)	28.2	18.4	5.7 - 72.9	37.4	23.1	4.9 - 85.3	0.2679

* Student's t test

4.2.2 Comparison between successful and unsuccessful exposed cases

As shown in Table 6a, there was a statistically significant difference in the proportion of successful and unsuccessful exposed cases with diarrhoea (p = 0.0427). In the questionnaire (appendix 1), however, diarrhoea was not defined. There was no way of determining whether the cause was viral, and thus whether it was relevant with regard to sister chromatid exchange frequency. A much larger proportion of unsuccessful cases had had an X ray in the last year (p = 0.0145). *In vivo* radiation, however, does not appear to increase the frequency of sister chromatid exchanges, as shown by Pant *et al.* (1976) and Waksvik *et al.* (1981b). No other statistically significant differences were found. More of the successful cases drank alcohol (71.0%) than did the unsuccessful cases (52.5%). As for the unexposed groups, most of the exposed subjects drank tea and/or coffee on a daily basis.

TABLE 6a. Comparison between successful and unsuccessful exposed cases

	Successful n = 31	Unsuccessful n = 80	p value*
Medical history:			
Influenza	9 (29.0%)	19 (23.8%)	0.5671
Diarrhoea	12 (38.7%)	16 (20.0%)	**0.0427**
Chicken pox	2 (6.5%)	2 (2.5%)	0.3106
Hepatitis	0	2 (2.5%)	1.0000
Glandular fever	0	7 (8.8%)	0.1873
Any viral disease	18 (58.1%)	36 (45.0%)	0.2187
Exposures:			
X rays	3 (9.7%)	26 (32.5%)	**0.0145**
Medication (non traditional)	14 (45.2%)	27 (33.8%)	0.2698
Medication (traditional)	8 (25.8%)	25 (31.3%)	0.6419
Immunizations	0	0	
Hair dye	5 (16.1%)	6 (7.5%)	0.2856
Artificial sweeteners	0	2 (2.5%)	1.0000
Environmental asbestos	2 (6.5%)	7 (8.8%)	1.0000
Smoking:			
Cigarettes (commercial)	20 (64.5%)	48 (60.0%)	0.6627
Cigarettes (hand rolled)	3 (9.7%)	8 (10.0%)	1.0000
Pipe	1 (3.2%)	3 (3.8%)	1.0000
Marijuana	8 (25.8%)	12 (15.0%)	0.1858
Caffeine:			
Tea or coffee	30 (96.8%)	79 (98.8%)	0.4824
Cold drinks	26 (83.9%)	70 (87.5%)	1.0000
Alcohol	22 (71.0%)	42 (52.5%)	0.0786

* Mantel-Haenszel Chi-square test or Fisher exact test

There were no statistically significant differences in age, cigarette smoking, caffeine consumption or alcohol consumption between the successful and unsuccessful exposed cases (Table 6b). The successful cases did, however, have a slightly higher mean daily caffeine consumption than the unsuccessful cases (226.0 and 194.2mg, respectively).

The conclusion reached from these analyses is that the successful cases did not differ from the unsuccessful ones with respect to factors influencing the frequency of sister chromatid exchange, and that the small sample of 52 successful cases is therefore representative of the total sample of 165.

TABLE 6b. Comparison between successful and unsuccessful exposed cases (cont.)

	Successful n = 31			Unsuccessful n = 80			p value*
	mean	SD	range	mean	SD	range	
Age (yrs)	34.3	13.2	23 - 68	37.9	12.7	20 - 66	0.1912
Cigarettes (no./day)	9.9	6.9	2 - 30	10.2	6.2	1 - 25	0.8943
Caffeine (mg/day)	226.0	135.7	14.3 - 547.9	194.2	101.8	50.0 - 612.1	0.1799
Alcohol (g/day)	40.2	33.7	9.7 - 174.9	40.8	38.0	2.9 - 187.1	0.9518

* Student's t test

4.3 Comparison between exposed and unexposed successful cases

The 31 exposed cases with successful sister chromatid exchange scoring in at least 15 metaphase cells were compared with the 21 successful unexposed cases.

The difference in the proportion of cases who had an X ray during the previous 12 months was statistically significant (Table 7a). As mentioned earlier, however, *in vivo* radiation has not been shown to influence the frequency of sister chromatid exchanges (Pant *et al.* 1976, Waksvik *et al.* 1981b). No other statistically significant differences were found, although a higher proportion of the unexposed group smoked cigarettes (76.2% compared to 64.5% of the exposed), and more of the exposed group consumed caffeine and alcohol.

TABLE 7a. Comparison between successful exposed and unexposed cases

	Unexposed n = 21	Exposed n = 31	p value*
Medical history:			
Influenza	7 (33.3%)	9 (29.0%)	0.7440
Diarrhoea	6 (28.6%)	12 (38.7%)	0.4552
Chicken pox	1 (4.8%)	2 (6.5%)	1.0000
Hepatitis	0	0	
Glandular fever	0	0	
Any viral disease	12 (57.1%)	18 (58.1%)	0.9479
Exposures:			
X rays	20 (95.2%)	3 (9.7%)	0.0000
Medication (non traditional)	10 (47.6%)	14 (45.2%)	0.9471
Medication (traditional)	4 (19.1%)	8 (25.8%)	0.7391
Immunizations	0	0	
Hair dye	2 (9.5%)	5 (16.1%)	0.6872
Artificial sweetener	1 (4.8%)	0 0.4038	
Environmental asbestos	3 (14.3%)	2 (6.5%)	0.6370
Smoking:			
Cigarettes (commercial)	16 (76.2%)	20 (64.5%)	0.3754
Cigarettes (hand rolled)	4 (19.1%)	3 (9.7%)	0.4205
Pipe	0	1 (3.2%)	1.0000
Marijuana	1 (4.8%)	8 (25.8%)	0.0670
Caffeine:			
Tea or coffee	18 (85.7%)	30 (96.8%)	0.2906
Cold drinks	15 (71.4%)	26 (83.9%)	0.2830
Alcohol	11 (52.4%)	22 (71.0%)	0.1762

* Mantel-Haenszel Chi-square test or Fisher exact test

TABLE 7b. Comparison between successful exposed and unexposed cases (cont.)

	Unexposed n = 21			Exposed n = 31			p value*
	mean	SD	range	mean	SD	range	
Age (yrs)	35.9	12.5	18 - 64	34.3	13.2	18 - 68	0.6791
Cigarettes (no./day)	9.4	5.9	1 - 20	9.9	6.9	2 - 30	0.8173
Caffeine (mg/day)	186.5	139.6	14.3 - 490.7	226.0	135.7	14.3 - 612.1	0.3144**
Alcohol (g/day)	28.2	18.4	5.7 - 72.9	40.2	33.7	9.7 - 174.9	0.1874

* Student's t test
** Kruskal-Wallis test

On average, the exposed group drank more caffeine (in the form of tea, coffee and cold drinks) and more alcohol, on a daily basis, than the

unexposed group (Table 7b). These differences, however, were not statistically significant. The mean ages of the two groups were very similar (34.3 and 35.9 years for the exposed and unexposed groups, respectively), as were the mean number of cigarettes smoked each day (9.9 and 9.4, respectively).

4.3.1 Frequency of sister chromatid exchanges in exposed and unexposed cases

Figure 3 is a photograph of a metaphase cell from a successful case, clearly showing the differentially stained sister chromatids of each chromosome, and eight SCEs.

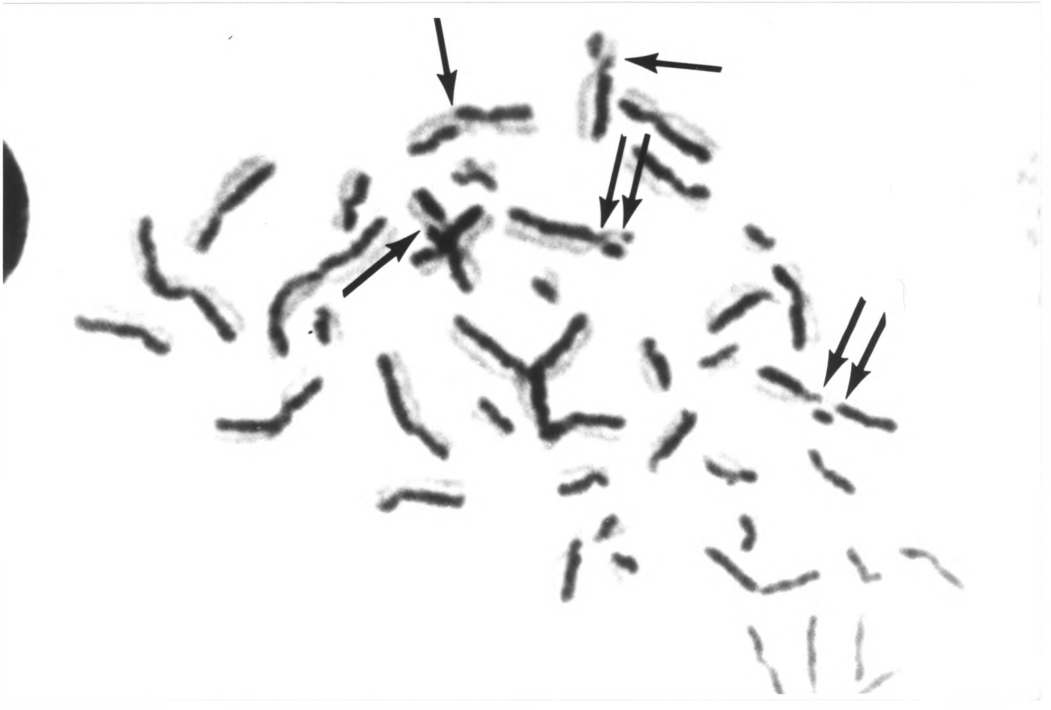


Figure 3. Photograph of a metaphase cell, showing differentiation of sister chromatids. Arrows point to exchanges.

The total number of cells examined for sister chromatid exchange frequency in the exposed group was 924, and that in the unexposed group, 681 (Table 8). An inclusion criterion for statistical analysis was that at least 15 cells should have been examined in each case. No more than 50 cells were examined in any one case. The mean number of cells examined was slightly higher for the unexposed than for the exposed group (32.4 and 29.8, respectively) but the difference was not statistically significant (p = 0.5153).

TABLE 8. Cells examined for sister chromatid exchange frequency - exposed and unexposed cases

	Exposed	Unexposed	Total
Total no. cells	924	681	1 605
Mean no. cells*	29.8	32.4	30.9
SD	12.5	13.7	12.9
Range	15 - 50	15 - 50	15 - 50
Median	27	27	27
Mode	50	50	50

* p = 0.5153

The total number of cells scored for each individual, as well as the total SCEs counted, the mean SCE frequency per cell, and the standard deviation and range, are presented in appendices 7 and 8.

The mean number of sister chromatid exchanges per chromosome (Table 9) and the mean number per cell (Table 10) were analysed for all chromosome groups (A to G). There were no statistically significant differences although, overall, the frequencies were slightly higher in the unexposed subjects.

The formula used to calculate the number of sister chromatid exchanges per chromosome for each group was:

$$\text{No. SCEs/chromosome} = \frac{\text{Total SCEs in group X}}{\text{Total no. group X chromosomes}}$$

TABLE 9. Mean number of sister chromatid exchanges per chromosome, per chromosome group

Chromosome group	Exposed mean	Exposed SD	Unexposed mean	Unexposed SD	p value*
A	0.412	0.096	0.433	0.101	0.5473
B	0.368	0.085	0.375	0.110	0.8096
C	0.250	0.059	0.257	0.061	0.7118
D	0.152	0.038	0.151	0.041	0.9025
E	0.124	0.034	0.137	0.040	0.2127
F	0.073	0.033	0.070	0.030	0.6817
G	0.034	0.018	0.037	0.025	0.6040
Total	0.214	0.043	0.220	0.050	0.6470

* Student's t test

For example, in a case where 21 metaphase cells were examined, a total of 126 (21 x 6) group A chromosomes would have been screened for sister chromatid exchanges. If a total of 49 SCEs were counted in those 126 chromosomes, the number of SCEs per chromosome, for group A, would be 0.39 (49/126).

The formula used to calculate the number of sister chromatid exchanges per cell for each group was:

$$\text{No. SCEs/cell} = \frac{\text{Total SCEs in group X}}{\text{Total no. cells}}$$

In the previous example where 21 metaphase cells were examined and a total of 49 SCEs were counted in the 126 group A chromosomes, the number of SCEs per cell, for group A, would be 2.33 (49/21).

TABLE 10. Mean number of sister chromatid exchanges per cell, per chromosome group

Chromosome group	Exposed mean	Exposed SD	Unexposed mean	Unexposed SD	p value*
A	2.457	0.571	2.579	0.601	0.5291
B	1.467	0.331	1.497	0.440	0.7787
C	3.731	0.876	3.829	0.927	0.7035
D	0.905	0.226	0.903	0.239	0.9670
E	0.740	0.198	0.808	0.234	0.2600
F	0.287	0.128	0.276	0.117	0.7606
G	0.167	0.090	0.187	0.122	0.5019
Total	9.752	2.006	10.078	2.259	0.5935

* Student's t test

There was no significant difference in the mean number of SCEs per cell between factory A (9.56; SD 2.40) and factory B (9.86; SD 1.81) (p = 0.7052).

The mean SCE frequency per cell was calculated for smokers and nonsmokers in each group (Table 11). The highest mean SCE frequency per cell was in unexposed smokers (10.83, SD 2.33), followed by exposed smokers (10.49, SD 1.98). Both exposed and unexposed nonsmokers had lower mean SCE frequencies (8.59, SD 1.47, and 8.58, SD 1.15, respectively). There was no difference in the mean SCE frequency per cell between the exposed and unexposed groups, even when controlling for smoking (p = 0.6555 for smokers, p = 0.9821 for nonsmokers). When the mean SCE frequencies were analysed

by smoking status, however, the differences were significant at the 95% level, both for the exposed (p = 0.0078) and unexposed (p = 0.0257) groups. The difference between smokers and nonsmokers, ignoring exposure status, was also highly statistically significant (p = 0.0006).

TABLE 11. Mean sister chromatid exchange frequencies per cell in exposed and unexposed cases, by smoking status

	Smokers			Non-smokers			p value*
	n	mean	SD	n	mean	SD	
Exposed group	19	10.49	1.98	12	8.59	1.47	0.0078
Unexposed group	14	10.83	2.33	7	8.58	1.15	0.0257
Overall	33	10.63	2.11	19	8.59	1.33	0.0006**

* Student's t test
** Kruskal-Wallis test

4.3.2 Regression analysis

The variables tested in the multiple linear regression analysis are discussed in chapter 2.

As shown in Table 12, there was a slightly higher proportion of exposed cases in the 20 to 39 year old age group (64.5% compared to 57.1% of unexposed cases), and a slightly higher proportion of unexposed cases in the under 20 year old group (9.5% compared to 3.2% of exposed cases); these differences were not statistically significant.

TABLE 12. Age categories of successful cases

Age group (yrs)	Exposed	Unexposed	Total
< 20	1 (3.2%)	2 (9.5%)	3 (5.8%)
20 - 39	20 (64.5%)	12 (57.1%)	32 (61.5%)
40 - 59	9 (29.0%)	6 (28.6%)	15 (28.9%)
> 59	1 (3.2%)	1 (4.8%)	2 (3.9%)
Total	31	21	52

The two groups were divided into smoking categories, according to the number of cigarettes smoked per day (Table 13). Light smokers smoked one to five cigarettes, moderate smokers six to ten, heavy smokers 11 to 20, and very heavy smokers more than 20 cigarettes per day.

There was a slightly higher proportion of non-smokers amongst the exposed group (38.7% compared to 33.3%). Of the smokers in the exposed group, most (52.6%) were moderate smokers. The unexposed smokers were fairly evenly spread across the light, moderate and heavy smoker categories; none were very heavy smokers.

TABLE 13. Number of cigarettes smoked per day

Smoking category	Exposed	Unexposed	Total
non smoker	12 (38.7%)	7 (33.3%)	19 (36.5%)
light	6 (19.4%)	5 (23.8%)	11 (21.2%)
moderate	10 (32.3%)	5 (23.8%)	15 (28.9%)
heavy	2 (6.5%)	4 (19.1%)	6 (11.5%)
very heavy	1 (3.2%)	0	1 (1.9%)
Total	31	21	52

Alcohol consumption was divided into three categories. Less than 20mg per day was considered to be low consumption; 20 to 79mg, moderate, and 80mg or more, high (Table 14). Most of the unexposed group drank little alcohol (61.9%), whereas the majority of the exposed group (58.1%) consumed a moderate amount of alcohol, daily. None of these differences was statistically significant.

TABLE 14. Daily amount of alcohol consumed

Alcohol (g/day)	Exposed	Unexposed	Total
low (0 - 19)	12 (38.7%)	13 (61.9%)	25 (48.1%)
moderate (20 - 79)	18 (58.1%)	8 (38.1%)	26 (50.0%)
high (> 79)	1 (3.2%)	0	1 (1.9%)
Total	31	21	52

TABLE 15. Daily amount of caffeine consumed

Caffeine (mg/day)	Exposed	Unexposed	Total
< 85	4 (12.9%)	4 (19.1%)	8 (15.4%)
85 - 169	12 (38.7%)	9 (42.9%)	21 (40.4%)
170 - 339	9 (29.0%)	5 (23.8%)	14 (26.9%)
>= 340	6 (19.4%)	3 (14.3%)	9 (17.3%)
Total	31	21	52

The daily amount (mg) of caffeine consumed, in the form of tea, coffee and cold drink, was categorised as equivalent to one cup of coffee (< 85 mg), one to less than two cups (85 to 169mg), two to less than four cups (170 to 339mg), and four or more cups (340 mg or more). As shown in Table 15, the

proportions were similar in both the exposed and unexposed groups. Most people drank the equivalent of two to four cups of coffee per day.

The regression model showed that the only significant predictor of SCE frequency was cigarette smoking. Asbestos exposure was a non-contributory variable, thus having no effect on the frequency.

CHAPTER 5: DISCUSSION

There was a small observed difference (3.4%) in the mean SCE frequency per cell, between the asbestos-exposed group and the unexposed group. This was not a statistically significant difference. Smoking, however, caused a significant increase in the SCE frequency per cell, in both the exposed and the unexposed groups. These findings are in accordance with three previously published SCE studies on the in vivo effects of asbestos exposure in humans (Table 16).

TABLE 16. *In vitro* sister chromatid exchange studies on the *in vivo* effects of asbestos exposure in humans

Reference	Setting	No. of subjects		SCE/cell exposed mean	exposed SD	unexposed mean	unexposed SD	p value
Rom *et al.* 1983	asbestos insulation workers	25 workers 14 controls	smokers:	10.51	1.33	10.05	1.66	> 0.0500
			nonsmokers:	9.39	1.30	7.79	1.20	**0.0020**
			overall:	10.07	1.40	9.08	1.84	0.0560
Kelsey *et al.* 1986a	asbestos-exposed construction workers	22 workers 10 controls	smokers:	7.40	0.30	7.10	0.30	> 0.0500
			nonsmokers:	6.30	0.20	6.10	0.20	> 0.0500
Fatma *et al.* 1991	asbestos cement factory	22 workers 12 controls	smokers:	8.16	0.45	5.73	0.16	**< 0.0010**
			nonsmokers:	6.63	0.50	3.61	0.14	**< 0.0010**
Nelson* 1994	asbestos products factory	31 workers 21 controls	smokers:	10.49	1.98	10.83	2.33	0.6555
			nonsmokers:	8.59	1.47	8.58	1.15	0.9821
			overall:	9.75	2.01	10.08	2.26	0.5935

* present study

The four studies in Table 16 are difficult to compare, quantitatively, because of the differences among laboratories in the SCE technique as discussed in chapter 3 (cell culturing methods, the concentration of BrdU used, the

number of cells read, etc). Nevertheless, there are some similarities in the findings and all mean SCE frequencies are within the range of "normal" baseline levels (Table 1 - chapter 1).

Rom *et al.* (1983) found the highest mean SCE frequency per cell in asbestos exposed smokers (10.51, SD 1.33) and the lowest in unexposed nonsmokers (7.79, SD 1.20). Differences between workers and controls were statistically significant for nonsmokers (20.5%; $p = 0.0020$) but not for smokers (4.6%; $p > 0.0500$). The overall difference between exposed and unexposed individuals was 10.9% ($p = 0.0560$). The difference between smokers and nonsmokers was statistically significant. The means calculated in the present study are very comparable with those of Rom *et al.*, i.e. 9.75 versus 10.49 in exposed workers, and 10.08 versus 9.08 in unexposed controls. When stratified by smoking status, the means in the two studies are even more comparable.

Kelsey *et al.* (1986a) found no statistically significant differences between asbestos-exposed workers and the unexposed group, for smokers or nonsmokers. They did, however, also find differences between smokers and nonsmokers ($p = 0.01$ for exposed, $p = 0.03$ for unexposed workers).

Fatma *et al.* (1991) also found the highest mean frequency in exposed smokers, and the lowest frequency in unexposed nonsmokers (8.16, SD 0.45, and 3.16, SD 0.14, respectively). There were statistically significant

differences between workers and controls in both the smokers and nonsmokers (42.4% and 83.7%, respectively; $p < 0.001$). As for the study by Rom *et al.* (1983), there was a statistically significant difference only between smokers and nonsmokers.

In contrast to the previous three studies, the highest mean SCE frequency per cell in the present study was found in unexposed smokers (10.83, SD 2.33). Exposed smokers had the second highest mean of 10.49. As in the previous studies, however, the differences between smokers and nonsmokers were statistically significant in both the asbestos-exposed group ($p = 0.0078$) and the unexposed group ($p = 0.0257$). The difference between smokers and nonsmokers, regardless of exposure status, was highly significant ($p = 0.0006$). There was no overall statistically significant difference between the exposed and unexposed groups, regardless of smoking status ($p = 0.6555$ for smokers, $p = 0.9821$ for nonsmokers). This finding is in accordance with that of Kelsey *et al.* (1986a).

The slightly higher mean SCE frequency in the unexposed group, compared to the exposed group, was not in accordance with any of the other studies. Perhaps the asbestos workers underwent a more rigorous preemployment examination than did the unexposed group, resulting in a healthier workforce. There were no demographic differences (e.g. age, sex and race) between the two groups.

The result of this study may be a true finding, i.e. perhaps asbestos does not cause genetic damage (at least not by a method reflected by the SCE technique). On the other hand, it is possible that, either the number of cells analysed for SCE, or the number of individuals in each group, was too small to allow for the detection of a statistically significant difference. Both these parameters affect the "sensitivity" of the study (Hirsch *et al.* 1984). The study must be sufficiently sensitive to allow meaningful differences to be detected as statistically significant.

Hirsch *et al.* (1984) have described a method to determine the minimum percentage increase in SCE frequency detectable at the 95% level, based on the number of individuals per group and the number of cells counted per individual. Based on their calculations, the present study would allow for the detection of a 12.4% difference in SCE frequency between the exposed and unexposed groups. This is calculated on the basis of an average of 25 individuals per group and an average of 30 cells counted per individual (Table 5 - chapter 4). In other words, with two independent groups of 25 subjects each, with 30 cells counted per individual, one would be able to detect, as statistically significant, a 12.4% or greater increase in baseline SCE rate. The observed difference was 3.4% and was therefore not statistically significant.

Other studies on the in vivo cytogenetic effects of asbestos (Table 16) have counted more cells per individual than were counted in this study, but have included fewer individuals per group. This latter parameter influences the sensitivity of the study to a greater degree than does the number of cells counted, as it increases the statistical power independent of the number of cells analysed (Hirsch *et al.* 1984). The sensitivities of the three previous studies in Table 16 (14.1%, 13.8% and 13.8%, respectively) are, in fact, lower than that of the present study (12.4%), indicating that perhaps the number of individuals and cells counted were sufficient, after all.

The concept of sensitivity of the study based on these parameters may be helpful, not only in planning future investigations, but also in interpreting "negative" results in SCE studies where too few cells were analysed and a small number of subjects included in each group, as well as "positive" results where very large numbers of cells were analysed per group and very large numbers of subjects included (Hirsch *et al.* 1984).

One must bear in mind the difference between statistical significance and biological significance. It is unlikely that the 3.4% difference detected in this study would be biologically significant, especially considering the wide variations in SCE frequency between individuals. A more realistic biologically significant difference would be closer to 10%, based on recommendations by Wulf (1990).

Wulf (1990) made certain proposals for action, based on the SCE frequencies of exposed individuals. In the case of extremely high levels (more than 100% above baseline or control level) in a single individual, he recommended that close relatives be examined for SCE frequencies. This is mainly for scientific reasons as no health effects have been found in family members with high SCE frequencies (Wulf *et al.* 1987). In the case of a high mean SCE frequency (more than a 50% increase) in groups of exposed employees, they should pass a health examination and the workplace should be examined to identify and, if possible, eliminate the toxic agent(s). Maximum immediate action should be taken to prevent exposure of the employees. For groups of employees with moderately elevated levels of SCE frequencies (10 to 50% above controls) the source should be searched for and suspected SCE inducing agents replaced. If the agent cannot be replaced, protection against exposure should be ensured, at least until an evaluation, together with other toxicological, epidemiological, and cytogenetic examinations, has been performed. If only a limited increase in the SCE rate (less than 10%) is observed, this should only give rise to concern if other tests indicate that the agent is hazardous.

The SCE technique provides an opportunity to study the acute consequences of exposure of man to agents already established as genotoxic (Ashby and Richardson 1985). Negative results are not necessarily synonymous with the absence of a mutagenic effect. If cytogenetic effects are evident in exposed populations, then it would seem plausible to assume that a possible

mutagenic or carcinogenic hazard exists. If such effects are absent, it may be that the agent in question is only genotoxic in vitro, or that the people exposed have been adequately protected.

An additional factor which may contribute to negative results is the potential repair of lesions. SCEs occur during the S phase of mitosis (Wulf 1990) which does not take place until the cells are stimulated to divide by a mitogen in vitro. For a chromosome lesion to be converted to an SCE, the lesion must be present during this DNA synthesis phase. This presents a potential problem for the measurement of SCEs induced in peripheral lymphocytes in vivo. Since the lymphocyte must be stimulated to undergo DNA synthesis (mitosis) in vitro after it is removed from the circulating blood, the SCE-inducing lesions must persist from the time of exposure in vivo until the time of DNA synthesis in vitro (Carrano 1982). During this time, the lesions could be removed through efficient cellular repair, resulting in a low or nonmeasurable increase in SCE frequency (Carrano 1982, Carrano and Natarajan 1988). Only the few lesions that remained could be expressed as chromosome aberrations or SCEs if they were misrepaired when they passed through the S phase in vitro. This is particularly true if SCEs are induced by low-level chronic exposures.

In past studies the greatest cytogenetic damage was observed at the early stages of surveillance; subsequent degrees of damage have reduced roughly in proportion to the industrial measures instituted (Ashby and Richardson

1985). Invariably, new genotoxicity assays are calibrated against the worst-case situation. When used later, in settings with lower exposures, the data may become equivocal.

The failure to detect an increase in SCE frequency in these asbestos workers is therefore probably not due to the inability of asbestos to induce SCEs in human lymphocytes, but rather due to low exposures. Fibre levels ranged from 0.2414 to 0.8500 fibres per cm^3 in the year preceding the study; and from 0.1320 to 0.1726 fibres per cm^3 in the year that the data were collected (V Yousefi - personal communication). According to the Machinery and Occupational Safety Act, the exposure limit for asbestos in the South African non-mining asbestos industry is 1 fibre per cm^3 (Department of Manpower 1987). Thus, the workers in the two asbestos-products factories had certainly been exposed to low levels of asbestos fibre during the period preceding this study.

On the other hand, the SCE technique may not be the ideal method for detecting subclinical adverse health effects from working with asbestos. These effects, such as cancer, may be induced by other mechanisms that do not involve mutagenic action as depicted by SCEs. Lechner *et al.* (1985) have suggested an alternative mechanism for the development of mesothelioma, an asbestos related cancer most often occurring in the pleura of the lung. They exposed pleural mesothelial cells from healthy adults to asbestos fibres

which penetrated the cells within two hours of exposure. The exposed cells were aneuploid and various chromosome abnormalities were found. The authors suggest that the uniquely fluid mesothelial cell cytoskeleton may be very easily disturbed by penetrating asbestos fibres which would cause chromosome instability, oncogenic activation and, ultimately, transformation.

Mahmood *et al.* (1993) recently suggested that the asbestos-mediated formation of free radicals in the presence of peroxides and hypoperoxides may be the causative factor of DNA damage (other chemical carcinogens either alkylate or make covalent bonds with DNA). These chromosome changes may, in turn, be related to the induction of lung cancer in asbestos exposed individuals.

Potential limitations of the study

1. Success rate

The success rate of the cultures, for SCE analysis, was very poor (31.5%). In some cases there were too few metaphase cells that met the criteria for SCE analysis (less than 15). This was sometimes due entirely to poor growth of the cultures. In other cases, however, there was good growth of the cultures and thus a sufficient number of metaphase cells, but the chromatids had not stained differentially. SCEs could therefore not be distinguished and the case had to be discarded as "unsuccessful". In some cases, not all of the

chromatids in a single cell were differentially stained. One explanation is that the culture period may not have been optimal. A 72 hour culture period was used, following standardised SCE methodology, but perhaps some of the cells passed through three, instead of only two, cell divisions. The result would be that the chromatids of only half of the chromosomes would be distinguishable (Fig. 4). Another problem that reduced the success rate was poor spread of the chromosomes in some cases. One of the criteria for a successful case was that there should be no more than four "overlaps", and cells with more than four overlapping chromosomes therefore had to be discarded.

Nevertheless, the successful cases did not differ, statistically, from the unsuccessful ones with respect to factors influencing the frequency of sister chromatid exchange. The small sample of 52 successful cases was therefore taken to be representative of the total initial sample of 165.

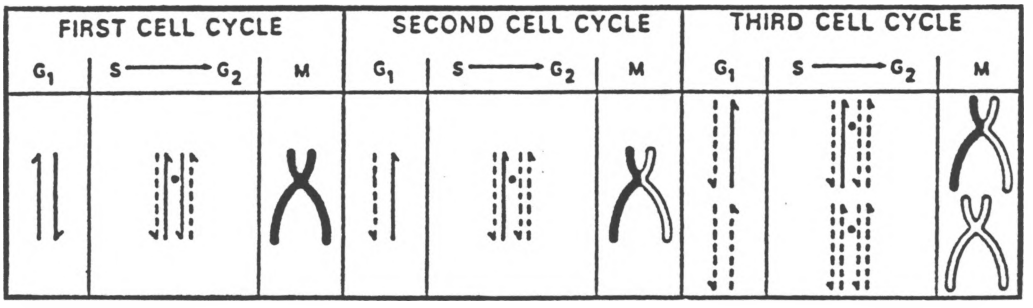


Figure 4. Progression of cells through three division cycles in the presence of BrdU. ——— Thymidine-bearing DNA strand. ---- BrdU-bearing strand. Black chromosomal regions indicate segments which stain darker with Giemsa. (Adapted from Schvartzman and Tice 1982).

2. Other exposures

The questionnaires were designed to detect exposure to as many factors known to influence SCE frequency as was possible. Nevertheless, it is possible that the asbestos workers, or even the unexposed group, were exposed to other genotoxic agents which could have affected the SCE frequency. Additional exposure histories of the asbestos-exposed group were not taken into account as all subjects had worked in the production side of the asbestos factory for at least six months. As SCE frequencies have been found to remain at a high level for four to 16 weeks after exposure, before returning to baseline levels (Vainio and Sorsa 1983), previous exposure to other chemicals would probably not have affected the SCE frequency in the lymphocytes at the time when blood was taken.

4. Control group

The unexposed subjects may have been an inappropriate choice for a control group. However, they were of similar socio-economic status to the exposed group, and the two groups did not differ in basic demographic characteristics, or with respect to exposure to factors known to influence SCE frequency.

3. Data analysis

A two-tailed Student's t test is commonly used for the statistical analysis of group means (Block 1982b). However, the means may not be normally

distributed and the t test may therefore be inappropriate (Carrano and Natarajan 1988). Analysis of variance or appropriate non-parametric statistics may be more rigorous for the particular population under study. Different studies have used various methods to transform the data in an attempt to normalise the data and thus, have more accurate estimates of the difference in SCE frequencies between populations. Murphy *et al.* (1992) explored the merits of various methods of data transformation in SCE studies. They concluded that common transformations such as natural logarithm, square root, etc. have little advantage in assessing whether an agent induces SCEs. As it appears that no statistical procedure can be recommended for universal application, the data in this study were not transformed. This also enabled comparison with other similar studies.

CHAPTER 6: CONCLUSION

There is controversy surrounding the rationale for measuring cytogenetic changes in humans occupationally or otherwise exposed (Carrano 1986). The main concern is whether chromosome damage, as measured in the human lymphocyte, predicts an ill health effect for the individual. It has been argued that, because there is not a clear understanding of the significance of increased chromosome damage, it is difficult to interpret the results of studies for the individuals being evaluated and, therefore, such studies should not be performed in an occupational setting.

The induction of SCEs in lymphocytes cannot be interpreted as a predictor of an ill-health effect for an individual. Nevertheless, much is known about the formation and significance of cytogenetic changes, and the prediction of an ill-health effect should not be the sole reason for applying cytogenetic tests in worker groups. At the least, cytogenetic changes in an individual are markers of personal exposure and may have an appropriate role in an industrial hygiene programme. Furthermore, positive correlations have been established between the induction of these endpoints and cytotoxicity, mutation, transformation, or tumour formation (Carrano 1986).

SCE analysis remains one of the more sensitive and widely applicable short-term tests. Its true value will be enhanced by a better understanding

of the mechanism of SCE formation. Real proof of the significance of these cytogenetic observations, however, can only be obtained in prospective epidemiological studies (Vainio and Sorsa 1983). Short-term, followed by long-term, genotoxicological monitoring of an exposed population would have several advantages. Early cancer diagnosis would be possible and genetic counselling could be offered (Brogger 1982e).

While the knowledge of mutagenicity of chemicals is gradually increasing, and cytogenetic methods are being better standardised to allow for interlaboratory correlations, studies of chromosome damage will most probably develop as an important tool in controlling unnecessary exposures to genotoxic agents in the occupational environment (Sorsa 1985). If the cytogenetic endpoint is sufficiently sensitive to levels of exposure occurring in occupational settings, the results could prompt the implementation of environmental controls or medical surveillance, even in the absence of direct evidence relating the chromosomal effects to adverse health outcomes (Stolley *et al.* 1984).

Finally, the failure to observe an increase in SCE frequency, as in the present study on asbestos exposure, does not rule out the agent as a genotoxin. Rather, it may help to resolve the limitations of the SCE method for detecting genetic damage.

REFERENCES

1. **Abdel-Fadil MR, Palmer CG, Heerema N.** Effect of temperature variation on sister chromatid exchange and cell-cycle duration in cultured human lymphocytes. *Mutat Res* 1982; 104: 267-273.

2. **Abe S, Sasaki M.** Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *JNCI* 1977; 58: 1635-1641.

3. **Abe S, Sasaki M.** SCE as an index of mutagenesis and/or carcinogenesis. In: Sandberg AA, ed. *Progress and Topics in Cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982: 461-514.

4. **Albertini RJ, Nicklas JA, O'Neill JP.** Somatic cell gene mutations in humans: biomarkers for genotoxicity. *Environ Health Perspect* 1993; 101 (Suppl 3): 193-201.

5. **Anderson D, Francis AJ, Godbert P, Jenkinson PC, Butterworth KR.** Variability in chromosome aberrations, sister chromatid exchanges, and mitogen-induced blastogenesis in peripheral lymphocytes from control individuals. *Environ Health Perspect* 1993; 101 (Suppl 3): 83-88.

6. **Andersson HC, Tranberg EA, Uggla AH, Zetterberg G.** Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory. *Mutat Res* 1980; 73: 387-401.

7. **Archard S, Perderiset M, Jaurand M.** Sister chromatid exchanges in rat pleural mesothelial cells treated with crocidolite, attapulgiote, or benzo 3-4 pyrene. *Br J Ind Med* 1987; 44: 281-283.

8. **Ardito G, Lamberti L, Ansaldi E, Ponzetto P.** Sister chromatid exchanges in cigarette smoking human females and their newborns. *Mutat Res* 1980; 78: 209-212.

9. **Ashby J, Richardson CR.** Tabulation and assessment of 113 human surveillance cytogenetic studies conducted between 1965 and 1984. *Mutat Res* 1985; 154: 111-113.

10. **Babu KA, Nigam SK, Lakkad BC, Bhatt DK, Karnik AB, Thakore KN, Kashyap SK, Chatterjee SK.** Effect of chrysotile asbestos (AP-1) on sister chromatid exchanges in Chinese hamster ovary cells. *Environ Res* 1981; 24: 325-329.

11. **Bajerska A, Wyszynska K, Szymczak W.** Evaluation of SCEs in bone marrow cells of mice after *in vivo* exposure to asbestos. *Polish J Occup Med* 1988; 1: 73-76.

12. **Bauchinger M, Dresp J, Schmid E, Hauf R.** Chromosome changes in lymphocytes after occupational exposure to pentachlorophenol (PCP). *Mutat Res* 1982; 102: 83-88.

13. **Bauchinger M, Schmid E, Dresp J, Kolin-Gerresheim J.** Chromosome aberrations and sister-chromatid exchanges in toluene-exposed workers. *Mutat Res* 1983; 113: 231-232.

14. **Bender MA, Preston RJ, Leonard RC, Pyatt BE, Gooch PC, Shelby MD.** Chromosomal aberration and sister-chromatid exchange frequencies in peripheral blood lymphocytes of a large human population sample. *Mutat Res* 1988; 204: 421-433.

15. **Bender MA, Preston RJ, Leonard RC, Pyatt BE, Gooch PC.** On the distribution of spontaneous SCE in human peripheral lymphocytes. *Mutat Res* 1992; 281: 227-232.

16. **Bianchi NO, Bianchi MS, Larramendy M.** Kinetics of human lymphocyte division and chromosomal radiosensitivity. *Mutat Res* 1979; 63: 317-324.

17. **Block AW.** Sister chromatid exchange methodology. In: Sandberg AA, ed. *Progress and Topics in Cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982a: 25.

18. ***ibid.*** 1982b: 27.

19. **Bradley MO, Hsu IC, Harris CC.** Relationship between sister chromatid exchange and mutagenicity, toxicity and DNA damage. *Nature* 1979; 282: 318-320.

20. **Brogger A.** Application of SCE to public health. In: Sandberg AA, ed. *Progress and topics in cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982a: 659.

21. ***ibid.*** 1982b: 657.

22. ***ibid.*** 1982c: 658.

23. ***ibid.*** 1982d: 656.

24. ***ibid.*** 1982e: 665.

25. *ibid.* 1982f: 666.

26. **Cairns A.** The origin of human cancers. *Nature* 1981; 289: 353-357.

27. **Carrano AV.** Sister chromatid exchange as an indicator of human exposure. In: *Banbury report 13. Indications of genotoxic exposure.* Cold Spring Harbor: Cold Spring Harbor Laboratory, 1982: 307-318.

28. **Carrano AV.** Chromosomal alterations as markers of exposure and effect. *J Occup Med* 1986; 28: 1112-1116.

29. **Carrano AV, Moore DH.** The rationale and methodology for quantifying sister chromatid exchange in humans. In: Heddle JA, ed. *Mutagenicity: new horizons in genetic toxicology.* New York: Academic Press, 1982: 277.

30. **Carrano AV, Natarajan AT.** ICPEMC publication no. 14. Considerations for population monitoring using cytogenetic techniques. *Mutat Res* 1988; 204: 379-406.

31. **Carrano AV, Minkler JL, Stetka DG, Moore DH.** II. Variation in the baseline sister chromatid exchange frequency in human lymphocytes. *Environ Mutat* 1980; 2: 325-337.

32. **Carrano AV, Thompson LH, Lindl PA, Minkler JL.** Sister chromatid exchanges as an indicator of mutagenesis. *Nature* 1978; 271: 551-553.

33. **Casey G.** Sister-chromatid exchange and cell kinetics in CHO-K1 cells, human fibroblasts and lymphoblastoid cells exposed *in vitro* to asbestos and glass fibre. *Mutat Res* 1983; 116: 369-377.

34. **Cassel DM, Latt SA.** Relationship between DNA adduct formation and sister chromatid exchange induction by 3H-8-methoxy-psoralen in Chinese hamster ovary cells. *Exp Cell Res* 1980; 128: 15-22.

35. **Cervenka J, Witkop CJ, Okoro AN, King RA.** Chromosome breaks and sister chromatid exchanges in albinos in Nigeria. *Clin Genet* 1979; 15: 17-21.

36. **Chaganti RSK, Schonberg S, German J.** A manyfold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes. *Proc Natl Acad Sci* 1974; 71: 4508-4512.

37. **Cheng W-S, Mulvihill JJ, Greene MH, Pickle LW, Tsai S, Whang-Peng J.** Sister chromatid exchanges and chromosomes in chronic myelogenous leukemia and cancer families. *Int J Cancer* 1979; 23: 8-13.

38. **Creech JL, Johnson MN.** Angiosarcoma of liver in the manufacture of polyvinyl chloride. *J Occup Med* 1974; 16: 150-151.

39. **Crossen PE.** In: Sandberg AA, ed. *Progress and Topics in Cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982a: 177.

40. ***ibid.*** 1982b: 175.

41. **Crossen PE, Drets ME, Arrighi FE, Johnston DA.** Analysis of the frequency and distribution of sister chromatid exchanges in cultured human lymphocytes. *Hum Genet* 1977; 35: 345-352.

42. **Davidson RL, Kaufman ER, Dougherty CP, Ouellette AM, DiFolco CM, Latt SA.** Induction of sister chromatid exchanges by BUdR is largely dependent of the BUdR content of DNA. *Nature* 1980; 284: 74-80.

43. **De Ferrari M, Artuso M, Bonassi S, Bonatti S, Cavalieri Z, Pescatore D, Marchini E, Pisano V, Abbondandolo A.** Cytogenetic biomonitoring of an Italian population exposed to pesticides: chromosome aberration and sister-chromatid exchange analysis in peripheral blood lymphocytes. *Mutat Res* 1991; 260: 105-113.

44. **Degrassi F, De Salvia R, Ricordy R, Tanzarella C, Benigni R, Palitti F.** Sensitivity of lymphocytes from vulcanizers in the *in vitro* induction of sister chromatid exchanges. *Environ Health Perspect* 1993; 101 (Suppl 3): 97-100.

45. **Department of Manpower, Republic of South Africa.** Machinery and occupational safety act 6 of 1983. *Asbestos regulations.* Government Gazette no. 4070. 10 April 1987; 262 (10700): 2. Pretoria: Government Printer, 1987.

46. **Duncan AMW, Evans HJ.** Molecular lesions involved in the induction of sister chromatid exchange. *Mutat Res* 1982; 105: 423-427.

47. **Du Frain RJ.** Probing sister chromatid exchange formation with halogenated pyrimidines. In: Tice RR, Hollaender A, eds. *Sister Chromatid Exchanges.* Part A. The nature of SCEs. New York: Plenum Press, 1984: 42.

48. **Edwards JW, Priestly BG.** Sister chromatid exchanges in lymphocytes of petroleum retailers. *Br J Ind Med* 1993; 50: 149-154.

49. **Emerit I.** Activated oxygen species at the origin of sister chromatid exchanges. In: Tice RR, Hollaender A, eds. *Sister Chromatid Exchanges.* Part A. The nature of SCEs. New York: Plenum Press, 1984: 127-140.

50. **Emerit I, Cerutti PA.** Tumour promotor phorbol-12-myritate-13-acetate induces chromosomal damage via indirect action. *Nature* 1981; 293: 144-146.

51. **Emerit I, Keck M, Levy A, Feingold J, Michelson AM.** Activated oxygen species at the origin of chromosome breakage and sister-chromatid exchanges. *Mutat Res* 1982; 103: 165-172.

52. **Enterline PE, Hartley J, Henderson V.** Asbestos and cancer: a cohort followed up to death. *Br J Ind Med* 1987; 44: 396-401.

53. **Evans HJ.** Molecular mechanisms in the induction of chromosome aberrations. In: Scott D, Bridges BA, Sobels FH, eds. *Progress in genetic toxicology.* Amsterdam: Elsevier-North Holland, 1977: 57.

54. **Fatma N, Jain AK, Rahman Q.** Frequency of sister chromatid exchange and chromosomal aberrations in asbestos cement workers. *Br J Ind Med* 1991; 48: 103-105.

55. **Fredga K, Davring L, Sunner M, Bengtsson B, Elinder C-G, Sigtryggsson O, Berlin M.** Chromosome changes in workers (smokers and nonsmokers) exposed to automobile fuels and exhaust gases. *Scand J Work Environ Health* 1982; 8: 209-221.

56. **Fujiwara Y, Kano Y, Tatsumi M, Paul P.** Effects of a tumor promotor and an anti-promotor on spontaneous and UV-induced 6-thioguanine-resistant mutagens and sister chromatid exchanges in V79 Chinese hamster cells. *Mutat Res* 1980; 71: 243-251.

57. **Funes-Cravioto F, Zapata-Gayon C, Kilmodin-Hedman B, Lambert B, Lindsten J, Norberg E, Nordenskjold M, Olin R, Swensson A.** Chromosome aberrations and sister-chromatid exchange in workers in chemical laboratories and a rotoprinting factory and in children of women laboratory workers. *Lancet* 1977; 2: 322-325.

58. **Galloway SM, Evans HJ.** Sister chromatid exchange in human chromosomes from normal individuals and patients with ataxia telangiectasia. *Cytogenet Cell Genet* 1975; 15: 17-29.

59. **Gebhart E, Kappauf H.** Bleomycin and sister chromatid exchange in human lymphocyte chromosomes. *Mutat Res* 1978; 58: 121-124.

60. **Gebhart E, Romahn R, Schneider A, Hoffman M, Rau D, Tittelbach H.** Cytogenetic studies in lymphocytes of patients with rectal cancer. *Environ Health Perspect* 1993; 101 (Suppl 3): 169-175.

61. **Goh K.** Sister-chromatid-exchange in the aging population. *J Med* 1981; 12: 195-198.

62. **Goto K, Maeda S, Kano Y, Sugiyama T.** Factors involved in differential Giemsa-staining of sister chromatids. *Chromosoma* 1978; 66: 351-359.

63. **Gu ZW, Sele B, Chmara D, Jalbert P, Vincent F, Marka C, Faure J.** Effects of trichloroethylene and its metabolites on the rate of sister-chromatid exchange. *Ann Genet* 1981; 24: 105-106.

64. **Hansteen I-L.** SCE as a monitor of industrial and environmental toxins. In: Sandberg AA, ed. *Sister Chromatid Exchange.* New York: Alan R Liss, 1982a: 680.

65. ***ibid.*** 1982b: 681.

66. **Hansteen I-L, Hillestad L, Thiis-Evensen E, Heldaas SS.** Effects of vinyl chloride in man. A cytogenetic follow-up study. *Mutat Res* 1978; 51: 271-278.

67. **Hart HP.** Asbestos in South Africa. *J S Afr Inst Min Metall* 1988; 88: 185-198.

68. **Hatcher NH, Brinson PS, Hook EB.** Sister chromatid exchanges in ataxia telangiectasia. *Mutat Res* 1976; 35: 333-336.

69. **Hedner K, Hogstedt B, Kolnig A-M, Mark-Vendal E, Strombeck B, Mitelman F.** Sister chromatid exchanges and structural chromosome aberrations in relation to age and sex. *Hum Genet* 1982; 62: 305-309.

70. **Hei TK, Geard CR, Osmak RS, Travisano M.** Correlation of *in vitro* genotoxicity and oncogenicity induced by radiation and asbestos fibres. *Br J Cancer* 1985; 52: 591-597.

71. **Hemminki K, Sorsa M, Vainio H.** Genetic risks caused by occupational chemicals. Use of experimental methods and occupational risk group monitoring in the detection of environmental chemicals causing mutations, cancer and malformations. *Scand J Work Environ Health* 1979; 5: 307-327.

72. **Hirsch B, McGue M, Cervenka J.** Characterization of the distribution of sister chromatid exchange frequencies: implications for research design. *Hum Genet* 1984; 65: 280-286.

73. **Hollander DH, Tockman MS, Liang YW, Borgaonkar DS, Frost JK.** Sister chromatid exchanges in the peripheral blood of cigarette smokers and in lung cancer patients; and the effects of chemotherapy. *Hum Genet* 1978; 44: 165-171.

74. **Hopkins JM, Evans HJ.** Cigarette smoke-induced DNA damage and lung cancer risks. *Nature* 1980; 388-390.

75. **Husum B.** Mutagenicity of inhalation anaesthetics studied by the sister chromatid exchange test in lymphocytes of patients and operating room personnel. *Dan Med Bull* 1987; 34: 159-170.

76. **Ikushima T.** Role of sister chromatid exchanges in chromatid aberration formation. *Nature* 1977; 268: 235-236.

77. **Ikushima T, Wolff S.** Sister chromatid exchanges induced by light flashes to 5-bromodeoxyuridine and 5-iododeoxyuridine substituted Chinese hamster chromosomes. *Exp Cell Res* 1974; 87: 15-19.

78. **Iserson KV.** Caffeine and nicotine. In: Haddad LM, Winchester JF, eds. *Clinical management of poisoning and drug overdose*, 2nd ed. Philadelphia: WB Saunders, 1990: 868.

79. **Ishii Y, Bender MA.** Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light-induced sister chromatid exchanges in Chinese hamster cells. *Mutat Res* 1980; 79: 19-32.

80. **Jacobson-Kram D, Albertini RJ, Branda RF, Falta MT, Iype PT, Kolodner K, Liou S-H, McDiarmid MA, Morris M, Nicklas JA, O'Neill JP, Poirier MC, Putman D, Strickland PT, Williams JR, Xiao S.** Measurement of chromosomal aberrations, sister chromatid exchange, *hprt* mutations, and DNA adducts in peripheral lymphocytes of human populations at increases risk for cancer. *Environ Health Perspect* 1993; 101 (Suppl 3): 121-125.

81. **Kaplan H, Renier A, Jaurand MC, Bignon J.** Sister chromatid exchanges in mesothelial cells cultured with chrysotile fibres. In: Brown RC, Chamberlain M, Davies R, Gormley IP, eds. *The in vitro effects of mineral dusts.* London: Academic Press, 1980: 251-253.

82. **Karelova J, Jablonicka A, Gavora J, Hano L.** Chromosome and sister-chromatid exchange analysis in peripheral lymphocytes, and mutagenicity of urine in anaesthesiology personnel. *Int Arch Occup Environ Health* 1992; 64: 303-306.

83. **Kato H.** Induction of sister chromatid exchanges by UV light and its inhibition by caffeine. *Exp Cell Res* 1973; 82: 382-390.

84. **Kato H.** Induction of sister chromatid exchanges by chemical mutagens and its possible relevance to DNA repair. *Exp Cell Res* 1974a; 85: 238-247.

85. **Kato H.** Possible role of DNA synthesis in formation of sister chromatid exchanges. *Nature* 1974b; 252: 739-741.

86. **Kato H.** Spontaneous sister chromatid exchanges detected by a BUdR-labelling method. *Nature* 1974c; 251: 70-72.

87. **Kato H.** Spontaneous and induced sister chromatid exchanges as revealed by the BudR-labelling method. *Int Rev Cytol* 1977; 49: 55-97.

88. **Kato H.** Evidence that the replication point is the site of sister-chromatid exchange. *Cancer Genet Cytogenet* 1980; 2: 69-77.

89. **Kato H, Sandberg AA.** The effect of sera on sister chromatid exchanges in vitro. *Exp Cell Res* 1977; 109: 445-448.

90. **Kelsey KT, Christiani DC, Little JB.** Enhancement of benzo[a]pyrene-induced sister chromatid exchanges in lymphocytes from cigarette smokers occupationally exposed to asbestos. *JNCI* 1986a; 77: 321-327.

91. **Kelsey KT, Yano E, Liber HL, Little JB.** The *in vitro* genetic effects of fibrous erionite and crocidolite asbestos. *Br J Cancer* 1986b; 54: 107-114.

92. **Kinsella AR, Radman M.** Tumor promotor induces sister chromatid exchanges; Relevance to mechanisms of carcinogenesis. *Proc Natl Acad Sci, USA* 1978; 75: 6149-6153.

93. **Klein G.** The role of gene dosage and genetic transpositions in carcinogenesis. *Nature* 1981; 294: 313-318.

94. **Knudson AG.** Genetics and etiology of human cancer. In: Harris H, Hirschhorn K, eds. *Advances in human genetics*, vol. 8. New York: Plenum Press, 1977: 1-66.

95. **Korenberg JR, Freedlender EF.** Giemsa technique for the detection of sister chromatid exchanges. *Chromosoma* 1974; 48: 355-360.

96. **Kowalczyk J.** Sister-chromatid exchanges in children treated with nalidixic acid. *Mutat Res* 1980; 77: 371-375.

97. **Kucerova M, Polivkova Z, Batora J.** Comparative evaluation of the frequency of chromosomal aberrations and the SCE numbers in peripheral lymphocytes of workers occupationally exposed to vinyl chloride monomer. *Mutat Res* 1979; 67: 97-100.

98. **Kurvink K, Bloomfield CD, Keenan KM, Levitt S, Cervenka J.** Sister chromatid exchange in lymphocytes from patients with malignant lymphoma. *Hum Genet* 1978a; 44: 137-144.

99. **Kurvink K, Bloomfield CD, Cervenka J.** Sister chromatid exchange in patients with viral disease. *Exp Cell Res* 1978b; 113: 450-453.

100. **Lambert B, Hansson K, Lindsten J, Sten M, Werelius B.** Bromodeoxyuridine-induced sister chromatid exchanges in human lymphocytes. *Hereditas* 1976; 83: 163-174.

101. **Lambert B, Lindblad A, Nordenskjold M, Werelius B.** Increased frequency of sister chromatid exchanges in cigarette smokers. *Hereditas* 1978; 88: 147-149.

102. **Lambert B, Ehrnst A, Hansson K, Lindblad A, Morad M, Werelius B.** Sister chromatid exchange in peripheral lymphocytes of subjects vaccinated against measles. *Hum Genet* 1979; 50: 291-296.

103. **Lambert B, Lindblad A, Holmberg K, Francesconi D.** Use of sister chromatid exchange to monitor human populations for exposure to toxicologically harmful agents. In: Wolff SS, ed. *Sister Chromatid Exchange.* New York: John Wiley, 1982a: 159-165.

104. ***ibid.*** 1982b: 177.

105. **Larripa I, Matos E, Labal de Vinnessa M, Brieux de Salum S.** Sister chromatid exchanges in a human population accidentally exposed to an organophosphate pesticide. *Rev Brasil Genet* 1983; 6: 719-727.

106. **Latt SA.** Microfluorometric detection of deoxyribonucleic acid replication in human metaphase chromosomes. *Proc Natl Acad Sci, USA* 1973; 70: 3395-3399.

107. **Latt SA.** Localization of sister chromatid exchanges in human chromosomes. *Science* 1974a; 185: 74-76.

108. **Latt SA.** Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction by mitomycin C. *Proc Natl Acad Sci, USA* 1974b; 71: 3162-3166.

109. **Latt SA.** Sister chromatid exchange: New methods for detection. In: Wolff S, ed. *Sister Chromatid Exchange.* New York: John Wiley and Sons, Inc., 1982: 17-40.

110. **Latt SA, Schreck RR.** Sister chromatid exchange analysis. *Am J Human Genet* 1980; 32: 297-313.

111. **Latt SA, Stetten G, Juergens LA.** Inductions by alkylating agents of sister chromatid exchanges and chromatid breaks in Fanconi's anemia. *Proc Natl Acad Sci, USA* 1975; 72: 4066-4070.

112. **Latt SA, Allen J, Bloom SE, Carrano A, Falke E, Kram D, Schneider E, Schreck R, Tice R, Whitfield B, Wolff S.** Sister chromatid exchange: a report of the Gene-Tox Program. *Mutat Res* 1981; 87: 17-62.

113. **Latt SA, Schreck RR, D'Andrea A, Kaiser TN, Schlesinger F, Lester S, Sakai K.** Detection, significance, and mechanism of sister chromatid exchange formation: past experiments, current concepts, future challenges. In: Tice RR, Hollaender A, eds. *Sister Chromatid Exchanges.* Part A. The nature of SCEs. New York: Plenum Press, 1984a: 20.

114. ***ibid.*** 1984b: 21-22.

115. ***ibid.*** 1984c: 19.

116. **Lechner JF, Tokiwa T, La Veck M, Benedict WF, Banks-Schlegel S, Yeager H, Banerjee A, Harris CC.** Asbestos-associated chromosomal changes in human mesothelial cells. *Proc Natl Sci Acad, USA* 1985; 82: 3884-3888.

117. **Livingston GK, Dethlefsen LA.** Effects of hyperthermia and X irradiation on sister chromatid exchange (SCE) frequency in Chinese hamster ovary (CHO) cells. *Radiat Res* 1979; 77: 512-520.

118. **Livingston GK, Rom WN, Morris MV.** Asbestos-induced sister chromatid exchanges in cultured Chinese hamster ovarian fibroblast cells. *J Environ Pathol Toxicol* 1980; 4: 373-382.

119. **Livingston GK, Cannon LA, Bishop DT, Johnson P, Finemen RM.** Sister chromatid exchange: variation by age, sex, smoking, and breast cancer status. *Cancer Genet Cytogenet* 1983; 9: 289-299.

120. **Loveday KS, Latt SA.** The effect of a tumor promotor, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), on sister chromosome exchange formation in cultured Chinese hamster cells. *Mutat Res* 1979; 67: 343-348.

121. **Mahmood N, Khan SG, Ali S, Athar M, Rahman Q.** Asbestos induced oxidative injury to DNA. *Ann Occup Hyg* 1993; 3: 315-319.

122. **Maki-Paakkanen J, Sorsa M, Vainio H.** Chromosome aberrations and sister chromatid exchanges in lead-exposed workers. *Hereditas* 1981; 94: 269-275.

123. **Margolin BH, Shelby MD.** Sister chromatid exchanges: A reexamination of the evidence for sex and race differences in humans. *Environ Mutagen* 1985; 4 (Suppl): 63-72.

124. **Mazrimas JA, Stetka DG.** Direct evidence for the role of incorporated BUdR in the induction of sister chromatid exchanges. *Exp Cell Res* 1978; 117: 23-30.

125. **McClintock B.** The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behaviour of ring-shaped chromosomes. *Genetics* 1938; 23: 315-376.

126. **McFee AF, Sherrill MN.** Mitotic response and sister chromatid exchanges in lymphocytes cultured in sera from different sources. *Experientia* 1981; 37: 27-29.

127. **Meiying C, Jiujin X, Xianting Z.** Comparative studies on spontaneous and mitomycin-C-induced sister-chromatid exchanges in smokers and non-smokers. *Mutat Res* 1982; 195-200.

128. **Miner JK, Rom WN, Livingston GK, Lyon JL.** Lymphocytes sister chromatid exchange (SCE) frequencies in coke oven workers. *J Occup Med* 1983; 25: 30-33.

129. **Moan J, Waksvik H, Christensen T.** DNA single strand breaks and sister chromatid exchanges induced by treatment with hematoporphyrin and light or by X-rays in human NHIK 3025 cells. *Cancer Res* 1980; 40: 2915-2918.

130. **Morgan WF, Crossen PE.** The incidence of sister chromatid exchanges in cultured human lymphocytes. *Mutat Res* 1977; 42: 305-312.

131. **Morgan WF, Crossen PE.** Factors influencing sister-chromatid exchange rate in cultured human lymphocytes. *Mutat Res* 1981; 81: 395-402.

132. **Mossman BT, Gee BL.** Asbestos-related diseases. *N Engl J Med* 1989; 320: 1721-1730.

133. **Murphy SA, Tice RR, Smith MG, Margolin BH.** Contributions to the design and statistical analysis of in vivo SCE experiments. *Mutat Res* 1992; 271: 39-48.

134. **Murthy PB.** Frequency of sister chromatid exchanges in cigarette smokers. *Hum Genet* 1979; 52: 343-345.

135. **Murthy PB, Prema K.** Sister-chromatid exchanges in oral contraceptive users. *Mutat Res* 1979; 68: 149-152.

136. **Murthy BP, Bhaskaram P, Srikantia SG.** Sister chromatid exchanges in protein-energy malnutrition. *Hum Genet* 1980; 55: 405-406.

137. **Nagasawa H, Little JB.** Effect of tumor promoters, protease inhibitors, and repair processes on X-ray-induced sister chromatid exchanges in mouse cells. *Proc Natl Acad Sci, USA* 1979; 76: 1943-1947.

138. **Nordic study group on the health risk of chromosome damage.** A Nordic data base on somatic damage in humans. *Mutat Res* 1990; 241: 325-337.

139. **Norppa H, Sorsa M, Vainio H, Grohn P, Heinonen E, Holsti LR, Nordman E.** Increased sister chromatid exchange frequencies in lymphocytes of nurses handling cytostatic drugs. *Scand J Work Environ Health* 1980; 6: 299-301.

140. **Nowell PC.** Phytohaemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res* 1960; 20: 462-466.

141. **O'Riordan ML, Evans HJ.** Absence of significant chromosome damage in males occupationally exposed to lead. *Nature* 1974; 247: 50-53.

142. **Otter M, Palmer CG, Baehner RL.** Sister chromatid exchange in lymphocytes from patients with acute lymphoblastic leukemia. *Hum Genet* 1979; 52: 185-192.

143. **Painter RB.** A replication model for sister chromatid exchange. *Mutat Res* 1980; 70: 337-341.

144. **Pant GS, Kamada N, Tanaka R.** Sister chromatid exchanges in peripheral lymphocytes of atomic bomb survivors and of normal individuals exposed to radiation and chemical agents. *Hiroshima J Med Sci* 1976; 25: 99-105.

145. **Park EH, Kim YJ, Byun DH, Lee JY, Lee JS.** Baseline frequency of sister-chromatid exchanges in 142 persons of the general Korean population. *Mutat Res* 1992; 268: 239-246.

146. **Pedersen C, Olah E, Merrild U.** Sister chromatid exchanges in cultured peripheral lymphocytes from twins. *Hum Genet* 1979; 52: 281-294.

147. **Perry P.** Chemical mutagens and sister chromatid exchange. *Chem Mutagens* 1980; 6: 1-39.

148. **Perry P, Evans HJ.** Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* 1975; 258: 121-125.

149. **Perry P, Wolff S.** New Giemsa method for the differential staining of sister chromatids. *Nature* 1974; 251: 156-158.

150. **Popescu NC, DiPaolo JA.** The relevance of sister chromatid exchange to the induction of neoplastic cell transformation. In: *Progress and Topics in Cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982: 425-460.

151. **Popescu NC, Amsbaugh SC, Larramendy ML, DiPaolo JA.** 12-O-tetradecanoyl-phorbol-13-acetate and its relationship to SCE induction in Syrian and Chinese hamster cells. *Environ Mutagen* 1982; 4: 73-81.

152. **Price-Jones M, Gubbings G, Chamberlain M.** The genetic effects of crocidolite asbestos; comparison of chromosome abnormalities and sister chromatid exchanges. *Mutat Res* 1980; 79: 331-336.

153. **Puntoni R, Vercelli M, Merlo F, Valerio F, Santi L.** Mortality among shipyard workers in Genoa, Italy. *Ann NY Acad Sci* 1979; 330: 353-377.

154. **Reidy JA, Annest JL, Chen ATL, Welty TK.** Increased sister chromatid exchange associated with smoking and caffeine consumption. *Environ Mol Mutagen* 1988; 12: 311-318.

155. **Reuterwall CA.** A Nordic data base on somatic chromosome damage in humans. *Mutat Res* 1990; 241: 325-337.

156. **Rom WN, Livingston GK, Casey KR, Wood SD, Egger MJ, Chiu GL, Jerominski L.** Sister chromatid exchange frequency in asbestos workers. *JNCI* 1983; 70: 45-48.

157. **Santesson B, Lindahl-Kiessling K, Mattsson A.** SCE in B and T lymphocytes. Possible implications for Bloom's syndrome. *Clin Genet* 1979; 16: 133-135.

158. **Sarto F, Faccioli MC, Cominato I, Levis AG.** Aging and smoking increase the frequency of sister chromatid exchanges (SCE) in man. *Mutat Res* 1985; 144: 183-187.

159. **Schneider El, Kram D, Nakanishi Y, Monticone RE, Tice RR, Gilman BA, Nieder ML.** The effect of aging on sister chromatid exchange. *Mech Aging Dev* 1979; 9: 303-311.

160. **Schuler D, Dobos M, Fekete G, Miltenyi M, Kalmar L.** Chromosome mutations and chromosome stability in children treated with different regimes of immunosuppressive drugs. *Hum Hered* 1979; 29: 100-105.

161. **Schvartzman JB, Postigo R, Gutierrez C.** Analysis of visible light-induced sister chromatid exchanges in 5-bromodeoxyuridine-substituted chromosomes. *Chromosoma* 1979; 74: 317-328.

162. **Schvartzman JB, Tice RR.** 5-Bromodeoxyuridine and its role in the production of sister chromatid exchange. In: Sandberg AA, ed. *Progress and Topics in Cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982: 124.

163. **Schwartz JL, Banda MJ, Wolff S.** 12-O-tetradecanoylphorbol-13-acetate (TPA) induces sister-chromatid exchanges and delays in cell progression in Chinese hamster ovary and human cell lines. *Mutat Res* 1982; 92: 393-409.

164. **Selikoff IJ, Hammond EC, Seidman H.** Mortality experience of insulation workers in the United States and Canada, 1943-1976. *Ann NY Acad Sci* 1979; 330: 91-116.

165. **Shafer DA.** Replication bypass model of sister chromatid exchanges and implications for Bloom's syndrome and Fanconi's anemia. *Hum Genet* 1977; 39: 177-190.

166. **Shafer DA.** Alternate replication bypass mechanisms for sister chromatid exchange formation. In: Sandberg AA, ed. *Progress and topics in cytogenetics*, vol. 2. Sister Chromatid Exchange. New York: Alan R Liss, 1982: 67-98.

167. **Shafer DA.** Replication bypass mechanisms and the induction of SCE by single-strand adducts or lesions of DNA. In: Tice RR, Hollaender A, eds. *Sister Chromatid Exchanges.* Part A. The nature of SCEs. New York: Plenum Press, 1984a: 245.

168. ***ibid.*** 1984b: 246.

169. **Shim J-S.** Sister-chromatid exchanges in 52 Korean women living in the vicinity of an industrial complex. *Mutat Res* 1989; 224: 511-515

170. **Sobti RC, Bhardwaj DK.** Cytogenetic damage and occupational exposure. I. Exposure to stone dust. *Environ Res* 1991; 56: 25-30.

171. **Solomon E, Bobrow M.** Sister chromatid exchanges - a sensitive assay of agents damaging human chromosomes. *Mutat Res* 1975; 30: 273-278.

172. **Soper KA, Stolley PD, Galloway SM, Smith JG, Nichols WW, Wolman SR.** Sister-chromatid exchange (SCE) report on control subjects in a study of occupationally exposed workers. *Mutat Res* 1984; 129: 77-88.

173. **Sorsa M.** Cytogenetic studies in relation to occupational exposures. In: Hemminki K, Sorsa M, Vainio H, eds. *Occupational hazards and reproduction.* Washington: Hemisphere Publishing Corporation, 1985: 177-178.

174. **Speit G, Vogel W, Wolf M.** Characterization of sister chromatid exchange induction by hydrogen peroxide. *Env Mutagen* 1982; 4: 135-142.

175. **Speit G, Hochsattel R, Vogel W.** The contribution of DNA single-strand breaks to the formation of chromosome aberrations and SCEs. In: Tice RR, Hollaender A, eds. *Sister Chromatid Exchanges.* Part A. The nature of SCEs. New York: Plenum Press, 1984a: 238.

176. ***ibid.*** 1984b: 229-234.

177. ***ibid.*** 1984c: 230-231.

178. ***ibid.*** 1984d: 240.

179. **Stella M, Montaldi A, Rossi R, Rossi G, Levis AG.** Clastogenic effects of chromium on human lymphocytes *in vitro* and *in vivo*. *Mutat Res* 1982; 101: 151-164.

180. **Stetka DG, Carrano AV.** The interaction of Hoechst 33258 and BrdU substituted DNA in the formation of sister chromatid exchanges. *Chromosoma* 1977; 63: 21-31.

181. **Stolley PD, Soper KA, Galloway SM, Nichols WW, Norman SA, Wolman SR.** Sister-chromatid exchanges in association with occupational exposure to ethylene oxide. *Mutat Res* 1984; 129: 89-102.

182. **Swierenga SHH, Heddle JA, Sigal EA, Gilman JPW, Brillinger RL, Douglas GR, Nestmann ER.** Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. *Mutat Res* 1991; 246: 301-322.

183. **Takeuchi Y, Hisanaga N, Ono Y, Shibata E, Saito I, Iwata M.** Modification of metabolism and neurotoxicity of hexane by co-exposure of toluene. *Int Arch Occup Environ Health* 1993; 65: S227-S230.

184. **Tates AD, Grummt T, Tornqvist M, Farmer PB, van Dam FJ, van Mossel H, Schoemaker HM, Osterman-Golkar S, Uebel C, Tang YS, Zwinderman AH, Natarajan AT, Ehrenberg L.** Biological and chemical monitoring of occupational exposure to ethylene oxide. *Mutat Res* 1991; 250: 483-497.

185. **Tatsumi K, Strauss B.** Production of DNA bifilarily substituted with bromodeoxyuridine in the first round of synthesis: Branch migration during isolation of cellular DNA. *Nucl Acids Res* 1978; 5: 331-347.

186. **Taylor JH.** Sister chromatid exchanges in tritium labeled chromosomes. *Genetics* 1958; 43: 515-529.

187. **Taylor JH.** Chromosome reproduction and the problem of coding and transmitting the genetic heritage. *Amer Sci* 1960; 48: 365-382.

188. **Taylor JH, Woods PS, Hughes ML.** The organisation and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. *Proc Natl Acad Sci, USA* 1957; 43: 122-128.

189. **Thompson LH, Baker RM, Carrano AV, Brookman KW.** Failure of the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate to enhance sister chromatid exchange, mitotic segregation or expression of mutations in Chinese hamster cells. *Cancer Res* 1980; 40: 3245-3251.

190. **Vainio H, Sorsa M.** Chromosome aberrations and their relevance to metal carcinogenesis. *Environ Health Perspect* 1981; 40: 173-180.

191. **Vainio H, Sorsa M.** Application of cytogenetic methods for biological monitoring. *Ann Rev Public Health* 1983; 4: 403-407.

192. **Vainio H, Sorsa M, Rantanen J, Hemminki K, Aitio A.** Biological monitoring in the identification of the cancer risk of individuals exposed to chemical carcinogens. *Scand J Work Environ Health* 1981; 7: 241-251.

193. **Vogel F.** "Our load of mutation": reappraisal of an old problem. *Proc Roy Soc London* 1979; 205: 77-90.

194. **Wagner JC, Sleggs CA, Marchand P.** Diffuse pleural mesothelioma and asbestos exposure in the northwestern Cape Province. *Brit J Indust Med* 1960; 17: 260-271.

195. **Waksvik H, Magnus P, Berg K.** Effects of age, sex and genes on sister chromatid exchange. *Clin Genet* 1981a; 20: 449-454.

196. **Waksvik H, Klepp O, Brogger A.** Chromosome analyses of nurses handling cytostatic agents. *Cancer Treat Rep* 1981b; 65: 607-610.

197. **Weinberg RA.** Oncogenes of spontaneous and chemically induced tumors. *Adv Cancer Res* 1982; 36: 149-163.

198. **Wen WN, Liew TL.** The effect of age and cell proliferation on the frequency of sister chromatid exchange in human lymphocytes cultured *in vitro*. *Mech Ageing Dev* 1983; 21: 377-384.

199. **Wolff S.** Sister chromatid exchange. *Ann Rev Genet* 1977; 11: 183-201.

200. **Wolff S.** Relation between DNA repair, chromosome aberrations, and sister chromatid exchanges. In: Hanawalt PC, Friedberg EC, Fox CF, eds. *DNA repair mechanism.* New York: Academic Press, 1978: 751-760.

201. **Wolff S.** Sister chromatid exchange as a test for mutagenic carcinogens. *Ann NY Acad Sci* 1983; 407: 142-153.

202. **Wolff S, Bodycote J, Painter RB.** Sister chromatid exchanges induced in Chinese hamster cells by UV irradiation of different stages of the cell cycle: the necessity for cells to pass through S. *Mutat Res* 1974; 25: 73-81.

203. **Wulf HC.** Monitoring of genotoxic exposure of humans by the sister chromatid exchange test. Methodology and confounding factors. *Dan Med Bull* 1990; 37: 132-143.

204. **Wulf HC, Niebuhr E.** Different sister chromatid exchange rates in XX and XY cells of a pair of human chimeric twins. *Cytogenet Cell Genet* 1985; 39: 105-108.

205. **Wulf HC, Niebuhr E, Lundgren K.** Extremely high sister chromatid exchange baseline levels in normal, healthy individuals. *Hereditas* 1987; 106: 115-118.

206. **Yager JW, Hines CJ, Spear RC.** Exposure to ethylene oxide at work increases sister chromatid exchanges in human peripheral lymphocytes. *Science* 1983; 219: 1221-1223.

207. **Zakharov AF, Egolina NA.** Differential spiralization along mammalian mitotic chromosomes. I. BUdr-revealed differentiation in Chinese hamster chromosomes. *Chromosoma* 1972; 38: 341-365.

208. **Zanzoni F, Bauman JW, Jung EG.** Sister chromatid exchanges (SCE) in human lymphocytes. Effect of UV C irradiation and age. *Arch Dermatol Res* 1979; 265: 283-287.

CONSENT FORM

NATIONAL CENTRE FOR OCCUPATIONAL HEALTH

P.O. BOX 4788, JOHANNESBURG. 2000

Tel.: 724-1844

DEPARTMENT OF OCCUPATIONAL MEDICINE

MEDICAL EXAMINATION AND STUDY OF PEOPLE EXPOSED TO ASBESTOS

Information Form

The National Centre for Occupational Health has been asked to give all employees at Mintex/Don and Capil full medical examination.

The purpose of this is to see whether employees may have picked up lung scarring from inhalaling asbestos.

We also want to do research to find out whether asbestos may affect certain cells in the body. This can be measured in the blood.

We therefore ask every employee to take part in the study.

This will involve :

1. Answering questions about symptoms and jobs ("questionnaire")
2. Chest X-ray
3. Chest examination by a doctor
4. Blowing test ("lung function")
5. Giving a small amount of blood.

There are no harmful effects from these tests, but the blood test may be slightly painful.

Any employee found to have a medical problem will be referred to a doctor. If you do not hear individually, you can assume your results did not show a medical proble.

All individual test results are confidential, and will be available to management only if needed to apply for workmen's compensation.

Group or "overall" results will be available to management and workers on request.

Taking part is entirely voluntary, and no employee will lose out by taking part. However it will be difficult to measure properly the effects of the asbestos exposure if many workers decide not to take part.

Any questions you may have will be answered by the team from N.C.O.H.

Proposed programme :

September - November	:	Questionnaires
(Every Monday)		Blood test
		This will take ± 30 minutes per employee
November 17th - 21st	:	Chest X-ray (± 15 minutes per employee)
November 24th -	:	Lung function tests
December 5th		Chest examination (± 30 mins. per employee)

Thank you for considering this request.

CONSENT FORM

I, _____

agree to participate in the study as set out in the Information Form.

Signed _____

Date: _____

Witness : _____

Researcher: _____

QUESTIONNAIRE 1 Card 1 [ ]$_1$

Clock/Company number [ ][ ][ ][ ]$_5$

DON INTERNATIONAL/CAPIL SURVEY 1986

This survey involves answering questions about your health and occupation, as well as taking a blood sample for tests. All your answers will remain confidential. You will receive a report (which you can discuss with your doctor, if necessary) after the lung function tests and X-rays have been completed. We appreciate your co-operation in this project.

a) DATE (dd/mm/yy): __ /__ / 1986 [ ][ ][ ][ ]$_9$

b) INTERVIEWER: (LM=1;RR=2)..... [ ]$_{10}$

c) LANGUAGE OF INTERVIEW..... [ ]$_{11}$

d) SURNAME.....

e) FIRST NAMES.....

f) DATE OF BIRTH (dd/mm/yy): __ /__ / 19__ [ ][ ][ ][ ][ ][ ]$_{17}$

g) AGE: [ ][ ] years [ ][ ]$_{19}$

h) HOME LANGUAGE (tick one): N Sotho [1] Xhosa [5]
S Sotho [2] Tsonga [6]
Zulu [3] Other [7]
Tswana [4] [ ]$_{20}$

i) SCHOOLING (tick one): nil [1] std.6-9 [4]
< std.1 [2] matric [5]
std.1-5 [3] post-matric [6] [ ]$_{21}$

j) PRESENT ADDRESS.....

.....

..... [ ]$_{22}$

k) ANY OTHER CONTACT ADDRESS.....

.....

..... [ ]$_{23}$

l) HAVE YOU EVER BEEN SEEN AT THE NCOH CLINIC IN JOHANNESBURG? Y [1] N [2] [ ]$_{24}$

IF YES, WHEN? month [ ][ ] year 19[ ][ ] [ ][ ]$_{26}$

1. FAMILY HISTORY

1.1 Are/were your parents cousins? Y [1] N [2] [ ]27

-- if yes,please specify ----------

..... [ ]28

1.2 Is your father still alive? Y [1] N [2] [ ]29

1.3 Does/did he suffer from any disease? Y [1] N [2] [ ]30

-- if yes,please specify ----------

..... [ ][ ]32

1.4 Is your mother still alive? Y [1] N [2] [ ]33

1.5 Does/did she suffer from any disease? Y [1] N [2] [ ]34

-- if yes,please specify ----------

..... [ ][ ]36

1.6 How many brothers do you have? [ ][ ] [ ][ ]38

1.7 How many sisters do you have? [ ][ ] [ ][ ]40

1.8 Are all your brothers and sisters still alive?
Y [1] N [2] [ ]41

1.9 Do/did any of them suffer from any disease?
Y [1] N [2] [ ]42

-- if yes,please specify ----------

1).....[ ][ ]44

2).....[ ][ ]46

3).....[ ][ ]48

4).....[ ][ ]50

1.10 Does/did any member of your immediate family have any of the following?

a) chest problems Y [1] N [2] [ ]51

-- if yes,please specify ----------

..... [ ][ ]53

..... [ ][ ]55

..... [ ][ ]57

b) cancer Y [1] N [2] [ ]58

-- if yes,please specify ----------

..... [ ][ ]60

..... [ ][ ]62

..... [ ][ ]64

1.11 Do you have any children? Y [1] N [2] [ ]65

if no, go to section 1.17

1.12 Are you related to your wife? Y [1] N [2] [ ]66

-- if yes,please specify ----------

..... [ ]67

Card 2 [ ]1

Clock/Company number [ ][ ][ ][ ]5

1.13 How many children do you have? [ ][ ] [ ][ ]7

1.14 What are their ages? (if fewer than 6 children, record ages of youngest 6)

[ ][ ]	[ ][ ]9
[ ][ ]	[ ][ ]11
[ ][ ]	[ ][ ]13
[ ][ ]	[ ][ ]15
[ ][ ]	[ ][ ]17
[ ][ ]	[ ][ ]19

1.15 Were any of your children born with a physical abnormality? Y [1] N [2] [ ]20

-- if yes, please specify ----------

abnormality:..... age of child:..... [ ][ ]22

abnormality:..... age of child:..... [ ][ ]24

abnormality:..... age of child:..... [ ][ ]26

1.16 Were any of your children born with a mental abnormality? Y [1] N [2] [ ]27

-- if yes, please specify ----------

abnormality:..... age of child:..... [ ][ ]29
abnormality:..... age of child:..... [ ][ ]31
abnormality:..... age of child:..... [ ][ ]33

1.17 Have any of your brothers/sisters/children ever had a child born with a physical or mental abnormality? Y [1] N [2] [ ]34

-- if yes, please specify ----------

abnormality:..... age of child:..... [ ][ ]36
abnormality:..... age of child:..... [ ][ ]38
abnormality:..... age of child:..... [ ][ ]40

1.18 Have any of your children been stillborn or died before 4 weeks of age? Y [1] N [2] [ ]41

-- if yes ----------

1.19 How many such children have you had? [ ][ ] [ ][ ]43

1.20 How many were stillborn before you started working in the asbestos factory? [ ] [ ]44

1.21 How many were stillborn after you started working in the asbestos factory? [ ] [ ]45

1.22 Has your wife ever had a miscarriage? Y [1] N [2] [ ]46

-- if yes ----------

1.23 How many miscarriages has she had? [ ] [ ]47

1.24 How many did she have before you started working in the asbestos factory? [ ] [ ]48

1.25 How many did she have after you started working in the asbestos factory? [ ] [ ]49

These questions relate mainly to your chest. Try and answer either "yes" or "no" to each question. If you are in doubt, answer "no".

2. COUGH

2.1 Do you usually cough first thing in the morning?
Y [1] N [2] [ ]50

2.2 Do you usually cough during the day, or at night?
Y [1] N [2] [ ]51

-- If yes to either 2.1 or 2.2 --

2.3 Do you cough like this on most days for as much as 3 months each year? Y [1] N [2] [ ]52

2.4 For how many years have you had this? [ ][ ] [ ][ ]54

3. PHLEGM

3.1 Do you usually bring up any phlegm from your chest first thing in the morning? Y [1] N [2] [ ]55

3.2 Do you usually bring up any phlegm from your chest during the day, or at night? Y [1] N [2] [ ]56

-- If yes to either 3.1 or 3.2 --

3.3 Do you bring up this phlegm like this on most days for as much as 3 months each year?
Y [1] N [2] [ ]57

3.4 For how many years have you had this? [ ][ ] [ ][ ]59

4. PERIODS OF COUGH AND PHLEGM

4.1 In the past 3 years, have you had a period of increased cough and phlegm lasting for 3 weeks or more? Y [1] N [2] [ ]60

-- If yes to 4.1 --

4.2 Have you had more than one such period?
Y [1] N [2] [ ]61

5. BREATHLESSNESS

5.1 Is the subject disabled from walking by any condition other than heart or lung disease?
Y [1] N [2] [ ]62

--- If yes to 5.1 ---

Describe condition.....

..... [ ][ ]64

GO TO QUESTION 6

5.2 Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill?
Y [1] N [2] [ ]65

--- If yes to 5.2 ---

5.3 Do you get short of breath when walking with other people of your own age on level ground?
Y [1] N [2] [ ]66

---If yes to 5.3 ---

5.4 Do you have to stop for breath when walking at your own pace on level ground? Y [1] N [2] [ ]67

6. WHEEZING

6.1 Does your chest ever sound wheezy or whistling?
Y [1] N [2] [ ]68

--- If yes to 6.1 ---

6.2 Do you get this on most days - or nights?
Y [1] N [2] [ ]69

6.3 Have you ever had attacks of shortness of breath with wheezing? Y [1] N [2] [ ]70

--- If yes to 6.3 ---

6.4 Is / was your breathing absolutely normal between attacks? Y [1] N [2] [ ]71

Card 3 [ ]$_1$

Clock/Company number [ ][ ][ ][ ]$_5$

7. CHEST ILLNESS

7.1 During the past 3 years, have you had any chest illness which has kept you away from your usual activities for as much as a week? Y [1] N [2] [ ]$_6$

--- If yes to 7.1 ---

7.2 Did you bring up more phlegm than usual in any of these illnesses? Y [1] N [2] [ ]$_7$

--- If yes to 7.2 ---

7.3 Have you had more than one illness like this in the past 3 years? Y [1] N [2] [ ]$_8$

7.4 Have you ever had, or been told that you have, any of the following illnesses or complaints?

a) injury or operation affecting chest? (eg. broken rib, stab wound) Y [1] N [2] [ ]$_9$

b) heart disease? Y [1] N [2] [ ]$_{10}$

c) bronchitis? (sternal pain, bad cough, sputum, 1 week) Y [1] N [2] [ ]$_{11}$

d) pleurisy? (sharp pain with breathing, no fever) Y [1] N [2] [ ]$_{12}$

e) pneumonia? (fever and sharp chest pain, doctor) Y [1] N [2] [ ]$_{13}$

f) tuberculosis (TB)? Y [1] N [2] [ ]$_{14}$

g) asthma? Y [1] N [2] [ ]$_{15}$

h) other chest trouble? Y [1] N [2] [ ]$_{16}$

details:..... [ ]$_{17}$

8. HISTORY OF CHEST ILLNESS IN CHILDHOOD

8.1 Did you have any chest illness before the age of 16? Y [1] N [2] [ ]18

-- if yes, please specify ----------

..... [ ]19

8.2 Did you have measles as a child? Y [1] N [2] [ ]20

8.3 Did you have whooping cough as a child? Y [1] N [2] [ ]21

9. HEALTH STATUS

9.1 Have you been told by a doctor that you have any of the following diseases?

a) heart disease (include high blood pressure) Y [1] N [2] [ ]22

b) kidney disease Y [1] N [2] [ ]23

c) arthritis (stiff, painful joints, for a long time) Y [1] N [2] [ ]24

d) sugar sickness / thyroid problems Y [1] N [2] [ ]25

e) rheumatic fever (swollen joints with heart disease) Y [1] N [2] [ ]26

f) meningitis (severe headache, fever, stiff neck, hospitalization) Y [1] N [2] [ ]27

9.2 Have you had any of the following illnesses in the last month?

a) 'flu/cold Y [1] N [2] [ ]28
b) diarrhoea Y [1] N [2] [ ]29
c) chickenpox Y [1] N [2] [ ]30

9.3 Have you had any painful swelling of the liver in the last 6 months which developed suddenly (hepatitis)? Y [1] N [2] [ ]31

9.4 Have you had glandular fever (swelling of the glands) in the last 6 months? Y [1] N [2] [ ]32

9.5 Have you had any vaccines/immunizations in the past 6 months? Y [1] N [2] [ ]33

-- If yes, please specify ----------

..... [ ][ ]35

9.6 Have you ever been treated for cancer?
Y [1] N [2] [ ]36

--- If yes ---

9.7 What type of cancer was it?

..... [ ][ ]38

9.8 What treatment were you given?

..... [ ][ ]40

9.9 When were you given this treatment? (mnth/yr - mnth/yr) [ ][ ][ ][ ]44 [ ][ ][ ][ ]48

9.10 Have you had an X-ray or been exposed to any X-rays (at work, in hospital or for treatment) within the last 12 months (including teeth X-rays)?
Y [1] N [2] [ ]49

9.11 Have you taken any medications (prescribed or non-prescribed) in the last 3 months? Y [1] N [2] [ ]50

--- If yes, please specify ---

desciption	reason for taking it	
.....	.....	[ ][ ]52
.....	.....	[ ][ ]54
.....	.....	[ ][ ]56
.....	.....	[ ][ ]58

9.12 Have you taken any medicines prescribed by a traditional doctor in the last 6 months?
Y [1] N [2] [ ]59

10. TOBACCO SMOKING

10.1 Have you ever smoked cigarettes? (Yes means more than 20 packs of cigarettes in your life or more than 1 cigarette a day for a year). Y [1] N [2] [ ]60

--- If yes to 10.1 ---

10.2 Do you now smoke cigarettes? (as of 1 month ago) Y [1] N [2] [ ]61

10.3 How old were you when you first started regular cigarette smoking? [ ][ ] years old [ ][ ]63

10.4 If you have stopped smoking cigarettes completely, how old were you when you stopped? [ ][ ] years old [ ][ ]65

10.5 How many cigarettes do you smoke per day now? [ ][ ] [ ][ ]67

10.6 On average of the entire time you smoked, how many cigarettes did you smoke per day? [ ][ ] [ ][ ]69

Card 4 [ ]1

Clock/
Company number [ ][ ][ ][ ]5

10.7 Have you ever smoked a pipe regularly? (Yes means for at least 1 year). Y [1] N [2] [ ]6

--- If yes to 10.7 ---

10.8 Do you now smoke a pipe? (as of 1 month ago) Y [1] N [2] [ ]7

10.9 How old were you when you started to smoke a pipe regularly? [ ][ ] years old [ ][ ]9

10.10 If you have stopped smoking a pipe completely, how old were you when you stopped? [ ][ ] years old [ ][ ]11

10.11 On the average over the entire time you smoked a pipe, how much pipe tobacco did you smoke per week? (a standard pouch of tobacco contains 50g) [ ][ ] g [ ][ ]13

10.12 How much pipe tobacco are you smoking now? [ ][ ] g per week [ ][ ]15

10.13 Have you ever smoked hand-rolled cigarettes regularly? (Yes means more than 1 hand-rolled cigarette per day for a year). Y [1] N [2] [ ]16

--- If yes to 10.13 ---

10.14 Do you now smoke hand-rolled cigarettes? (as of 1 month ago) Y [1] N [2] [ ]17

10.15 How old were you when you started smoking hand-rolled cigarettes regularly? [ ][ ] years old [ ][ ]19

10.16 If you have stopped smoking hand-rolled cigarettes completely, how old were you when you stopped? [ ][ ] years old [ ][ ]21

10.17 How many hand-rolled cigarettes do you smoke per day now? [ ][ ] [ ][ ]23

10.18 On average of the entire time you smoked hand-rolled cigarettes, how many hand-rolled cigarettes did you smoke per week? [ ][ ] [ ][ ]25

10.19 Have you smoked marijuana, more often than once a month, in the last 12 months? Y [1] N [2] [ ]26

11. CAFFEINE AND ALCOHOL CONSUMPTION

11.1 Do you drink tea or coffee? Y [1] N [2] [ ]27

--- If yes ---

11.2 How many cups of tea per day? (on average) [ ][ ] [ ][ ]29

11.3 How many cups of coffee per day? (on average) [ ][ ] [ ][ ]31

11.4 Do you drink fizzy cold-drinks? (coke, fanta, etc.) Y [1] N [2] [ ]32

--- If yes ---

11.5 How many cans of cold-drink per week? (on average) [ ][ ] [ ][ ]34

11.6 Do you drink alcohol? Y [1] N [2] [ ]35

--- If yes ---

11.7 How many cartons of beer/home-brew per week? (on average) [ ][ ] [ ][ ]37

11.8 How many cans of beer per week? (on average) [ ][ ] [ ][ ]39

11.9 How many glasses of wine per week? (on average) [ ][ ] [ ][ ]41

11.10 How many tots/shots of hard liquor per week? (eg. brandy, whisky) [ ][ ] [ ][ ]43

12. OTHER

12.1 Have you used hair dye in the last 3 months? Y [1] N [2] [ ]44

12.2 Have you used artificial sweeteners in the last 3 months (eg. saccharin, sweetex, etc)? Y [1] N [2] [ ]45

13. COOPERATION OF SUBJECT good [1] poor [2] [ ]46

14. COMMENTS

.....

.....

..... [ ]47

QUESTIONNAIRE 2

Card 5 [ ]$_1$

Clock/
Company number [ ][ ][ ][ ]$_5$

OCCUPATIONAL AND EXPOSURE HISTORIES

16. DATE OF INTERVIEW (dd/mm/yy): __ /__ / 1986 [ ][ ][ ][ ]$_9$

17. OCCUPATIONAL HISTORY AT DON/CAPIL

17.1 Information from: subject [1]
records [2]
subject and records [3] [ ]$_{10}$

17.2 List all departments/jobs at Capil/Don/Cape, starting from the earliest

DEPARTMENT/JOB	PERIOD from month,year	to month,year
1. [ ][ ]$_{20}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{18}$
2. [ ][ ]$_{30}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{28}$
3. [ ][ ]$_{40}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{38}$
4. [ ][ ]$_{50}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{48}$
5. [ ][ ]$_{60}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{58}$
6. [ ][ ]$_{70}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{68}$

Card 6 [ ]$_1$
Co. No. [ ][ ][ ][ ]$_5$

7. [ ][ ]$_{15}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{13}$
8. [ ][ ]$_{25}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{23}$
9. [ ][ ]$_{35}$	[ ][ ],19[ ][ ] -	[ ][ ],19[ ][ ]$_{33}$

18. OTHER ASBESTOS EXPOSURE

18.1 Have you ever lived or spent time in any of the following places?

a) North-Western Cape (eg. Kuruman, Prieska, Postmasburg, Danielskuil) Y[1] N[2] [ ]36

-- If yes --

When were you first there? (year) 19 [ ][ ] [ ][ ]38

For how long were you there? (months) [ ][ ][ ] [ ][ ][ ]41

b) North-Eastern Transvaal, near asbestos mines (eg. Penge, Bewaarskloof, Kromellenbogen)? Y[1] N[2] [ ]42

-- If yes --

When were you first there? (year) 19 [ ][ ] [ ][ ]44

For how long were you there? (months) [ ][ ][ ] [ ][ ][ ]47

18.2 Have you ever lived in the same house/hostel room as someone working with asbestos? Y[1] N[2] [ ]48

-- If yes --

When did this start? (year) 19 [ ][ ] [ ][ ]50

How long did this last? (months) [ ][ ][ ] [ ][ ][ ]53

18.3 Have you ever cut through asbestos boards, sheeting, roofing, etc? Y[1] N[2] [ ]54

-- If yes --

When did you first do this? (year) 19 [ ][ ] [ ][ ]56

For how long? (months) [ ][ ][ ] [ ][ ][ ]59

18.4 Have you ever had any other asbestos exposure outside work? Y[1] N[2] [ ]60

-- If yes, specify ----------

1..... [ ]61

From m[ ][ ] y[ ][ ] to m[ ][ ] y[ ][ ] [ ][ ][ ][ ]65
[ ][ ][ ][ ]69

2..... [ ]70

From m[ ][ ] y[ ][ ] to m[ ][ ] y[ ][ ] [ ][ ][ ][ ]74
[ ][ ][ ][ ]78

Card 7 [ ]$_1$
Clock/Company number [ ][ ][ ][ ]$_5$

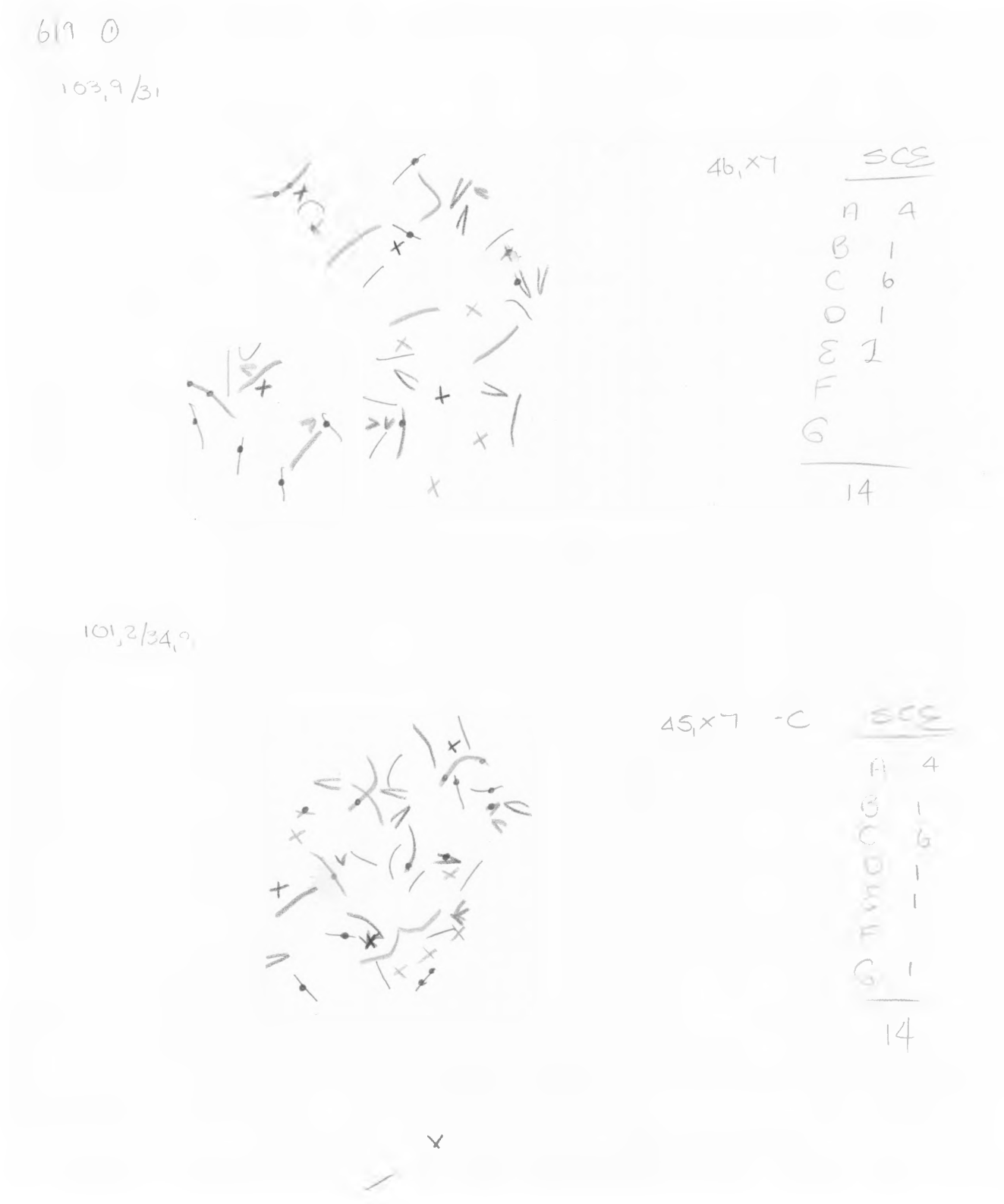
19. OTHER JOBS

List in order, starting from earliest

	COMPANY/ MINE	TYPE OF INDUSTRY	SPECIFIC JOB	TYPE(S) OF: DUST	FUMES/VAPOUR/ GAS	PERIOD YEAR to YEAR	
1.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	13
2.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	21
3.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	29
4.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	37
5.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	45
6.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	53
7.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	61
8.		[ ]	[ ]	[ ]	[ ]	19 [ ][ ] to 19 [ ][ ]	69

APPENDIX 4

EXAMPLE OF METHOD FOR SCORING SISTER CHROMATID EXCHANGES



DATA CAPTURE SHEET FOR RECORDING RAW DATA

Company number: _____

	Slide ID	Vernier	Chrom. no.	Number of SCEs A	B	C	D	E	F	G	Tot
1.											
2.											
3.											
4.											
5.											
6.											
7.											
8.											
9.											
10.											
11.											
12.											
13.											
14.											
15.											
16.											
17.											
18.											
19.											
20.											
21.											
22.											
23.											
24.											
25.											
	subtotal										

	Slide ID	Vernier	Chrom. no.	Number of SCEs A	B	C	D	E	F	G	Tot
26.											
27.											
28.											
29.											
30.											
31.											
32.											
33.											
34.											
35.											
36.											
37.											
38.											
39.											
40.											
41.											
42.											
43.											
44.											
45.											
46.											
47.											
48.											
49.											
50.											
		Total									

DATA CAPTURE SHEET FOR RECORDING CALCULATED MEANS

Company no. [ ][ ][ ][ ] 6

[ ][ ] 8

Total no. cells: _____

Chromosome Group	Total Chromosomes	Total SCEs	No. SCEs/ Chromosome	No. SCEs/ Cell	
A	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	20
B	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	32
C	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	44
D	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	56
E	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	68

Card no. [ ][ ] 2
Company no. [ ][ ][ ][ ] 6

F	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	18
G	[ ][ ][ ]	[ ][ ][ ]	[ ],[ ][ ]	[ ],[ ][ ]	30
Total	[ ][ ][ ][ ]	[ ][ ][ ][ ]	[ ],[ ][ ]	[ ][ ],[ ][ ]	45

APPENDIX 7

STATISTICS FOR INDIVIDUAL ASBESTOS-EXPOSED SUBJECTS

Study No.	No. cells scored	Total SCEs	SCEs/cell Mean	SD	Range
139	34	494	14.53	7.15	1 - 30
153	15	182	12.13	5.64	6 - 25
182	27	269	9.96	3.10	4 - 16
194	19	153	8.05	3.22	3 - 14
271	18	208	11.56	4.55	5 - 20
304	50	597	11.94	4.44	4 - 24
333	50	603	12.06	4.42	4 - 24
338	48	433	9.02	3.96	2 - 20
339	21	233	11.10	3.41	5 - 17
343	19	146	7.68	3.29	4 - 14
347	31	440	14.19	5.15	5 - 28
400	31	257	8.29	3.14	3 - 18
422	38	446	11.74	4.78	5 - 25
512	16	99	6.19	3.84	2 - 18
560	50	471	9.42	3.50	3 - 19
568	21	185	8.81	3.39	3 - 16
594	16	111	6.94	3.85	1 - 13
675	38	254	6.68	3.61	1 - 16
733	20	154	7.70	3.59	1 - 17
743	23	195	8.48	3.62	3 - 18
950	46	453	9.85	3.41	4 - 16
1109	26	243	9.35	3.10	3 - 15
1307	49	486	9.92	3.61	2 - 20
1436	15	137	9.13	4.56	1 - 22
1437	50	481	9.62	3.92	2 - 20
1443	20	207	10.35	4.07	3 - 20
1447	32	277	8.66	2.88	3 - 13
1450	17	158	9.29	3.64	5 - 18
1451	30	249	8.30	3.79	3 - 16
1470	35	389	11.11	4.15	3 - 22
1492	19	195	10.26	4.06	5 - 21

STATISTICS FOR INDIVIDUAL UNEXPOSED SUBJECTS

Study No.	No. cells scored	Total SCEs	SCEs/cell Mean	SD	Range
67	21	186	8.86	3.58	2 - 15
138	17	127	7.47	3.36	1 - 14
195	50	591	11.82	5.97	2 - 28
221	27	287	10.63	4.75	2 - 23
334	23	234	10.17	3.97	3 - 18
340	31	226	7.29	3.18	2 - 14
456	22	273	12.41	6.24	3 - 26
474	31	257	8.29	3.89	1 - 18
490	15	127	8.47	3.86	3 - 14
553	15	127	8.47	3.54	3 - 15
569	26	356	13.69	5.21	6 - 22
741	50	460	9.20	3.41	2 - 18
749	50	423	8.46	3.38	1 - 15
797	23	365	15.87	5.69	7 - 29
834	50	562	11.24	4.36	4 - 20
954	50	620	12.40	4.71	3 - 24
981	41	425	10.37	4.22	2 - 21
1509	23	167	7.26	2.61	2 - 11
1551	47	533	11.34	5.56	5 - 26
1642	50	444	8.88	2.96	3 - 18
2219	19	172	9.05	2.89	4 - 14