FINAL REPORT

to the

RESEARCH PROJECT EXTENSION

Development of a new mineral dust monitoring method incorporating genotoxicity assessment

FIELD MEASUREMENTS

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1. Executive Summary

Occupational dust exposure in mine and quarry operations is known to be linked to lung disease, notably fibrosis and lung cancer. The toxicological mechanisms are still unclear but a widely held view is that certain minerals have the ability to induce significant "oxidative stress" on lung tissue by virtue of their physical characteristics and surface chemistry. Toxicologically important features include length, width and aspect ratio, solubility, surface charge, surface area, and the presence of metal ions capable of catalysing the formation of cell-damaging oxy radicals. There is little reason to believe that these properties are restricted to asbestos and crystalline silica, and a variety of basic and applied investigations are under way to identify other potentially hazardous minerals that have not been the subject of epidemiological study. Preliminary studies are also being carried out for relatively common mineral combinations, such as farm soils, to examine health risks.

This project sought to investigate the surface reactivities of a wide range of minerals extracted from South Australian mines and quarries. Conventional occupational hygiene dust sampling methods were combined with a laboratory assay which measures the production of DNA-damaging hydroxyl radicals. A standard set of laboratory analytical conditions were established, and in this way the relative reactivities of airborne and bulk dust samples were compared.

By and large, the data from numerous mines and quarries indicate low reactivities. Exceptions were samples from a granite quarry and a copper uranium mine, and it is recommended that control measures be upgraded and/or maintained. The results are variable, even for a single worksite, but are broadly consistent with the international literature and support the hypothesis that the most toxic exposures arise from freshly fractured mineral surfaces containing ferrous and cuprous ions.

The method used in this project is relatively straightforward and can be applied more widely. It is likely to be useful as an aid in the prevention of fibrotic and malignant lung disease. However, caution should be adopted in interpreting reactivity data since oxidative stress is only one component of the disease process.

2. Synopsis of Original Research Project Report (March 1993)

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.

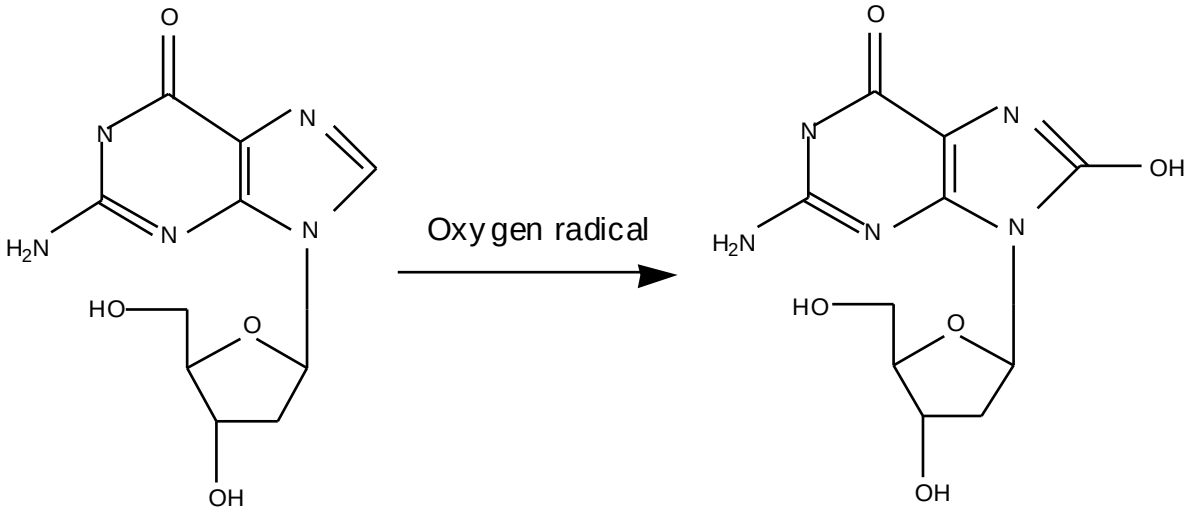
In conclusion, the study has provided new insights into mineral surface toxicity, the results of which should be of some reassurance to the local mining and quarrying industry.

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.

3. Objectives

This project is an extension of work previously carried out and reported on in March 1993; specifically an extension of *Phase 3: On-site air sampling*.
The results of the earlier work indicated that airborne dust samples collected on-site may be more reactive than those prepared in the laboratory. However, time and financial constraints limited the number of sites visited.

The objectives of this extension project were:

(1) To undertake (personal and fixed position) airborne dust sampling at selected worksites. Bulk mineral samples would also be collected for comparison.

(2) To evaluate the potential genotoxicity of the dust samples using the previously-developed HPLC/deoxyguanosine method.
Comparisons of the reactivities of the airborne dust and bulk mineral samples would be made.

(3) To summarize and interpret the findings in the context of the most recent scientific literature on mineral dust toxicity.

The data presented here are from a range of mine and quarry sites.

4. Review of Scientific Literature since 1993

4.1 Introduction

A proactive approach to the characterisation of the risk associated with exposure to airborne dust generally entails research on a number of fronts, as in Figure 1.

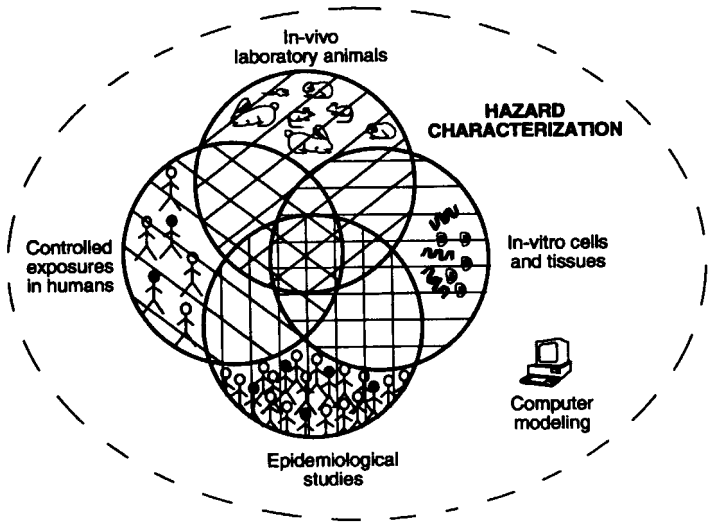


Figure 1:A proactive approach to hazard or risk characterisation requires use of complementary approaches (from McClellan, JOHS (ANZ), 1996; 12, 253)

The recent scientific literature on dust toxicity follows this pattern, and indeed, numerous *in vitro*, *in vivo* and epidemiological studies have been reported. In the cases of crystalline silica and fibrous minerals, the proceedings of three major symposia have been published (Corn, 1995; Goldsmith et al, 1995; Berry, 1996).

4.2 Genotoxicology: In-vitro and basic chemical studies

The cancer risks associated with asbestos exposure are well known, but those arising from exposure to crystalline silica are less well defined. Recent epidemiological studies are providing stronger evidence of risk for crystalline silica and so the current IARC classification of 2A is soon to be reviewed. The risk for lung cancer among silicotics is very strong, moderate among those with silica exposure without fibrosis, and both backed up positive animal carcinogenicity, including DNA breaks in vitro (Corn, 1995; Goldsmith et al, 1995).

The manner in which silica and asbestos induces DNA damage is still only partly understood but various direct and indirect mechanisms have been proposed (Mossman et al, 1995; Shi et al, 1995; Daniel et al, 1995) as in Figure 2.

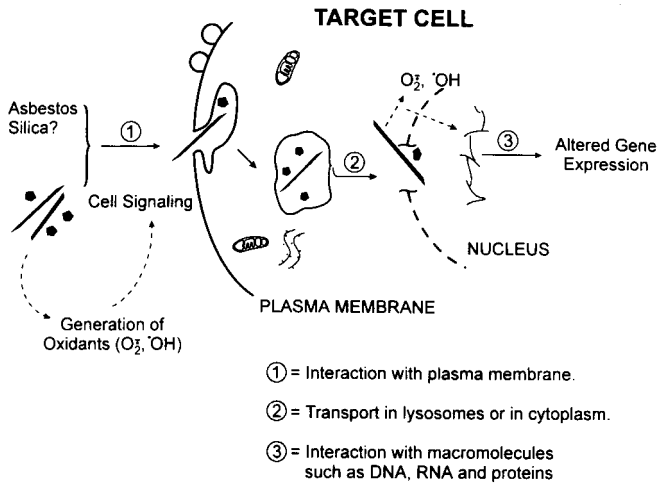


Figure 2: Diagram showing plausible mechanisms of interaction of silica and asbestos with target cells of disease. (from Mossman et al, 1995)

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). However, Mossman (1993) has also argued that asbestos seems to have more of a co-carcinogenic and promoter-type role in the development of lung cancer. By contrast, chemical carcinogens in cigarette smoke cause damage to DNA by multiple mechanisms consistent with their classification as initiators of carcinogenesis.

The notion that freshly fractured mineral surfaces may be more toxic has received further support in the literature (Shoemaker et al, 1995; Shi et al, 1995). In the study reported by Shoemaker and co-workers, inhalation of freshly milled quartz

resulted in greater cytotoxicity and pulmonary inflammation, compared with aged quartz. Interestingly, the Shoemaker study utilised the deoxyguanosine assay to assess hydroxyl radical formation.

Similarly, the role of ferrous ion in enhancing oxy radical formation at surfaces has been emphasized (Nejjari et al, 1993). There is evidence that the presence of ferrous ion, Fe^{2+}, rather than ferric, Fe^{3+}, plays a significant role in free radical generation by acting as a Fenton catalyst. It has been shown that partial conversion of Fe^{3+} to Fe^{2+} can activate UICC crocidolite (Gulumian et al, 1993). However, for most iron-containing minerals, the predominant form of iron is Fe^{3+}, such that various iron-rich minerals display low reactivity, as evidenced by electron spin resonance (Zalma et al, 1987a). The situation is somewhat more complicated since the activity is expected to also depend on solubility, pH, surface area, density of surface active sites accessible to molecular oxygen and redox potential, which will be a function of metal ion stereochemistry. In addition, the presence of other Fenton catalysts such as cuprous ion, Cu^{+} , or nickel(I), Ni^{+} , may augment reactivity. For example Dean and co-workers (1993) have suggested an important role for copper in the oxidative damage caused by asbestos.

5. Methods

5.1 Air Sampling

Personal and area airborne dust samples were collected using 25-mm *Gelman DM 800* filters in *Casella Higgins* sampling heads for respirable dust and *IOM* or *7-hole* heads for inhalable dust, in accordance with Australian Standards 2985 and 3640 respectively.

Initial experiments indicated that a filter deposit of at least 0.2 mg would be required for reliable HPLC analysis. Thus, the fieldwork sampling strategy was oriented towards worst-case sampling, with the primary objective being to characterise the intrinsic reactivity of the airborne dust. It was noted that operators of equipment were often working inside air-conditioned cabins with little dust exposure, and so area sampling heads were often fixed to the sides of vehicles, rather than being located inside the cabin.

Some examples are illustrated in Figures 3-5.

Figure 3:Komatsu WA 200 Front Loader loading into screening plant

Figure 4:Location of inhalable and respirable dust sampling equipment for Komatsu WA 200 Front Loader (left side)

Figure 5:Arrow indicates location of air sampling pump for Volvo dump truck

5.2 Laboratory Assays

After field sampling and weighing, the membrane filters were placed in glass petri dishes. Buffer solution (2.4 ml of phosphate buffer at pH 7) and 100 microlitres of a 10 mM 2-deoxyguanosine solution were then added. The 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 microlitres injected into a high performance liquid chromatography (HPLC) system fitted with electrochemical (EC) and ultraviolet detectors. The amount of 8-OH-deoxyguanosine (8 OHdG) produced was evaluated by comparison of the observed EC signal area with that for an external standard solution (Floyd et al, 1986). These experiments were run in duplicate, and results were expressed as percent conversion to 8 OHdG, normalised to an equivalent 10 mg sample of dust.

Bulk samples of the minerals were also analysed using an analogous procedure. Here known weights (about 10 mg) were placed in 4 ml glass vials, prior to the addition of the buffer and 2-deoxyguanosine solution.

HPLC conditions:

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. 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

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

A solution containing 8 OHdG 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 OHdG was dissolved in distilled water (to form a stock reference solution) and an ultra violet absorbance spectrum run in the region λ = 200-320 nm. Two absorbance maxima were observed at λ = 245nm and 293 nm in the spectrum. The molar extinction coefficient for 8 OHdG at 293nm is, λmax(293nm) = 10300 (Kasai, 1984). From the measured absorbance at this wavelength, the concentration of 8 OHdG in the stock solution was calculated. Subsequent HPLC analysis of this solution then gave UV and EC peak areas equivalent to this concentration; this EC value was then used to calculate the percent conversion of 2-deoxyguanosine to 8 OHdG for the unknown samples.

6. Results and Discussion

A wide range of South Australian mines and quarries were proposed in the original grant application. Due to unusally damp conditions and other logistical constraints, there were some delays in conducting the on-site sampling work and not all of the proposed worksites could be sampled.

6.1 List of worksites investigated

Boral Resources: Murray Bridge (granite), Para Hills (sand), Stonyfell (quartzite), Linwood, Seacliff Pk (dolomite)

Penrice Soda Products: Angaston (marble)

Kanmantoo Quarry: Kanmantoo (stone/slate)

Martin's Granite Quarries: Walker's Flat (granite)

Rocla Quarry Products: Maslins Beach (sand), Golden Grove (sand)

N.B. Rocla's quarry at Rowland Flat was too wet to sample.
Rocla's quarry at Black Hill was adjacent to Martin's Granite Quarry, and was not sampled, since the materials were essentially the same.

Commercial Minerals: Williamstown Quarry (kaosil)

CSR: Victor Harbor quarry (greywacke)

Western Mining Corporation: Olympic Dam Operations (copper/uranium ore)

BHP: Iron Duke and Iron Knob (iron ore)

6.2 Field observations, sampling and results of laboratory assays

Most of the workers spent the majority of their time working in air conditioned (filtered) cabins of earth moving or fixed-location equipment. Under these circumstances, dust exposures should be low, unless there is leakage into the cabin via, for example, inadequate seals. In general, this was not evident.

Given that the primary purpose of dust collection was to assess the intrinsic reactivity of the dust, the collection of (external to the cabin) area airborne dust samples was considered appropriate.
Where workers were obviously exposed to airborne dust generated at the sites, personal samples were collected.
Furthermore, most samples collected were inhalable dust samples, as the quantity of dust collected using respirable samplers was small, often less than 0.2 mg, which was judged to be the practical threshold for subsequent HPLC analysis.
Bulk samples of the minerals were also collected and were ground in a mortar and pestle prior to being weighed out and tested in the same manner as described.

6.2.1 Boral Resources

Area samples were collected at all sites. The area samples at the Linwood site were collected near the conveyor lines and the primary crusher. The roadways were all well watered and no dust was generated by traffic. At the Stonyfell site, the area samples were collected along the conveyor line and screens as well as near the crushers. For the Murray Bridge site, samplers were placed in the cabin of the primary crusher operator and along the conveyor and screen sites as well as the truck loading areas. Samples at the Para Hills site were collected at the boundaries of the property, near the weigh bridge on the exit road and under the crusher line conveyor belt system.

The results from the analysis of the samples are given in Table 1. Overall, these data suggest low reactivities for the Boral products. The highest values were for granite (0.085%) at Murray Bridge and quartzite (0.086%) at Stonyfell.

Table 1: The percentage of 8-OH-deoxyguanosine formed for respirable and inhalable samples at Boral sites.

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Murray Bridge	Inhal #1	67.5	0.003
"	Inhal #2	201.7	0.001
"	Inhal #3	77.8	0.001
"	Inhal #6	6.94	0.085
"	Inhal #8	181.9	0.001
"	Inhal #12	70.0	0.019
"	Resp	20.9	0.031
"	Lab	11.10	0.02
Linwood	Inhal #1	8.06	0.005
"	Inhal #2	16.00	0.019
"	Inhal #7	16.78	0.007
"	Inhal #8	93.07	0.001
"	Resp	0.445	0.046
"	Lab	11.90	0
Salisbury East[A]	Inhal #3	2.444	0.011
"	Inhal #6	0.547	0.039
"	Inhal #8	0.322	0.024
"	Inhal #12	10.80	0.002
"	Lab	11.00	0.002
Stonyfell[B]	Inhal #1	0.712	0.086
"	Inhal #2	1.877	0.011
"	Inhal #3	1.872	0.000
"	Inhal #6	4.081	0.001
"	Inhal #8	1.080	0.006
"	Inhal #12	1.265	0.040
"	Lab	10.30	0.001

[A] Two inhalable and one respirable sample collected with dust weights < 0.2 mg.
[B] One respirable sample collected with dust weights < 0.2 mg.
[C] % conversion normalised to equivalent 10mg sample.
Lab = bulk sample, crushed and weighed in the laboratory

6.2.2 Penrice Soda Products

At Penrice, marble was quarried and crushed. The area samples were collected at various locations around the plant, including along conveyor lines and near crushers.

Table 2: The % of 8-OH-deoxyguanosine formed for respirable and inhalable dust samples at the Penrice quarry, Angaston

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Penrice	Inhal #1	22.44	0.001
"	Inhal #2	41.64	0.038
"	Inhal #6	92.26	0.002
"	Inhal #8	2.19	0.027
"	Inhal #12	2.64	0.017
"	Resp	0.845	0.025
"	Lab	11.0	0.047

[C] % conversion normalised to equivalent 10mg sample.

The free radical generating ability of the airborne samples collected at the Penrice site were all relatively low.

6.2.3 Kanmantoo Stone Quarry

On the day of collecting the dust samples at the Kanmantoo site the quarry was still damp. Airborne dust samples were collected around the quarry site and where the workers were chipping the stone and splitting the stone/slate material.

Table 3: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples collected at Kanmantoo Stone Quarry

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Kanmantoo	Inhal #1	0.282	0.047
"	Lab	11.00	0.001

C % conversion normalised to equivalent 10mg sample.

Most of the area samples collected gave too small a weight to be analysed (< 0.2mg).
The results from the sample which gave a sufficient sample of dust for analysis is higher than the laboratory sample . However, the hydroxyl radical generating ability for both samples are low.

6.2.4 Martin's Granite Quarries, Black Hill

At Martins Black Hill Granite Quarry, Walkers Flat, personal samples were collected for all the workers at the site. The workers were involved in drilling the granite, hand cutting and general work such as moving granite blocks using a forklift. On the day of collecting the airborne dust samples it was a dry cool day.

Table 4: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples collected at Martins Granite Quarry

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Walkers Flat	Inhal #1	5.393	0.046
"	Inhal #2	4.045	0.051
"	Inhal #6	1.572	0.09
"	Inhal #8	1.400	0.087
"	Lab	6.00	0.058

[C] % conversion normalised to equivalent 10 mg sample.

A problem occurred in the analysis of one of the inhalable samples collected. All other samples collected from this granite quarry gave consistently higher formations of 8-OH-deoxyguanosine. The granite dust produced during the work carried out at the site appears to have a greater free radical generating ability when compared to the samples collected at other quarries.

6.2.5 Rocla Quarry Products

Area and Personal Samples were collected at the Maslins Beach sand quarry. Area samples were collected at the Golden Grove quarry.

Table 5: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples collected at two Rocla quarry sites.

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Maslins Beach[A]	Inhal #12	0.317	0.018
"	Lab	10.00	0.001
Golden Grove[B]	Inhal #2	0.336	0.00
"	Lab	10.00	0.00

[A] Two inhalable area samples collected with dust weights < 0.2 mg.

[B] One respirable and one inhalable area sample were collected with dust weights < 0.2 mg.

[C] % conversion normalised to equivalent 10mg sample.

At the Maslins Beach quarry two area samples were collected, however the quantity of airborne dust was less than 0.2 mg. The result shown in the table above was for a personal sample of a worker carrying out some general duties. All other employees worked in filtered airconditioned cabins. For the Golden Grove site very little dust was found to be airborne and for the sample collected the analysis gave no conversion to 8-OH-deoxyguanosine when compared to blank samples.

6.2.6 Commercial Minerals

At the Williamstown quarry site the main ore body is kaosil, but damourite occurs around the edges. The overburden is clay and quartzite.

Table 6: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples collected at the Commercial Minerals Williamstown quarry

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Williamstown[A]	Inhal #2	32.87	0.002
"	Inhal #5	14.48	0.005
"	Inhal #6	3.174	0.010
"	Resp C9	0.267	0.068
"	Lab Damourite	11.80	0.000
	Lab Kaosil	11.30	0.001

[A] Two respirable sample collected with dust weight < 0.2 mg.

[C] % conversion normalised to equivalent 10 mg sample.

The airborne dust samples were collected by placing the samplers on front end loaders or the outside of dump trucks. The conversion reported for the samples collected at the Williamstown site are low with the higher value found for the respirable sample collected.

6.2.7 CSR Victor Harbor

The area samples at the Victor Harbor site were collected near the primary and secondary crushers and the conveyor lines. The workers were not exposed to dust on the site except when checking the operation of the crusher which was for very short periods of time. All other operations were carried out in earth moving equipment or control rooms.

Table 7: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples collected at the CSR Readymix quarry at Victor Harbor

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Victor Harbor	Inhal #2	46.31	0.002
"	Inhal #6	175.99	0.002
"	Inhal #12	9.918	0.013
	Inhal #8	43.16	0.005
"	Inhal #5	16.90	0.006
"	Resp C1	1.719	0.013
"	Lab Greywacke	12.00	0.002

[C] % conversion normalised to equivalent 10mg sample.

The hydroxylating ability of the samples collected at the Victor Harbor greywacke site were all low including the laboratory sample.

6.2.8 WMC Olympic Dam

Area samples were obtained from WMC at Olympic Dam. The inhalable and respirable samples were collected on consecutive days. The samples from "Outside Crusher Station" were obtained from outside the underground crushing station. The samples labelled "UL106" are samples from an underground loader. The "Upcasting" samples were collected from ventilation shafts in the mine.

Table 8: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples collected at WMC ODO, Roxby Downs

Mine Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Olympic Dam	Inhal Outside ULO16	2.647	0.181
"	Resp Outside ULO16	5.37	0.062
"	Inhal Outside Crusher Station	1.545	0.314
"	Resp Outside Crusher Station	1.617	0.082
"	Resp Upcasting	0.49	0.080
"	Inhal Upcasting	0.783	0.250
"	Lab	10.75	0.009

[C] % conversion normalised to equivalent 10 mg sample.

All the results, except for the laboratory sample, indicate high hydroxyl radical generating activity for these samples. Several points should be made regarding these results, firstly no personal exposure to the dust occurred as these were area samples, and respiratory protection was worn by the operator of the underground loader . Secondly the ore body is very variable in its composition, the haematite and copper ore concentrations on any day can vary from 0% to 50%. The metal ions Cu^{+} or Fe^{2+} (the latter present in haematite) will make considerable contributions to the hydroxyl generating activity of the samples tested and may account for the high hydoxyl generating ability of these samples.

6.2.9 BHP Whyalla

Respirable and inhalable area samples from rail sidings during the unloading of iron ore from Iron Duke and Iron Knob deposits were collected.

Table 9: The % of 8-OH-deoxyguanosine formed for respirable and inhalable samples from BHP iron ore deposits

Quarry Site	Inhal/Resp Lab samples	Weight of Sample (mg)	% Conversion [C] to 8-OH-Deoxyguanosine
Whyalla	Iron Duke Inhal	4.860	0.016
"	Iron Duke Resp	0.629	0.031
"	Iron Knob Inhal	57.80	0.000
"	Iron Knob Resp	2.059	-----
"	Lab Iron Duke	10.00	0.000
"	Lab Iron Knob	11.20	0.000

[C] % conversion normalised to equivalent 10mg sample.

The generation of hydroxyl free radicals for all these iron ore samples is low. This is despite the high iron content.

6.3 Interpretation

The data for locally extracted minerals are variable, but those for the samples obtained from the Walkers Flat granite quarry are consistently high. The airborne samples are personal exposures, recorded when workers were drilling granite, hand cutting and moving granite blocks. It is tempting to suggest that the drilling created freshly-fractured mineral surfaces, thereby generating more free radicals. Alternatively, granite is an igneous rock, and may contain relatively higher proportions of ferrous ion.

It is of interest to note that the airborne dusts arising from the Roxby Downs ore display the greatest reactivity. The ore body is very variable in its composition, particularly with respect to haematite and copper ore. Thus the observed reactivities may be explained by the crushing processes and the combined presence of Fe^{2+} and copper, with a possible contribution from ionising radiation.

Finally, the iron ore samples displayed low reactivity. Surface analyses of some bulk samples by X-ray Photoelectron Spectroscopy showed Fe^{3+} present in the surface of the minerals. The amount of Fe^{2+} appears to be too small to be detected by this method.

7. Conclusions and Recommendations

This research project has demonstrated the application of a new mineral dust monitoring procedure, in which an assessment can be made of surface reactivity. The minimum quantity of dust required for the deoxyguanosine assay appears to be roughly 0.2 mg, and consequently the procedure would be more suitable for measurements of inhalable dust, unless dust levels are very high.

This study is the most wide ranging of its type anywhere in the world, covering minerals as diverse as dolomite and uranium ore. The reactivity data are variable, even within a single worksite, but are broadly consistent with results published in the scientific literature, and support the contention that minerals having freshly-fractured surfaces and/or surface ferrous ions are probably more toxic.
In addition, the data for samples of copper/uranium ore from Roxby Downs are suggestive of an important role for copper, via a Fenton-type reaction.

It is simplistic to infer that mineral surface reactivity serves as an index of toxic potential. Oxidative stress is only one possible contributor to carcinogenesis, and even if this pathway is important, significant oxidative stress also arises from endogenous oxidant sources, dietary iron and copper and cigarette smoke.
Various researchers have attempted to correlate mineral surface reactivity with epidemiological evidence of toxicity. These attempts have met with mixed success and further basic research is required to unravel the complex toxicological mechanisms.

In the absence of more diagnostic information, precuations should be taken when exposure to freshly fractured minerals might occur, e.g. drilling or blasting rock containing crystalline silica, ferrous or cuprous ions.
If one assumes that the measured dust reactivities are predictive of toxicity, then the data in Section 6 suggest generally low risk. However, it appears that good dust control measures should be introduced and/or maintained at Martins Granite Quarry and WMC Olympic Dam Operations.

8. References

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