Final Report

to

Research Project

Development of a new mineral dust monitoring method
incorporating genotoxicity assessment:
an aid in cancer prevention

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March 1993

Executive Summary

ABSTRACT

The mechanisms of lung disease induced by inhalation of mineral dusts are only partly understood. Chemical oxidative stress has been strongly implicated in lung fibrosis and cancer. For example, it is thought that deposited asbestos fibres may lead to the formation of DNA-damaging oxygen radicals directly, or indirectly, by inducing an inflammatory reaction. If this oxidative stress mechanism is generally important, it is possible that a variety of minerals, not just asbestos, may be capable of catalysing the formation of free radicals and thereby cause lung damage.

This project addressed the issue of the surface catalytic formation of DNA-damaging radicals with regard to a range of locally extracted minerals. Using a laboratory assay, based on the hydroxylation of deoxyguanosine, it was found that all of the local mineral samples displayed a low reactivity, and nearly all displayed less reactivity than the asbestos reference minerals. Various experimental conditions were used, including different pHs, concentrations and buffer systems.

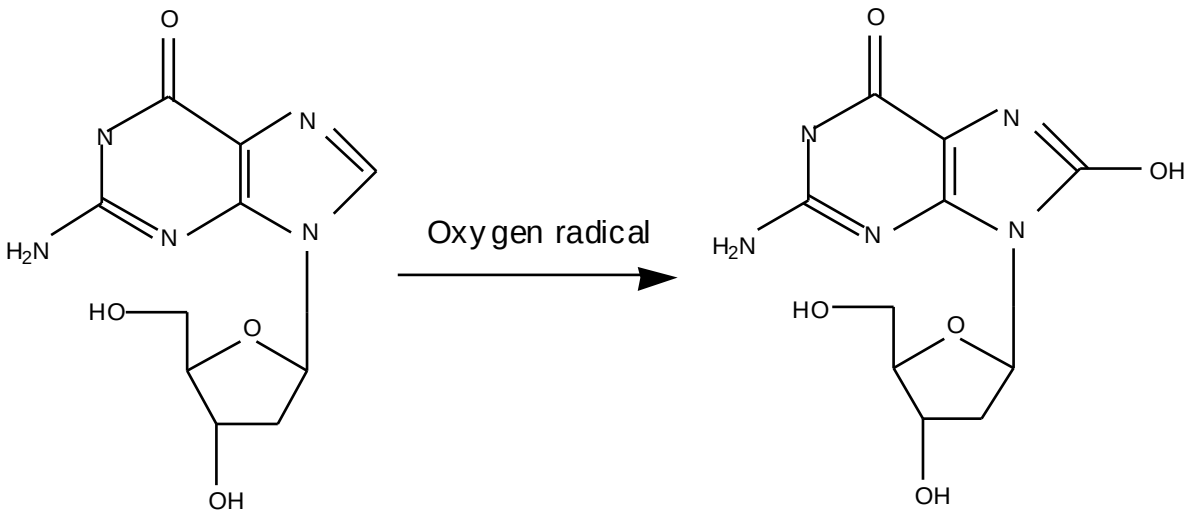
The investigations of airborne dust necessitated the construction of an experimental dust chamber. This facility was used in preliminary studies of the influence of airborne particle size and different dust collection approaches on observed surface reactivity. Data on respirable and inhalable size fractions were variable and the results do not demonstrate that smaller particles are always more reactive. Airborne dust sampling using a membrane filter method proved to be satisfactory but there were practical problems with the alternative liquid impinger method. In general, it appears that samples of suspended dust are not markedly more reactive than bulk samples of finely divided minerals.

In view of the laboratory results and other constraints, limited on-site air sampling was conducted (talc, kaosil and shellgrit). The field data are also variable but indicate that some increase in reactivity may be expected when airborne dust is mechanically generated.

In conclusion, the study has provided new insights into mineral surface toxicity, the results of which should be of some reassurance to the local mining and quarrying industry.

BACKGROUND

There is increasing evidence that oxidative stress, leading to cellular DNA damage and lipid peroxidation, is linked with chronic disease, such as heart disease, cancer and even ageing itself. In the case of asbestos related lung diseases, such as asbestosis and lung cancer, it has been argued that one of the principal steps in cancer initiation is DNA alteration, which may arise from the hydroxylation of deoxyguanosine residues by reactive oxygen species, particularly hydroxyl radicals. Thus it may be hypothesised that the carcinogenic effect of asbestos is due to free radical mediated DNA damage and that the carcinogenic potential of different minerals may be assessed by measuring their ability to hydroxylate deoxyguanosine to 8-hydroxydeoxyguanosine, as below.



Deoxyguanosine 8-Hydroxydeoxyguanosine

The hydroxylation reaction may be studied in the laboratory using a technique which involves mixing the mineral with a buffered solution of deoxyguanosine under controlled conditions and looking for the presence of 8-hydroxydeoxyguanosine with high performance liquid chromatography (HPLC). The more hydroxyl free radicals generated from the mineral surface, the more 8-hydroxydeoxyguanosine is produced.

It must be noted, however, that asbestos related lung cancer cannot be simply be inferred from this single cancer initiation step. The body has natural defence mechanisms to deal with the presence of oxygen radicals and DNA damage. The most recent work on carcinogenesis suggests that at least two chromosome "hits" are required for a cell to exhibit uncontrolled growth. Furthermore, subsequent progression to a clinically important cancer would seem to depend on various factors such as the state of the immune system and chance. Therefore, while "test tube" methods such as the deoxyguanosine assay described above find use in preliminary screening for the genotoxicity of mineral dusts, they are likely to have limited predictive power for clinical disease because they fail to take into account potentially important

variables such as particle dimensions, clearance rates, smoking and the state of the immune system.

Nevertheless, it was considered that meaningful comparisons could be made of potential genotoxicity between minerals. Such assessments would be very relevant if they could be related to inhalable dust and locally-extracted minerals.

On the basis of these results, management and unions would have more information for the purpose of deciding upon appropriate dust exposure controls. It may be possible to more clearly define those processes warranting extra surveillance.

PROJECT DEVELOPMENT

This project was conducted in three distinct phases, which are now briefly described.

Phase 1: Setting up and systematic investigations of reference minerals and locally-extracted minerals.

The initial work in the project involved the refinement of the deoxyguanosine assay, and studies of the influence of various conditions on the extent of hydroxylation for asbestos reference samples. Typically, this work involved incubation of a known weight of the mineral with deoxyguanosine under physiological conditions (pH 7, 37 C) for variable periods. Subsequently, the extent of hydroxylation would be determined by HPLC with electrochemical detection. One of the difficulties encountered during the setting up phase was temperature control within the laboratory, since the baseline noise on the electrochemical detector was temperature dependent. Satisfactory thermal control was achieved by the installation of a dedicated room air conditioner.

Factors which were examined in the deoxyguanosine assay included the reaction period, the influence of shaking, bubbling of air, mineral washing, crushing and the dependence on reagent concentration and mineral weight. It was found that the conversion of deoxyguanosine to 8-hydroxydeoxyguanosine was low (less than 1%), with little further reaction after about 18 hours. Mechanical agitation and bubbling of air through the solution improved the yield somewhat. Eventually, for reasons of practicality, sensitivity and comparability, a set of standard conditions was adopted and used for subsequent experiments involving reference minerals and other mineral samples. The reference minerals were International Union Against Cancer (UICC) standard asbestos reference samples, kindly provided by the Institute of Occupational Medicine (UK). Other minerals were obtained from local mines and quarries and other sources, with the cooperation of the management.

By using the standardized approach it was possible to compare the hydroxyl radical producing capacities of a range of bulk mineral samples. In general, the reactivities were low, even for samples containing large amounts of iron, such as iron ore. Of the local minerals tested, magnesite was found to be most active. There was some correlation of activity with

the amount of ferrous ion which could be mobilised into solution by bipyridyl but little correlation with surface area. It would appear that iron in the form of Fe^{2+} is an important factor, but there may be variable activity even for minerals containing Fe^{2+}. The presence of structural and surface Fe^{2+}, with sufficient electron donor capability to reduce oxygen, along with some degree of solubility are features which may lead to enhanced production of radicals. From the results of this work, it seems, fortunately, that these properties are shared by very few minerals.

Phase 2: Development of the industrial hygiene dust monitoring method

It was necessary to be able to generate stable concentrations of various dusts, and a laboratory dust generating device was specially constructed and tested for this work. A recirculating system was used for the chamber, which was capable of housing a variety of occupational hygiene air sampling devices. Some delays were experienced in obtaining materials for the construction of the chamber and there were some problems in commissioning the unit (e.g. sealing of the viewing panels and entry ports).

Several mineral dusts, which displayed reasonable activity in the bulk mineral assays, were selected for the chamber investigations. Respirable and inspirable (inhalable) dust collection systems were set up in the chamber. The traditional membrane filter technique was used. In addition, a liquid impinger technique was also tried where deoxyguanosine solution was used as a trapping medium. While the membrane technique proved to be satisfactory, there were some practical difficulties with the impinger approach, e.g. trapping efficiency. Samples of respirable and inhalable dust, collected on membrane filters, were analysed. However, the results were variable, indicating that smaller sized particles were not always more active. In general, there was not a markedly increased reactivity when compared with corresponding bulk mineral samples. This may be partly due to the fact that airborne dust in the chamber was simply being recirculated, and not freshly generated by mechanical means.

Phase 3: On-site air sampling

In view of the observed low reactivity of the minerals in the laboratory, there was only limited on-site air sampling. Furthermore, to improve sensitivity, some samples were collected from atypical exposure situations, e.g. sampling device located near obvious dust emission sources. The results of the on-site sampling suggest that there is somewhat increased reactivity, on a weight basis. Unfortunately, due to time and financial constraints it has not been possible to extend this work, in order to cover a wider range of exposure situations.

CONCLUSIONS

The project has demonstrated that occupational hygiene dust sampling methods may be combined with simple *in vitro* laboratory assays to provide further information about the relative genotoxicities of mineral dusts. The results do not suggest that there is an obvious problem with any of the local mineral products examined and thus no special action would seem to be required. It would be useful, however, to apply the method more widely to a comprehensive range of minerals extracted in South Australia and elsewhere. Finally, it would be desirable to conduct further on-site air sampling, particularly personal dust monitoring, in order to confirm the preliminary results obtained so far.

Consultation

Since the project was largely laboratory-based, there was limited interaction with the mining and quarrying industry. Mineral samples were sought from and generously supplied from a number of organizations, including BHP, ETSA, Olympic Dam Operations, Commercial Minerals Ltd, CSIRO Division of Soils and the Institute of Occupational Medicine (UK). In addition, there has been direct collaboration with the S.A. Department of Labour.

Acknowledgements

I thank Dr Michael Tkaczuk who has been responsible for conducting the bulk of the experimental work of this project. I acknowledge the cooperation of Dr John Addison (Institute of Occupational Medicine, Edinburgh) and Mr Alan Rogers (National Institute of Occupational Health and Safety, Sydney) in providing UICC asbestos reference samples. I also acknowledge the technical support of the South Australian Department of Labour, Occupational Health Division, and in particular Dr Czes Grygorcewicz. The University of Adelaide provided some financial support for the project, through the University Research Grants Scheme.

Finally, I thank Dr Catherine Skinner (Department of Geology, Yale University) for useful comments and advice.

Chapter 1

Introduction

1.1 Background

Inhalation of silica, asbestos, clay, talc, mica, zeolite and other silicate dusts can result in a wide variety of lung diseases, which include pneumoconiosis, irritant bronchitis, focal emphysema, bronchogenic carcinoma and mesothelioma (Ghio *et al*, 1990). The mechanism of toxic action is only partly understood, but is thought to be associated with particle dimensions (Stanton *et al*, 1981) and characteristics of the silicate surface, such as durability (Scholze and Conradt, 1987), surface area (Gormley and Addison, 1983), charge (Bagchi, 1992), and the presence of reducing surface sites containing ferrous ion (Bonneau *et al*, 1986; Zalma *et al*, 1987a).

Asbestos and man-made mineral fibres have been extensively studied using *in vivo* and *in vitro* techniques (Liddell and Miller, 1991). *In vivo* experimentation is unwieldy because of the practical difficulties and costs associated with long term animal studies, and because of methodological problems associated with inappropriate animal models and low incidence rates of cancers.

The more practical *in vitro* investigations have involved cell-containing systems as well as cell-free systems (Leanderson *et al*, 1988). The cell-containing systems have involved (a) the use of established cell lines (sometimes with an origin unrelated to the respiratory tract) to screen various types of asbestos and other mineral fibres for their relative toxicities; (b) short term culture of macrophages (alveolar and peritoneal), which has allowed investigation into the phagocytic process and factors released from these cell types during asbestos-induced inflammation; and (c) cell and organ cultures of differentiated “target” cells, i.e., the lung fibroblast, mesothelial cell and tracheobronchial epithelial cell. These three approaches have enabled studies of the mechanisms of fibre-cell interactions related to the induction of cell proliferation, genotoxicity, and cytotoxicity.

There is increasing support for the “oxidant stress” model of disease causation (Halliwell and Gutteridge, 1985). In this model, substances capable of catalysing the formation of DNA-damaging oxy radicals, especially hydroxyl radicals, are potential carcinogens. In particular, it is thought that the toxicity of inhaled asbestos fibres may be partly explained by the ability of asbestos to generate local high concentrations of hydroxyl radicals directly, or indirectly following interactions between fibres and macrophages (Case *et al*, 1986). The local high concentrations of DNA-damaging radicals may be sufficient to overwhelm the natural defence mechanisms, leading to DNA strand breaks and pyrimidine base modifications. Indeed, the generation of hydroxyl radicals at mineral surfaces assists in rationalizing the observed

synergism between cigarette smoking and asbestos exposure in the induction of lung cancer (Leanderson *et al*, 1989; Jackson *et al*, 1987).
While not the only possible indicator of the presence of hydroxyl radicals (Vallaythan *et al*, 188), the hydroxylation reaction of deoxyguanosine to 8-hydroxydeoxyguanosine is sensitive and has been used to evaluate genotoxicity (Leanderson *et al* , 1992).

Given that cellular oxidative stress plays a role in the development of cancer, it is likely that a variety of minerals, not just asbestos, will be able to catalyse the formation of hydroxyl radicals and thus be genotoxic. There is some evidence to support this argument (Gormley and Addison, 1983; Pezerat, 1989; Fontecave *et al*, 1990) and hence further investigations are required.
The cell-free deoxyguanosine assay was chosen for this research project, since it is relatively quick and may be used to "screen" a large number of minerals for subsequent further investigation using other approaches, e.g. *in vivo* laboratory methods and epidemiological studies.

1.2 Objectives of Project

(1) Investigate a range of reference minerals commonly extracted in South Australian mines and quarries, e.g. quartzite, clay minerals, talc. The techniques of analysis would include measurement of surface area and surface element composition. Their genotoxicity (potential for damage to genetic material, i.e. DNA) would be assessed by the deoxyguanosine method. Comparisons between minerals would be made.

(2) Develop a dust monitoring method using the deoxyguanosine hydroxylation reaction as an indicator of the carcinogenic potential of the inhaled dust.

(3) To carry out dust monitoring at selected sites, chosen mainly on the outcome of (1), and with the cooperation of management and employees.

1.3 Significance of Project

This project is potentially significant because

(a) a variety of locally-extracted minerals would be investigated for their ability to catalyse the formation of DNA-damaging free radicals, hence giving an indication of potential carcinogenicity

(b) it would enable a relatively quick and inexpensive genotoxic endpoint to be applied to conventional industrial hygiene air sampling for inorganic dusts

(c) it would serve as a good screening and monitoring tool for a variety of inorganic dust situations, e.g. “excavation” and “building” dust. *Hence, it would be useful for deciding on the extent of the control measures (engineering, administrative or personal protection) necessary for a given dust-generating operation*

(d) the results could be used to refine epidemiological studies investigating the relationship between cancer and exposure to mineral dust.

Chapter 2

Systematic Investigations of Reference Samples and Locally Extracted Minerals

2.1 Hydroxyl Free Radical Generating Ability of International Union Against Cancer (UICC) Asbestos and Other Asbestos Samples

The ability of a mineral to catalyse the formation of hydroxyl radicals was assessed using the deoxyguanosine method (Floyd, 1986).

Experimental

The following asbestos samples were used :

Amosite (South Africa)	UICC Chrysotile "B"
Chrysotile (Canadian)	UICC Crocidolite
UICC Amosite	Tremolite Korea
UICC Anthophyllite	Tremolite Standard Reference
UICC Chrysotile "A"	Crocidolite (Wittenoom)

Pre-treatment of asbestos mineral samples

All UICC and tremolite asbestos samples were used without any pre-treatment.

The Wittenoom crocidolite, South African amosite and Canadian chrysotile specimens were treated as follows: Samples were placed in a blender with distilled water and blended for 10 minutes in order to wash and break up clumps. They were subsequently filtered and air-dried before use.

Description of the typical[@] reaction method (Leanderson *et al*, 1988):

Known weights (about 10 mg) of the mineral samples were placed[#] in 4 ml glass vials. Buffer solution (2.4 ml of acetate or phosphate buffer at pH 4 or 7) and 100 µL of a 2-deoxyguanosine solution (usually 10 mM) were then added to the mineral. These samples and blanks (containing only the buffer and 2-deoxyguanosine) were placed in an incubation oven at 37^{o}C (with gentle mechanical agitation) for 18 hours. Samples were then filtered and 20 µl injected into a high performance liquid chromatography (HPLC) system fitted with electrochemical and ultraviolet detectors. The amount of 8-hydroxydeoxyguanosine produced was evaluated by comparison of the observed signal area with that for an external standard solution (see below). These experiments were run in duplicate.

[@] specific experimental conditions are given in relevant subsequent sections of this chapter

[#] all asbestos mineral samples were handled in a fume cupboard

High performance liquid chromatography (HPLC)

The system consisted of a *ICI Instruments LC 1500* HPLC pump, *TC 1900* HPLC temperature controller, *DP 600* chart recorder, *DP 800* data station, *Kortec* K95 variable wavelength UV detector, *Rheodyne 7125* injection valve (20 microlitre sample loop) and a *BAS LC4B/LC17A* electrochemical (EC) detector. A 25 cm x 4.6 mm *Spherisorb* ODS2 (C18) column, at 30 C was used. This system was connected via an interface to an IBM PC clone 386 computer (see Plate 1 below).

Chromatographic conditions:

Mobile phase**:** Consisted of a 10% methanol, 90% water, 10 mM acetic acid, 12.5mM citric acid, 25mM sodium acetate, and 30mM sodium hydroxide solution.

Flow rate**:** 1.0 ml/min with helium sparging.

Detector setup**:** UV at 254 nm; EC at +0.6V

Reagents:

2-Deoxyguanosine (99%) was purchased from SIGMA Chemical Co. Acetic acid, citric acid, sodium hydroxide, sodium acetate, ortho phosphoric acid and potassium hydrogen phosphate were all Analytical Reagent grade chemicals. Distilled water and HPLC grade methanol were used in the preparation of the mobile phase.

Plate 1 : *HPLC in use for the analysis of 8-OH-deoxyguanosine*

Calculation of the percentage conversion to 8-OH-deoxyguanosine:

A solution containing 8-hydroxydeoxyguanosine in 10% methanol/90% water, obtained by liquid chromatographic separation of an ascorbic acid/H_2O_2 reaction (Kasai, 1984), was evaporated down under nitrogen. The residual 8-hydroxydeoxyguanosine was dissolved in distilled water (to form a stock reference solution) and an ultra violet absorbance spectrum run in the region $\lambda = 200\text{-}320$ nm. Two absorbance maxima were observed at $\lambda = 245$nm and 293 nm in the spectrum. The molar extinction coefficient for 8-OH-deoxyguanosine at 293nm is, λ_{max}(293nm) = 10300 (Kasai, 1984). From the measured absorbance at this wavelength the concentration of 8-OH-deoxyguanosine in the stock solution was calculated. Subsequent HPLC analysis of this solution then gave an electrochemical peak equivalent to this concentration; this value was then used to calculate the percent conversion of 2-deoxyguanosine to 8-OH-deoxyguanosine for the mineral sample being tested.

2.1.1 The effect of weight of asbestos sample and 2-deoxyguanosine concentration on the quantity of 8-OH-deoxyguanosine formed.

Samples of Canadian chrysotile weighing 40,10, or 1 mg were added to 4 ml sample vials, to which was also added 2.4 ml of phosphate buffer (pH=6.95) and 100 µl of 5 or 10 mM 2-deoxyguanosine. These samples were then incubated at 37 ^{o}C for 20 hours while being mechanically agitated. The samples were then removed and filtered before being analysed by HPLC. Triplicate samples were run.

Table 2.1.1: The effect of weight and 2-deoxyguanosine concentration on the percentage of 8-OH-deoxyguanosine formed for Canadian chrysotile

		% 8-OH-deoxyguanosine formed		
		Weight of Chrysotile (mg)		
		40	10	1
Conc. of 2-Deoxyguan added.	10mM	0.358	0.184	0.012
	5mM	0.281	0.114	0.008

A nearly-linear increase in the amount of 8-OH-deoxyguanosine formed (with respect to increase in weight) was observed. The 10mM 2-deoxyguanosine solution, however, did not give twice as much 8-OH-deoxyguanosine as the 5mM solution.

2.1.2 The effect of Chrysotile, Amosite and Crocidolite on hydroxyl free radical formation.

Triplicate 10mg samples of Wittenoom crocidolite, South African amosite and Canadian chrysotile were weighed into 4ml vials to which a phosphate buffer at pH=6.95 and 100 μl of 10mM 2-deoxyguanosine were added.
The samples were then incubated at 37 ^{O}C for 20 hours while being mechanically agitated prior to being filtered and analysed by HPLC.

Table 2.1.2: The effect of asbestos type on the % of 8-OH-deoxyguanosine formed

	Chrysotile	Amosite	Crocidolite
% 8-OH-deoxyguanosine formed	0.145	0.026	0.002

The results of this experiment indicates that the asbestos type is of importance in determining the quantity of hydroxyl free radicals being generated. The Canadian chrysotile sample was the most active.

2.1.3 The effect of reaction time on hydroxyl free radical generating capacity for different weights of chrysotile

Triplicate samples of 10, 1 and 0.3 mg of Canadian chrysotile were weighed out into 4 ml sample vials: 2.4 ml of phosphate buffer (pH=6.95) was added to each vial along with 100 μl of 10mM 2-deoxyguanosine before being incubated for 20 or 44 hours at 37^{O}C respectively. After 20 hour, some samples were filtered and analysed by HPLC. The filtrate was returned to the incubation oven for a further 18 hours to see if the reaction continued.

Table 2.1.3: The effect of reaction time and asbestos on the % of 8-OH-deoxyguanosine formed for chrysotile asbestos

		% of 8-OH-deoxyguanosine formed		
Wt. Chrysotile(mg)		10	1	0.3
Time	20 hours	0.148	0.010	0.007
of	20+18 hrs with no fibres	0.145	0.010	0.007
Exposure	44 hours	0.163	0.009	0.008

The results of this experiment indicate that virtually no change in the amount of the hydroxyl free radicals formed occurs once the chrysotile fibres have been removed from the solution. Furthermore, only a small increase in the quantity of hydroxyl free radicals occurs after twenty hours of reaction.

2.1.4 The effect of pH and type of buffer on hydroxyl free radical generating activity of asbestos samples

Triplicate 10 mg samples of Wittenoon crocidolite, South African amosite and Canadian chrysotile in 4ml sample vials containing 2.4 mls of acetate or phosphate buffers at pH's 4.00 or 6.95 respectively, along with 100 µl of 10 mM 2-deoxyguanosine were incubated at 37^{O}C for 20 hours.

Table 2.1.4: The effect of pH and buffer type on the % of 8-OH-deoxyguanosine formed

	% of 8-OH-deoxyguanosine formed			
Buffer	Acetate		Phosphate	
pH	4.00	6.95	4.02	6.95
Crocidolite	0.008	0.005	0.015	0.007
Amosite	0.156	0.026	0.169	0.029
Chrysotile	0.072	0.015	0.266	0.148

A comparison of the activity between the two buffers at equivalent pH's indicates that there are more hydroxyl free radicals generated in the phosphate buffer. For both buffer systems, a lower pH increases the amount of hydroxyl free radicals generated.

2.1.5 The effect of time of reaction on the amount of 8-OH-deoxyguanosine formed.

Five 4 ml sample vials containing 10mg of Canadian chrysotile, 2.4 ml of phosphate buffer (pH=6.95) solution and 100 μl of 10mM 2-deoxyguanosine were placed in an incubation oven at 37 ^{O}C for periods of 2, 4, 6, 8 and 20 hours respectively. The blank sample was incubated for 20 hours.

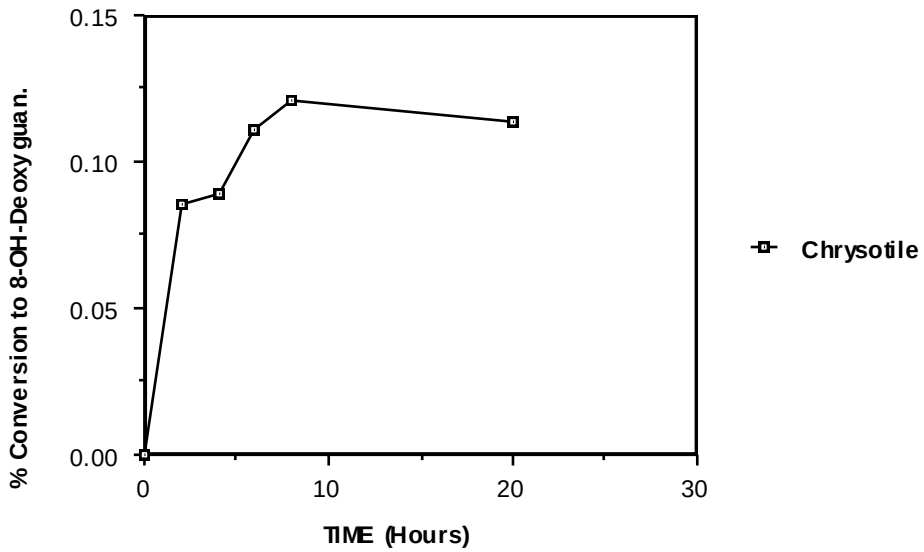


Figure 2.1.5(a): The effect of incubation time on the % of 8-OH-deoxyguanosine formed for chrysotile.

Five 4ml sample vials containing 10mg of Wittenoom crocidolite or South African amosite, 2.4ml of phosphate buffer (pH=6.95) solution and 100 μl of 10mM 2-deoxyguanosine were placed in an incubation oven at 37^{O}C for periods of 4, 8, 24, 32 and 48 hours and 2, 4, 6, 8 and 24 hours respectively. Blank samples were incubated for periods of 24 and 48 hours.

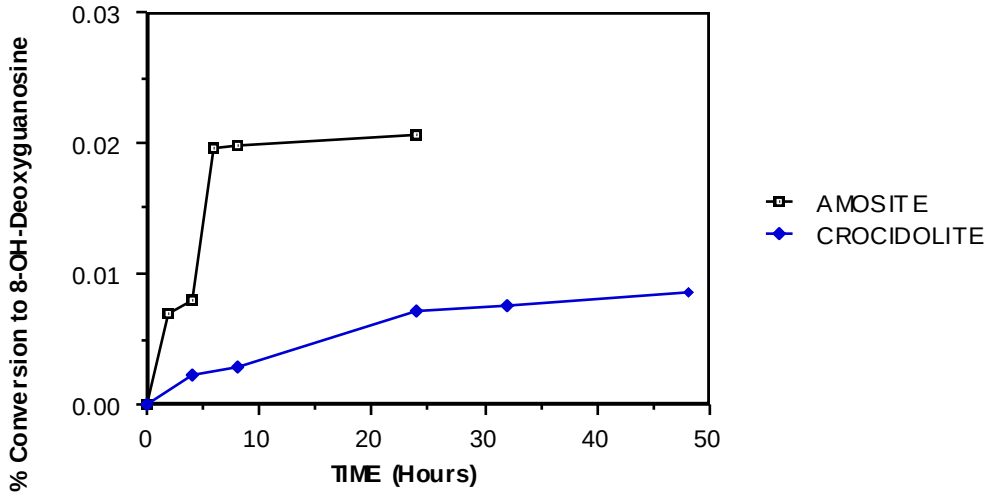


Figure 2.1.5 (b): The effect of incubation time on the % of 8-OH-deoxyguanosine formed for amosite and crocidolite

The results of these three, and earlier, experiments indicate that after about 20 hours of incubation no more significant quantities of hydroxyl free radicals are generated by most of the asbestos samples. Only the Wittenoon crocidolite appears to continue to generate a small quantity of hydroxyl free radicals after the 20 hours of incubation.

2.1.6 The effect of bubbling air through a 2-deoxyguanosine/asbestos suspension.

Canadian chrysotile and Wittenoon crocidolite samples (10mg) were placed in 4 ml vials with phosphate buffer at pH = 6.95 (2.4mls) and 100 µl of 10mM 2-deoxyguanosine. These were placed in an incubation oven with air bubbling through the solutions for periods of 2, 4, 6, 7.5 and 24 hours and 4, 7.5, 24, 31 and 48 hours respectively .

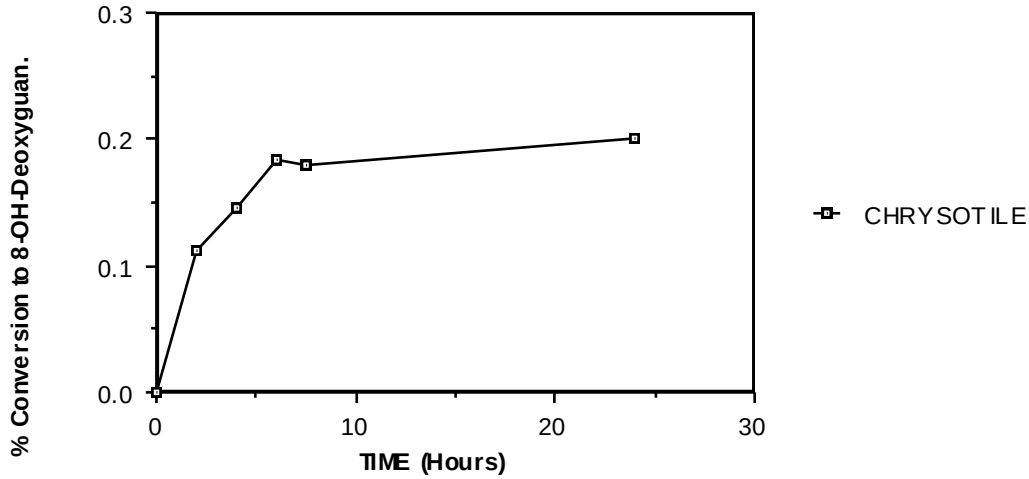


Figure 2.1.6(a) The effect of incubation time and air on the % of 8-OH-deoxyguanosine formed for chrysotile.

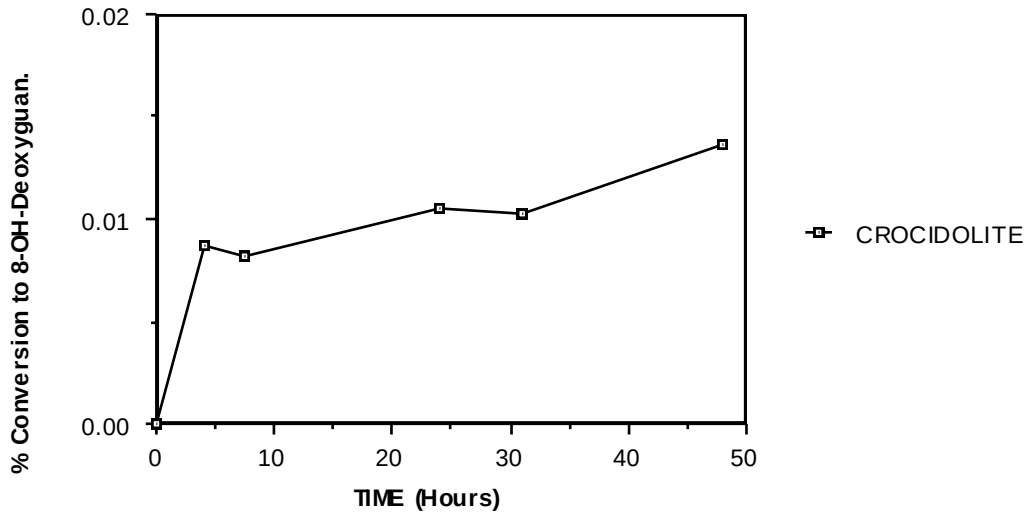


Figure 2.1.6(b): The effect of incubation time and air on the % of 8-OH-deoxyguanosine formed for crocidolite

Results of these experiments are displayed graphically above and are similar to that observed in previous experiments. However, the quantity of 8-OH-deoxyguanosine formed is slightly greater when air has been bubbled through the samples, compared to the earlier experiments (no bubbled air).

For each type of sample two other experiments were tried; 1) an open container with no air bubbling through the solution and 2) a closed container as used previously .

After 48 and 24 hours in the incubation oven the following results were obtained respectively:

Table 2.1.6 The effect of the availability of air on the % of 8-OH-deoxyguanosine formed for chrysotile and crocidolite asbestos

	% Conversion to 8-OH-Deoxyguanosine		
SAMPLE	**AIR**	**OPEN**	**CLOSED**
Crocidolite (48 hrs)	0.014	0.009	0.009
Chrysotile (24 hrs)	0.201	0.168	0.156

These results indicate that there is no deficiency in oxygen in the open or closed systems tested compared to the samples which had air bubbled through them. There is only a minor increase in the quantity of hydroxyl free radicals generated when air (oxygen) is bubbled through the asbestos/2-deoxyguanosine/buffer suspension.

2.1.7 The influence of chrysotile type and acid washing on the amount of 8-OH-deoxyguanosine formed.

UICC Chrysotile “A”(South Rhodesian), UICC Chrysotile ”B”(Canadian), acid-washed Canadian chrysotile and Canadian chrysotile samples (10mg) were placed in 4 ml vials with phosphate buffer at pH =6.95 (2.4mls) and 100µl of a 10mM 2-deoxyguanosine solution. These were placed in an incubation oven for 20 hours at 37^{O}C, prior to filtration and HPLC analysis. (The acid-washed chrysotile samples were washed in pH=2 nitric acid in an ultrasonic bath for 15 minutes. They were then rinsed in distilled water and dried before being used in the experiments.)
All experiments were run in triplicate.

Table 2.1.7: The effect of the type of chrysotile on the % of 8-OH-deoxyguanosine formed

Sample	% Conversion to 8-OH-Deoxyguanosine
UICC Chrysotile “A”	0.085
Acid washed Can. chrysotile	0.099
Canadian chrysotile	0.143
UICC Chrysotile ”B”	0.330

Acid washing appears to inhibit the hydroxylating activity of the Canadian chrysotile. UICC Chrysotile “B” is considerably more active than the other chrysotile samples. The higher

hydroxyl free radical generating ability of the UICC Chrysotile ”B” samples is likely to be due to the presence of magnetite and nemalite impurities (Zalma *et al*, 1987) in this material. The absence of the nemalite impurity in the UICC Chrysotile “A” may explain the lower activity observed for this sample.

2.1.8 Surface active iron(II) content of asbestos samples and its relation to hydroxyl free radical generation using 2-deoxyguanosine in phosphate and acetate buffers at pH=4.02 and pH=6.92.

To analyse for chelatable iron(II) on the surface of the asbestos samples, 2.5 ml of a solution of bipyridyl (5mM, which was made up in the appropriate buffer) was added to a known weight of the mineral sample and incubated for 20 hours at 37^{o}C. The solution was then filtered and the quantity of mobilised iron was measured by monitoring the absorption at 520 nm (Fontecave *et al*, 1990). The molar extinction coefficient of the iron-bipyridyl complex was 8400 M^{-1} cm^{-1}.
The percentage of mobilised iron(II) given in the following sections of this report have been calculated on a weight by weight basis for the samples.

Duplicate samples of the UICC asbestos weighing about 10 mg were placed in 4 ml vials to which were added phosphate buffer at pH=4.02 or 6.92 , or acetate buffer at pH=4.02 or 6.92 and 100 µL of 2-deoxyguanosine (10mM). These samples were then incubated for about 20 hours before being filtered and analysed by HPLC. The results of these experiments are given below in the following tables.

Table 2.1.8(a): The assay results for mobilised iron(II) and the % of 8-OH-deoxyguanosine formed in phosphate buffer at pH= 4.0 and 6.9 for asbestos samples

	% Fe(II) Mobilised[a]		% 8-OH-Deoxy. formed	
SAMPLE	pH=4.0	pH=6.9	pH=4.0	pH=6.9
Tremolite SR	<0.001	<0.001	0.010	0.005
Tremolite Korea	0.00241	0.00042	0.009	0.006
UICC Chrysotile "A"	0.028	0.00438	0.068	0.019
UICC Anthophyllite	0.055	0.00036	0.136	0.012
UICC Chrysotile "B"	0.105	0.0743	0.269	0.134
UICC Amosite	0.141	0.0259	0.155	0.011
UICC Crocidolite	0.106	0.0295	0.036	0.011

[a] calculated on weight by weight basis (wt of Fe/wt of sample).

A comparison of the quantities of the 8-OH-deoxyguanosine formed by the various asbestos samples at the lower pH demonstrates that more hydroxyl free radicals are being formed. This is consistent with the observation that more iron(II) is being mobilised into solution. The greatest increase in hydroxyl free radical formation is observed for amosite and anthophyllite. These results suggest that the hydroxyl free radical generating activity of these asbestos samples is due to the presence of iron(II), which is more easily brought into solution under acidic conditions.

It is also possible that some of the discrepancies between hydroxylating activity and mobilised iron(II) content are due to iron(II) species still being bound to the surface of the mineral.

Similar experiments were carried out on the same samples using acetate buffers. The results are given below.

Table 2.1.8(b) Assay results for mobilised iron(II) and the % of 8-OH-deoxyguanosine formed in acetate buffer at pH= 4.0 and 6.9 for asbestos samples

	% Fe(II) mobilised[a]		% 8-OH-Deoxy formed	
SAMPLE	pH=4.0	pH=6.9	pH=4.0	pH=6.9
Tremolite SR	0.00153		0.00112	0.007	0.003
Tremolite Korea	0.00709	0.00005	0.001	<0.001
UICC Chrysotile "A"	0.152		0.00196	0.029	0.012
UICC Anthophyllite	0.349		0.00034	0.065	0.010
UICC Chrysotile "B"	0.210		0.00171	0.111	0.003
UICC Amosite	0.803		0.0212	0.027	0.018
UICC Crocidolite	0.484		0.0134	0.007	0.011

[a] calculated on weight by weight basis.

A comparison of the amount of 8-OH-deoxyguanosine formed at the two pH's, ie hydroxyl free radicals generated, indicates that for all cases (except crocidolite) more hydroxyl free radicals are generated at the lower pH.

A comparison of the results for the two types of buffer at pH=6.9 shows that only crocidolite has about the same activity and that amosite increases in activity, all the other samples show a decline in activity towards the formation of hydroxyl free radicals in the acetate buffer. At the lower pH, all the samples tested in the acetate buffer show lower hydroxyl free radical activity compared to the phosphate buffer results.

2.1.9 Influence of pH and buffer on hydroxyl free radical generation for re-tested samples

An additional experiment was run in which a UICC Chrysotile "B" and UICC Anthophyllite samples which had been tested in the phosphate buffer, were washed with water before being tested again under the same conditions (ie phosphate buffer pH=6.9 and pH=4 in the presence of 2-deoxyguanosine). These experiments were repeated using acetate buffer at pH=6.9.

This was done to test whether a further release of iron(II) would occur for the same samples.

Table 2.1.9(a) Influence of pH on the % of 8-OH-deoxyguanosine formed for retested asbestos samples.

SAMPLE	% 8-OH-Deoxyguanosine formed (Phosphate Buffer)			
	pH=6.9		pH=4	
	1st run	2nd run	1st run	2nd run
UICC Chrysotile "B"	0.134	0.006	0.269	0.080
UICC Anthophyllite	0.012	-----	0.136	0.072

Table 2.1.9(b) Influence of the buffer type on the % of 8-OH-deoxyguanosine formed for retested asbestos samples.

SAMPLE	% 8-OH-Deoxyguanosine formed			
	Acetate Buffer		Phosphate Buffer	
	pH=6.9		pH=6.9	
	1st run	2nd run	1st run	2nd run
UICC Chrysotile "B"	0.003	0.007	0.134	0.006
UICC Anthophyllite	0.010	0.014	0.012	-----

These results indicate that for the phosphate buffer (pH=4) a greater number of hydroxyl free radicals are generated when the asbestos samples are rerun using the same reaction conditions.
This could be due to the breakdown of the asbestos fibre under the acidic conditions leading to the release of more iron(II) into solution or making more iron (II) available at the surface of the asbestos.

For the acetate buffer at pH =6.9 more hydroxyl free radicals are generated on the retested samples than was the case in the original experiment. This may indicate a lower dissolution rate in the acetate buffer. A comparison of the quantity of hydroxyl free radicals generated indicates that the asbestos is still less active in the acetate buffer than in the phosphate buffer.

2.1.10 The effect of added hydrogen peroxide on the formation of hydroxyl free radical generation for UICC asbestos samples

Duplicate samples of 10 mg of the UICC asbestos were placed in 4 ml sample vials to which 2.3ml of phosphate buffer at pH=6.9 was added, 100 µl of 10mM 2-deoxyguanosine and 100 µl of solutions of hydrogen peroxide to give final concentrations of hydrogen peroxide of $1x10^{-5}$M and $1x10^{-3}$M.

Table 2.1.10 Influence of hydrogen peroxide on the % of 8-OH-deoxyguanosine formed for asbestos samples.

SAMPLE	**% Fe[a]**	**% 8-OH-Deoxyguanosine formed**		
	pH=6.9	pH=6.9 Phosphate buffer		
		Hydrogen peroxide concentration		
		0	$1x10^{-5}$	$1x10^{-3}$
UICC Crocidolite	0.0295	0.011	0.025	0.010
UICC Chrysotile “A”	0.0043	0.019	0.021	0.019
UICC Chrysotile “B”	0.0743	0.134	0.247	0.042
UICC Amosite	0.0259	0.012	0.015	0.032
UICC Anthophyllite	0.0003	0.012	0.022	0.053
Tremolite (Std Ref)	<0.002	0.005	0.007	0.070
Tremolite Korea	0.0004	0.006	0.002	0.026

a mobilised iron(II) on a weight by weight basis

From the table above it can be seen that the effect of the adding hydrogen peroxide is variable and no systematic trend appears. It is interesting to note that the activitity of Chrysotile “B” is lowest at the highest concentration of hydrogen peroxide. Such effects have also been observed by other researchers, without explanation (Pezerat *et al*, 1989).

2.1.11 Conclusions

The above experiments on the standard UICC asbestos and other asbestos samples were carried out to improve understanding of the deoxyguanosine assay and to optimize reaction conditions for subsequent testing of locally extracted mineral samples.

Suitable reaction conditions were found to be :

a) A 10 mg mineral sample was appropriate - this gave an adequate 8-OH-deoxyguanosine signal.

b) An incubation time of about 20 hours at 37^{o}C was satisfactory - very little further reaction was observed.

c) A phosphate buffer rather than an acetate buffer should be used. This is because larger amounts of hydroxyl free radicals were formed when the phosphate buffer was employed, ie more 8-OH-deoxyguanosine was formed.

d) It was unnecessary to bubble air through the suspension - bubbling did not appear to greatly increase the quantity of hydroxyl free radicals formed. There appeared to be sufficient oxygen available from the headspace of the closed reaction vial.

e) No hydrogen peroxide should be added - addition of hydrogen peroxide did not appear to be uniformly beneficial in generating more hydroxyl free radicals.

Re-use of tested asbestos samples with fresh buffer and 2-deoxyguanosine solution shows that further hydroxyl free radicals can be generated. This is despite the results showing little or no extra 8-OH-deoxyguanosine is formed after 20 hours of incubation of the first test of the sample. (This may indicate a breakdown of the structure of the material revealing more iron(II) at the surface.)

The presence of iron (II) on the surface of the mineral tends to correlate reasonably well with the ability of the mineral to generate hydroxyl radicals. Monitoring the level of available iron (II) in the mineral samples may give some indication of free radical generating ability. It should be noted, however, that iron(II) can be structurally bound to the surface of the mineral. This may result in a sample that has a lower amount of mobilised iron but a high hydroxylation activity.

2.2 The Hydroxyl Free Radical Generating Ability of Mineral Samples

The following range of mineral samples, many of which were locally mined, were collected and tested for their ability to generate hydroxyl free radicals using the deoxyguanosine assay.

Quartz
Freshly crushed Quartz
Silica Fume
Silicon

Birdwood Clay
Kaosil
Barytes
Cosmetic Talc
Damourite
Magnesite
Industrial Grade Talc
Gumeracha Talc

Fly Ash Z1(Pt Augusta Power Station)
Fly Ash Z2+3(Pt Augusta Power Station)
Vermicullite Young River
Brick Dust

Marion Lake Gypsum (Yorke Peninsula)
Milled Gypsum
Plaster
Dolomite (Marino)
Sand (Stonyfell Quarry)
Shellgrit

Leigh Creek Brown Coal
Leigh Creek Shale Overburden
Olympic Dam Mullock Heading
Olympic Dam Ore Heading

HG1 Iron Princess (High Grade Ore)
HG2 Iron Knob “C”(Hematite)
HG3 The Slot (High Mn)
HG4 Iron Monarch High Grade Ore
HG5 Iron Monarch High Mn
Iron Duke Hematite
Iron Duke Limonite-Goethite
Iron Duke Goethite-Hematite

Sample pretreatment: All samples were initially ground with an agate mortar and pestle for 10 minutes prior to the experiment.

2.2.1 The ability of locally mined mineral samples to generate hydroxyl free radicals

Duplicate ten milligram samples of the minerals were weighed into 4 ml vials, to which was added a buffer (2.4 ml of phosphate buffer at pH=7) and 100 µl of a 2-deoxyguanosine solution (10 mM).
These samples were incubated in an oven at 37^{O}C (while being mechanically agitated) for 18 hours prior to being filtered and analysed by HPLC.

Table 2.2.1: The % of 8-OH-deoxyguanosine formed for quartz, silica fume and silicon samples

SAMPLE	8-OH-deoxyguanosine Percentage conv.	% Fe(II) Mobilised[a]
Quartz	0.021	1.3×10^{-3}
Crushed Quartz	0.016	
Silica Fume	0.008	
Silicon	0.348	5.7×10^{-3}

a mobilised iron(II) on a weight by weight basis, using the bipyridyl assay.

These experiments show that the quartz forms hydroxyl free radicals to a greater extent than the crushed quartz and silica fume.
Furthermore, silicon is about twenty times as active as the quartz in generating hydroxyl free radicals, yet there is only 4.4 times as much mobilised iron(II) available.

2.2.2 Further experiments on silicon to investigate the effects of phosphate and acid washing on the formation of 8-OH-deoxyguanosine

Triplicate 10 mg samples of the silicon were weighed out and 2.4 ml of pH=6.92 phosphate buffer and 100 µl of 10mM 2-deoxyguanosine added prior to being incubated in an oven at 37^{O}C overnight.
The acid-washed silicon sample was prepared by placing the sample in an ultrasonic bath and washing for 15 minutes in nitric acid (pH=1.0). The sample was then washed with distilled water and dried before use. A phosphate-washed silicon sample was prepared in a similar

way to the above sample except that pH=6.9 phosphate buffer was used. The filtrate of the phosphate wash was also then similarly tested for its hydroxyl free radical generating activity.

Table 2.2.2: The % of 8-OH-deoxyguanosine formed for treated silicon and $FeSO_4$ samples

Sample	**pH**	**% 8-OH Deoxy formed**
Silicon	6.9	0.332
Phosphate washed Silicon	6.9	0.211
Acid washed Silicon	1.0/6.9	0.082
Filtrate (Phosphate washed)	6.9	0.005
$FeSO_4$	6.9	0.034

The bulk analysis of the silicon gives the composition as Si 99.35% , Fe 0.18%, Al 0.23%, Ca 0.02% and Ti 0.02%.

The iron(II) sulphate/buffer solution was prepared so that the equivalent amount of iron(II) as would be found in a 10mg silicon sample would be present. This was done to test whether the hydroxyl free radical generating activity was due only to the presence of iron(II) or some other feature peculiar to the silicon.

A comparison of the results in the table above for the silicon with the acid and phosphate washed silicon samples indicates that the silicon has intrinsic hydroxyl free radical forming activity and that washing it may remove some iron(II) which contributes to this activity.

The hydroxyl free radical activity of the filtrate suggests that some iron(II) is being removed from the silicon.

The activity of the $FeSO_4$ solution indicates that hydroxylation of the 2-deoxyguanosine can be caused in part by the presence of iron(II) in solution Furthermore the silicon must intrinsically have some hydroxyl free radical generating ability not due to the iron(II) as the percentage of 8-OH-deoxyguanosine is far greater than the activity of the total possible equivalent quantity iron(II) in the silicon.

2.2.3 Experiments to examine the hydroxyl free radical forming ability of processed minerals

The following minerals were obtained and tested for their ability to form hydroxyl free radicals: Barytes (Oraparinna, Flinders Ranges), Cosmetic talc (Mt Fitton), Industrial grade talc (Mt Fitton), Damourite (Williamstown), Birdwood clay, Kaosil (Williamstown), Gumeracha talc and Magnesite (Myrtle Springs,Flinders Ranges).

Triplicate 10 mg samples were weighed out and 2.4 ml of pH=6.9 phosphate buffer and 100 µl of 10mM 2-deoxyguanosine added prior to being incubated in an oven at 37^{o}C overnight. The samples were then filtered and analysed by HPLC.

Table 2.2.3: The % of 8-OH-deoxyguanosine formed & the % of mobilised iron(II) for processed minerals samples

Sample	**8-OH-deoxyguanosine % conv.**	**%Fe(II) Mobilised[a]**
Birdwood Clay	0.003	
Kaosil	0.003	
Barytes	0.006	1.1×10^{-3}
Cosmetic Talc	0.003	9.7×10^{-5}
Damourite	0.003	
Magnesite	0.056	2×10^{-4}
Industrial Grade Talc	0.006	
Gumeracha Talc	0.009	1×10^{-4}

a mobilised iron(II) on a weight by weight basis.

The magnesite is the most active hydroxyl free radical generating mineral of this group. Although the amount of mobilised iron(II) is small, having only twice the amount of mobilised iron(II) compared to the Gumeracha talc sample, the hydroxyl free radical generating activity is 5 times greater. It was noted that after doing the analysis for iron(II) in solution the magnesite was pink in colour indicating that iron (II) is also present on the surface of this sample and must contribute to its activity.

The surface areas of silicon, quartz and magnesite were measured to see if this parameter had any correlation with the hydroxylation ability of these mineral samples. From the table below it can be seen that the surfaces areas are low and are not correlated.

Table 2.2.3: The % of 8-OH-deoxyguanosine formed & the % of mobilised iron(II) for processed minerals samples

Sample	**Surface Area[a] (m^2/gm)**	**% Mobilised iron (II)[b]**	**% 8-OH-deoxy. formed**
Silicon	2.2	5.7×10^{-3}	0.348
Quartz	2.2	1.3×10^{-3}	0.021
Magnesite	2.5	2×10^{-4}	0.056

[a] Surface Areas: The BET method, with nitrogen as the adsorbing gas, has been used. A known volume of nitrogen at a known pressure is admitted to the sample which is held at liquid nitrogen temperature. Gas adsorbs on the support, lowering the pressure. When equilibrium is reached the number of moles of gas adsorbed at that pressure can be calculated. If the process is repeated at successively higher pressures an isotherm can be obtained. Surface areas can then be calculated using the isotherm.
[b] mobilised iron(II) on a weight by weight basis.

2.2.4 Experiments to examine the hydroxyl free radical forming ability of iron ore minerals

Seven iron ore samples were obtained; these had a range of minerals present and were of variable composition. Duplicate 10 mg samples were weighed out and 2.4 ml of pH=7 or 4 phosphate or acetate buffers and100µl of 10mM 2-deoxyguanosine added prior to being incubated in an oven at 37^{o}C overnight. The samples were then filtered and analysed by HPLC.

Table 2.2.4: The % of 8-OH-deoxyguanosine formed for iron ore samples at pH's 4 and 7 in phosphate and acetate buffers.

	% Conversion to 8-OH-deoxyguanosine			
Sample	**Phosphate Buffer**		**Acetate Buffer**	
	pH=7	pH=4	pH=7	pH=4
HG1 Iron Princess High Grade Ore	0	0	0	0
HG2 Iron Knob "C" Hematite	0.015	0	0.004	0
HG3 The Slot High Mn	0	0	0	0
HG4 Iron Monarch High Grade Ore	0.015	0	0.003	0
HG5 Iron Monarch High Mn	0.009	0	0	0
1001 Iron Duke Hematite	0.009	0	0.003	0
1002 Iron Duke Limonite-Goethite	0.012	0.003	0.005	0.009
1003 Iron Duke Goethite-Hematite	0.015	0.003	0.004	0.007

The bulk iron content of these iron ore samples ranged from 55% for HG3 The Slot High Mn to 69.8% for the Iron Monarch High Grade Ore.

Analyses for mobilised iron(II) in all these samples showed no iron (II) to be present in solution. It is possible, though, that surface bound iron(II) was present.

The results in the table above indicate that the hydroxyl radical generating ability of these samples is less than that of the quartz and greater than that of the Gumeracha talc. Samples HG1 and HG3 showed no activity at all under any of the conditions tested.

Surface analysis of the iron ore samples HG2, HG3, 1003 as well as magnesite silicon and quartz using X-ray Photoelectron Spectroscopy shows iron(III) present in the surface of the minerals. The quantity of iron(II) in the surface of the minerals appears to be to small to be detected by this method. The mobilised iron(II) assay method appears to give better results so long as the iron(II) is not structurally bound on the surface of the mineral. However, if the iron(II) is bound structurally on the surface of the mineral then a true indication of the iron(II) available to generate hydroxyl free radicals will not obtained.

2.2.5 An examination of the hydroxyl free radical forming ability of fly ash, vermiculite and brick dust.

Duplicate 10mg samples were weighed out and 2.4 ml of pH=7 phosphate buffer and100µl of 10mM 2-deoxyguanosine added prior to being incubated in an oven at 37^{o}C overnight. The samples were then filtered and analysed by HPLC.

Table 2.2.5: The % of 8-OH-deoxyguanosine formed for fly ash, vermiculite and brick dust samples

	% Conversion to 8-OH-deoxyguanosine	
Sample	**Phosphate buffer pH=7**	**Acetate buffer pH=4**
Fly Ash Z1 Unit 2, Pt Augusta	0.010	----
Fly Ash Z2 Unit 2, Pt Augusta	0.004	----
Vermiculite, Young River	0.003	----
Brick Dust	0.006	0.018

All these samples generated less hydroxyl free radicals than the quartz sample and have very low activity.

2.2.6 An examination of the hydroxyl free radical forming ability of gypsum, plaster, sand, dolomite and shellgrit.

Duplicate 10 mg samples were weighed out and 2.4 ml of pH=7 phosphate buffer and 100µl of 10mM 2-deoxyguanosine added prior to being incubated in an oven at 37^{O}C overnight. The samples were then filtered and analysed by HPLC.

Table 2.2.6: The % of 8-OH-deoxyguanosine formed for iron ore samples at pH's 4 and 7 in phosphate and acetate buffers.

	% Conversion to 8-OH-deoxyguanosine
Sample	**Phosphate Buffer** pH=7
Marion Lake Gypsum (Yorke Pen.)	0.007
Milled Gypsum	0.007
Plaster	0.006
Dolomite (Marino)	0.014
Sand (Stonyfell Quarry)	0.009
Shellgrit(Pt Parham)	0.023

All but one of these samples were less active than quartz in generating hydroxyl free radicals, the exception being the shellgrit which was slightly more active than the quartz..

2.2.7 An examination of the hydroxyl free radical forming ability of Leigh Creek and Olympic Dam samples

Duplicate 10 mg samples were weighed out and 2.4 ml of pH=7 phosphate buffer and 100 µl of 10mM 2-deoxyguanosine added prior to being incubated in an oven at 37^{O}C overnight. The samples were then filtered and analysed by HPLC.

Table 2.2.7: The % of 8-OH-deoxyguanosine formed for Leigh Creek & Olympic Dam samples.

% Conversion to 8-OH-deoxyguanosine

Sample	Phosphate Buffer pH=7
Leigh Creek Shale Overburden	0.008
Leigh Creek Brown Coal	0.011
Olympic Dam Mullock Heading	0.015
Olympic Dam Ore Heading	0.018

All these mineral samples were less active than the quartz sample in generating hydroxyl free radicals.

2.2.8 Conclusions

Nearly all of the locally mined samples do not generate large amounts of hydroxyl free radicals when compared to the UICC asbestos sample tested under similar conditions.
Of the samples tested in the laboratory, magnesite and silicon had the greatest hydroxylating ability.

Chapter 3

Dust Chamber Construction and Development of Industrial Hygiene Dust Monitoring Methods

3.1 Description and Operation of the Dust Chamber

A dust chamber was constructed for this project, with some technical assistance from the S.A. Department of Labour, Occupational Health Division.

The chamber has internal surfaces of stainless steel or glass. It is about 1.5m high, with a volume of about 0.3 m^3 and a rectangular external frame to provide structural support. It is essentially a cube with an inverted square pyramid on its base (see Plate 2 below). This design was adopted to ensure that recirculation of the dust within the chamber could be achieved. The dust chamber was built essentially as a recirculating dust unit rather than a continuos feed system, which would have required the purchase of expensive dust generating equipment.

Several delays in its construction occurred due to problems in obtaining the materials required and it took about eight weeks to modify the dust chamber and have it operating as a functional unit[#] .

The detachable front and top, with viewing windows, allowed the chamber to be thoroughly cleaned between the testing of different dust samples.

Eight ports (four on each side) were drilled into the sides of the chamber; all were fitted with Swagelok fittings. Two of the ports were used to allow air to escape from the chamber - these had a double filtering arrangement so as to stop any of the dust contaminating the laboratory. The other six ports were to be used for sampling the dust within the chamber.

Filtered air was supplied to the chamber at rates up to 12L/minute. This was passed through the bottom of the chamber over a filter which had the dust of interest placed on it. This then allowed the dust to become airborne within the chamber. Any dust that settled in the chamber eventually ended up on the filter again and was then recirculated within the chamber. A pneumatically driven propeller was used to create turbulence within the chamber to achieve an even distribution of the dust throughout the chamber.

[#] The dust chamber was extensively tested for leaks. Several pinholes in the welds and leaks in the rubber seals and fittings were detected. These were sealed using a flexible silicone sealant or a two-pack putty.

Plate 2 below shows the dust chamber in use for a dust sample collection trial.

Plate 2 The dust chamber being prepared for an experiment

3.2 Methods of Collecting Airborne Dust Samples.

Two methods of collecting the dust samples in the field were considered to be potentially useful namely:

Method 1 (Membrane filter method) - In this technique the sample is collected using inhalable and respirable dust sampling heads, incorporating membrane filters - these could be accurately weighed before and after the dust collection to quantify the amount of dust present on the filter. The dust deposit plus filter could then be placed into a petri dish container with the 2-deoxyguanosine solution/buffer, incubated/agitated and then analysed.

Method 2 (Liquid impinger method) - In this technique the sample is collected into a midget impinger containing 2-deoxyguanosine solution/phosphate buffer. A parallel inhalable dust sample, as above, would also needed in order to determine the amount of dust collected, since the impinger is not weighed.

Plate 3 below shows the chamber set up to collect respirable and inhalable dust samples.

3.2.1 The hydroxyl free radical forming ability of membrane filter materials

To evaluate method 2, the ability of the membrane filters (to be used to collect dust samples) to generate hydroxyl free radicals needed to be tested and corrected for.
To this end, six types of 25mm filters, suitable for gravimetric analysis of dust samples, were tested using 2.4ml of a phosphate buffer pH=7 and100µl of 10mM 2-deoxyguanosine. Duplicate samples were placed in 30mm glass petri dishes and incubated for 18 hours overnight at $37^{O}C$. Two blanks containing only the buffer/ 2-deoxyguanosine solution were also incubated.

Table 3.2.1: The % of 8-OH-deoxyguanosine formed from dust sample collection membrane filters

FILTER	**% Conv.**	**CORRECTED % Conv.**
Blank	0.003	0.000
Glass Fibre *(Gelman)*	0.003	0.000
DM-800 (Gelman)	0.003	0.000
PTFE *(Sartorius)*	0.005	0.002
PVC VM1*(Millipore)*	0.003	0.000
PVC *(Nuclepore)*	0.003	0.000
Polycarbonate *(Nuclepore)*	0.003	0.000

Effectively only the PTFE(teflon) filter gave a response in terms of causing hydroxylation of the 2-deoxyguanosine. From these results it was decided that any of the filters used in experiments would suffice for the collection and testing of dust samples, excepting for the PTFE(teflon) filters.

Plate 3 Dust sample collection using inhalable and respirable collectors in the dust chamber

3.3 Trials of the two methods for dust sample collection

Magnesite dust was loaded into the dust chamber and respirable and inhalable dust samples were collected using *Casella Higgins* cyclone separators (used according to Australian Standard 2985 - 1987) and seven hole samplers (used according to AS 3640-1989) respectively using SKC portable air sampling pumps (Model 224-30). The collected samples were then placed in petri dishes and 2-deoxyguanosine solution/phosphate buffer added; they were then incubated overnight at 37^{o}C.

Two midget-impingers each containing 4.8 mls of 2-deoxyguanosine solution in pH=7 phosphate buffer were also attached to ports, fitted with *Casella Higgins* cyclone separators

(without a filter) in order to collect the respirable dust sample directly. These samples were then also incubated overnight at 37^{o}C.

The results of this series of experiments on magnesite are given in the Table below.

Table 3.3(a): The % of 8-OH-deoxyguanosine formed for magnesite samples tested using the two sampling methods.

Sample Type	**% Conv[a] to 8-OH-Deoxy.**
Impinger	0.008
Resp.	0.052
Inhal.	0.071
Inhal.	0.084
Inhal.	0.066
Lab. test	0.056

a % conversion normalised to equivalent 10mg sample.

A comparison of the results obtained from these dust chamber experiments with those for the earlier laboratory tests indicates that the inhalable samples produced a somewhat larger amount of hydroxyl free radicals than did the respirable or impinger samples.
Quartz and silicon dust were also loaded into the dust chamber individually, and respirable and inhalable dust samples were collected. They were processed in the same manner as described earlier and the samples analysed for the presence of 8-OH-deoxyguanosine.

Table 3.3(b): The % of 8-OH-deoxyguanosine formed for respirable and inhalable quartz and silicon samples.

Sample Type	**% Conv to[a] 8-OH-Deoxy.** (Resp.)	**% Conv to[a] 8-OH-Deoxy.** (Inhal.)
Quartz		0.072
Quartz		0.049
Quartz		0.025
Quartz	0.016	
Quartz	0.015	
Quartz (Lab Tested)	0.020	0.020
Silicon		0.200
Silicon	0.266	
Silicon (Lab Tested)	0.348	0.348

a % conversion normalised to equivalent 10mg sample

These results indicate that the inhalable quartz and magnesite produce more hydroxyl free radicals than either the respirable or laboratory tested samples. However this does not apply to the silicon samples where the respirable and inhalable samples were less active than the laboratory tested samples.

3.4 Conclusions

Method 1, collecting the dust samples using respirable and inhalable dust samplers and then reacting them with 2-deoxyguanosine gave a better indication of the ability of these samples to generate hydroxyl free radicals than did the impinger method.

The reason why method 2, using the impinger, did not give as high a value for the production of hydroxyl free radicals may be due to aerosol losses and variable sampling and trapping efficiencies. Incomplete trapping of the dust particles might occur with the high flow rate of air required and the 2-deoxyguanosine solution might also be lost and become airborne.

The impinger method does not appear to be practical, but further work would be necessary to fully evaluate it.

A comparison of the results obtained for the inhalable and respirable samples are variable, indicating that smaller sized particles were not always more active. Generally, there was not a large increase in reactivity when compared to the bulk mineral samples tested. This in part may be due to the airborne dust within the chamber being recirculated and not being freshly generated as one might expect the case to be for work situations.

Finally, this dust chamber is a useful piece of equipment that can be utilised for occupational health teaching and research purposes.

Chapter 4

On-site Dust Sampling

4.1 Field Sampling

Laboratory testing indicated that nearly all of the mineral samples had a low hydroxylation activity and hence only limited on-site sampling was done.

Respirable and inhalable samples of shellgrit, kaosil, talc and micronised talc were collected on-site using *Casella Higgins* cyclone separators and seven hole sampling heads respectively. The collected membrane filter samples were then placed in petri dishes, 2-deoxyguanosine solution and pH=7 phosphate buffer added, and incubated overnight at 37^{o}C.

Table 4.1: The % of 8-OH-deoxyguanosine formed for respirable and inhalable on-site collected samples

Sample Type	**% Conv to[a] 8-OH-Deoxy.** (Resp.)	**% Conv to[a] 8-OH-Deoxy.** (Inhal.)
Shellgrit	0.097	
Shellgrit		0.003
Shellgrit		0.010
Shellgrit(Lab Tested)	0.023	0.023
Kaosil	0.023	
Kaosil	0.017	
Kaosil		0.011
Kaosil		0.015
Kaosil (Lab Tested)	0.003	0.003
Talc	0.021	
Talc		0.030
Talc		0.026
Talc (Lab Tested)	0.006	0.006
Micronised Talc		0.014
Micronised Talc		0.017

a % conversion normalised to equivalent 10mg sample.

Plates 3 and 4 below show the on-site dust sampling near a talc microniser and kaosil bagging operation respectively. These samples and the shellgrit samples were collected as fixed position area samples.

Plate 4 On-site dust collection near a talc microniser

Plate 5: On-site dust collection near a kaosil bagging operation

4.2 Conclusions

The respirable shellgrit samples were more active in generating hydroxyl free radicals than either the inhalable or laboratory tested samples. The respirable shellgrit sample also had greater activity than the magnesite samples on a weight basis. For the kaosil and the talc, the on-site samples were more active in generating hydroxyl free radicals than the laboratory tested samples.

The results of the on-site testing suggest that there is increased reactivity on a weight basis compared to the laboratory tested mineral samples.

The extent of the on-site work carried out in this project was limited by time and financial constraints. Further sampling work, particularly personal monitoring, is recommended in order to characterize a greater number of exposure situations in the mining and quarrying industry.

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APPENDIX 1

List of mineral samples tested, their compositions and location.

Birdwood Clay	Hydrous aluminium silicate, Birdwood
Kaosil	Kaolinised sillimenite, Williamstown
Barytes	$BaSO_4$, Oraparinna, Flinders Ranges
Cosmetic Talc	$Mg_3(Si_4O_{10})(OH)_2$, Mt Fitton
Damourite	contains mica, Williamstown
Magnesite	$MgCO_3$, Myrtle springs, Flinders Ranges Industrial
Grade Talc	$Mg_3(Si_4O_{10})(OH)_2$, Mt Fitton
Gumeracha Talc	$Mg_3(Si_4O_{10})(OH)_2$, Gumeracha
Fly Ash Z1	Pt Augusta Power Station
Fly Ash Z2+3	Pt Augusta Power Station
Vermicullite Young River	$K(Mg,Fe)_3\ (AlSi_3O_{10})(OH)_2$
Brick Dust	
Gypsum	$CaSO_4.2H_2O$, Marion Lake, Yorke Peninsula
Milled Gypsum	$CaSO_4.2H_2O$, Marion Lake, Yorke Peninsula
Plaster	$CaSO_4$
Dolomite	Marino
Quartzite Sand	SiO_2, Stonyfell Quarry
Shellgrit	$CaCO_3$, Pt Parham
Quartz	SiO_2, WA
Freshly crushed Quartz	SiO_2,WA
Silica Fume	SiO_2,WA
Silicon	Si,WA
HG1 Iron Princess (High Grade Ore)	
HG2 Iron Knob “C”(Hematite)	Fe_2O_3
HG3 The Slot (High Mn)	
HG4 Iron Monarch High Grade Ore	
HG5 Iron Monarch High Mn	
Iron Duke Hematite	Fe_2O_3
Iron Duke Limonite-Goethite	$FeO(OH).nH_2O$-$HFeO_2$
Iron Duke Goethite-Hematite	$HFeO_2$-Fe_2O_3
Leigh Creek Brown Coal	
Leigh Creek Shale Overburden	
Olympic Dam Mullock Heading	
Olympic Dam Ore Heading	