Respirable crystalline silica - Phase 1

Variability in fibrogenic potency and exposure-response relationships for silicosis

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ISBN 978 0 7176 2374 7
Price £7.50

This hazard assessment document examines whether or not the fibrogenic potency (i.e the ability to cause silicosis) of crystalline silica is variable and, if so, what are the factors that influence its variability.

It is aimed at a technical audience and reports on the scientific information which underpins the hazard assessment of a specific substance.

Contents

Foreword

This Hazard Assessment Document has been published by the UK Health and Safety Executive (HSE). It is aimed at a technical audience and reports on the scientific information which underpins the hazard assessment of a specific substance.

In Great Britain, substances which cause harm to health are subject to the Control of Substances Hazardous to Health Regulations (COSHH) 1999. These Regulations require employers to prevent, or if that is not reasonably practical, adequately control employees' exposure to hazardous substances.

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■ Exposure Assessment Document – EH74 series
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Respirable crystalline silica:

Variability in fibrogenic potency

Exposure-response relationships for silicosis

Front summary

This document addresses two issues (i) whether or not the fibrogenic potency (i.e ability to cause silicosis) of crystalline silica is variable, and if so, what are the factors that influence its variability (ii) in the light of currently available information, what is the most reliable view that can be formed of the exposure-response relationship(s) for the development of silicosis.

The analysis presented in this document reveals that there are a number of uncertainties and information gaps relating to these issues; for example the potential changes in the surface chemistry of crystalline silica following long-term residence in the lungs, and the possible toxicological consequences of such changes, is a particular area of uncertainty. However, based on a synthesis of the available evidence drawn from both human experience and from experimental research a number of conclusions can be drawn.

It is important to note that the evidence presented in this document reveals that all forms of respirable crystalline silica dusts of occupational relevance have the potential to cause silicosis. This is an irreversible and progressive condition in which healthy lung tissue becomes replaced with areas of fibrosis. However, human experience and experimental evidence both indicate that at specified levels of exposure, the potential to cause silicosis may be influenced by the type of industrial processing and by the presence of surrounding minerals associated with the crystalline silica. Such factors are capable of modifying the surface chemistry and thus the biological effects of crystalline silica, as well as changing the particle size characteristics. Thus, in different occupational settings, exposures to the same airborne mass concentrations of respirable crystalline silica might pose greater or lesser risks to health depending on the influence of such factors, referred to as "Potency Factors". The main conclusions on these issues are briefly summarised below, supported by a more detailed account of the arguments and supporting evidence in the main document.

Overview of experimental and human evidence on the factors which influence the toxicity of crystalline silica

Variability according to polymorphic type of crystalline silica

Experimental evidence indicates that the toxicity of crystalline silica varies according to polymorphic form; cristobalite, tridymite and quartz appear more reactive and more cytotoxic than coesite and stishovite. Quartz is by far the most commonly encountered polymorph of crystalline silica, although in some circumstances there can also be occupational exposure to cristobalite e.g from the conversion of quartz in the high temperature conditions of industrial furnaces and kilns. There has been a widespread belief that cristobalite is more toxic than quartz, largely based on an early study now considered to be unreliable by modern standards. Experimental evidence from studies *in vitro* shows no differences in the cytotoxic, inflammatory, or fibrogenic properties of these polymorphs. Furthermore, there are no theoretical grounds based on a knowledge of surface chemistry to suggest that cristobalite would be more toxic than quartz, both having the same density of surface silanol groups. The relative toxicity of different polymorphs of crystalline silica has been

poorly studied in animals, and no reliable conclusions can be drawn from the limited data available. The two studies in humans which inform on the effects of exposure to cristobalite provide no indication that cristobalite is more fibrogenic than quartz.

Overall, it is concluded that there are no theoretical grounds or any convincing scientific evidence to indicate any differences in the toxic properties of cristobalite and quartz. This should be borne in mind in considerations of the need for targetting regulatory activities at particular industries where exposure to cristobalite might occur.

Variability due to the presence of other minerals

Occupational exposure to quartz may occur as a result of its close geological association with aluminium-containing clay minerals, such as muds, marls or shale-based clays. Such materials are used in the heavy clay industry to make bricks, tiles and pipes. Aluminium-containing minerals are also encountered in the pottery industry. In some coalmines, some of the the quartz present may be coated with aluminium-containing clay minerals such as kaolinite and illite, found in dirt bands associated with coal deposits. There is experimental, animal and human evidence all consistently pointing in the same direction to indicate that the toxic effects of quartz are reduced in the presence of such aluminium-containing clay minerals. It has been suggested that this is due to the binding of aluminium ions ($Al^{3}+$) to the surface silanol groups of quartz. Over the millions of years of geological formation of coal, the surface of quartz grains in dirt bands associated with coal strata can become coated or intergrown with clay minerals. This reduces the amount of "free" or unexposed quartz surface present. Note that these quartz grains are merely liberated and not fractured during coal-getting activities, and retain their clay mineral coating. It may well be that it is the amount of "free" quartz surface, rather than the total amount of quartz present in respirable coalmine dust which is of relevance to the risk of coalworkers pneumoconiosis.

However, there is evidence from a number of animal studies indicating that the protective effect of aluminium-containing minerals is not permanent. This is presumably due to the differential clearance of $Al^{3}+$ and quartz from the lungs. The retained "cleaned" quartz eventually begins to express its pathogenic properties. This may partly explain the progression of pneumoconiosis in retired coalminers, but there is a lack of clear human evidence on this point.

Overall, the available evidence indicates that aluminium-containing minerals found in close geological association with quartz will protect against the toxicity of quartz as long as exposure to these minerals continues. However, on cessation of exposure, this protective effect is likely to wear off. This caveat should be borne in mind in any considerations of the regulatory stance that should be taken in relation to situations where there is co-exposure to quartz and to clay minerals.

Furthermore, it is conceivable that the health consequences of ceasing co-exposure to quartz and aluminium-containing clay minerals may be greater in younger workers. The retained lung burden of quartz has more time to instigate silicosis development in younger workers, compared to those workers who continue to be co-exposed to quartz in the presence of aluminium up until retirement age. Information from radiographic follow-up of workers leaving the industry concerned e.g heavy clay industry or coalmining would help to clarify this issue.

Variability due to particle number, size and surface area

Current knowledge suggests that regardless of the type of dust, the total surface area of dust retained in the lungs is an important determinant of toxicity. Surface area is related to particle size; smaller particles possess a larger surface area per unit mass compared to larger sized particles. Hence, smaller particle size fractions (very fine dusts) of respirable crystalline silica would be expected to produce more lung damage than equal masses of larger respirable size fractions. The available experimental evidence, although limited in extent, supports this conclusion. The available epidemiology studies do not directly inform on this issue. Overall, in view of the theoretical considerations outlined above, and the limited experimental support, it would be prudent to consider that there would be a greater risk of silicosis in workers exposed to very fine particles of crystalline silica, such as might be found in silica flours, compared to exposure to equal masses of larger size respirable particles. Again, if considering what stance to take in relation to occupational risk management, it might be helpful to bear this consideration in mind when faced with exposure situations where very fine dust of crystalline silica may be generated.

Variability between freshly fractured and "aged" surfaces

Cleavage of crystalline silica particles into smaller fragments results in the formation of reactive radical species at the newly generated particle surfaces. This leads to an increase in cytotoxicity in short-term *in vitro* tests independent of particle size reductions. However, the activity of the free radicals decays with time, a process referred to as "ageing". This occurs slowly in air, but rapidly (within minutes) in water. This phenomenon has not been well studied in animals, but the available evidence does demonstrate enhanced lung damage with freshly fractured quartz. Inferences drawn from human studies are consistent with the contention that exposure to aged surfaces may be less hazardous than exposure to freshly cut surfaces of quartz. Overall, there are enough grounds to conclude that occupational exposures to freshly cut surfaces of crystalline silica will pose greater health risks than exposures to "aged" surfaces. From what is understood about particle ageing, the use of "wet-processes" will help to reduce the reactivity of any freshly cut quartz surfaces, by quenching the formation of free radicals at the cut surfaces. However, this will depend on the effectiveness of the wetting process and the time interval between dust generation and inhalation. In the seconds (or less) between generating the dust and the deposition of the dust into the lungs, it is unlikely that wet processes could completely alleviate the enhanced toxicity of freshly cut surfaces. Freshly cut surfaces may be generated in abrasive processes such as grinding, drilling and crushing. These considerations should be borne in mind in deciding what regulatory stance to take in particular industrial settings where such processes occur.

Exposure-response relationship(s) for silicosis

Widely different estimates have been reported for the risk of developing silicosis in different epidemiological studies covering a range of industries in which exposure to crystalline silica occurs. Much of this variation is considered to be due to inaccuracies in the assessments of past exposure, uncertainties in the diagnosis of silicosis, and differences in study design. However, in some studies, it is possible that the low observed prevalences of silicosis relative to other studies may be due to co-exposure to aluminium-containing minerals as well as the absence of significant exposure to freshly fractured surfaces.

In order to come to the most reliable view possible on the exposure-response relationship (s) for silicosis, the approach taken in this assessment has been to identify the most robust study in terms of the reliability of the exposure data and diagnosis of silicosis, and to use this study as a starting point for characterising the exposure-response relationship for silicosis development. The study selected is a study in Scottish coalminers in which workers encountered major seams of sandstone (almost pure quartz), which generated relatively high exposures to freshly cut surfaces of respirable quartz, uncontaminated with other minerals. It is emphasised that exposures to quartz in this study are not typical of most coalmining situations, where quartz is more closely admixed with coal minerals.

The numerical risk estimates derived from the Scottish coalminers study are shown below in Table 1. Note that they apply to the risks of developing silicosis 15 years post-exposure, which reflects the long period of radiographic follow-up in this workforce.

It should also be noted that these risk predictions only apply when individual exposures do not exceed absolute concentrations of 2 $mg.m^{-3}$. If average exposures exceed this value, even for periods of just a couple of months, then the risks of developing silicosis are likely to rise to exceptionally high levels. This is presumably due to an overwhelming of lung defense mechanisms at high rates of dose delivery.

Table 1 Predicted risks of developing silicosis based on a study in Scottish coal-miners

15 years exposure to crystalline silica (8-hour TWA) $mg.m^{-3}$	Equivalent cumulative exposure $mg.m^{-3}.years$	Risk of developing silicosis 15 years post-exposure as indicated by ILO score 2/1+
0.02	0.3	0.25%
0.04	0.6	0.5%
0.1	1.5	2.5%
0.3	4.5	20%

Table 1 shows the predicted exposure-response relationship for the risk of developing silicosis in terms of Category 2/1+ profusion of opacities on the ILO (1980) scale. Category 2/1+ has been selected as the index of response because it is regarded as the most reliable basis for identifying true cases of silicosis in large-scale occupational studies. In most studies that have investigated exposure-response relationships for silicosis, lower radiographic scores, either Category 1/0 or Category 1/1, have been used as indicators of silicosis development. However, Category 1/0 represents only a minor radiographic abnormality, and is not necessarily indicative of the development of silicosis; nor would it be expected to be associated with any functional impairment. A key difficulty associated with the ILO score Category 1/1 relates to the subjective nature of the scoring process. There is a particularly high degree of inter-reader variability associated with Category 1/1 which makes it difficult to reach agreement on the number of X-ray films that should be assigned this score. Hence, reaching agreement on what should be regarded as the most reliable position on the exposure-response relationship based on Category 1/1 scores is correspondingly difficult and uncertain.

In contrast, the scoring of Category 2/1 is less subject to problems of reader variability. Furthermore, compared to Categories 1/0 and 1/1, the ILO score Category 2/1 is a more specific, though less sensitive indicator of the presence of silicosis in workers with a history of occupational exposure to crystalline silica. However, the predicted risks of developing ILO Category 1/0+ based on the study in Scottish coal-miners are provided in order to allow a comparison with other studies. Statistical analysis of the results of this study suggest that 15 years exposure to respirable crystalline silica at 0.02, 0.04, 0.1 and 0.3 mg.m^{-3} (8-hour TWA) would lead to risks of developing Category 1/0+ profusion of opacities of 16, 18, 25 and 54% respectively, 15 years after exposure ceased.

Due to the exposure durations that applied in the Scottish coalminer's study, there are uncertainties in extrapolating the risks to a 40-year working lifetime. However, observations in retired workers from the Vermont granite industry have shown that a very low risk (<1%) of developing silicosis (Category 2/1) results from a 20-40 year exposure to respirable crystalline silica when exposures are controlled to 0.06 mg.m^{-3} (8-hour TWA).

It needs to be considered to what extent the risk estimates presented above would be representative of silicosis risks in other industries? The risk estimates shown apply to conditions in which workers are exposed to freshly cut uncontaminated surfaces of quartz. Such conditions occur in many industries where abrasive processes take place (eg grinding, drilling). Hence, it is suggested that the scope of the risk estimates above should be extended, such that they can be considered to be well-founded approximations of the dose-response relationship for the development of silicosis (of different radiographic categories) in all such situations. However, in some industries, greater or lesser risks (at specified exposure levels) might pertain, depending on the presence of factors ("potency factors") that could influence the toxicity of crystalline silica.

The following "Potency Matrix" proposes how various factors might influence the *fibrogenic potency* of respirable crystalline silica *i.e its ability to cause silicosis* in different circumstances of occupational exposure. The evidence underpinning the Matrix has been derived from a synthesis of the findings from human experience and from experimental studies. In relation to the latter, evidence for reactivity and cytotoxicity *in vitro*, and evidence for markers of pulmonary damage/inflammation in studies *in vivo* have been taken as supportive evidence for the ability to cause silicosis. In this Matrix, the risks from exposure to dry freshly cut uncontaminated surfaces of crystalline silica are taken as a reference against which other circumstances of exposure can be compared. This is partly because the most reliable risk estimates for the development of silicosis reflect such an exposure scenario, and also because this scenario is of relevance in a high proportion of occupational situations. It should be emphasised that although the Matrix is based on the variable potency contention, it should not be used as a basis for suggesting relaxation in control standards in any industry or process involving exposures to crystalline silica. Rather, it serves to highlight those situations where the highest risks of developing silicosis are thought to occur.

Respirable crystalline silica (RCS) Potency matrix

POTENCY FACTORS	COMMENT	RELEVANT EXPOSURE SITUATIONS
Dusts of extremely small particle size	Enhanced potency compared with exposures to same mass of larger size respirable particles	**High energy grinding and abrasive processes. Exposure to silica flours.**
Production of dry freshly cut surfaces of RCS	This form of RCS is presented as the "reference point" against which the potency of forms is compared.	**Drilling, blasting, grinding and all other abrasive processes**
Wetting of freshly cut surfaces	Reduced potency compared to dry freshly cut surfaces. *Effect depends on efficiency of wetting and how long the surfaces have been wetted prior to inhalation.* *Note that the main risk management benefit of wetting is dust suppression rather than potency reduction.*	**Wet extraction or handling processes.**
Exposure to "aged" dusts Dusts that have not been freshly cut or ground	Reduced potency compared to freshly cut or freshly ground dusts.	**Handling or non-abrasive processing of dusts after storage**
Presence of aluminium-containing clay minerals which coat the surfaces of the silica particles	Reduced potency compared to freshly fractured uncoated surfaces. *The clay coating may wear off during residence in the lungs allowing the toxicity of the quartz particles to be expressed.*	**Work in the heavy clay industry or in mines extracting low rank coals.**
Cristobalite	Equivalent potency to quartz for equivalent conditions of exposure.	**Heating of quartz-containing materials in furnaces and kilns.**

Background

A MEL for crystalline silica of 0.4 mg.m^{-3} (8-hour TWA) was introduced in 1992, following consideration of all the relevant information available at that time by WATCH and ACTS. In 1997, the value of the MEL was adjusted to 0.3 mg.m^{-3} following adoption of the ISO/CEN sampling convention for respirable dusts. The principal health concern underlying the MEL for crystalline silica was silicosis.

The application of the MEL and the requirement to reduce exposures as low as reasonably practicable below the MEL value covers all UK occupational exposure situations in which exposure to crystalline silica occurs. In practice, there is a wide spectrum of situations in which such exposures occur, and the surrounding features of the exposures (in terms of the crystalline silica polymorph present, particle size, particle surface area relative to mass, the presence or absence of freshly-cleaved crystalline surfaces and the presence or absence of other materials along with the crystalline silica) also vary widely. There has been widespread belief that these variables can exert considerable influence on the toxicity of "crystalline silica". This has caused HSE's Field Operations Directorate (FOD) to ask what is the state of the scientific evidence supporting the contention that the toxicity of crystalline silica is variable. This has implications for the possibility of targetting attention on those industries and exposure situations meriting most concern, in relation to the risk of silicosis.

Since the introduction of the MEL, a further issue surrounding crystalline silica has been its potential to cause lung cancer. Many analyses and commentaries on this issue have appeared in the scientific literature in the last few years. WATCH debated the evidence for the carcinogenicity of crystalline silica at its meeting in January 1998. FOD has now added carcinogenic potential and potential variations in its expression as an additional issue for consideration in connection with different occupational exposure scenarios.

These considerations prompted FOD to ask HSE's Industrial Chemicals Unit (Toxicology Unit as it was at the time) to undertake a project to address the following issues:

(i)   Does the fibrogenic capability of crystalline silica vary depending upon its source?
(ii) Are there any good dose-response data for silicosis that might be of use in making judgements on sector-related industries and to provide guidance on what is reasonably practicable in different industries or processes?
(iii) Is there any evidence to indicate that the potential carcinogenic activity of crystalline silica may vary depending on its source?
(iv) Is fibrosis (silicosis) a necessary precursor for the development of lung cancer such that controlling for silicosis would minimise any risk of cancer?

It has been decided to deal with these issues in two phases. This document (Phase 1) represents an attempt to explore the first two of these issues. It focuses on the cytotoxic, inflammatory and fibrogenic effects of crystalline silica; the issues surrounding carcinogenicity are considered separately in Phase 2. The rationale for this approach is that the database for the former properties is far more extensive and (in some ways) clearer, than for carcinogenicity, and therefore better facilitates the ability to address the "variable potency" issue. Furthermore, if crystalline silica does have the potential to cause cancer, then the fibrogenic and carcinogenic potencies of crystalline silica are both likely to derive from similar biological processes involving macrophage activation and chronic inflammation; hence any relationships established for variability in fibrogenic potency might be reasonably assumed to hold for carcinogenic potency.

There have been many analyses of the health effects of crystalline silica in recent years. Although the total extent of the primary literature is huge, only a relatively small proportion of the studies available contain information of sufficient quality and relevance to inform on the key questions explored in this project. The approach that has been used here is to focus on those more informative studies, using recently published reviews as a guide to identifying the most useful primary literature. These selected original studies have then been critically appraised.

This document is divided into 3 sections. Section 1 deals with the experimental evidence from studies *in vitro* relating to factors which might influence the cytotoxic properties of crystalline silica; this section is important in helping to determine whether there is scientific plausibility to the concept that the toxicity of crystalline silica might be of variable potency. Section 2 deals with evidence from studies in animals informing on factors which might influence the cytotoxic and fibrogenic effects of crystalline silica. Section 3 analyses epidemiological studies to determine whether there is evidence from human experience supporting the contention that crystalline silica presents a fibrogenic hazard of variable potency. Also, Section 3 explores the various risk estimates for the risk of developing silicosis in different studies, to inform on what the most reliable exposure-response (or range of exposure-response) relationship(s) might be. The main aim of Sections 1 and 2 is to inform on the "variable potency" issue; these Sections have not directly contributed to the construction of a view on the exposure-response relatonships for silicosis. This is because most lifetime exposure studies in animal models have focussed on carcinogenic endpoints rather than silicosis development. Furthermore, there are marked differences in the fibrotic responses to inhaled dusts, including crystalline silica, between different animal species, and which animal species might be of most relevance to human health is uncertain. Therefore, the analysis of exposure-response relationships for silicosis in this document is based entirely on studies in humans.

Section 1 - Studies conducted *in vitro*

This section outlines the evidence from *in vitro* studies relating to the factors that might influence the toxicity of crystalline silica. Most of the data comparing the *in vitro* toxicity of the various silica polymorphs and the influence of contamination or chemical treatments on toxicity have been reviewed previously (Driscoll, 1995). In general, various commercially available forms of quartz are used in experimental studies, the two main types being DQ-12 and Min-U-Sil. DQ-12 is extracted from a kaolinitic sand deposit in Germany, and is typically around 87% pure quartz (Robock, 1973). No specific information concerning the source of Min-U-Sil has been found, but it is also reported to be a naturally occurring form of quartz. A survey of studies utilising Min-U-Sil suggests that it is typically 99% pure quartz (IARC, 1997). Both Min-U-Sil and DQ12 can be contaminated with iron and possibly other elements (Guthrie and Heaney, 1995; IARC, 1997). 'Fused' silica, produced by heating and then rapid cooling of crystalline silica, is essentially indistinguishable from amorphous silica, and therefore is not included in the scope of this review which only covers crystalline forms of silica.

1.1 Theoretical considerations - surface chemistry

Silica is the common name for silicon dioxide, SiO_2. It is a solid material that can exist in either a crystalline or non-crystalline (amorphous) form. Crystalline forms of silica consist of regularly arranged SiOx units. The arrangement and molecular dimensions of these units can vary, resulting in a variety of polymorphs that are identical in chemical composition, but differ in crystalline structure. The most common crystalline forms are quartz and cristobalite; other forms such as tridymite, stishovite and coesite are rare and unlikely to be encountered occupationally.

Theoretical considerations suggest that the surface characteristics of silica particles rather than bulk chemistry are likely to be the main determinants of toxicity. Surface chemistry is influenced by the crystalline structure of the different polymorphs, by the generation of freshly fractured surfaces, by particle "ageing" (discussed below), and by the presence of other minerals. Recent toxicological evidence with other dusts suggests that particle size and total surface area exposed to the lungs, may also be important determinants of particulate toxicity.

Silica is essentially insoluble in the lung. As such, it can be predicted that the surface characteristics of silica particles rather than bulk chemistry would be the main determinants of toxicity. The surface of silica contains a number of surface moieties, including siloxane bridges (Si-O-Si), peroxybridges (Si-(O)x-Si), and silanol groups (Si-OH). In the presence of water, silanol groups are partially ionised in an approximate 30 (SiOH) :1 (Si-O-) ratio. Silanol (SiOH and SiO-) groups are by far the most abundant chemical moieties on the silica surface and are thought to play a role in mediating the toxicity of silica particles. Molecular models of silica polymorphs indicate that quartz, cristobalite and tridymite have relatively open structures which allow the silanol groups to protrude from the crystal surface, making them relatively chemically reactive/accessible. In contrast, stishovite, which is formed under conditions of very high temperature and pressure, has a relatively closed structure from which the silanol groups do not protrude.

Cleavage of crystalline silica particles into smaller fragments (eg by grinding or blasting) can result in homolytic or heterolytic breakage of the Si-Si and Si-OH bonds at the surface of the cleavage planes, resulting in the formation of highly reactive radical species at the particle surface. These reactive species include Si. and Si-O., as well as surface ionic groups, Si+ and SiO-, and strained siloxane bridges (Si-O-Si). Radicals are highly reactive species and may produce cytotoxicity by causing direct chemical damage or indirectly by instigating chemical cascades producing other cell damaging agents. Although the generation of freshly fractured silica particles is thought to lead to an increase in the number of chemically reactive species at the particle surface, the fracturing process will also cause an increase in surface area per unit mass (referred to below as specific surface area) due to the decrease in particle size.

1.2 Comparisons of quartz derived from different sources

A recent study (Clouter *et al*, 2001) compared the *in vitro* and *in vivo* toxicities of DQ12 quartz with two different workplace samples of high purity quartz. The *in vivo* data are presented in Section 2 of this report. The feedstock for one workplace sample, designated OM, was medium hard sandstone, which had been crushed then milled to produce silica flour. The other workplace sample (RH1), was collected from a similar site location but in this case the feedstock to the plant was a soft, loosely consolidated sand, which had not been crushed prior to milling.

Chemical analysis showed that DQ12, OM and RH1 consisted of 89%, 85% and 95% quartz respectively. Iron was present at less than 0.5% in any sample, but to a 10-fold greater extent in RH1 than OM. Aluminum showed differences, but again was present in all samples at less than 1%. Although the samples did not differ markedly with respect to chemistry, mineralogically they were distinct. DQ12 was said to contain a small amount of kaolin. The OM sample contained a "high level" of alumino-silicate minerals in the form of microcline feldspar and some illite.

Scanning electron microscopy showed all three quartz samples to have a similar appearance, with a suggestion that some DQ12 particles may be aggregates of smaller particles. This aggregation of DQ12 particles is likely to have caused inconsistencies in surface area and particle size analyses. The specific surface areas of DQ12, OM and RH1 as measured using a standard nitrogen adsorption technique (BET) were 10.1, 7.99 and 5.22 m^2/g respectively, suggesting smaller individual particle size for DQ12. However, particle size analysis by transmission electron microscopy suggested that the DQ12 particles were larger than the other particles; with this method, 50% of OM and RH1 particles were < 0.6 m diameter, but 50% of DQ12 were < 3 μm. In contrast, using standard Coulter methods the DQ12 sample appeared to be the finest (data not shown). This apparent inconsistency was suggested to be due to clumping of the DQ12 particles during preparation for the TEM.

The ability of the quartz samples to release iron at neutral and acid pH was compared by a spectrophotometric method that detects the colour change in desferrioxamine when it combines with Fe^{3+}. The results showed a substantial release of soluble iron from RH1, but no difference between DQ12 and OM in the small amounts of iron released.

The surface reactivity of the quartz samples was compared in a red blood cell haemolysis assay. This assay measures the ability of substances to lyse red cells, and is a measure of the membrane-damaging potential or direct surface reactivity of the test substance. When tested at equal mass concentrations, the activity of the workplace quartz samples was negligible in this assay; OM and RH1 produced 6.4% and 3.9% of the degree of haemolysis produced by a positive control detergent, compared to 34.7% with DQ12 quartz. This was surprising because the surface generation of hydroxyl radicals, as measured by ESR, was greater in the workplace samples than with the DQ12.

The cytotoxicity of the quartz samples was compared in A549 epithelial cells and primary rat alveolar macrophages using an MTT assay. This assay provides an indication of the number of metabolically active cells using a label, MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) which is converted by oxidative metabolism to the dye formazan. The cells were incubated with the test samples (25, 50, 100 and 200 µg/ml) for 1, 20 and 48 hours before the MTT assay. The epithelial cells were not particularly sensitive to the quartz exposures. However, in the alveolar macrophages there was a clear dose-response with all quartz samples at all time points in terms of decreases in MTT activity, but DQ12 was clearly more active in this assay than either OM or RH1.

In summary, the three quartz samples were all of high purity and were broadly similar in chemical composition. A true indication of their particle size distributions is difficult to establish, due to the apparently greater tendency of the DQ12 particles to aggregate. The BET measurements showed that the DQ12 particles had a greater specific surface area than the workplace samples (surface areas of 10.1, 5.22 and 7.99 m^2/g) for the DQ12, RH1 and OM samples respectively. This supports the view that the individual particle sizes of DQ12 were likely to be slightly smaller than for the workplace samples. The three samples of quartz showed differences in their surface reactivity and cytotoxicity. When tested at equal mass doses, DQ12 was substantially more active than the workplace samples in causing red cell haemolysis, and was more cytotoxic in rat alveolar macrophages. These effects were not related to the ability to release soluble iron (RH1 was the most active), or to the ability to generate surface hydroxyl radicals (both workplace samples generated a stronger ESR signal than DQ12). As discussed in Section 2 DQ12 also caused more pulmonary inflammation in rats following single intratracheal instillation compared with the workplace quartz samples. Whether the observed differences could be related to the differences in the specific surface areas of the three quartz samples, or to the differences in their mineralogy, is uncertain. Furthermore, the relevance of the findings to the effects of long-term inhalation has not been established. Overall, the most likely property responsible for the observed differences in reactivity of these samples was the smaller particle size of the DQ12 quartz compared to the workplace samples of quartz.

1.3 Variability in toxicity of silica according to polymorph

A review by Driscoll (1995) indicates that the relative toxicities of the various polymorphs have been investigated using tests of membranolytic activity and toxicity towards macrophages, but provides only limited information relating to experimental design and study findings. Therefore the key studies identified in the Driscoll review are presented in detail below. However, it should be noted that these *in vitro* studies only measure short-term acute toxicity towards cells, possibly mediated by direct chemical attack, and as such provide findings of uncertain relevance to the development of chronic conditions such as silicosis.

1.3 a Membranolytic studies comparing polymorphs

A study by Stalder and Stöber (1965) compared the haemolytic activity of several polymorphs towards human and sheep erythrocytes. Each polymorph was prepared using a slightly different method, i.e. quartz by grinding pure rock crystal, cristobalite and tridymite were produced by sintering vitreous silica with a flux (to increase the extent of conversion) followed by grinding, and coesite and stishovite by 'isolation' from sandstone from a crater with no mention of grinding, resulting in samples of different specific surface areas (assessed by argon adsorption). Grinding silica leads to an increase in the number of radical species on the crystal surface, potentially increasing the chemical reactivity of the particle surface; however, these radicals react rapidly in aqeous solution, a process known as 'ageing', quenching the radical species and hence lowering the chemical activity of the surface (described in detail in below). Given that these samples were prepared in aqueous solution for the experiments, it is likely that the ground samples were aged when used in the experiments. Following incubation with erythrocytes at a surface area of $0.02m^2$ silica/incubation (achieved by adjusting the mass dose as appropriate) quartz, cristobalite and tridymite were found to have similar haemolytic activity (causing 90-92, 64-85 and 91-100% haemolysis respectively), and were more active than coesite and substantially more active than stishovite (55 and 8% haemolysis respectively). The findings from this study provide reasonably reliable evidence to demonstrate the high haemolytic activity of quartz, cristobalite and tridymite and the low activity of stishovite, a range of activity consistent with the relative chemical availability of surface groups, particularly silanol groups, on the silica surface.

A similar study by Wiessner *et al*. (1988) also compared the haemolytic activities of various silica polymorphs when tested at the same applied surface area ($0.023m^2$) in human erythrocytes. Crystals were reduced in size (80% of particles less than 2 µm) by freeze milling followed by boiling in HCl to remove metal contaminants and finally heating to 250ºC to destroy endotoxins. The effect of freeze milling on the surface characteristics of silica is not known, but the authors indicated that the membranolytic activities of milled or non-milled crystals boiled in HCl was the same, suggesting that the preparation process did not significantly alter the surface characteristics (no data presented). Quartz, cristobalite and tridymite were of similar membranolytic activity (around 60% haemolysis) which was much greater than that of coesite (15% haemolysis).

A study by Kozin *et al*. (1982) also compared the haemolytic activity of Brazilian quartz, Min-U-Sil-5 and -10 µm quartz, natural and synthetic cristobalite, tridymite and stishovite towards human erythrocytes. The study utilised particles less than 40 µm. To achieve this particle size, natural and synthetic cristobalites were ground and sieved; the other silicas were supplied to the authors with particle sizes below 40 µm and were not subject to grinding. The specific surface area of the Min-U-Sil quartz crystals was determined using inert gas adsorption, but the surface area of the other crystals was estimated by microscopic comparison with other crystals of known specific surface area; the authors did not address the accuracy of this method. These polymorphs were tested at an equal mass and the haemolytic activity presented in absolute terms, and also normalised in terms of specific surface area (findings for surface area presented in section 3.1.2). When presented in absolute terms, the findings demonstrated a considerable range of haemolytic activity between the silicas tested, with percentage haemolysis rates of around 2, 30, 52, 25, 1 and 0.3 for Brazilian quartz, Min-U-Sil-10, Min-U-Sil-5, natural cristobalite, synthetic cristobalite and stishovite respectively. Thus, these findings demonstrated considerable differences in haemolytic activity between different polymorphs and also between different samples of the same polymorph, as with quartz and cristobalite, when compared on a mass basis.

When the level of haemolytic activity was normalised for surface area, Brazilian quartz, tridymite and synthetic cristobalite caused a similar level of haemolysis (around 20-25% membranolysis/cm2). However, two samples of Min-U-Sil (particle sizes of 5 and 10 μm) were approximately two-fold more membranolytic than these forms (50% membranolysis/cm2). The reasons for these differences are unclear and were not addressed by the authors. Natural cristobalite, reported to be contaminated with quartz (no further details presented), had a very high membranolytic activity (780% and 25%/cm^2 for natural and synthetic cristobalite respectively). The reason for this dramatic difference in activity is not clear. Both forms of cristobalite were prepared by grinding to give samples of identical estimated surface areas (approximately 0.07 m^2/g). It is possible that the high activity of natural cristobalite may be a result of differences in the preparation of each sample, for instance more extensive grinding, or due to the presence of quartz contamination, although insufficient details were presented to address these possibilities. In common with findings from other studies, stishovite was relatively inactive compared to the other polymorphs tested (7% membranolysis/cm^2). Overall, these findings indicate that Brazilian quartz is of similar haemolytic activity to synthetic cristobalite and tridymite, although the relative activity of each polymorph may also vary substantially dependent on its source.

A study by Ottery and Gormley (1978), described below, demonstrated that at equivalent particle size and specific surface area, quartz and cristobalite had similar haemolytic activity.

1.3 b Toxicity to macrophages
The review by Driscoll (1995) states that quartz, cristobalite and tridymite are toxic to macrophages under *in vitro* conditions, whereas stishovite is not. However, only one study is cited which provides comparative information on the polymorphs. This study by Marks and Nagelschmidt (1957) demonstrated that quartz, tridymite and cristobalite are toxic to macrophages, as assessed by leakage of lactate dehydrogenase, but did not provide any reliable information on the relative toxicities of these polymorphs. Overall, there is no reliable evidence to inform on the relative *in vitro* toxicity of silica polymorphs to macrophages.

Summary of studies with polymorphs
The relative *in vitro* toxicity of polymorphs of crystalline silica has not been extensively researched. However, tests of membranolysis have been used by a number of researchers to provide some relevant information. The findings obtained are reasonably consistent and show that quartz, cristobalite and tridymite possess significant and similar membranolytic activity whereas stishovite is virtually inactive. This clear difference in toxicity is consistent with the greater chemical availability of silanol groups on the surface of the active polymorphs by virtue of their more open crystalline structures. In addition, the findings do not provide any consistent evidence to support the contention that cristobalite is of greater toxicity than quartz.

1.4 Studies with surface modified silica

The influence of surface modification on silica toxicity has been extensively reviewed (Donaldson and Borm, 1998; Driscoll, 1995; Brown and Donaldson, 1996). Driscoll (1995) cites a number of studies that have demonstrated that the *in vitro* toxicity of quartz, assessed by haemolytic activity and toxicity to macrophages, can be reduced by contamination of the surface with a range of agents. A review by Brown and Donaldson (1996) presents evidence indicating that aluminium (as Al^{3+}) contamination on the surface can reduce the toxicity of silica.

Various studies have demonstrated that agents that bind to protonated or ionised silanol groups can reduce the toxicity of quartz. Agents that bind protonated silanol groups, such as polyvinylpyrolidine-N-oxide (PVPNO), act to reduce the haemolytic activity and toxicity to macrophages. Although PVPNO is not of industrial relevance it does illustrate the role of silanol groups in mediating such toxicity. Similarly, agents that bind to ionised silanol groups, such as $Al3+$, also act to reduce haemolytic activity and toxicity to macrophages. Other studies not cited by Driscoll (1995) have also demonstrated similar reductions in toxicity in similar test systems following treatment with other substances, such as protein and surfactant. In contrast, Driscoll (1995) also highlights data indicating that iron contamination might increase the toxicity of silica by catalysing the production of reactive oxygen species. In this context, as discussed by Donaldson and Borm (1998), the valence state and amount of iron may be important factors. Overall, the data discussed in these reviews provide strong and consistent evidence to indicate that the toxicity of silica can be modified by surface treatment or contamination. In general, contamination of the silica surface with agents that bind or interact with silanol groups appears to reduce the toxicity of the silica particles under *in vitro* conditions, probably by reducing the chemical availability and reactivity of these groups. It should be noted however that these tests are only short-term and provide no information on the permanence or reversibility of such inhibitory effects.

1.5 Effect of particle number, size and surface area

The effect of particle size or specific surface area on *in vitro* toxicity has not been extensively studied. However, a number of the haemolysis studies cited above included experiments to investigate the role of numbers of particles or surface area on haemolytic activity. It should also be noted that the results of the study outlined in section 1.2 suggested that the smaller particle size of DQ12 was the most likely property responsible for the greater *in vitro* reactivity of this sample compared to two workplace samples of quartz.

Additional experiments in the study by Stalder and Stöber (1965), described above, were conducted to assess the influence of surface area on toxicity. Quartz, coesite and stishovite were incubated with erythrocytes at a range of surface areas/incubation, obtained by varying the mass of polymorph present in the incubation. The haemolytic activity was found to increase rapidly with increasing specific surface area for both quartz and coesite although quartz was more active than coesite at any given surface area, with each producing 90% haemolysis at approximately 0.05 and 0.15 m^2/incubation respectively. The membranolytic activity of stishovite increased only slightly with increasing surface area. These findings demonstrated an apparent relationship between extent of haemolysis and surface area. However, given that the range of surface areas used was obtained by varying the mass dose, and hence particle numbers, these findings cannot distinguish between the effects of surface area or particle number on resultant haemolytic activity.

Ottery and Gormley (1978) investigated the membranolytic activity towards sheep erythrocytes of quartz from various commercial sources (X7488, DQ-12) and cristobalite. Only particle size information was presented for these silicas which indicated that the number of particles per unit mass was considerably greater for DQ-12 than for X7488 or cristobalite (>245, >68 and 88 particlesx10^6/mg). When tested at equivalent mass doses, the membranolytic activity of X7488 and cristobalite was the same. However, DQ12 was considerably more active than the other two forms for any particular mass dose tested (approximately 60% haemolysis with DQ12 and 20% haemolysis with X7488 and cristobalite at the highest dose tested of 0.25 mg/ml). Interestingly, for similar particle sizes, cristobalite was of similar toxicity to X7488, suggesting no differences in the toxicity

between these two polymorphs. The greater toxicity of DQ12 is likely to be due
to its smaller particle size distribution, yielding a greater number of particles and
consequential higher surface area present at any given mass dose. Therefore,
these results suggest that under *in vitro* conditions, the toxicity of crystalline silica is
related to the surface area and particle number present.

In another part of this study, the relationship between membranolytic activity and
particle number and surface area was further investigated using fractions of
Min-U-Sil with different particle numbers per unit mass ($6\text{-}70 \times 10^6$ particles/mg).
These samples had been prepared by successive sedimentations with no grinding.
When tested at the same mass concentrations, the extent of membranolysis
increased significantly with increasing particle number per unit mass; for
example, when tested at 3 mg/ml, there was approximately 60, 30, 15 and 5%
membranolysis for preparations containing 70, 30, 17 and 6×10^6 particle/mg
respectively. Clearly, there was more toxicity with the smaller particle size fractions.
When percentage haemolysis was plotted against the number of particles in the
incubation an almost linear relationship was found between these two parameters,
indicating a direct relationship between the extent of haemolysis and number of
particles present. However, there would be covariability with surface area, given
that for a given mass, as the particle number increases, the total surface area also
increases.

Overall, these two sets of experiments within the same study all suggest that for
a given mass dose of crystalline silica, toxicity increases with increasing particle
number and particle surface area. However, the authors of the study attempted
to distinguish between the influence of particle numbers from surface area, and
calculated for each size fraction the number of particles and the particle surface
area (calculated by multiplying the square of the median volume diameter by the
number of particles) present in an incubation that would cause 5% haemolysis.
However, HSE considers that this method of estimating the surface areas is very
crude; ideally, specific surface areas should be measured with a gas adsorption
technique. The results of this analysis suggested that each size fraction would
cause 5% haemolysis at roughly the same number of particles, around 15×106
particles/ml incubation. In contrast, there was no relationship between this level
of haemolysis and surface area present in the incubation. HSE considers that little
reliability can be placed on these calculations. Overall, the observations from this
study suggest that for a given mass dose of crystalline silica, toxicity increases with
increasing particle number and particle surface area.

A study by Wiessner *et al*. (1989) investigated the effect of particle size on the
membranolytic activity of quartz. Fractions of Min-U-Sil quartz of various average
particle sizes (1, 5, 8 and 11 μm) were obtained by sedimentation and specific
surface area determined by inert gas adsorption (5.9, 1.9, 1.2 and 0.75 m^2/g for
1, 5, 8 and 11 μm-sized particles respectively). Each fraction was incubated with
human erythrocytes at a mass concentration designed to give a surface area
of 95 cm^2/incubation (1.6, 5, 8.2 and 13 mg/incubation for 1, 5, 8 and 11 μm,
respectively). The extent of membranolysis was found to be greatest for particles of
1 m and roughly similar for the other sizes (25% haemolysis for 1 μm and around
15% for 5, 8 and 11 μm). Hence, for four different particle size fractions of quartz
tested at the same applied surface area, the smallest size fraction showed a
greater haemolytic activity than the three larger particle size fractions, suggesting
that surface area alone is not sufficient to account for toxicity. Information on
particle number was not presented, so it is not possible to distinguish the possible
influence of particle size and number from these results. In general these studies
demonstrated that toxicity increased with increasing particle number per unit mass,
but were unable to inform on the relative importance of particle size or surface
area. However, these two properties are so closely interlinked that there may be no
practical distinction between the two.

1.6 Effect of comminution

Comminution is the term given to the process of reducing the size of crystals by physical processes such as milling, grinding or blasting. The effect of comminution on silica surface properties and toxicity has been well studied and a consistent body of evidence has been established (reviewed by Castranova *et al*., 1996).

Comminution of silica involves the homolytic or heterolytic breakage of silicon-oxygen chemical bonds, resulting in the formation of radical species (Si·, Si-O· and Si-O$_2$·) and surface charges (Si$^+$ and SiO$^-$) on the cleavage surfaces. A consequence of these surface changes is the enhanced production of reactive radical species. The effect of comminution on radical production has been investigated using Electron Spin Resonance (ESR). Non-comminuted silica particles exhibit a small ESR signal, indicating that the surface contains very few free radicals. However, similar particles fractured in a jet mill or by grinding exhibited a significantly increased ESR signal indicating the formation of radicals. The extent of radical formation is proportional to the amount of comminution; for example, in one study, grinding in a ball mill for 8 minutes gave a 7-fold increase in the ESR signal over grinding for 1 minute. Such radicals are highly reactive and can react on contact with tissue, water or air. However, following comminution, the radical yield decreases in a process known as ageing. The process of ageing follows an exponential decline with radicals remaining detectable on the silica surface for extended periods of time. The rate of aging depends on the particles' environment. In air, the half-life for the disappearance of such radicals is 24-30 hours. In contrast, in aqueous environments, such as would be found in the lung, the half-life is much shorter, at just a few minutes. However, although freshly fractured silica may age rapidly in the lung, it would be expected that some increased toxicity would be expressed before significant aging had occurred.

Radicals are known to be highly reactive and damaging to tissues. Thus, it is reasonable to postulate that comminuted (freshly fractured) silica might be more toxic than equivalent aged or non-fractured silica as a result of increased radical yield. To inform on the effect on toxicity of comminution a number of *in vitro* studies have been performed using end-points such as lipid peroxidation and membranolysis. Several good quality studies have demonstrated that freshly fractured quartz causes increased lipid peroxidation, haemolysis and cell toxicity; the activity then decreases relatively rapidly with time post-grinding as aging occurs (Vallyathan *et al*., 1988; Castranova *et al*., 1995). In addition, when incubated *in vitro* with alveolar macrophages, freshly fractured quartz causes greater stimulation of oxidant production than aged quartz (approximately 80% greater production of superoxide and hydrogen peroxide) and approximately 9-fold greater chemiluminescence. The correlation of toxicity with the presence of surface radicals and decrease in toxicity as aging occurs indicates that the increased toxicity following comminution is due to the presence of radicals, independent of the decreased particle size.

The effect of comminution on the toxicity of other polymorphs has not been investigated but it can be predicted that the effect would be generally similar for all polymorphs. Overall, these findings demonstrate that comminuted quartz is more toxic in *in vitro* systems than non-comminuted quartz due to the increased presence of surface radicals.

1.7 Summary of in vitro experimental data

Evidence from *in vitro* studies shows that various factors can influence the potency of crystalline silica; these factors include polymorphic type, surface contaminaiton, particle size/surface area, and the presence of "freshly fractured" surfaces. Quartz, cristobalite and tridymite have been shown to be of significantly higher toxicity *in vitro* than stishovite. These polymorphs differ in that silanol groups protrude from the crystal surfaces of quartz, cristobalite and tridymite making them chemically available, in contrast to stishovite where these groups do not protrude to the same extent. The presence of silanol groups on the crystal surface has been implicated as playing a role in mediating the toxicity of crystalline silica. Masking the surface silanol groups with synthetic polymers or with aluminium salts generally reduces haemolytic activity, consistent with the view that these groups play a key role in the interaction with cellular membranes. Particle size, number and surface area per unit mass are related properties which may influence toxicity by virtue of a greater surface area presenting more reactive silanol groups to exposed tissue. The importance of these factors for toxicity *in vitro* has been poorly investigated, but when tested at equal mass doses *in vitro*, in general, reactivity and cytotoxicity increase with increasing particle numbers and specific surface areas.

Comminution processes that generate freshly fractured surfaces enhance the *in vitro* toxicity of quartz by increasing the yield of highly reactive radicals at the particle surface. This increase in toxicity is independent of the concurrent reduction in particle size by comminution. However, the increased reactivity of such freshly fractured silica is only transient; the enhanced toxicity declines with time (ageing) due to the decay of the surface radicals. Ageing occurs very rapidly in aqueous media, but occurs more slowly in air.

Section 2 - Studies conducted *in vivo*

2.1 Comparisons of quartz derived from different sources

Inhalation data
There are no inhalational studies that have compared the toxicities of quartz derived from different sources.

Intratracheal data
In a recent study, the pulmonary effects of a commercial sample of DQ12 quartz were compared with those of two different workplace sample of quartz (Clouter *et al* 2001). The characteristics of these quartz samples were described earlier in this report (Section 1.2). Male rats (4 per group) were given an intratracheal dose of either 250 or 1000 μg of the different quartz samples and then sacrificed after either 3 or 14 days. Analysis of the cell population from bronchoalveolar fluid (BALF) was undertaken to measure the recruitment of inflammatory cells into the lung, and the cell-free supernatant was tested for protein and lactate dehydrogenase (LDH) activity as markers of an inflammatory response in the lungs. The results showed no differences in any of these inflammatory markers compared to controls with either of the workplace samples of quartz. However, compared to controls and to the workplace samples of quartz, DQ12-exposed rats showed increases in the total cell count in BALF and increased proportions and total numbers of neutrophils at all time points and doses, and increased macrophage numbers at the high dose. Similarly, BALF from DQ12-exposed rats had raised protein levels after 3 days at the high dose, and after 14 days at both doses. LDH activities were not raised in

comparison with controls at any dose, or at any time point, for any of the quartz samples.

In conclusion, for three different samples of quartz all of high purity, when tested at equal mass doses, only the DQ12 sample produced evidence of a pulmonary inflammatory response. The specific surface area (cm^2/g) of the DQ12 sample was somewhat higher than that of the workplace samples, but whether this was the reason for the observed differences in biological response is uncertain. All samples contained less than 1% aluminium, but the mineralogical form of the aluminium was different in these samples (see 3.1.1), and whether this could have influenced the results is unclear. It should be noted that these results derive from a single dose with short-term follow up; whether the pattern of findings would be similar with longer-term repeated exposures is uncertain.

2.2 Comparison of different polymorphs of crystalline silica

Most of the fundamental toxicological research on silica has been carried out using standard commercial sources of quartz, such as Min-U-Sil and DQ12. Consequently, few studies are available with which to compare the relative toxicities of the various silica polymorphs in animals.

2.21 Inhalation exposure studies

Only two studies were located that investigated the relative toxicity of cristobalite and quartz using inhalation exposure (Hemenway *et al*. 1986 and 1990).

In the study by Hemenway *et al*. (1986), rats were exposed to 0, or 36 or 81 mg.m^{-3} quartz or 73 or 58 mg.m^{-3} cristobalite for 8 days followed by observation for 180 days. The study also included exposures to an amorphous silica sample. Very little information was provided concerning the properties of the dust samples, both the cristobalite and quartz being obtained from commercial suppliers. Particle sizes were reported to be less than 5 μm, but no information on the particle size distributions/specific surface areas was provided. Quartz and cristobalite were contaminated with aluminium at 21.4 and 2.4 μg/g respectively. The lung response was assessed throughout the observation period by bronchoalveolar lavage (BAL) and histopathological examination.

The findings for each polymorph at both dose levels tested were combined and presented as a single set of results. The findings demonstrated that cristobalite caused a significant lung response. The total number of cells in BAL fluid increased steadily over the observation period, reaching a maximum at day 60 (2.5-fold increase over control levels immediately on cessation of exposure). Similarly, the proportions of neutrophils (up to 70%) in BAL fluid and levels of lung hydroxyproline (up to around 150% of control) were also substantially increased at all time points (hydroxyproline is a constituent of collagen and hence is a marker for fibrosis). In contrast, the response to quartz was similar to control findings in terms of total and differential cell counts in BAL fluid. Even the amorphous silica produced a greater lung response than the quartz. Histopathological examination also revealed a considerable difference betwen cristobalite and quartz. Cristobalite was reported to cause an early and sustained severe inflammatory response characterised by influx of inflammatory cells, enlargement of lymphatic tissue and deposition of connective tissue. With quartz, findings were limited to a 'modest' diffuse interstitial cellular response. Quartz and cristobalite were not tested at the same exposure concentrations, and it is unclear what the relative particle size distributions may have been. Also the results from two dose levels were presented in combined form making it difficult to reliably compare the relative toxicities of these two polymorphs. However, it was clear that under the conditions of this study, cristobalite caused a significant lung response whereas quartz was relatively inactive.

The body of experimental research on quartz indicates that quartz would normally be expected under these experimental conditions to cause a significant lung response and so its apparent inactivity in this study is surprising. The reason for this was not addressed by the authors, but it may be speculated that the greater presence of aluminium on the surface of the quartz sample used compared to cristobalite may have attenuated the toxicity.

A later study by the same workers (Hemenway *et al*., 1990) utilised the same study protocol, as described above. However, in this study rats were exposed to 7, 18 or 44 mg.m^{-3} cristobalite (98% pure with 2% quartz and amorphous silica), or to approximately 55 mg.m^{-3} Min-U-Sil and observed for 180 or 120 days for cristobalite or quartz respectively. The particle size distributions were similar for each type of silica (mass median aerodynamic diameters of 0.7-0.9 μm). Cristobalite and quartz were said to be contaminated to a similar extent with <0.2% 'non-toxic' metals and <20 mg heavy metals/g silica (no further details presented). Lung response was assessed as described above and, in addition, particle clearance was assessed for 125 days post-exposure by determination of silica burden in the lung.

The lung burdens of silica were similar in rats exposed to 55 and 44 mg.m^{-3} Min-U-Sil or cristobalite respectively on cessation of exposure, and at 125 days post-exposure (around 1000 and 600 μg silica/lung respectively). However, the profile of the rate of clearance appeared to vary between the two forms. The lung burden of Min-U-Sil decreased relatively constantly during the observation period. In contrast, for cristobalite, the most rapid clearance occurred during the first 10 days with very little clearance thereafter. It is uncertain to what extent this initial rapid clearance was due to clearance of particles from the tracheobronchial region by the muco-ciliary escalator as opposed to clearance from the deep lung by alveolar macrophages. Tracheo-bronchial clearance is effectively complete within 24 hours post-exposure for particles deposited in this region, whereas clearance of non-cytotoxic particles from the deep lung has a half-time of about 60 days in the rat.

The lung response, indicated by the presence of neutrophil, macrophage and lymphocyte cells in BAL fluid, differed significantly between cristobalite and Min-U-Sil. The two highest concentrations of cristobalite caused a peak inflammatory response at 60 days which decreased to the end of the study. In contrast, Min-U-Sil caused a progressive increase in cell numbers in BAL fluid over the entire observation period. At day 60 and 120, the inflammatory response to 44 mg.m^{-3} cristobalite was approximately 3-fold and 2-fold greater respectively than with 55 mg.m^{-3} Min-U-Sil. Cristobalite caused increased production of hydroxyproline above control and that caused by Min-U-Sil (approximately 3, 4 and 6 mg hydroxyproline/lung for control, Min-U-Sil and cristobalite at 120 days post-exposure). With Min-U-Sil, hydroxyproline was only increased above control levels at 120 days post-exposure.

These two studies were similar in terms of study protocol but did not provide particularly consistent findings. In each study cristobalite caused a significant lung response. However, the total cell influx in BAL fluid was around 5-fold higher in the later study compared to the earlier study despite relatively similar exposure concentrations, although the increase in lung hydroxyproline appeared to be roughly similar in each study. Similarly, the response to quartz was not consistent between studies, causing a clear lung response in the later study but being relatively inactive in the early study. The inconsistency of the findings, particularly with quartz, limits the ability to draw any reliable conclusions regarding the relative toxicities of cristobalite and quartz.

Intratracheal exposure studies

An early study by King *et al*. (1953) compared the pulmonary effects of several silica polymorphs in rats. This study seems to be largely responsible for the long established belief that cristobalite is more toxic than quartz, and hence is reported in some detail below.

In this study, cristobalite was prepared by heating sand for 1 hour at 1620°C for 1 hour without a flux; tridymite was obtained from a silica cement that had long service at approximately 1380°C; fused (amorphous) silica was obtained commercially; the quartz was a Belgian glass sand. Fractions below 2 μm were obtained by repeated water sedimentation; prior to this, the cristobalite, tridymite and fused silica samples were ground in an agate mortar. The composition and purity of the dusts was confirmed using x-ray diffraction.

In relation to particle size characteristics, it appears that samples of a few hundred particles of each type were manually counted and sized. The cristobalite sample showed a slightly smaller particle size distribution than the quartz, and was also reported to have a slightly greater surface (2.6 m^2/g and 2 m^2/g for cristobalite and quartz respectively), but how surface areas were determined was not stated.

Rats (30 males per group) were administered a single dose of approximately 50 mg of fused silica, quartz, cristobalite or tridymite by intra-tracheal injection through an incision in the trachea, followed by serial sacrifices over a 12 month period for histopathological examination. There did not appear to be a control group included in the study. It should be noted that the dose level used, 50 mg, would now be considered excessively large for a study of this nature. Studies in rats have shown that retained lung dust burdens of between 0.5 –1.5 mg (depending on particle size) of "low toxicity" dusts such as titanium dioxide are around the threshold for the onset of the lung overload condition (Muhle *et al* 1990, Morrow 1988). A study presented above in Section 2.2 (Clouter *et al* 2001) showed that 1 mg of DQ12 quartz produced a lung inflammatory response following a single instillation in rats.

There was a high mortality in all groups within the first 120 days of the study, with 10, 17, 18 and 11 deaths in the quartz, cristobalite, tridymite and fused silica group respectively. Because of these deaths it was not possible to obtain equal numbers of rats from each group at each time point for histopathological examinations, which were carried out on both the dead and killed rats. To illustrate this point, there was only one quartz-exposed rat examined between 61-90 days, and this showed grade 2 fibrosis. Over this time period, 14 cristobalite-exposed rats were examined, 13 showed grade 1 fibrosis, and 1 showed grade 3.

The lungs of rats that died were dark and haemorrhagic, some of the animals had lung abscesses. In a few cases the abscesses almost completely replaced the lung parenchyma and these animals were discarded. It is unclear whether these deaths were due to infection or were dust-related, or a combination of both. The tracheo-bronchial lymph nodes of the decedents were enlarged to "several" times their normal size, and were firm and gritty.

The authors used their own descriptive grading system to report the severity of fibrosis observed at each time point; grade 1 indicated the presence of fine reticulin fibrils and grade 5 corresponded to extremely severe fibrotic lesions.

Tridymite appeared to be the most fibrogenic, causing grade 2 fibrosis at 1 month post-instillation, which progressed to grade 5 by 31-60 days. In contrast, the development of fibrosis with cristobalite and quartz progressed considerably more slowly, causing grade 2 fibrosis at 1 and 2 months post-instillation respectively and grade 5 fibrosis at 8 and 9 months respectively. Both the cristobalite and the quartz-exposed rats developed classical silicotic nodules; in the quartz-exposed rats the nodules showed a tendency to agglommerate by 240 days (grade 4 fibrosis); in the cristobalite group, there were confluent areas of fibrosis at this time (grade 5). These findings at this time-point were based on examination of only 1 cristobalite-exposed rat, and 2 quartz exposed rats. In the few rats per group examined at time points after 240 days there was no difference between the fibrosis scores between the cristobalite and the quartz groups; in both groups fibrosis grade 5 was usually scored at these later time points. Fused silica produced grade 3 fibrosis by 210 days, but this did not progress to a higher grade at later time points.

In the authors' discussion, they state that "*quartz and cristobalite acted similarly. Grade 3 fibrosis existed from two or three months up to about seven or eight months, and there was regular progression to grade five. The cristobalite acted slightly faster than the quartz, as all grades were reached about a month earlier*". However, it needs to be borne in mind that very generally very few rats were examined per time point.

This study has a number of shortcomings; the administered dose of 50 mg would now be regarded as excessively high by current standards, and may well have contributed to the high mortality rate in all groups in the first few months of the study; very few rats were available for histopathological examinations over time; and there were unequal numbers of rats per treatment group compared at each time point. Under the conditions of this study, the tridymite sample appeared to cause a more rapid fibrogenic response than either cristobalite or quartz. The fused silica was not as fibrogenic as any of the crystalline silicas; both cristobalite and quartz produced classical silicotic nodules that progressed in size with time, and no clear differences could be discerned between these two polymorphs.

A series of early studies by Brieger and Gross (1966 and 1967) was conducted to investigate the fibrogenicity of quartz, coesite and stishovite in rats following intra-tracheal instillation. In each study, rats (30-50 per group) received a single intra-tracheal instillation of 0 or 30 mg of each polymorph in aqueous suspension and were then sacriced at intervals over a period of 12 months for histopathological examination of the lungs. No information was presented on the preparation of the samples but the average particle diameters were reported to be 0.14, 0.44 and 0.07 µm for coesite, quartz and stishovite respectively. Quartz and coesite, which were tested concurrently in the same study, were reported to cause an initial inflammatory response followed by a gradual development of fibrotic lesions over the observation period. The authors indicate that there were no clear qualitative differences in the extent of fibrosis or production of collagen.

Lung response to stishovite was reported to be minimal, limited to alveolar wall thickening with no evidence of fibrosis. The findings from this study suggest that quartz and coesite were of similar toxicity when instilled at the same mass dose. However, the comparison of the toxicity of quartz and coesite is confounded by different particle sizes resulting in a higher number of coesite particles being instilled. Therefore, from these results it can be concluded that quartz and coesite are both fibrogenic, but no reliable conclusions can be drawn regarding their relative toxicities. The study also provided more evidence to support the view that stishovite is of low toxicity.

Summary of in vivo data comparing polymorphs

Very few *in vivo* studies have been conducted to compare the fibrogenic potencies of different silica polymorphs. Results from a study involving intratracheal dosing indicate that stishovite is less fibrogenic than quartz, and this is consistent with what would be expected based on a knowledge of the surface chemistry of these polymorphs. However, stishovite is of no real occupational significance; it is only quartz and cristobalite to which workers are exposed to a significant extent. In attempting to analyse the limited information available concerning the relative potencies of quartz and cristobalite, it has to be borne in mind (as shown in other sections in this document) that even samples of quartz from different sources show clear differences in their ability to cause pulmonary toxicity. Hence, assuming that as a generality, quartz and cristobalite were of similar fibrogenic potency, it would not be surprising if some samples of quartz were more active than cristobalite in some tests, and less active in others, depending on the source of the quartz and the ways in which the test samples were prepared.

There has been a widely held belief that cristobalite is more toxic than quartz; this can be traced back to an early study involving intratracheal administration of high single doses of these polymorphs. The results of this study indicate that the nature and severity of the lung fibrosis produced by cristobalite and quartz were very similar, and no real differences in the time of onset of these effects could be discerned.

The only other information available on the relative potency of cristobalite and quartz comes from a series of two inhalation studies. However, despite the high airborne concentrations of quartz used in the first study (up to 81 mg.m^{-3}) no pulmonary inflammation was observed. In contrast, similar exposures to cristobalite did cause pulmonary changes. In fact, completely contrary to what would be expected, the quartze was even less active than amorphous silica in this study. The negative results for quartz are not consistent with what would be expected based on the general pattern of evidence for quartz, and it is uncertain whether this single study could be viewed as "representative" of the general comparison between quartz and cristobalite. In the second study, a different quartz sample was tested (Min-U-Sil) and this did produce a pulmonary inflammatory and fibrogenic response, although to a lesser extent and with a different time course of action compared to the cristobalite sample.

The surfaces of quartz and cristobalite polymorphs are similar, possessing a similar surface density of silanol group. Hence, there is nothing in their surface chemistries to suggest there would be a general difference in their biological effects. The very limited amount of animal data available do not point to any significant differences in the fibrogenic potencies of quartz and cristobalite.

2.3. Effect of Other Minerals

2.3a Aluminium

As indicated in Section 1 aluminium cations can bind to ionised silanol groups and mask the toxicity of silica particles *in vitro*. There is consistent and reliable evidence that aluminium can also reduce the *in vivo* toxicity of quartz (reviewed in Donaldson and Borm, 1998 and Brown and Donaldson, 1996). As described in these reviews, a number of studies have demonstrated that quartz-induced lung damage in rats, guinea pigs and rabbits is attenuated, and in some cases eliminated entirely, by simultaneous treatment with aluminium salts. Several informative studies have been conducted in sheep. Compared to pure quartz, aluminium-coated quartz caused a significantly less pronounced inflammatory and fibrotic response following intra-tracheal administration. Indeed, studies in rats and sheep demonstrate that the pulmonary inflammatory response to quartz can be ameliorated by aluminium

even when it is administered after inflammation is established. The presence of aluminium has also been shown to significantly improve the clearance rates of particles from the lungs, clearance half lives of 30 and 145 days being reported for aluminium-coated and pure quartz respectively. The enhanced clearance is suggestive of a lower toxicity towards macrophages facilitating more efficient clearance.

It has been speculated that since clays contain aluminium, the pulmonary toxicity of quartz found in the presence of clay is likely to be reduced. From experimental data available on aluminium this is plausible, although little direct experimental evidence with clays containing quartz is available.

LeBouffant *et al*. (1982) investigated the chronic toxicity of various sands contaminated with clay, in rats. The results provide some useful information on the influence of clay on quartz toxicity during long-term residence in the lung. Sands (over 95% quartz), from various regions in France and Germany, were subject to grinding followed by separation to give a size fraction of less than 3 µm for use in the study. Pure "Madagascar quartz" of the same particle size was used as a reference quartz, although it wasn't specified if this size fraction was obtained by grinding. The sands were reported to contain 'small' quantities of clay, particularly kaolinite and illite, containing various elements, most notably aluminium and iron (up to 12.1 and 1.5 mg/g sand respectively).

Rats received a single intra-tracheal dose of 0 or 30 mg quartz or 30 mg of one of five types of sand followed by serial sacrifices over a 12 month period for assessment of lung weight and lung lipid and collagen formation and lung histopathology. Quartz induced a substantial lung response, causing increased lung weight and lipid (a marker of alveolar epithelial cell inflammation or toxicity) and collagen formation throughout the observation period. In contrast, at 3 months, the sands had only exerted a minimal lung response compared to the pure quartz. However, at 6 months there was evidence of more marked toxicity with the sands, and at 12 months the sands had produced increases in lung weight, lipid and collagen formation considerably above control, approaching levels obtained with pure quartz, as well as causing overt fibrotic lesions. There was no clear relationship between the levels of aluminium contamination of the sands and the latency or severity of pulmonary changes. However, the study report mentions that on-going unpublished studies have demonstrated that removal of aluminium from these sands increases their toxicity, suggesting an important role of aluminium (no further information presented). Overall, this study clearly demonstrated the delayed toxicity of silica-sand, suggesting that the toxicity of the sand was initially attentuated, but that the attenuation was gradually lessened during residence in the lung. The authors speculated that the attenuating agents were clay materials associated with the sand. This conclusion is plausible. However, there was no clear relationship between the degree of toxicity of a sand and its relative level of contamination with aluminium or iron. Therefore, no firm conclusions can be drawn regarding the identity of the agent responsible for the attenuation of the toxicity.

LeBouffant (1982) conducted an additional series of experiments to further investigate the influence of lung residence on the toxicity of 'Nemours' sand. Rats were intracheally instilled with 50 mg 'Nemours' sand or pure quartz. After one year (or 3 months for pure quartz), the dust was recovered from the lung by incineration of lung tissue followed by washing in acid. Of this extracted dust, 30 mg was instilled into additional rats that were assessed at 3 months for lung response. Additional rats were exposed to 0 or 30 mg pure quartz or unadulterated 'Nemours' sand. Sand recovered from the lung immediately following instillation by this extraction method was found to have low toxicity similar to that of unadulterated sand indicating that the extraction method did not significantly alter the surface properties of the sand.

Recovered sand caused an increase in lung weight, lipids and collagen, similar to that of pure quartz, at 3 months, whereas the control Nemours sand caused only a minimal response. These findings provide additional evidence to indicate that prolonged residence in the lung can remove protective agents from the surface of quartz, resulting in an increase in toxic potential.

Taken together these findings suggest that aluminium in clays may attenuate the toxicity of silica to some extent but that this effect is transient and that over time, the protective effect is lost, possibly as a result of aluminium being removed from the surface during residence in the lung.

2.3 b Iron

The paper by Donaldson and Borm (1998) reviews the evidence on the influence of iron on the toxicity of quartz. The inflammatory response in rats following instillation of 3.9 mg quartz was substantially decreased when the quartz was coinstilled with 50 mg iron carbonyl (around 90% less neutrophil influx compared to quartz alone). However, given that iron carbonyl is a synthetic organic compound of iron, not found naturally, this finding cannot be extrapolated to inform on the potential effects of iron found naturally contaminating silica.

Iron in certain valence states can catalyse the production of reactive oxygen species (ROS), via the so-called Fenton reaction. Such increased production of ROS would be anticipated to significantly increase toxicity. In support of this, it has been reported that inhalation of a mixture of iron and freshly fractured quartz caused a greater inflammatory response than of freshly fractured quartz alone. The quantity of contaminating iron is reported to be an important factor, since trace amounts of iron may promote the production of radical species whereas a large excess may reduce such activity, although the details of this phenomena have not been fully elucidated (Donaldson and Borm, 1998).

2.3 c Coal

A number of studies have been conducted to assess the toxicity of quartz when associated with coal dust. These have been reviewed briefly in Donaldson and Borm (1998) and are presented below in more detail.

Inhalation data

LeBouffant *et al*. (1982) investigated the toxicity of quartz associated with coal by inhalation in rats. Rats were exposed to 100 mg.m^{-3} low-quartz coal (0.5% quartz; referred to below as coal), coal occuring naturally with 5 or 15% quartz content (referred to below as 5- or 15%-coal), or to a low quartz coal doped with pure quartz to give an equivalent 5% or 15% quartz content (referred to as "quartz-coal") for 12 months. Serial sacrifices were conducted for 12 months post-exposure for determination of lung weight and collagen formation. The coal dusts were prepared by crushing, and the quartz was reportedly mixed in with this coal (no further details presented). No details on particle sizes were presented.

The amount of quartz in the lung after 12 months exposure was around 8 mg/lung with both types of coal containing 15%-quartz and 3 mg/lung with both coals containing 5% quartz, demonstrating no difference in accumulation of quartz for natural coal or quartz-coals. However, despite the similar lung quartz burden the pattern of lung response differed significantly in the natural and quartz-coal groups.

The lung response to low-quartz, 5 and 15%-coals was similar at all time points. For coals containing 5%-quartz, the lung response was similar at 12 months. However, at subsequent time points the response to 5% quartz-coal was more pronounced (from graphical representation approximately 200 and 300% increase in lung weight and collagen respectively over 5%-coal level at 24 months). The lung response to 15% quartz-coal was substantially greater than 15%-coal at all time

points and the extent of the difference tended to increase with time (from graphical representation approximately 170 and 300% increase lung weight and collagen respectively over 15%-coal level at 24 months). The severity of lung response was greater with 15% quartz-coal than with 5% quartz-coal (approximately 110 and 150% higher lung weight and collagen formation respectively).

Overall, this study clearly demonstrated that the presence of coal can attentuate the toxicity of quartz, but that the coal needs to be very closely associated with the quartz. In coals with a natural quartz content, up to 15%, there was only slight pulmonary toxicity over a 24 month period. In contrast, there were marked increases in lung weight and collagen production caused by coal that had been artificially mixed with 5 and 15% quartz. Hence, the protective effect of coal appears to require a close physical association with quartz.

In a poorly described study by Martin *et al*. (1977), groups of rats were exposed to air, coal dust or coal dust doped with 10% quartz. No details were presented regarding the method of preparation of the coal-quartz mixture or on the particle sizes in the resulting dust. Rats were exposed to 200 mg.m^{-3} coal or 10% quartz-coal daily for a week on alternate weeks for 24 months. At 12 months, coal and quartz-coal both caused a similar increase in lung weight and collagen formation compared to air-exposed control rats. These markers continued to increase at later time points in both groups but the increases were markedly greater in animals exposed to quartz-coal (approximately 180% increase over coal for lung weight and collagen formation). Histopathological findings with coal were limited to the formation of some reticulin fibres with no fibrotic masses. In contrast, with quartz-coal massive nodular lesions with collagen fibres were evident after 18 months exposure. These findings suggest the possibility that the presence of coal may have attenuated the toxicity of quartz initially, but after a period of residence in the lung this protective effect was lost.

In a further poorly reported study by Martin *et al*. (1972), the toxicity of quartz when associated with coal was investigated in rats exposed by inhalation or intratracheal injection (described in next section). In the inhalation part of the study, rats were exposed to 300 mg.m^{-3} quartz-free coal dust mixed with 0 (referred to below as coal), 4, 7 or 18% quartz for 3 months and lung weight and collagen formation were assessed for up to 18 months post-exposure. No positive control with quartz alone was included. At 3 months, lung weights and collagen formation were increased above control levels to a similar extent in all quartz-coal and coal groups. However, at 6 and 12 months clear differences between the 7 and 18% quartz-coal and coal began to emerge, with the increase in lung weight and collagen formation increasing in proportion to quartz level. With 4% quartz-coal, an increase in toxicity compared to that produced by coal alone was only evident at 18 months. At 18 months post-exposure, lung weights and collagen increased compared to that produced by coal in all quartz-coal groups i.e. approximately 1.2, 2.4, 3.8 and 5g for lung weight and 0, 35, 50 and 80 mg collagen for control, 4, 7 and 18% quartz respectively. Histopathological assessment revealed increasing severity of tissue response with increasing quartz content. 'Simple cellular reactions' were seen in lymphatic sheaths with low collagen formation with 4% quartz-coal. Marked fibrosis was observed with 7% quartz-coal, and fibrotic nodules with 18% quartz-coal.

Overall these findings demonstrated that quartz associated with coal expressed toxicity in lung after a period of around 12 months residence in the lung, in proportion to the quartz content of the coal. The delayed expression of quartz toxicity may suggest that the presence of coal transiently attenuated the toxicity of quartz. However, given the absence of a pure quartz exposure group for comparison, no conclusion can be drawn regarding the relative toxicity of quartz in coal compared to pure quartz.

Ross *et al*. (1962) investigated the toxicity of quartz associated with coal by inhalation in rats. Rats were exposed to 0 or 60 mg.m^{-3} low-quartz natural coal mixed with 5, 10, 20 or 40% quartz for up to 17 months and serially sacrificed for histopathological examination. No air-only control or quartz-only exposures were performed. The 5 and 10% quartz-coal mixtures were prepared by grinding the components together, giving particles mostly less than 5μm; 20 and 40% quartz-coal mixtures were prepared by 'thorough' manual mixing.

The amount of lung collagen generally increased with exposure time in proportion to quartz content (approximately 37, 61, 125 and 260 mg/rat in control, 5-10, 20 and 40% quartz respectively after 400 days exposure). In animals exposed to 5 or 10% quartz-coal there was no histopathological evidence of fibrosis after 300 days exposure, but after 500 days exposure there was a slight increase in reticulin formation in the lung and some fibrosis in the lymph nodes. In animals exposed to 20 or 40% quartz-coal for 10 months (longest exposure duration) there was clear fibrosis in the lung and lymph nodes that increased in severity with exposure time and was more severe with 40% quartz-coal. At the end of the exposure period, the lung dust burden was 80-120 and 100-120 mg in the 20 and 40% quartz-coal groups respectively (data not presented for 5 and 10% quartz-coal groups), with no evidence for differential retention of quartz in the lung. However, the authors did suggest that there was more transport of quartz to the lymph nodes in the 40% quartz-coal group (no data presented). Overall, this study demonstrated a clear fibrotic response to quartz-coal mixed dusts. There was clear evidence that coal with high levels of quartz (20 and 40%) caused a marked fibrotic response. At lower quartz contents (5 and 10%) there was a less pronounced response. The lack of a concurrent quartz only exposed group prevents the ability to determine whether this minimal response was due to quartz or a response to inhaled dust. Thus, it is reasonable to conclude that this study demonstrated that the toxicity of quartz is not completely attenuated by coal, even at a relatively low percentage content.

Intracheal instillation

A previous inhalation study by LeBouffant *et al*. (1982), described above, demonstrated that quartz found naturally with coal was of lower toxicity than quartz simply added to coal. To further inform on the nature of this protective effect, quartz was extracted from coal from two different mines by incineration and chemical treatment to remove all traces of coal or other contaminating material. A fraction of extracted quartz particles <10 μm was isolated using water sedimentation. Rats received a single intra-tracheal instillation of 30 mg of the quartz extracted from coal or pure quartz and the lung response was assessed at 3 and 12 months in terms of lung weight, lipid and collagen formation. There was no concurrent control group. The quartz extracted from coal was found to be of similar toxicity to pure quartz at 3 and 12 months. These findings demonstrate that the coal factors responsible for reducing the toxicity of quartz found naturally in coal can be removed and are not irreversibly bound to quartz.

Martin *et al*. (1972) investigated the toxicity of quartz when associated with coal by intratracheal injection in rats, but the study suffered from a poor standard of reporting. Rats received a single intra-tracheal instillation of 50 mg natural quartz-free coal mixed with approximately 0, 4 (8%), 8 (13%) or 20 mg (29% by mass) quartz, or 4, 8 or 20 mg pure quartz. A non-dust exposed control group was not included. Lung weight and collagen content were assessed at 3 months post-instillation. No details were presented regarding the method of preparation of these coal-quartz mixtures and so it is unclear if quartz was simply added to or ground in with the coal dust. The text indicated that the particles used were <3 μm.

Quartz caused a dose-related increase in lung weight and collagen formation, with the increase in collagen being particularly marked at 20 mg compared to that obtained with 50 mg quartz-free coal (80 against 9 mg collagen/lung). Coal-quartz mixtures also caused increases in these parameters in proportion to quartz content (0.84, 1, 0.96 and 1.33 g/100g bodyweight for lung weight respectively and 9.3, 12, 15.8 and 27.1 mg collagen/lung at 0, 8, 13 and 29% quartz respectively), but these increases were much lower than those obtained following instillation of an equivalent mass of quartz alone.

The coal and pure quartz instillations involved significantly different numbers of particles. To investigate whether or not the difference in lung response to coal and pure quartz was not simply a reflection of the different number of particles instilled, an additional instillation experiment was performed. The experiment was poorly described but appeared to utilise the same instillation protocol described above, but with coal substituted by titanium dioxide (TiO_2). The quartz-titanium dioxide dusts caused increases in lung weight and collagen formation of similar magnitude to those obtained with quartz alone, demonstrating that TiO_2 did not attentuate the toxicity of quartz. This finding demonstrates clearly that the attenuation of quartz toxicity by coal is due to coal-associated factors rather than due to a general effect of mixed dust or number of particles instilled.

Additional experiments were conducted to further investigate the nature of the protective agents. Coal and quartz were held for 4 weeks in water-filled chambers separated from each other by a semi-permeable membrane that only allowed dissolved products to pass between the two samples. A quartz positive control sample was similarly treated but in the absence of coal in the other chamber. The resulting quartz samples were instilled into rats (dose not specified) and lung weight and collagen assessed at 3 months post-instillation. Quartz exposed to the water-soluble products from coal was found to cause a substantially less pronounced increase in lung weight and collagen formation at 3 months following intra-tracheal instillation (43% and 6% respectively of that obtained with an equivalent dose of pure quartz). This finding suggests that coal contains water-soluble products which can be removed from coal and adsorb to the surface of the silica particles, inhibiting its toxicity. However, the study did not attempt to identify the chemical nature of these factors. Overall, this study clearly demonstrated that quartz in the presence of coal gave a less pronounced inflammatory response than an equivalent amount of pure quartz following instillation.

2.4 Particle size and surface area

The influence of particle size on the *in vivo* toxicity of crystalline silica has not been well researched. Two recent reviews by Mossman and Churg (1998) and Fubini (1998) cite only one relatively old animal study that provides little reliable information on the influence of particle size. Recent toxicological findings from animal studies with poorly soluble dusts such as titanium dioxide and carbon black suggest that particle size may be an important factor in pulmonary toxicity. It is likely that particle size and surface area are also important for crystalline silica but very little useful information from *in vivo* studies is available to inform on this. The intratracheal study with DQ12 and two workplace samples of quartz outlined in section 2.1 suggest that the greater lung response seen with DQ12 might have been due to its smaller particle size and hence larger specific surface area.

2.5 Freshly fractured versus "aged" quartz

The relative *in vivo* toxicities of freshly fractured and aged quartz have not been studied in detail and only a single relevant study is available.

In a study by Vallyathan *et al*. (1995; following on from a similar pilot study by the same workers, Shoemaker *et al*., 1995) rats were exposed to 0 or 20 mg.m^{-3} freshly fractured (median particle diameter of 0.46 µm) or aged quartz (median particle diameter of 0.53 µm) for 5h/d for 10 days over a 2 week period. Animals were then sacrificed immediately for analysis of BAL fluid and lung histopathology. Quartz was prepared from a single batch by acid washing, to remove any metal contamination, and then fractured in a jet mill before immediate entry into the exposure chamber. Aged quartz was prepared using the same method and stored in air for 2 months. The ageing process caused a statistically significant reduction in surface radicals (by 40%), and the ability of particles to generate reactive oxygen species such as hydrogen peroxide (by 58%) and the hydroxyl radical in aqueous solution (reduced by 20% compared to that for freshly fractured quartz).

No information was presented on particle surface area, but given that freshly fractured and aged quartz were prepared using an identical method from the same batch material it can be assumed that the specific surface areas were the same. It was noted that the jet mill preparation process caused an increased contamination of dust with chromium (58 against <1.2 µg/g), iron (222 against 7 µg/g), manganese (6.6 against 0.93 µg/g) and nickel (25 against 0.56 µg/g) compared to the bulk source material.

Reporting of histopathology findings was limited to details of the severity and distribution of cellular infiltrates in the lung. The authors indicated that freshly fractured quartz caused a more severe and more diffuse cellular influx than aged quartz. Findings in BAL fluid indicated that freshly fractured quartz induced a greater oxidative stress and inflammatory response than aged quartz. For example, although there was a very pronounced, statistically significant, influx of neutrophils, lymphocytes and erythrocytes in both quartz groups compared to unexposed control rats, the influx of cells with freshly fractured quartz was approximately twice as great as that for aged quartz. Similarly, other indicators of cytotoxicity such as protein, lactate dehydrogenase (LDH; a marker of cytotoxicity) and N-acetylglucosaminidase (NAG; a marker of toxicity to macrophage) levels in BAL fluid were consistently greater in the rats exposed to freshly fractured quartz compared with aged quartz. There was also evidence to indicate that freshly fractured quartz induced more oxidative stress in the lung than aged quartz, as indicated by a higher level of lipid peroxidation, greater production of reactive oxygen species (ROS) by phagocytes (35% greater than with aged quartz or control). Also, levels of the anti-oxidant enzymes, glutathione peroxidase, catalase and superoxide dismutase were increased in BAL cells from animals exposed to aged and freshly-fractured quartz although the increase was lower in the latter case (65-80% of levels with aged quartz), an observation which the authors interpreted as indicating a compromising of anti-oxidant defense in rats exposed to freshly-fractured quartz.

The exposures in this study simulated occupational exposures to freshly fractured quartz surfaces, with inhalation of the dust occurring immediately after the fracturing process. Overall, this study demonstrated that freshly fractured quartz caused a significantly greater inflammatory response than equivalent aged quartz. The presence of increased surface radical activity in freshly fractured quartz together with the evidence for increased oxidative stress in the lung, such as lipid peroxidation and depletion of antioxidants, suggests that the increased radical activity on freshly fractured quartz is responsible for its increased toxicity. However, it should be borne in mind that this study was only of two weeks duration and it is uncertain what the longer term consequences of exposure to the freshly fractured and aged quartz might be. It could be predicted that once in the aqueous medium of the lung, freshly fractured quartz is likely to "age" rapidly, becoming indistinguishable from "pre-aged" quartz. Nonetheless, with continual repeated exposures, both theoretical considerations and limited experimental evidence suggests that quartz which has been freshly subject to industrial comminution processes, grinding, blasting etc., will be more toxic than "aged" quartz.

2.6 Summary of evidence from in vivo studies

Few *in vivo* studies have been specifically designed to investigate factors that might influence the cytotoxic and fibrogenic potency of crystalline silica. However, the studies available provide a reasonable body of evidence in support of the view that the potency of crystalline silica does exhibit variability. A single study compared three samples of quartz all of high purity; the two workplace quartz samples appeared to be inactive in the lung, whereas the DQ12 sample clearly elicited an inflammatory response. The reason for this variability is unclear, but (speculatively) may be due to the fact that the DQ12 particles appeared to consist of aggregates of smaller particles, and this may have provided a somewhat greater specific surface area compared to the workplace samples. These results suggest that even among different quartz samples of high purity there are differences in biological activity. However, it needs to be borne in mind that this finding derives from a short-term study, and whether differences in lung toxicity would be observed after long-term repeated exposures remains uncertain.

There is considerable evidence to suggest that surface contamination of crystalline silica can influence fibrogenic potency. Results from studies in sheep and rats reveal that the toxicity of quartz can be reduced by the presence of aluminium salts. Also, studies in rats show that quartz found naturally with coal, or quartz vigorously mixed with coal, has lower fibrogenic activity than unadulterated quartz. The factors responsible for this effect have not been identified but are probably non-coal minerals, such as aluminium-containing clays, which can coat the silica surface. However, the protective effect of these agents is only temporary, and is removed during extended residence in the lung.

Evidence from a single short-term study in rats shows that freshly-fractured quartz causes a more pronounced inflammatory response in the lungs than an equivalent exposure to 'aged' quartz. However, there are no experimental data concerning the comparative lung response to long-term repeated exposure to freshly fractured quartz. Nonetheless, it is likely that continual repeated exposure to freshly fractured quartz would cause greater chronic toxicity than with similar exposures to aged quartz.

There are no studies that have been designed to compare the fibrogenic potency of samples of crystalline silica of different particle sizes. However, from what is known about the toxicology of other dusts in animals, it can be predicted that particles of smaller size and hence greater specific surface areas would have a greater fibrogenic potency than equal masses of coarser particles.

Very few *in vivo* studies have been conducted to compare the fibrogenic potencies of different polymorph of crystalline silica. Results from a study involving intratracheal dosing indicate that stishovite is less fibrogenic than quartz, and this is consistent with what would be expected based on a knowledge of the surface chemistry of these polymorphs. However, stishovite is of no real occupational significance; it is only quartz and cristobalite to which workers are exposed to a significant extent. When considering the question of the relative potencies of quartz and cristobalite, it has to be borne in mind that even samples of quartz from different sources show clear differences in their ability to cause pulmonary toxicity. Hence, it would not be surprising if some samples of quartz were more active than cristobalite in some tests, and less active in others, depending on the source of the quartz and the ways in which the test samples were prepared. The results from a single isolated study could be misleading in terms of providing a "generic" comparison of cristobalite and quartz.

There has been a widely held belief that cristobalite is more toxic than quartz. However, this derives from an early study in rats that study would be considered unreliable by modern standards. The results showed that the nature/severity of the lung fibrosis produced by cristobalite and quartz was very similar; the authors felt that the onset of fibrosis showed a slightly faster progression with cristobalite than quartz. However, given the disparity in the numbers of rats examined per time-point (for example, there was only one quartz-exposed rat examined between 61-90 days, and this showed grade 2 fibrosis. Over this time period, 14 cristobalite-exposed rats were examined, 13 showed grade 1 fibrosis, and 1 showed grade 3) no meaningful comparison of the rate of progression of fibrosis can be made based on such data.

The surfaces of quartz and cristobalite possess a similar density of silanol groups and there is nothing in their surface chemistries to suggest there would be a general difference in their biological effects. Consistent with this, the body of evidence from *in vitro* studies does not show any differences in the biological reactivity of these polymorphs. The limited evidence from *in vivo* experimental studies does not point to any meaningful differences in the fibrogenic potencies of quartz and cristobalite.

Section 3 - Epidemiological evidence

Introduction

This section has two inter-related aims; to determine whether there is actual human experience to support the contention that crystalline silica presents a fibrogenic hazard of variable potency, depending on factors such as the presence of other minerals or the nature of the industrial process. Also, to determine whether a coherent perspective can be assembled from the variety of different risk estimates for silicosis reported in different studies.

The starting point for constructing this section was to make use of reviews by Pilkington *et al*. (1996) and Finkelstein (2000). These authors identified and critically evaluated the studies that in their opinion represent the most reliable sources of information on the exposure-response relationships for silicosis in relation to cumulative exposure (a measure of the sum of exposures to time-weighted concentrations over a period of time). There is reasonably good agreement between Pilkington *et al* and Finkelstein in terms of which studies were considered appropriate for analysis. Therefore this section is largely confined to a consideration of the same studies, with the exception of a couple of more recently published studies (one in Chinese tin miners and an update on the Vermont granite workers, and also a study in UK pottery workers reported in 1998). Also, given that much of the "variable hazard" claims for crystalline silica were founded on observations in coalminers (Donaldson and Borm 1998), this section goes somewhat beyond the scope of the Pilkington *et al* and Finkelstein reviews, and presents a brief overview of the nature of the evidence that there are factors associated with coalmine dust which influence the toxicity of quartz.

The reviews by Pilkington *et al*. and Finkelstein also discuss the evidence for lung cancer in relation to crystalline silica, but this topic is not considered in this document. Almost all of the available epidemiological studies refer to quartz; the two exceptions are the study in diatomaceous earth workers in which there was exposure to cristobalite, and there was also some reference to exposure to cristobalite in the study in UK pottery workers.

The data available derive from studies carried out in a variety of industries. These include coal mining; diatomaceous earth work; tin mining; hard rock mining (including either gold/uranium/molybdenum/lead or zinc); iron foundry work; granite workers; and work in the potteries and the heavy clay industry. A recent study has been reported in US industrial sand workers, this has investigated mortality due to silicosis and lung cancer, and therefore is not included in this review (McDonald *et al* 2001).

As can be seen from the reviews by Pilkington *et al* and Finkelstein, widely different estimates for the risk of developing silicosis have been reported in different studies (see Table 1 in this Section). However, Pilkington *et al* and Finkelstein both indicate that all of the selected studies suffer from limitations/weaknesses either in relation to imprecise estimates of exposure, diagnosis of health outcome, or both. In addition, factors such as differences in study design (cross-sectional or cohort, length of follow-up etc) may also contribute to the apparent variability in reported estimates of risk. It needs careful consideration to determine whether these weaknesses and differences are sufficient in themselves to account for the observed variability in the risks of silicosis reported in different studies.

In a number of studies, estimates of cumulative exposure to crystalline silica were constructed from limited and incomplete exposure data. In some studies, the crystalline silica content of respirable dust was not measured, and assumptions were made about the likely percentages present. In most studies, early dust exposures were measured in terms of particle counts (either by thermal precipitators, impingers or konimeters) which had to be subsequently converted to estimates of gravimetric concentrations; there are likely to be uncertainties associated with the conversions. Many of the available studies refer to underground mining operations (coal, gold, tin etc) and exposure measurements were likely to be largely if not entirely derived from area sampling methods, introducing uncertainty as to how well the data are representative of personal inhalation exposures. The accumulation of factors such as these introduces considerable uncertainty into the estimates of exposure to respirable crystalline silica, and therefore the ability to meaningfully compare quantitative risk estimates from different studies.

In relation to weaknesses in the reported health outcomes, a number of studies suffered from an inadequate period of follow-up for the diagnosis of silicosis, leading to possible underestimates of health risk, given that silicosis is a progressive disease with a long "latent period" for development. In most studies, the diagnosis of silicosis is based on scoring the profusion of pulmonary opacities on chest radiographs, but this is a subjective process, and different readers within the same study often reported very different scores for the same radiographs, raising uncertainties about the health diagnosis. The scoring system used in most studies follows the ILO (1980) 12-point classification system. However, despite this standardised system, there is variability in the definition of silicosis used in different studies. Some studies define silicosis as the presence of ILO category 1/0 or more, others use 1/1 or more, and one study takes the cutoff for scoring as 2/1 or more.

The ILO classification system is based on scoring the profusion of opacities observed in chest radiographs. However, the opacities may or may not be due to fibrotic changes. The scores obtained are used to indicate the degree of pneumoconiosis, and are not specific for silicosis. Scores below Category 2, although indicative of some abnormality, may not necessarily be due to pneumoconiosis, but may be due to age, cigarette smoking, or scar tissue from previous lung infections or diseases. Furthermore, radiographic changes in Category 2 are rarely associated with any functional impairment, although they may well progress to a higher category in the absence of continued exposure. A score for profusion of opacities of Category 2/1 or more has much less diagnostic uncertainty compared to lower categories, and particularly in workers with a history of dust exposure, presents a more reliable diagnosis of pneumoconiosis. Given the importance of the ILO scoring system to this review, a brief explanation of the system is given in Appendix 1 to this document.

All the available studies represent mixed dust exposure situations, in which crystalline silica is only a proportion of the respirable dust present; the reported proportions range from about 10% in Vermont granite sheds to 30% in South African gold mines, and in one study in coalminers there were periods of exposure to respirable dust containing up to 60% quartz generated by cutting into a sandstone seam. The non-crystalline silica content of the dust exposures is often poorly (if at all) characterised in most studies, but most mineral dusts have the potential to cause pulmonary opacities on chest radiography, whether due to fibrosis or radio-opaque dust accumulation. These opacities cannot be distinguished from those caused by nodular fibrosis due to silica. Hence, in some studies there can be doubts as to what extent the scores for pulmonary opacities reflect silicosis as opposed to mixed dust fibrosis. The potential contribution of non-crystalline silica exposures to pulmonary opacities may well have led to an over-estimation of the effects due to crystalline silica in some studies.

Several of the studies selected for review by Pilkington *et al*. and Finkelstein are of cross-sectional design. Such studies provide useful information on the prevalence of silicosis in a given population, but as they represent only a "snapshot" in time, they cannot inform on when silicosis first developed in any one individual, and so are limited in their ability to relate cumulative exposure to disease development. The design of cross-sectional studies may well preclude their ability to reliably inform on the exposure-response relationship for a progressive disease such as silicosis. However, these criticisms do not warrant a complete dismissal of the results of cross-sectional studies from this analysis; it is considered that the results can be used to build up the body of evidence of the risks of silicosis in different industries, as long as care is taken to recognise the limitations of such studies.

There is also an issue concerning the use of the simple metric 'cumulative exposure' which implies 40 years exposure to 0.1 mg.m^{-3} is equivalent to 2 years exposure to 2.0 mg.m^{-3} (both exposure patterns give a cumulative exposure of 4 mg.m^{-3} yrs). The rate of dose-delivery may be an important factor for a cytotoxic dust such as crystalline silica; high absolute concentrations could overwhelm lung clearance mechanisms leading to a greater than expected degree of lung damage than the same total dose delivered more gradually. Thus, absolute exposure concentrations as well as cumulative exposures need to be borne in mind when comparing different studies.

Table 1 Table showing risks of developing silicosis from cumulative exposures to 2 and 4 mg.m^{-3}.years respirable crystalline silica. Modified from a table presented by Finkelstein (2000). Note that the results are not strictly comparable due to differences in ILO category used to define silicosis.

Study (and ILO category of silicosis)	Risk of silicosis (%) with cumulative exposure to		Comment
	2	4	
	(mg.m^{-3}.yrs)		
Miller *et al* **(2/1+)** Scottish coalminers	6 (5*)	30 (15*)	Extensive follow-up. Subsequent reanalysis suggests lower risks at lower exposures*.
Muir *et al* **(1/1+)** Ontario hard rock miners	0.4	1.2	Retrospective cohort study. Cumulative exposures lagged 5 yrs. No follow-up. Lack of agreement among readers. Workers possibly inhaled aluminium oxide as a prophylactic.
Kreiss and Zhen **(1/1+)** Colorado hard rock miners	11	53	Small scale study. X-sec design. Possibly a biased cohort selection. Weak exposure data.
Hnizdo and Sluis-Cremer **(1/1+)** South African gold miners	5	52	Retrospective cohort study. Cumulative exposure likely to be underestimated thus risks may be overestimated. Extensive follow-up.
Steenland and Brown US Goldminers	8	53	Unreliable diagnosis of silicosis - death certificates/bias. Weak exposure assessment. Extensive follow-up.
Chen *et al* **(1/1+)** Chinese tin miners	11	45	Retrospective cohort study. Good followup. Clearly high rate of silicosis but exposure assessment very uncertain.
Graham *et al* **(1/0+)** Retired Vermont granite workers	4 (HSE estimate)	Beyond range of data	Exposures given mainly in years of granite. Data suggest 20-40 years exposure to 0.06 mg.m^{-3} gives very low risk of silicosis.
Ng and Chan **(≥1/1)** Hong Kong granite workers (*irregular opacities*)	6	15	X-sec study. Weak assessments of exposure and of silicosis. Some follow-up.
Love *et al* **(1/0+)** UK Heavy clay industry	0.9 (estimated by Pilkington *et al*)	-	X-sec study. No follow-up. Co-exposures to aluminium from clay minerals.
Cherry *et al* **(1/0+)** UK Pottery industry	0 (n=109)	2% (n=449)	Raw data show very low prevalence of silicosis of low severity.
Rosenman *et al.* **(≥1/0)** Iron foundry workers	2	10	X-sec study. No follow-up - prevalence of silicosis appeared high in long term current workers.
Hughes *et al.* **(≥1/0)** Diatomaceous earth workers	1.1* (low intensity) 3.7 (high intensity)	4 (low) 12 (high)	Retrospective cohort study. Uncertainties as to accuracy of past exposures. No follow-up of leavers. *Low intensity = absolute exposures <0.5 mg.m^{-3}, high intensity = >0.5 mg.m^{-3}.

3.1 Coal Mining

The literature on coal mining is extensive, yet the debate concerning the role of quartz in the development of coal workers pneumoconiosis (CWP) and Progressive Massive Fibrosis (PMF) is not completely resolved. The key issue for the purposes of this section is whether there are factors associated with coalmine dust that protect against the toxicity of quartz. An early theory was that the quartz content of coalmine dust was likely to be the main factor responsible for the development of CWP and PMF. However, this theory was shown not to be supported by observations in the UK, France and Germany, of lower prevalences of CWP in mines with a higher % quartz content compared with mines with a lower % quartz, even at otherwise similar levels of exposure to respirable dust. A review by Donaldson and Borm (1998) highlights the apparent discrepancy between the quartz content of coal and its ability to cause pneumoconiosis.

In an effort to understand more fully the unexplained reasons for colliery-specific differences in the prevalences of CWP, an extensive programme of research was initiated in Europe in the 1970's to investigate the "specific harmfulness" of respirable coalmine dust, which included the aim of elucidating the role of quartz. A brief overview of the research programme is presented by Reissner *et al* (1982) and many papers on the progress of individual studies within the programme were published in Inhaled Particles V (1982). The programme included epidemiological studies, autopsy studies, mineralogical analyses, and *in vivo* and *in vitro* toxicity studies.

The main conclusion to emerge from the epidemiological studies is that the rank of coal (% carbon) appears to play an important role in the development of pneumoconiosis. This was independently concluded by Walton *et al* (1977) in a study of the progression of pneumoconiosis over a period of 10 years in over 3000 miners from 20 UK collieries, and by Reissner *et al* (1982) in studies of the prevalence of CWP and numbers of compensation cases for CWP in ~250 coalmines from different regions in Germany. In general, the prevalence of CWP was found to be higher in mines extracting high rank coal from older carboniferous strata, and decreased with younger coal strata yielding lower rank coals. Respirable dust from the younger strata was found to be higher in ash and quartz content. The explanation put forward to explain these findings is that organic and inorganic materials associated with coal, particularly aluminium-containing clay minerals, can become adsorbed onto the quartz surfaces, thereby reducing its toxicity. It has been suggested that it is the amount of "free" or clean quartz surface not intergrown with other minerals which is of importance in terms of pathogenic potential. This may account to some extent for the generally lower rates of CWP in mines extracting low rank coals. However, the report by Reissner *et al* (1982) also contains a suggestion that the respirable dust from some mines extracting high rank coals is of smaller particle size; it may be the case that there are a number of variables which may affect the expression of quartz toxicity in coal mines; these would include particle size, amount of "free" or clean quartz surface, and extent of contamination with coal and other minerals.

Furthermore, although the pattern of evidence pointed to a role for coal rank in the development of CWP, Walton *et al* (1977) noted that the progression of pneumoconiosis can also vary significantly in workers from collieries extracting coal of equal rank, indicating that there are as yet other unexplained reasons for colliery-specific differences in pneumoniosis rates. These authors advised that until the reasons for the differences between collieries were elucidated, the simple mass of respirable coal dust, unadjusted for composition, should remain the most suitable index of dust hazard as long as the quartz content does not exceed 7.5%. The cut-off of 7.5% was chosen because the highest percentage of quartz in any of the 20 collieries in this UK study was 7.8%. HSE notes that the study of Walton *et al* (1977) was probably not robust enough to reliably support this conclusion, in particular, the study only covered a 10-year period and given the long latency for silicosis development a longer follow-up would have been preferable. Therefore, the recommendation can be seen as more of a pragmatic approach than one firmly supported by scientific evidence.

HSE notes that weaknesses can be identified in the epidemiological studies in this programme, in particular, in relation to the assessments of exposure. Well founded exposure assessments are needed to support any meaningful comparisons of the risks of CWP from one colliery to another. However, there is a lack of reliable measured quantitative data concerning cumulative exposures to respirable quartz in coalmines (with the exception of only one atypical study by Miller *et al* 1998 outlined below). The data that exist for any particular mine on the % quartz in respirable coalmine dust generally do not reflect the marked day-to-day variations that can occur. Data from different parts of the mines were often pooled together to give average estimates of the proportions of quartz present, despite the fact that quartz concentrations were known to vary a lot, even from different faces in the same mine. Therefore, it is not surprising that a distinct role for quartz in CWP has been difficult to discern. To some extent, the lack of evident relationship between CWP and the percentage of quartz present in coalmine dust may simply reflect the fact that the quartz measurements were not very accurate, and/or that the wrong metrics were being used (particle counts generally up to the 1960s).

Nonetheless, despite the weaknesses and difficulties with the available data outlined above, the consistency of the findings from the UK and German studies is reasonably convincing for a role of coal rank in pneumoconiosis development, and there seems to be a plausible explanation to account for the seeming paradox of reduced toxicity with increasing quartz content. Therefore, it would seem inappropriate to totally dismiss the consistency of the findings from the UK and German studies.

Overall, the situation seems to prevail that a clear and reliable view of the risk of silicosis due to the specific presence of quartz in coalmine dust cannot be articulated from current knowleddge. The risks of CWP in different mines do not appear to relate simply and directly to the presence of quartz, and there may well be some interaction between quartz and other minerals. It is also possible that differences in particle size distributions of respirable quartz dust from different mines may also be a factor capable of influencing the risk of CWP, but this possibility has not been adequately investigated. Epidemiological obserations support the view that there are factors or contaminants associated with coal that may reduce the toxicity of quartz. However, during long-term residence in the lungs, it is possible that surface contaminants could be removed from quartz particles, particularly in the aggressive environment within alveolar macrophages. Experimental evidence from *in vitro* and animal studies suggests that with time, the protective effect of non-quartz minerals eventually wears off (see section 2.3c). This may help to explain the progression of CWP observed in retired miners.

3.2 Study in Scottish Coalminers

This study informs on the consequences of periods of exposure to respirable dust containing very high percentages of quartz. Respirable coalmine dust normally contains a small proportion (usually <10%) of quartz or silicates, mostly from dirtbands within the coal stratum. The source of the increased quartz exposures in this study was sandstone strata between which the coal lay. Therefore, it should be emphasised that this study is quite unrepresentative of exposures to quartz that occur in typical coalmining situations, where the quartz particles have been more closely admixed with the coal minerals. The study was originally reported by Miller *et al* (1995, 1998), and the results were subject to further statistical analysis by Buchanan *et al* 2001).

A series of medical surveys was carried out at a particular Scottish colliery between 1954-78 as part of the British coal board's Pneumoconiosis Field Research programme; the mine closed in 1981. Detailed exposure measurements dating back to the 1950's were available for coalmine dust and respirable quartz. Up to 1965, dust particles were counted using a standard thermal precipitator, and after that using MRE gravimetric samplers for respirable dust. A mass-number conversion calibration method was used to convert earlier particle counts into gravimetric mass. Compositional analysis of the respirable dust samples for each occupational group was carried out with infra-red spectrophotometry. Estimated (quarterly) mean quartz concentrations were available for each occupational group from the product of quarterly dust concentrations and the proportion of quartz in the dust samples for each occupational group. A total of 1416 men attended the 1970, 1974, and 1978 surveys. At the last survey in 1978, attended by 622 men, a high rate of progression of pneumoconiosis scores was observed which prompted follow-up investigations. The unusually rapid progression of the radiographic scores was thought to be more suggestive of silicosis than of simple CWP.

In 1990-91, 547 workers of the 1032 men thought to be alive after the 1978 survey, were traced and agreed to chest radiography. Records showed that in the 1970's, exceptionally high levels of respirable quartz were generated by mechanical cutting into sandstone strata above and below a coal seam (seam B), where >10% of mean exposures exceeded 1 mg.m^{-3} respirable quartz (some exposures as high as 10 mg.m^{-3}). By contrast, at the other seam (A), <10% of exposures exceeded 0.3 mg.m^{-3}. These concentrations refer to mean quarterly values measured over full shift periods, and hence will be equivalent to 8-hour TWAs. The percentages of quartz in the respirable coalmine dust varied over time and according to seam. In seam A, the proportion of quartz never exceeded 15%, but reached up to a maximum of 60% for occupational groups in seam B, and were highest between about 1970-78. Individual cumulative exposures to respirable dust (both quartz and the non-quartz content) were determined for each worker based on knowledge of past occupational history.

Chest radiography revealed that 203 men (38%) showed progression of at least one category on the 12-point ILO scale since 1978, and 47 men had a profusion of at least 2/1. A variety of logistic regression models were fitted to the response 2/1+ using different combinations of variables; the findings showed that profusion of small opacities related more strongly to respirable quartz exposures than to the non-quartz fraction of the dust; inclusion of smoking status did not improve the fit of the regression models. Overall, the results suggested that the radiological progression related to quartz exposures encountered between 1970-78, and that some earlier quartz exposures may also have contributed to the risks. Most of the progression took place after exposure ended, a known feature of silicosis. The results of this study indicate a high risk of silicosis relative to a number of other studies, as indicated in Table 1. Risk predictions from the modelled data suggested that exposure to an average of 0.1 mg.m^{-3} (8-hour TWA) for 15 years would incur a 5% risk of silicosis of category 2/1 or more (note that the later re-analysis of the data described below, led to changes to these risk predictions).

Finkelstein comments that interpretation of this study is difficult because only about 50% of the original cohort was examined at the 1990-91 follow-up, and selection bias may have influenced the risk estimates. However, Miller *et al* noted that the size and direction of the effects observed make it implausible that they could be artefacts of selection or response bias or of imprecision in the exposure estimates which were felt to be much better differentiated than in most other occupational epidemiology. HSE notes that in underground mining, exposure measurements are obtained using area sampling methods, and the relation to personal inhalation exposures may be uncertain. However, in this study, exposure measurements were not entirely based on the statutory area sampling methods, but many were taken as part of the Pneumoconiosis Field Research programme, and personal communication with the study authors reveals that the air sampling equipment used in this programme was suspended above the heads of the workers from support beams, and hence provided a good surrogate of personal inhalation exposure. Furthermore, the airborne dust in the mine was well mixed and was not generated from a point source, again reducing the possibility of unrepresentative air samples.

HSE notes that the measure of radiological pneumoconiosis in this study (2/1+), is more severe than used in other comparable epidemiological studies. This was justified by the authors on the grounds that mis-diagnosis of pneumoconiosis is far less likely with category 2/1 than with lower categories, giving greater specificity, although perhaps less sensitivity, to the results. Furthermore, the authors noted that Category 2 pneumoconiosis is rarely associated with any functional disability, although it is likely to progress in the absence of further exposure. However, given that at the time of the 1990/91 survey (10 years after the mine closure), most ex-workers at that time were 50-75, it is unlikely that there would have been many potential cases missed, as most of the progression would have occurred by then. Overall, HSE feels it is appropriate to use Category 2/1 as indicative of silicosis, given the uncertainties surrounding the interpretation of lower categories.

Finkelstein also comments that due to the very high quartz exposures in this study, lung clearance mechanisms may have been affected. This implies that the use of a simple index of cumulative exposure may be inappropriate, as it does not take into account the potential importance of the rate of dose delivery. However, a more detailed analysis of the exposure-response data from this Scottish colliery has since been conducted which addresses this issue (Buchanan *et al* 2001). This analysis focuses on identifying the risks of silicosis at low levels of exposure, and also considers in detail the additional contribution to risk caused by periods of exposure to high absolute concentrations of respirable quartz (>2 mg.m^{-3}). Logistic regression methods were used to compare the predictive ability of a variety of exposure metrics. Indices based on the square of the concentration gave the best fits. Compared with conventional cumulative exposure indices, the exposure measure based on squared concentrations estimated higher risks of radiographic abnormalities at higher exposures. Thus, cumulative exposure to quartz concentrations above 2 mg.m^{-3} produced over four times the effect of exposure to concentrations below this level. The converse of this is that lower risks are predicted at lower levels of exposure compared to conventional models.

From the study population, the lowest exposure associated with 2/1 pneumoconiosis in any observed case was equivalent to 12.25 years average exposure to 0.1 mg.m^{-3}. From the regression model providing the best fit to the data (which focused on the 37 cases aged 50-75 at the 1990/91 survey), a 2.5% risk of developing 2/1+ pneumoconiosis was predicted from an average exposure to 0.1 mg.m^{-3} over 15 years (cf 5% risk with the previous conventional model). This risk estimate applies to 15 years after exposure ends. To illustrate the additional effects of exposure to higher concentrations, the model predicts a 1.7% risk from an average exposure to 0.1 mg.m^{-3} over 10 years; however, an additional 2 month exposure (presumably 8-hour TWA) to 2 mg.m^{-3} would approximately double this risk, or an additional 12-month exposure to 2 mg.m^{-3} would incur a 63% risk. The analyses suggest that even brief exposures to high concentrations of quartz could produce significant risks of pneumoconiosis. A flexible statistical function (Generalised Additive Model) was used to extrapolate to the risks from lower exposures; the model predicted risks of category 2/1+ opacities of 0.26% and 0.5% for 15 years exposure to 0.02 mg.m^{-3} and 0.04 mg.m^{-3} respirable quartz respectively. The model also predicted a 0.13% risk even at zero exposure. Overall, this study indicates high risks of silicosis in a workforce where exceptionally high airborne concentrations of respirable quartz were generated over an 8-year period, and that the risks were not linearly related to simple estimates of cumulative exposure.

In relation to the variable potency issue, the results from the Scottish coalworkers pertain to a situation in which high concentrations of quartz were generated from a sandstone seam. Exposures would have been to freshly cut surfaces of quartz, which would not have been coated with non-coal minerals under these circumstances. Both the proportions of quartz in the total dust, as well as the absolute concentrations of respirable quartz, were thought to be much higher than in typical coal mining situations. In comparison with the results of other studies, these results are consistent with the possibility that the fibrogenic hazards of quartz are greater where there is exposure to freshly cut surfaces. The results also support the concept of a disproportionate increase in risk where there is a relatively high rate of dose-delivery to the lung. Hence, short-term average exposures to high concentrations (>2 mg.m^{-3}) of respirable crystalline silica are likely to be more detrimental to health than longer-term exposures to lower absolute concentrations.

Figure 1 Graph showing predicted risks of small opacities 2/1+ in Scottish coal miners in relation to cumulative exposure to respirable quartz for workers exposed to absolute concentrations < 2 mg.m^{-3}. Adapted from Buchanan *et al* 2001.
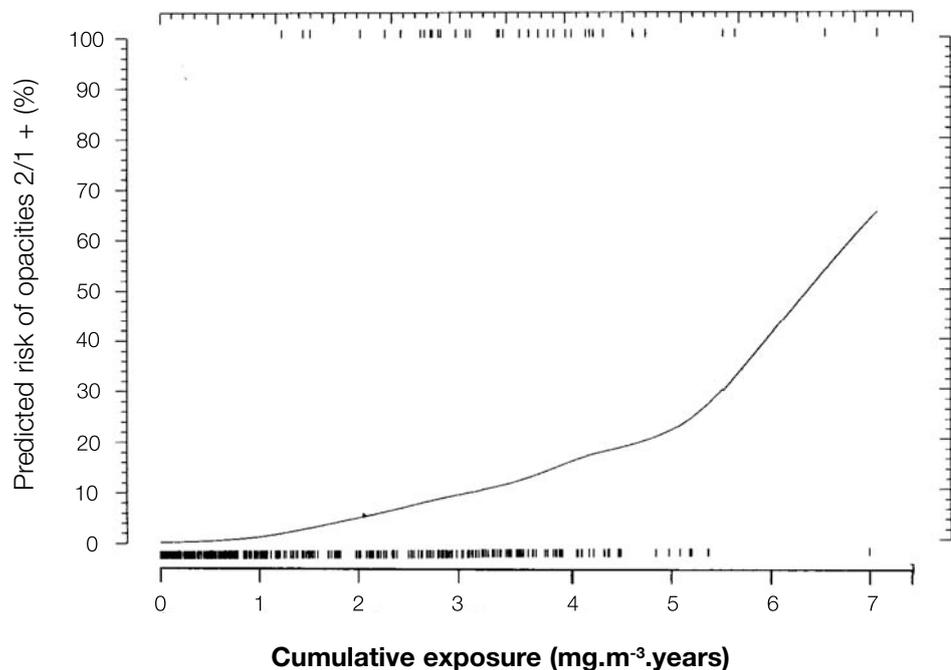
The ticks at the bottom of the graph represent non-cases of silicosis, the ticks at the top represent cases (all for workers aged 50-75 at the last survey in 1990/91). The cumulative quartz exposures refer to exposures over Inter-Survey Periods (ISP) 3-7, which cover 1964-78. Inclusion of earlier ISP cumulative exposures (ISP 0-7, going back to the 1950's) did not improve the fit of the model, suggesting that it was quartz exposures from ISP 3-7 which were associated with the radiographic changes.

3.3 Studies in Hard Rock Miners

Two studies have been described as being from the hard rock mining industry, a retrospective cohort study by Muir *et al*. (1989), and a study by Kreiss and Zhen (1996), described as a population-based cross-sectional survey.

Study in Ontario hard-rock miners
The study by Muir *et al* (1989 a, b) was based on a cohort of 2109 miners from 21 mines in Ontario where ores of uranium and gold were mined. The workers were first employed in the mines between 1940-59, and were followed through to the end of 1982. Chest radiography was undertaken annually on all miners during this time. The films were evaluated by five readers. Silicosis was indicated as the presence of small round opacities at 1/1 on the ILO scale. As pointed out by Pilkington *et al* and Finkelstein there was considerable variability among the readers in their interpretation of the X-ray films. One reader identified 7 cases of silicosis (1/1), but another identified 24, all from the same set of films. Overall, among the 5 readers, 32 cases of silicosis were identified, although not all of the 32 were unanimously agreed.

Historically, dust measurements had been carried out sporadically using konimeters, which basically give an instantaneous reading of the numbers of acid insoluble particles per unit volume of air. It was acknowledged in an accompanying paper (Verma *et al* 1989), that there was some measurement error associated with the konimeter measurements. Over a period of 2 years an extensive side-by side sampling survey had been carried out, comparing konimeter and cyclone/ filter measurements (the latter providing gravimetric measures of respirable crystalline silica using infra-red spectrophotometry). Miners were fitted with a separate cyclone/filter connected to a personal sampling pump for specific tasks, and konimeter samples were taken close to the miner every 15 minutes. The gravimetric sampling did not correspond to full shift 8-hour samples - rather, it was task specific. This meant that quartz analysis had to be based on pooled samples. There was also an attempt to recreate high dust exposures of the past to compare konimeter counts and gravimetric measures in both the gold and uranium mine.

From the side-by side survey, the mean percentage of crystalline silica in the respirable uranium mine dust was estimated to be 8.4%, and in gold mines was 6%. Estimates of cumulative exposure to crystalline silica were constructed for each worker based on individual job history, past konimeter measurements, and using the appropriate conversion factors. No comment was made about the composition of the non-crystalline silica content of the respirable dust exposures, other than to note that the miners were exposed to a number of pollutants, including cigarette smoke, diesel fumes, and a wide range of silicotic rock compounds.

As the authors of the study pointed out, the early konimeter counts in these mines tended to be undertaken because of specific concerns about high dust levels; hence, this may have led to overestimation of true cumulative exposures, and thereby an underestimate of risk.

In the calculations of cumulative risk, cumulative exposures to the time of first diagnosis of silicosis (1/1) were lagged 5 years, as it was assumed that the previous 5 years exposure could not have contributed significantly to radiographic changes. The statistical analysis was a survival analysis using cumulative exposure as the continuum instead of time. Because of the low frequency of silicotics, parametric failure time models were fitted and the Weibull model appeared best suited to the data. Separate cumulative risk estimates were derived for each reader as well as from pooled readings. The pooled readings suggested that the cumulative risk of developing category 1/1 silicosis would be 1.2% with 40 years exposure to 0.1 $mg.m^{-3}$ respirable crystalline silica (i.e a cumulative exposure of 4 $mg.m^{-3}$ years).

However, it should be pointed out that in view of the generally low exposures to quartz in this study, there were few members of the cohort with cumulative exposures greater than 2 $mg.m^{-3}$.years. Therefore, the extrapolated estimates for the risk of silicosis at higher categories of cumulative exposure are extremely uncertain. This should be borne in mind when considering the graph of the predicted exposure-response curve from this study as shown in Figure A4 in the Pilkington *et al* review.

Compared to other studies, this study yields the lowest risk estimates for the development of silicosis in relation to cumulative exposure to quartz. However, the diagnosis of silicosis was only made in current workers, with no follow-up of leavers. It is widely observed that the true risks of silicosis in this study are likely to have been underestimated due to an inadequate period of time for disease development (<18 yrs) which may not have been sufficient to diagnose all eventual cases of silicosis. The authors of the study pointed out the possibility that some miners whose annual radiography revealed abnormalities may have chosen to leave the industry. Again, as there was no follow-up of leavers it is uncertain to what extent this possibility may have led to underestimates of the true burden of silicosis in this cohort. Furthermore, Muir *et al* (1989b) note that workers in this industry used to inhale aluminium powder as a prophylactic against silicosis, although no further information on this practise was provided. The possible influence of this on the incidence of silicosis in this workforce is uncertain.

Overall, this was a well reported study but there are uncertainties in the exposure estimates, the diagnosis of silicosis, the use of aluminium powder as a prophylatic, and the lack of follow-up of leavers. Therefore, the estimates of risk deriving from this study are of doubtful reliability. Finally, in relation to the variable hazard issue, the work activities in these hard rock mines may have involved the generation of freshly fractured surfaces of quartz. However, in view of the limitations in this study noted above, it cannot be used to inform on the question of whether freshly fractured quartz is more hazardous than aged quartz.

Study in Colorado hard-rock miners
The study by Kreiss and Zhen was based on a group of Colorado hard-rock miners aged over 40 years, many of whom had left mining employment. This study appears to show high risks of silicosis relative to other studies (Table 1). However, it should be borne in mind that this was basically a survey carried out at one point in time, and the ability of such a study to accurately identify exposure-response relationships for silicosis is limited. HSE also notes that there are major weaknesses in the exposure assessments for this study that strongly undermine the quantitative risk estimates for silicosis.

The study population consisted of residents of a mining town who were invited to take part in this survey in 1986. 100 of the respondents had worked in mining, and 34 had not, and served as an internal control group. The ex-miners were identified from 149 participants of a previous respiratory survey that had been carried out in this town. No indication was given in the paper concerning what proportion of the retired mining population the study group represented, but it is stated that some of the participants already had a physicians diagnosis of silicosis so there may have been some element of selection bias in this study. The major mine in the area was a molybdenum mine, and smaller mines operated for lead, zinc and gold. No differentiation was given in the paper as to which mines the subjects had worked in.

The only exposure data had been supplied by the owners of the molybdenum mine, and were assumed to be applicable to the other mines for similar job titles (no discussion was given of the likely validity of this assumption). Measurements of respirable dust and crystalline silica had been taken in the larger mine from 1941-1982, just before underground mining operations ceased. Midget impingers were used for sampling until 1976, and gravimetric sampling began in 1974. Due to the absence of any correlation between impinger and gravimetric data for similar tasks, the impinger data were not used, and instead the gravimetric data were retrospectively applied to exposures pre-1974 with no allowance made for the fact that exposures were considered to have been higher in the 1940s. No information was given in the paper concerning how the silica content was analysed, and no information on sampling strategies, durations of sampling, whether sampling was personal or area etc.. Exposures to crystalline silica were estimated in two ways; one was from actual measurements of airborne silica (no details given), and the other was by assuming that silica comprised 12% of respirable dust. This estimate derived from results of 483 paired silica and respirable dust measures, but no details of the side-by side sampling procedures were given. These two methods yielded very different estimates of exposure to silica. Cumulative exposure estimates for each subject in the study were constructed based on applying the exposure data to a knowledge of their job histories.

Among the 100 men in the survey who had worked in hardrock mining, 32 showed pulmonary opacities of 1/0 or more on the ILO (1980) scale, among these men, 18 had ILO 1/1, and 5 showed large opacities. None of the 34 subjects from the control group showed an abnormal X-ray. The risks of developing silicosis were estimated in relation to cumulative exposure to respirable crystalline silica (two sets of estimates for this as explained above, one for "measured" and one for "estimated" silica) as well as to "average silica exposure". A logistic regression model for the risk of silicosis (ILO 1/0) indicated that with an average exposure to 0.05 mg.m^{-3} for 25 years, there would be a 9% risk at the end of employment, rising to a 36% risk at 20 years post-exposure. For comparison with most other studies which determined risks in relation to an X-ray profusion of >1/1, the authors also calculated the risks for this more stringent classification. Using "measured silica data" there was an 80% risk of developing category 1/1 silicosis with a cumulative exposure to 4 mg.m^{-3}.years, falling to a 53% risk assuming a 12% silica content of dust.

Overall, HSE considers that the risk estimates from this study are of very doubtful reliability, primarily because of uncertainties in the exposure estimates, but also because it was a small-scale study, with possible problems in terms of selection bias of the study population. Finkelstein also identified possible selection bias due to over-representation of individuals with lung disorders. However, it should also be noted that this study included a long period of time since first exposure, with an average of 36 years, and therefore probably detected most cases of silicosis in this study population. Hence, this would tend to give rise to higher risk estimates compared to studies with shorter follow-up periods.

In the discussion section of their paper, Kreiss and Zhen discuss the same potential biases as were highlighted by Finkelstein. The authors indicate that historical exposures may have been higher in the past than those used in the calculation of the cumulative silica exposure indices and therefore the resulting risk may have been overestimated. However, they were unable to estimate the extent of possible inaccuracy in these assessments. Therefore, HSE maintains the conclusion that the risk estimates deriving from this study are of very doubtful reliability.

In relation to the variable potency issue, few conclusions can be drawn due to the limitations in this study. Hard-rock mining is likely to involve processes in which workers would be exposed to freshly fractured quartz surfaces generated by processes such as blasting, drilling and crushing. This may partly account for the high risk estimates for silicosis estimated in this study, but would not explain the very low risk estimates derived from the previous study in Ontario hard-rock miners.

3.4 Studies in Gold Miners

There are two studies in gold miners, the study of white South African gold miners by Hnizdo and Sluis-Cremer (1993), and the study of US gold miners by Steenland and Brown (1995). Both studies generate remarkably similar risk estimates, but as discussed below, this is more likely to be a spurious coincidence rather than any meaningful observation.

Study in South African gold miners
The study by Hnizdo and Sluis-Cremer was a retrospective cohort study of 2,235 gold miners with an average 24 years service from 1940 to the early 1970s, and who were followed up to 1991 for the radiological diagnosis of silicosis (ILO category 1/1 or more). The miners had an annual chest X-ray during employment, and most received occasional chest X-rays post-employment. Autopsies revealed the presence of silicosis in 326 of the 934 miners that died since 1971. The radiographs from each miner were read by two readers and the onset of silicosis was defined as the year when opacities of 1/1 of higher were first read. Only the reader whose scores correlated most strongly with the autopsy findings was used for further analysis. For the purposes of comparing this study with the study in Scottish coalminers, the differences in the definitions of silicosis used in these studies need to be borne in mind. The less stringent category of pneumoconiosis used in this study (1/1+) is likely to account significantly for the apparently greater risks of silicosis observed in this study compared to the Scottish study. This comment would of course apply to most other studies in this analysis.

In total, 313 of the miners were identified with radiological category 1/1 (somewhat fewer than identified at autopsy, suggesting a lesser sensitivity of X-ray diagnosis). The onset of silicosis occurred on average after 27 years of service, and in 57% of workers the onset occurred on average after 7 years of leaving employment.

The key problem with this study is that it is limited by substantial weaknesses in the exposure estimates. Estimates of cumulative exposure were based on a single survey of respirable dust levels conducted in the 1960s. The survey was conducted in a random sample of 20 gold mines using a standard thermal precipitator. No information was provided by the authors, or in any of the references cited by the authors, as to whether personal or area sampling methods were used, or the durations of the sampling periods. The numbers and surface areas of the incombustible and acid insoluble particles collected by thermal precipitators were measured. The results of these parameters were used to estimate the mass of respirable dust in mg.m^{-3}. The reliability of the conversion to gravimetric units is uncertain, but is considered by HSE to represent a likely source of error in the exposure estimates. Respirable quartz concentrations were estimated by presuming a quartz content of 30% in respirable dust, quoted by the authors as being typical in South African gold mines. This led to an estimated mean quartz concentration of 0.09 mg.m^{-3}. The assumption of a constant percentage of quartz of 30% is another possible source of error in the estimates of cumulative exposure to respirable quartz, particularly as a report cited by the authors noted that airborne dust levels and the percentages of quartz varied greatly from mine to mine and from one working place to another. The authors indicated that if conditions prior

to the 1960s had been dustier then this exposure estimate would lead to an underestimate of exposure, and hence an overestimate of the risk of silicosis. This possibility is supported by an earlier report cited by the authors which stated that from 1938 through the 1970s the average respirable silica concentrations in these mines would have been between 0.3 and 0.5 mg.m^{-3}, considerably above the 0.09 mg.m^{-3} value used for miners in this study. Since miners in this study were employed from 1947 for an average of 24 years, many in this population would probably have received higher exposures in the past than indicated from the 1960s survey.

Measures of cumulative dust exposure (CDE) in mg.m^{-3}.yrs were constructed based on a knowledge of job history and estimates of respirable dust exposure. Risk estimates for the cumulative risk of silicosis were calculated using similar methods of statistical analysis to those followed by Muir *et al* (1989) were followed. From a graph in the paper, and assuming that the quartz content of CDE was 30%, it can be seen that the cumulative risk of developing silicosis in relation to cumulative exposure to respirable quartz would appear as 5 and 53% for exposures of 2 and 4 mg.m^{-3}.yrs respectively.

In view of the considerable uncertainties surrounding the exposure assessment the silicosis risk estimates are considered relatively unreliable. It seems highly likely that early quartz exposures in this study were underestimated probably by 2-3 fold. Hence, while it is clearly apparent that there was a high proportion of silicosis cases (1/1+) among this workforce, it seems fairly probable that the quantitative predictions of risk in relation to cumulative exposure to respirable quartz are likely to have been overestimated.

Study in US goldminers
The study by Steenland and Brown (1995) was a retrospective cohort study in 3330 US gold miners. The average year of first exposure was 1945; average duration of exposure was 9 years; average length of follow-up was 37 years, with follow-up through 1990. By this time, 1551 workers in the cohort had died, and death certificates were available for almost all decedents. Silicosis cases were identified either by death certificate (if silicosis, silico-tuberculosis, respiratory tuberculosis, or pneumoconiosis was listed as a cause of death or an underlying/contributory cause), as well as from two cross-sectional chest X-ray surveys conducted in 1960 and 1976.

In total, 170 cases of silicosis were identified, 128 by death certificate alone, 29 by X-ray only, and 13 by both death certificate and X-ray. However, about 75% of the living cohort members were not X-rayed in either of the two surveys, so the study may have missed a number of cases. The death certificate information was not backed up by autopsy data, and a physician's diagnosis on the death certificate may well have been influenced by knowledge of past occupational history. Hence, the assumption that silicosis could be reliably diagnosed by death certificate is a major weakness in the study, and means that the results cannot be easily compared to other studies in which the diagnosis of silicosis was based entirely on the standard ILO classification criteria for pulmonary opacities. One further problem with the use of death certificate data is that the time of onset of silicosis could not be determined. Hence, for the purposes of the exposure-response analysis, time of death was taken as the time of silicosis onset, an assumption that seems highly improbable, and is likely to have considerably biased the cumulative risk estimates.

The exposure assessment was only briefly reported. The exposure measurements were in terms of millions of particles per cubic foot (mppcf), and had been converted to gravimetric measures of respirable crystalline silica based on the results of two side-by side sampling surveys conducted in the 1970's. It was assumed that 10 mppcf = 0.1 mg.m^{-3} respirable dust, and the percentage of respirable crystalline silica in the dust was 13% (range of 1-48% based on 82 paired samples). There was no mention of durations of sampling, sampling strategies, or whether it was area or personal sampling, but given that exposures were mainly in underground mining (there was some above ground work such as crushing), it is possible that the underground exposure data derives from area sampling methods. Although the mine continued to operate after 1975, it was said that exposure levels after this time were low, and only 14% of the cohort were still employed after this time. Thus, no job history data were collected after 1975, and estimates of cumulative exposure were based on job history up to 1975. Estimates of cumulative exposure were derived for each worker based on job-specific exposure estimates and job-exposure matrices.

The cumulative risk of silicosis was calculated according to level of cumulative exposure based on standard survival analysis techniques. The results predicted cumulative risks of silicosis of about 8 and 60% for cumulative exposures of <2 and 4 mg.m^{-3}.years respectively. In the opinion of HSE, there are too many weaknesses associated with this study to permit any confident predictions of the risk of silicosis in relation to cumulative exposure.

In relation to the variable potency issue, it is unknown whether there would be exposure factors associated with gold mining which might enhance the toxicity of quartz, nor is it known whether there were co-exposures to aluminium-containing clay minerals which may have reduced the toxicity of the quartz present. Overall, no reliable conclusions can be drawn in relation to this issue.

3.5 Study in Chinese Tin Miners

A recent cohort study reported risk estimates for the cumulative risk of developing silicosis in 3010 workers from 4 Chinese tin mines (Chen *et al* 2001). Each worker was followed up to the end of 1994 with respect to the diagnosis of silicosis. Exposures in the cohort started as early as the 1920s, with most starting before 1960. However, only 5% of the cohort (101 workers with silicosis and 57 without) was first exposed to silica dust before 1950.

From 1963, annual chest radiography was undertaken; workers continued to be radiographed every 2-3 years even after exposure ended. Diagnoses of silicosis were made by radiologists using the Chinese classification system, that was shown in a validation exercise in 1991 to have good correspondence with the ILO scoring system.

Measurements of airborne total mixed mine dust (Chinese total dust - CTD) had been gathered since the 1950s. Concentrations were said to be dramatically reduced after wet dust suppression and ventilation systems were introduced in 1958. Dust samples were gathered using battery operated area samplers operating at a very high flow rate (25 l/min) for periods of about 15 minutes during dust-producing activities.

Cumulative exposure estimates to CTD for each worker were created based on the dust sampling data, and the development of job exposure matrices based on each person's work history. For people without silicosis, exposure to CTD was calculated from the start of mining to the end of employment or to the end of 1994. For people with silicosis, cumulative exposure to total dust was calculated to the time of diagnosis by radiography. Estimates of cumulative exposure to CTD were converted to estimates of cumulative exposure to respirable crystalline silica dust using a conversion factor of 3.6%. The conversion factor was based on a special side-by side area sampling survey in 1989-9 in which the ratio of the concentration of respirable crystalline silica obtained from American nylon cyclones (8-hr TWA) to the paired CTD concentration measured by traditional Chinese samplers (15-min TWA) was determined.

At the end of 1994, 833 of the miners had died, and 16 (<1%) were lost to follow up. Among the cohort, 1015 (33.7%) developed silicosis, of which, 684 (67.4%) developed silicosis after exposure ended (a mean of 3.7 years after). The results showed a clear relationship between the severity of silicosis (category I, II or III) with cumulative exposure to CTD. The cumulative risk of silicosis in relation to exposure to CTD (mg.m^{-3}.years) was estimated using the Weibull distribution model. Cumulative risk increased from 0.001 to 0.917 as exposure to CTD increased from <10 to >150 mg.m^{-3}.years. Using the conversion factor of 3.6%, the range of cumulative exposure to respirable crystalline silica was about 0.2-6 mg.m^{-3}.years. The authors calculated that the risk of silicosis (corresponding to category 1/1 on the ILO scoring system) was less than 0.1% with cumulative exposure to 0.36 mg.m^{-3}.years respirable crystalline silica, rising to 55% with 4.5 mg.m^{-3}.years. From a graph presented in the paper, it appears that cumulative exposures to 2 and 4 mg.m^{-3}.years incur risks of silicosis of 11 and 45% respectively.

Although this was a large-scale study with an extensive follow-up of workers, HSE feels that there are major limitations in the exposure assessments. No information was given concerning the geological conditions in the 4 mines, or on the working conditions (degree of mechanisation etc.) No measured exposure data were available for the period before 1950 although only 5% of the cohort was exposed before 1950 and so this was not thought to introduce a significant bias into the cumulative exposure estimates. A conversion factor of 3.6% was used to determine the percentage of respirable crystalline silica in CTD, but how much mine-to mine or day-to day variation surrounds this figure is uncertain. The percentage is likely to be highly variable, depending for example, on whether workers encounter a sandstone seam or the metal ore itself. The authors acknowledged that the conversion of CTD to respirable crystalline silica was likely to have introduced some uncertainties into the risk estimates, but felt that the risk estimates were similar to those from other large-scale epidemiological studies such as those found in South African gold miners by Hznido and Sluis Cremer (1993).

HSE also notes that dust measures were derived from area sampling, and the relation to personal inhalation exposures is unknown. No precise information was provided concerning the nature of the mining work, but in these underground Chinese tin mines it is likely to have involved (at least to some extent) hand-held tools with workers being in close proximity to the sites of dust generation. The area samplers (said to be placed in three monitoring stations in each mine) may well have underestimated personal exposures, but to what degree is unknown. To some extent this may be counter-balanced by the fact that sampling was only undertaken for short-term peak exposures, which may over-estimate average work-shift exposures. No information was provided on the composition of the remaining ~96% of the total airborne mixed dust, but presumably much of it was tin oxides. Given the high cumulative CTD exposures, the radiographic findings in these workers possibly reflect a certain amount of mixed dust fibrosis, rather than opacites purely due to crystalline silica. No information on general hygiene

conditions in the mines was given, but the possibility that workers lived all their lives in dusty mining areas, taking home workplace dust on their work clothes cannot be ignored. Such factors may lead to an apparent overestimate of the risks attributed to silica.

Overall, this study shows relatively high risks of silicosis in relation to cumulative exposure to respirable crystalline silica, with broadly similar quantitative risk estimates to those reported in South African gold miners (Hnizdo and Sluis-Cremer 1993). Both of these studies included a lengthy period of post-exposure follow-up suggesting that few cases of silicosis would have been missed. This may help to explain the apparently high risk of silicosis in these studies relative to other studies. However, as noted above, there are considerable uncertainties regarding the exposure estimates for both of these studies. The similarities in risk estimates may therefore be a spurious coincidence.

In relation to the variable potency issue, there is no information to suggest that mixed exposure to other minerals may have ameliorated the intrinsic fibrogenicity of crystalline silica in this workforce. The activities in this study involved such processes as drilling, excavating and crushing, which are likely to generate freshly fractured surfaces of crystalline silica. Wet drilling was said to be introduced from 1958, but nonetheless, a significant amount of exposure to freshly cut surfaces of respirable crystalline silica dust is likely to have occurred. This may partly explain the relatively high percentage of the workforce that developed silicosis (~34%).

3.6 Studies in Granite workers

The studies available derive from a cross-sectional study in Vermont granite workers (Graham *et al* 1991), with a follow-up study in retired workers from this industry (Graham *et al* 2001). In addition, there is a cross-sectional study in Hong Kong granite workers by Ng and Chan (1994). Granite is a coarse-grained rock consisting of variable proportions of grains of feldspar, mica and quartz.

Studies in Vermont granite workers
Graham *et al* (1991, 2001) note that a programme of improvements in dust controls (such as wet processing, suction devices, use of non-quartz abrasives) in the Vermont granite industry had been comprehensively introduced after 1938-1940 following a detailed study in that industry published in 1929. The latter study highlighted an epidemic of tuberculosis and silicosis. Although the programme of improvements began in 1938-40, dust levels only gradually stabilised to uniformly low levels within a period of about 15 years.

The study by Graham *et al* (1991) presents the results of an industry-wide radiographic survey conducted in 1983. At this time, all current quarry, stone shed and office workers were offered chest X-rays. Out of a total of 1400 workers, 972 received an X-ray, and work and smoking histories were taken. The X-rays were read by three readers in accordance with the ILO (1980) scoring system. Dust measurements were taken as part of a stratified sampling plan, said to provide a representative distribution of samples from small, medium and large sheds in different occupational groups over a full calendar year (from this, it appears that there were no measurements of exposure in the quarries). Personal samplers were run over an entire shift, and sampled the mass of respirable dust deposited on PVC filters. Efforts to analyse quartz using X-ray diffraction were unsuccessful.

The average dust concentration from 417 samples was 0.6 (+ 0.3) mg.m^{-3}. Using the quartz estimate of 10% in granite dust published by other workers, this suggests that the average exposure to respirable quartz was 0.06 mg.m^{-3} in this workforce. Although historical exposure data were not provided, the impression given in the paper is that working conditions would have changed little since the 1940's, and most workers in the study would have commenced employment after 1940.

The radiographic findings showed that 28 (3%) workers showed abnormalities consistent with pneumoconiosis. This was judged on the basis of 2 or 3 of the readers scoring a profusion of rounded or irregular opacities of 1/0 or more. Most of the opacities were of the irregular type and the grades of profusion were extremely low. Only 4 X-ray films were judged to have profusion scores of 2/1 or higher, and all of these films showed irregular opacities. The authors considered that the opacities might not have been typical of early silicosis, suggesting that they have been due to smoking, age or other causes.

Of the 28 workers with ILO scores of 1/0 or more, the average number of years worked in granite was 31, versus 18 years in those 922 workers with normal radiographs. The workers with abnomal scores were generally older (mean age = 54 yrs) compared with those with normal scores (mean age = 42), and had smoked more. The authors applied various logistic regression models to examine the effects of variables such as age, pack-years of cigarettes smoked, smoking status and years worked in granite on the probability of developing an abnormal radiographic score. The model that appeared the most promising was based on years worked in granite and pack-years smoked. The predicted percentages of abnormal radiographic findings (for 0 pack-years of smoking) were 0.1, 0.2, 0.7, 2.7, and 9.8% for 7.5, 10, 20, 30 and 40 years of granite work respectively. No cumulative exposure indices for respirable quartz were presented. Assuming past exposures to have been relatively constant, and that the percentage of quartz in the granite did not substantially vary from 10%, then it can be roughly judged that there would be an extremely low risk (<1%) of developing silicosis with exposure to 0.06 mg.m^{-3} for up to 20 years. This is particularly so given the mainly low severity of the pneumoconiosis scores in this study.

One limitation of this study is that there was no follow-up of leavers, and the potential for further progression of the severity/prevalence of abnormal radiographs was not known. Another problem is that 23% of the current workforce did not participate in the study. The non-participants were mainly quarry workers and office workers, and it is uncertain whether this may have introduced some bias into the findings.

Subsequently, a study was carried out to assess the prevalence of radiographic abnormalities in 600 retired workers from the Vermont granite industry (Graham et al 2001). In 1996, files of regional clinics and hospitals were searched for chest X-rays taken on this group. Although the most recent X-ray available for each person was used, in some cases this had been taken while the person was still employed in the granite industry.

After excluding women and office workers, 408 X-ray films were read by 3 readers according to the ILO (1980) scheme. The authors note that the 408 X-ray films read for this study represent only a small fraction from a population that numbers in the thousands. However, this group of pensioned ex-workers also represents a group with long life, long latency, and long exposure to quartz, and therefore are probably the most likely ones to develop radiographic silicosis. A diagnosis of silicosis was made based on 2 or 3 readers rating a radiograph as having predominantly upper lobe rounded opacities with a profusion of 1/0 or more. Of the 408 films, 58 were taken in workers hired before 1940, a time prior to the introduction of dust controls

in this industry. Abnormalities were detected in 26% of these films. In contrast, of the 350 X-ray films of workers hired at/after 1940, 20 (5.7%) showed abnormalities. Of the 20 retirees in this group with abnormalities, 17 were of category 1/0 or 1/1, and 3 were category 2 (no further details given, but presumably these were 2/1). Overall therefore, the prevalence and severity of silicosis in the retired men hired after 1940 was quite low. Of the X-ray films from the 350 retirees hired after 1940, 269 were taken during retirement, but the most recent radiograph available for 81 retirees was taken while they were still in employment (pre-retirement). Only one of the 81 X-ray films taken during employment was positive, versus 19/269 taken during retirement. Among the 81 retirees, the average time from hire to date of radiography was 34 years versus 40 years for the 269 retirees.

Among the 350 retirees hired at/after 1940, there was a consistent decline in the prevalence of silicosis with increasing date of hire; in the 28 men hired 1940-44 there were 5 (17.9%) abnormalities; in 106 men hired 1945-49 there were 8 (7.5%) abnormalities; in 96 men hired 1950-54 there were 4 (4.2%) abnormalities; in 52 men hired 1955-59 there were 2 (3.8%) abnormalities; in 68 men hired >1959 there was 1 (1.5%) abnormalities. The mean years worked across these groups declined steadily from 37.6 - 20.5 years. The authors concluded that as dust levels were known to have stabilized after 1955 to 0.05-0.06 $mg.m^{-3}$ respirable quartz, that prolonged exposures to these low levels were likely to lead to only a very low prevalence of silicosis.

There are some limitations to this follow-up survey, one being that 81 of the 350 X-ray films of retired workers were actually taken pre-retirement, and so there may have been some underestimate of the eventual development of silicosis. Nonetheless, the overall picture to emerge is one of a low cumulative prevalence of silicosis in workers in an industry where dust exposures had been well controlled for many years. The cases of silicosis identified by chest radiography were also of a generally low severity as judged by the profusion of opacities. Given that exposures to respirable quartz appeared to be almost uniformly low in these Vermont granite shed workers, the findings from these two studies cannot usefully inform on the likely risks of silicosis development from higher exposures. However, the findings are of use in helping to characterise the effects of long-term cumulative exposure to low average airborne concentrations of respirable quartz.

Study in Hong Kong granite workers
The study by Ng and Chan is well described in the Pilkington *et al* review. In summary, 206 current workers from two granite quarries (91% of total workforce) and 132 of former workers (49% of surviving past workers) agreed to take part in this survey in 1985. One quarry began operating in 1946, and the other in 1955. Individual estimates of cumulative exposure to respirable silica were constructed based on knowledge of job history and job-specific measures of dust exposure. Exposure measurements were based on a limited number of surveys, and prior to 1980 were obtained with impingers; the particle count data were converted to estimates of respirable dust using a conversion factor derived from findings in the Vermont granite quarries. No indication of how relevant the conversion factor might be for the Hong Kong granite quarries is given, which introduces considerable uncertainty into the exposure assessment. The mean quartz content of the respirable dust was determined to be 27% based on a 1982 survey of both quarries.

The average estimate of cumulative exposure to respirable quartz was 5.35 mg.m^{-3}.yrs, with a maximum of 17 mg.m^{-3} years. The average age of the 338 current and ex-workers in the study was 48 years, suggesting that even though the average duration of employment was 17 years, there may not have been adequate time for the development of silicosis. No information was given in the paper concerning the average length of time since first exposure.

The prevalences of chest X-ray scores for profusion of opacities >1/1 were given separately for rounded and irregular opacities for each of the 3 readers, and there were quite marked differences in the scores reached across the 3 readers. The reported prevalences of rounded and irregular opacities were 10% and 16% respectively across the entire study population, and for those >50 yrs old were18 and 26% respectively. In men over 50, the prevalence of small opacities was only related to cumulative exposure; no smoking effect was shown for 1/1 opacities whether rounded or irregular.

Separate linear and logistic models were used to determine the risks of rounded and irregular opacities only for workers >50 years old. The linear model was thought to provide a better fit to the data, but both models suggested about a 6% risk of rounded opacities (1/1+) at 2 mg.m^{-3} years. However, both models predicted about a 5% risk even at zero exposure; this did not seem to be explained by smoking because the risks of opacities, whether rounded or irregular, appeared to be independent of smoking habit. The authors suggested that this non-threshold effect may have been due to imprecision in the historical exposure estimates. A graph was presented in the paper of the predicted risks according to the linear and logistic models; it was unclear which lines represented which model, but from the line which appeared to give the best fit to the data (presumably from the linear model) it can be seen that cumulative exposures of 2, 4, 6 and 8 mg.m^{-3}.years are predicted to lead to risks of rounded opacities (1/1+) of 6, 9, 12 and 15% in workers >50 yrs. Higher risks were reported for irregular opacities; 6, 15, 20 and 25% respectively for the same levels of cumulative exposure.

Finkelstein makes the comment that only 49% of surviving ex-workers agreed to take part in the study, so that there was an incomplete follow-up of workers. Furthermore, it was known that silicosis was cited as the cause of death in 17 of 53 former deceased workers, suggesting possible underestimates of risk in the high exposure groups. HSE agrees with this comment, particularly as the average duration of follow-up appeared inadequate in this study for true identification of all eventual cases of silicosis. There are limitations in this study in relation both to the diagnosis of health outcome (unclear position regarding the disparity between the rounded/irregular opacities, no other study considered these opacities separately, and it is unclear why a single overall score for the combined profusion of opacities was not presented), and the exposure assessment. The cross-sectional design of the study precludes the ability to determine the precise nature of the exposure-response relationship. Therefore HSE feels that little confidence can be attached to the numerical risk estimates.

The prevalence of silicosis in this Hong Kong workforce was higher than for the Vermont granite shed workers; this is likely to be due to better control of dust exposure in the latter industry. It is difficult to compare the risk estimates from these two workforces for similar levels of exposure mainly due to the uncertainties in the exposure data for the Hong Kong study, but also because the Vermont study does not usefully inform on the consequences of exposures above absolute concentrations of about 0.06 mg.m^{-3}.

Exposures in both workforces are likely to have involved some exposures to freshly fractured surfaces of quartz, which might suggest a greater degree of hazard than with "aged" surfaces or surfaces coated with clay containing minerals. However, in view of the limitations attaching to both studies, little can be said regarding the variable potency issue for exposures to quartz in the granite industry.

3.7 Study in the Heavy Clay Industry

One study is available for workers in the heavy clay industry (Love *et al* 1994, 1999). This was a cross-sectional survey of 1925 workers in 18 heavy clay sites in Britain, conducted in 1990-91. The factories included in this survey had been selected because working processes had changed little during recent decades, thereby giving some credence to the estimates of cumulative exposure. The factories manufactured non-refractory products such as bricks, pipes and tiles. The factories were described as using either soft clay, marl, soft mud, or harder shale-based clays. There was unlikely to have been any conversion of quartz present in the clays to cristobalite, as the products would not have been held in the kilns and furnaces for long enough for this process to occur. More than 1400 personal samples were collected for respirable dust and the quartz content was determined (mainly by either infra-red spectrophotometry or by x-ray diffraction). Chest-radiography was carried out on current workers at each factory. A respiratory questionnaire was also administered to investigate symptoms such as chronic bronchitis but details of this are not covered in this document. The average age of the workers was 40 years; information on durations of employment in the heavy clay industry was not specifically provided.

Concentrations of respirable dust and quartz (presumably 8-hr TWA) ranged from means of 0.4 and 0.04 mg.m^{-3} for non-process workers, to 10 and 0.62 mg.m^{-3} for kiln demolition workers respectively, with a mean of 0.11 mg.m^{-3} for respirable quartz. 10% of all respirable quartz exposures exceeded 0.4 mg.m^{-3}. The percentage of quartz in the dust samples ranged from 6-20%, depending on the feedstock and processes carried out at each site. Estimates of cumulative exposure to respirable dust and quartz were determined for each worker, taking account of the exposures in each occupational group, kiln type, and the factory sites. Cumulative exposures to respirable quartz ranged from 0.01 –10 mg.m^{-3}.yrs.

Chest radiographs were interpreted by 3 experienced readers in accordance with the ILO (1980) scoring system. There was some divergence of scores across the 3 readers, but overall, it was reported that in 25 workers (1.4% of workforce), the profusion of small opacities was graded >1/0. In 7 of these 25 workers, the profusion was > 2/1. These 7 men had worked in the heavy clay industry for between 9 and 37 years, and were aged 29-61 years. Review of the abnormal chest radiographs by a further chest physician indicated that the pattern of small opacities was not typical of silicosis, but was more like a mixed dust type of pneumoconiosis. Statistical analyses revealed that the risks of having opacities >1/0 differed by site, and were influenced by age, smoking and cumulative exposure to dust and to quartz. Although exposures to dust and to quartz were highly correlated, logistic regression suggested that radiological abnormality was associated more so with quartz rather than dust.

As described in the review by Pilkington *et al*, the predicted prevalences of opacities 1/0+ in non-smoking workers exposed to 0.1 mg.m^{-3} respirable quartz for 10 and 20 years were 0.2 and 0.9% respectively.

Only 1.4% of the workforce showed evidence of opacities >1/0, which given that 10% of exposures exceeded 0.4 mg.m^{-3} respirable quartz, may suggest a relatively low risk of silicosis in this industry. The authors suggested that this might be due to the presence of the clay minerals used in this industry such as illite and kaolin, which may reduce the toxicity of quartz. However, it is important to note that the chest X-rays were only taken on current workers, with no followup of leavers. The study did not clearly report information on durations of past employment, so whether or not there was adequate time for the development of silicosis is unclear. From a table in the report it can be seen that >80% of the workforce was less than 55 years old, so this adds to the concern that there may have been insufficient time allowed for silicosis development across this workforce. For the small numbers of workers who did show radiological abnormalities >1/0 in this study, the point in time at which these abnormalities developed is not known and hence the cumulative exposures to quartz which may have caused the appearance of these abnormalities is uncertain. Overall, it is doubtful whether any reliable risk estimates for the development of silicosis in relation to cumulative exposures to quartz can be derived from this study.

In terms of the variable potency issue, the very low prevalence of opacities as well as the low severity of the radiological scores observed in this workforce is consistent with the view that aluminium from the clays used as raw materials reduces the toxicity of quartz. Also, there is unlikely to have been significant exposure to freshly cut surfaces of quartz in this workforce. Therefore, the combination of exposure to "aged" surfaces and co-exposure to aluminium may be factors that are to some extent, responsible for the low observed prevalence of silicosis in this workforce. However, this was only a cross-sectional study with no follow-up of leavers, and therefore may have underestimated the true potential for eventual silicosis development. It is uncertain whether the protective effect of aluminium (if real) might wear off post-employment, due to the possibility of improved pulmonary clearance of aluminium from the lungs compared to quartz. Longer-term follow up of leavers from this workforce would be needed to address these questions.

3.8 Study in Pottery Workers

A study was undertaken to investigate the risk of lung cancer in a cohort recruited from numerous UK pottery companies (Cherry *et al* 1998). Within the study, a sub-cohort of 1080 workers was identified and used to test whether the exposure estimates were related to radiographic changes in a reasonable way. This "pneumoconiosis sub-cohort" was restricted to men with at least 10 years of service within the potteries, beginning before 1960. Although this study was not primarily designed to investigate the quantitative relationships between silicosis development and cumulative exposure to crystalline silica, it has been included within this assessment because it is the only study informing on the risks of silicosis in the pottery industry, and as such, helps to extend the available body of evidence concerning the risk of silicosis in different occupational settings.

The entire cohort for this study was identified from records held by the Department of Social Security (DSS), which had been obliged to keep a register of any employee working in specified trades/processes exposed to dust. Workers on the register were given chest radiographs every 4 years from 1948. Men were excluded from the cohort if it was identified that they had been exposed to dusts outside of the potteries e.g. in coal mining.

In relation to exposure estimates, the study report indicates that extensive records were available covering various parts of the industry. Prior to the late 1960s, dust sampling methods relied upon static (breathing area) samples for particle counting. After this time personal sampling methods were used, involving a cyclone to collect respirable dust followed by analysis of gravimetric silica mass. Measurements of airborne respirable crystalline silica fell in the range 0-0.8 mg.m^{-3}, but were mainly between 0.05 -0.2 mg.m^{-3}, (8-hour TWA). In early years, records were sparse, but it was stated that all samples were randomly taken and did not reflect exceptionally high exposures. Particle counts from the static samples were converted to gravimetric measures by assuming that 1 million particles per cubic foot equals 0.09 mg.m^{-3} respirable dust. This conversion has been reported in the literature for the dusty trades of North Carolina, and has been used in a number of studies. Job titles were used to indicate similar levels of exposure, and an exposure matrix was established covering 11 different process groups for the each decade between 1930-1992. The matrix showed that in general, there was a downward trend in exposure levels with time, presumably reflecting improvements in control measures. It was unclear from the study report how the percentage of crystalline silica in respirable dust was estimated.

For the members of the pneumoconiosis sub-cohort, work histories (derived from clinical records) were used to determine cumulative exposures to crystalline silica up to the time of the first positive reading (> 1/0) or, if normal, to the end of employment or to 1992 if still employed. Work at any time in a firing or a postfiring occupation (secondary shaping or glazing) was also recorded as these jobs potentially entailed exposure to cristobalite or tridymite caused by sustained heating above 1000°C.

In the pneumoconiosis sub-cohort, 64 (5.9%) of the 1080 men had at least one radiograph read as > 1/0 for small opacities on the ILO classification. Of the 64, 21 had a reading > 2/1. No information was provided on the numbers of medical readers used to score the chest radiographs, or on the distribution of the shapes (rounded or irregular) of the opacities. Some scores dated back to radiographs taken prior to 1980, and the ILO classification criteria were revised after that time. It might have been better had the earlier radiographs been re-read so that all scores were based on the same classification criteria.

On average, across this sub-cohort, there was about 30 years between starting employment and the date of the first positive radiograph. The overall results for smokers and non-smokers combined showed prevalences of opacities > 1/0 of 0, 2, 6.4, and 16% for cumulative exposure categories of <2, 2- 3.99, 4 - 4.99, and >6 mg.m^{-3}.years. Within the results however, it was clear that the prevalences were about twice as high as in non-smokers as in smokers. Unconditional logistic regression analysis was used to determine the odds of developing small opacities (>1/0) using cumulative exposure as a continuous variable. This showed that cumulative exposure was strongly related to the risk of small opacities. The odds ratio was 1.38 (95% CI 1.24 - 1.53) for each increase in cumulative exposure of 1 mg.m^{-3}.years. Adjusting for smoking status slightly reduced the exposure effect but did not change the conclusions drawn. Interestingly, in this sub-cohort, work in the firing and post-firing jobs did not seem to increase the probability of radiographic changes. Hence, there was no evidence for any increased risk with exposure to cristobalite compared to quartz.

As noted above, this study was not primarily designed to investigate silicosis risks, rather, the pneumoconiosis sub-cohort was constructed to test the validity of the exposure matrix used in the lung cancer investigation. In relation to the accuracy of the exposure assessment, from the paper itself it is unclear what proportion of the respirable dust in the workplace was crystalline silica, and it is uncertain how much error may have been associated with the conversion of past particle counts

to gravimetric measures of crystalline silica exposure. There is also uncertainty regarding the accuracy of case identification for pneumoconiosis in this study. From the way the risk of small opacities has been calculated in relation to cumulative exposure (odds ratios), it is difficult to compare the risk estimates with those from other studies, but from the raw results, it does seem that the prevalences of pneumoconiosis scores for particular categories of cumulative exposure are low compared to most other studies (only a 2% prevalence of >1/0 pneumoconiosis in workers with cumulative exposures up to 3.99 mg.m^{-3}.years cf Table 1 in this section). It is uncertain whether or not this may reflect a possible overestimate of past silica exposures.

Alternatively, when considering the "variable potency" contention, it is possible that the relatively low prevalence of pneumoconiosis observed in this sub-cohort may reflect the fact that most exposures to crystalline silica in this industry are likely to have involved "aged" surfaces, which may be less reactive and cytotoxic than freshly cut surfaces of crystalline silica. Furthermore, in the pottery industry, the clays used as raw materials are likely to have contained aluminium silicates; the presence of aluminium-containing minerals appears to have the potential to reduce the toxicity of crystalline silica. Also in relation to the variable potency issue, the results showed no suggestion of an increased risk with exposure to cristobalite compared to quartz.

3.9 Study in Iron Foundry Workers

Rosenman *et al* (1996) investigated the risks of silicosis in iron foundry workers in a cross-sectional study. The foundry had been producing automotive engine blocks since 1949. The cohort comprised 549 current hourly paid workers, 29 current salaried workers (presumably "white-collar" or office staff, but who had been formerly hourly paid workers) and 497 retired workers. About half of the cohort had worked at the factory for at least 20 years. "Early" (not defined) exposure data were obtained using midget impingers to yield estimates of total dust for specific tasks or in certain areas. These were converted to gravimetric estimates of airborne silica exposure based on the average percentage of quartz in bulk samples, and assuming that 1 mppcf = 0.09 mg.m^{-3}. No information on how more recent exposures were measured was given. Individual estimates of cumulative exposure to quartz (mg-day/m^3) were calculated from the converted impinger data and knowledge of job-histories.

No chest X-ray was available for 107 subjects, and 13 radiographs were unreadable. The radiographs were interpreted by three readers according to a NIOSH standard form; it was not stated whether this corresponds to the ILO scoring system, although from the scores presented it seems to correspond. It was stated that the "most recent" radiograph was interpreted for each worker, but no further details were given (hence it is unknown just how "recent" the X-rays were). The chest X-ray findings of 8 workers were consistent with asbestosis (linear opacities in the lower lobe), and 24 had pleural changes again suggesting asbestos exposure. Round opacities in the upper lobes (>1/0) were found in 28 workers, of which, 25 were consistent with "simple" silicosis and 3 with progressive massive fibrosis. The X-ray changes consistent with asbestosis showed no relation to estimates of cumulative exposure to quartz, whereas the X-ray changes consistent with silicosis showed a trend of increasing prevalence and severity with increasing cumulative exposure and with increasing absolute mean exposure levels. For workers with cumulative exposures greater than 2160 mg-days/m^3 the prevalence of silicosis was about 12%. Assuming 286 working days per year this cumulative exposure would be about 7.5 mg.m^{-3}.yrs (equivalent to about 30 years exposure

to 0.25 mg.m^{-3}). In relation to duration of employment, 6% of workers with 20-29 years of service, and 12% of workers with 30 years of service had X-ray changes consistent with silicosis.

The risks of silicosis were not evenly distributed among the jobs and departments within the foundry. The "high risk" areas were identified as the cleaning room removing sand from the metal products, making cores, making molds, and performing core knockout. It was also noted that the prevalence of silicosis was notably higher in smokers than in non-smokers in this cohort.

The authors commented upon weaknesses in the study. In particular, it could not be ascertained from the chest X-rays just when silicosis first developed in any one individual. Nonetheless, calculations of cumulative exposure were estimated up to the time of the survey, and therefore could have overestimated the true cumulative exposure that caused the silicotic changes. This is a common problem with cross-sectional studies, and generally precludes their ability to reliably identify exposure-response relationships for a chronic disease such as silicosis.

HSE considers that no reliable quantitative risk estimates can be drawn from this study, partly because of the inherent design of the study, but also because of a weak exposure assessment, and a lack of clarity in the paper concerning precisely when the chest X-rays were taken for each individual. However, qualitatively, the findings suggest that prolonged exposure to crystalline silica in this occupational setting leads to substantial risks of developing silicosis. In relation to the variable potency issue, exposures to quartz in this industry are likely to have comprised exposures to "aged" quartz used in the casting of molds, but also to freshly fractured silica surfaces generated in the fettling process. The authors also inferred that silica flour had not been used in this foundry. It is likely that there would have been exposures to iron in this workforce, and there is some experimental evidence that iron can influence the toxicity of crystalline silica (see Section 2.3b). However, the nature of this influence appears to vary with the form of the iron (valence state) and the amount present. Hence, it is impossible to judge whether the co-exposures to iron in the iron-foundry workers may have had an interaction with exposures to quartz. Overall, no reliable conclusions can be drawn concerning the variable potency issue from this particular study.

3.10 Study in Diatomaceous Earth Workers

Occupationally, quartz is by far the most commonly encountered polymorph of crystalline silica. However, in industries or processes that expose silica to high temperatures (~1000°C) such as in furnaces or kilns (for instance in the pottery industry) or during calcination of diatomaceous earth (DE), there is also the likelihood of exposure to significant amounts of cristobalite.

The only relevant quantitative information on the risks of silicosis from exposure to cristobalite is from a study in the Californian DE industry (Hughes *et al*., 1998). In this industry, DE (amorphous silica from the skeletons of microscopic organisms) is calcined to form a product typically consisting of 10-30% crystalline silica, predominantly in the form of cristobalite. If a flux is added during calcination the proportion of cristobalite rises to 40-60%. Early studies in this industry, cited by Hughes *et al*, showed high prevalences of pneumoconiosis in DE workers, particularly in those known to be more highly exposed to the calcined dust.

The study by Hughes *et al* (1998) was a retrospective cohort study of 1809 workers from a single plant in California and from whom chest X-rays had been obtained periodically during employment. The cohort members had worked at the plant for at least 1 year between 1942 and 1987. The details of how individual estimates of cumulative exposure to respirable dust and to crystalline silica were derived were given in another paper (Seixas *et al* 1997). Briefly, routine exposure measurements based on a combination of personal and area sampling were undertaken from 1948 in the plant, and consisted of a mixture of particle counts, as millions of particles per cubic foot (mppcf) determined using light microscopy from impinger samples, and gravimetric measures of total and respirable dust. Conversion factors were used to convert measures in mppcf to mg.m^{-3} respirable dust. These were modified to produce estimates of exposure to respirable crystalline silica based on knowledge of the product mix (DE, calcined, or calcined with a flux) and job histories for each worker. Exposures prior to 1948 were estimates, taking into account historical records suggesting exposures were substantially higher in the early 1940's. Although an extremely thorough explanation was provided on how the exposure estimates were derived, the authors noted a considerable number of uncertainties due to working from occasionally incomplete datasets of uncertain quality. However, a more detailed exposure profile was provided compared to most other studies. It was known that there had been various uses of chrysotile asbestos at this plant, but there was a low prevalence of pleural changes seen at chest X-ray (1.3% and 2.6% in those exposed and unexposed to asbestos respectively), and the prevalence of opacities was greater in those unexposed to asbestos. These findings suggest that asbestos exposure was unlikely to have affected the scores for pulmonary opacities.

The results revealed a relatively low prevalence of silicosis overall (opacities of ILO category 1/0 or more in only 4.5% of workers). The risk of opacities showed a clear relation to cumulative exposure to respirable crystalline silica. However, the risk increased more steeply for the <35 year age category than for the 45+ age category. It was reasoned that workers could only enter the high cumulative exposure categories at a relatively young age if they had been exposed to high absolute concentrations of respirable dust. Hence, a more detailed analysis was undertaken, which indicated that the risk increased more steeply in workers exposed to high absolute concentrations of cristobalite (>0.5 mg.m^{-3}) compared to those exposed to lower levels (<0.5 mg.m^{-3}). For cumulative cristobalite exposures of 2 and 4 mg.m^{-3}.y, the risk of opacities was 1.1 and 3.3% respectively in workers exposed to concentrations < 0.5 mg.m^{-3} (referred to as low intensity), and was 3.7 and 12.4% respectively in those exposed to concentrations > 0.5 mg.m^{-3} (high intensity).

However, an alternative explanation for the findings noted by the study authors is that there may have been an underestimation of the absolute exposure levels for the high exposure group. Furthermore, as pointed out by Finkelstein, the risk estimates from this study may be underestimates of the true risk because the period of follow-up for diagnosis of silicosis in the DE workers in this study was too low, with no follow-up of retired workers. HSE feels that there may be further difficulties with the interpretation of this study; the chest radiographs in these workers showed poorly defined opacities. A previous cross-sectional study in this same workforce (Harber *et al* 1998) supported this observation. Harber *et al* suggested that the radiographic findings were unlikely to be silicosis, but were more likely to represent a mixed dust fibrosis. However, Hughes *et al* clearly indicated that they interpreted the radiographic findings as silicosis.

HSE considers that due to the acknowledged weaknesses in the exposure estimates, the uncertainty as to whether or not the chest radiographs indicated silicosis as opposed to mixed dust fibrosis, and the fact that there was no follow-up of leavers, no firm reliability can be placed upon the risk estimates derived from this study.

In relation to the variable potency issue, this is the only study providing quantitative information on the risks of silicosis in workers exposed to cristobalite. There is a widespread belief that cristobalite is more fibrogenic than quartz, but the relatively low prevalence of ill-defined pulmonary opacities in this workforce, even allowing for uncertainties in the exposure estimates and the lack of long-term follow up to allow radiographic progression, certainly does not point to any evidence for a greater degree of toxicity with cristobalite compared with quartz. It is unknown whether there would have been exposures to aluminium-containing materials or other factors that may have influenced the expression of toxicity of cristobalite in this occupational setting.

3.11 Analysis of epidemiological evidence

The aim of this section is to determine what conclusions can be drawn from the available epidemiological evidence concerning the "variable potency" contention. A subsequent section (3.11b) attempts to construct the most reliable view possible on the exposure response relationships for silicosis.

Marked differences in the risk of developing silicosis have been reported in different studies. To illustrate this point, the predicted risk of developing silicosis with a cumulative exposure of 2 mg.m^{-3}.years (equivalent to 20 years exposure to 0.1 mg.m^{-3}) was estimated at 0.4% (in Ontario hard rock miners) and 11% (in Colorado hard rock miners). Such variation in risk estimates might be considered to reflect the contention that the fibrogenic potency of crystalline silica is variable depending on the presence of other minerals and the nature of the industrial process. However, most of the variation is considered to be due to inaccuracies in the exposure assessments and/or diagnosis of silicosis as well as differences in the definitions of silicosis used. This is emphasised by the fact that even some studies from the same type of industry yielded very different risk estimates.

3.11a Summary of epidemiological evidence relating to the "variable hazard" issue

Occupational exposure to crystalline silica occurs in a wide variety of industrial settings, in which the surrounding features of exposure, such as the generation of freshly cut surfaces, particle size and presence of other minerals are believed to be factors which are all capable of influencing its pathogenic potential. However, in general, the importance of these key variables has not been specifically investigated in epidemiological studies. The only possible exception to this relates to coalmining; a number of studies were conducted in Europe in the 1970's that provided some information on how the toxicity of quartz might be reduced by the presence of other minerals. The key conclusions which can be drawn from epidemiological studies are bulleted below:-

Particle size: There is no direct documented information from any epidemiological study, or from comparisons of different epidemiological studies, concerning how differences in particle size might influence the ability of crystalline silica to cause silicosis in workers.

Polymorphic form: Two occupational studies inform on the ability of cristobalite to cause silicosis. One study in diatomaceous earth workers suffered from certain limitations, mainly the lack of adequate time for follow-up of workers, but certainly showed no suggestive evidence for any enhanced fibrogenic potential of cristobalite compared to quartz. A study in pottery workers showed no increased risk of silicosis in those workers with the greatest potential for exposure to cristobalite compared to those mainly exposed to quartz. Overall, there is no evidence from human experience for any differences in the fibrogenic potency of quartz and cristobalite.

Freshly fractured surfaces: Most studies derive from industries in which there would have been abrasive processes such as grinding, blasting, drilling, all of which would have generated freshly cut surfaces of crystalline silica. Hence, the ability to discriminate between the effects of aged" as opposed to "freshly generated" surfaces is extremely limited. However, studies in heavy clay and pottery workers both reported relatively low prevalences of pneumoconiosis, and both are unlikely to have involved significant exposure to freshly cut surfaces of quartz. Hence, there is limited support for the contention that there would be lower risks of silicosis development from exposure to aged surfaces compared to freshly cut surfaces of quartz. However, the confounding influence of co-exposure to aluminium-containing minerals in these studies weakens the strength of this conclusion.

Influence of other minerals: There are a number of strands of human evidence pointing to the ability of aluminiun-containing minerals to reduce the fibrogenic potency of quartz. The most convincing evidence derives from observations in coalminers, but even here the evidence is not definitive, and suffers from a lack of reliable data on cumulative exposures to respirable quartz in different mines. The pattern of findings suggests there are factors found particularly in association with low rank coals, which reduce the pneumoconiotic potential of quartz. These factors are believed to be aluminium ions from clay minerals. The potential for the "protective" effect of aluminium to wear off with time post-exposure has not been specifically studied in coalworkers. This possibility might explain the progression of pneumoconiosis in retired coalminers. Low prevalences of silicosis were observed in workers in the heavy clay and the pottery industry (aluminium is present in the clay minerals used in these industries), and in one study in hard rock miners who had inhaled aluminium oxide powder as a prophylactic against silicosis. Although there are limitations and weaknesses in the data available, most signficantly the lack of follow-up to investigate possible radiographic progression in the heavy clay and hard rock workers, the consistency of the observations lends support to the contention that co-exposure to aluminium-containing minerals can reduce the toxicity of crystalline silica.

The Front Summary to this document combines the human and experimental evidence to give an overview of the total body of evidence available on the "variable potency" issue.

3.11 b Exposure response relationships for silicosis

For the purposes of this analysis, it has been decided to identify the most reliable of all of the available studies, and to take this as a starting point for constructing a view of the exposure-response relationships for silicosis development. The study selected is the study in Scottish coalminers (Miller *et al* 1998, Buchanan *et al* 2000); the quality of the exposure data for this study is more detailed and better documented compared to other studies. Furthermore, there was extensive post-exposure follow-up for the detection of silicosis. Also, this is the only study in which the identification of silicosis was based on ILO category 2/1+, and this is judged by HSE to be most appropriate "definition" for silicosis identification based on chest radiography.

As with all of the available studies, there are certain limitations associated with the study in Scottish coalminers; one limitation is the fact that the most of the exposures to quartz were accumulated over a time period of around 8 years, and there is uncertainty about extrapolating the measured cumulative exposure indices to periods of time much beyond this. However, the risk predictions made by the authors of this study have not been extrapolated to periods of time beyond 15 years, which at least allows a more meaningful comparison with other studies.

A recent re-analysis of the exposure-response data focussed on estimating the risks at low exposures, as well as analysing the consequences of periods of exposure to high absolute concentrations. The lowest exposure associated with any case of 2/1 pneumoconiosis observed among the workers in this study was equivalent to 12.25 years average exposure to 0.1 $mg.m^{-3}$. Modelling the exposure response data led to a predicted risk of 2.5% of developing 2/1 pneumoconiosis following exposure to 0.1 $mg.m^{-3}$ respirable quartz over 15 years. This risk applies to a time at 15 years post-exposure, reflecting the period of radiographic follow-up in this cohort. This predicted risk clearly involves some extrapolation beyond the existing data, in the sense that the exposures of relevance were mainly condensed into a time period of about 8 years, but does not involve much extrapolation below the observed cumulative exposure range. Modelling of the data suggested there would be about a 0.5% risk of silicosis with a 15-year exposure to 0.04 $mg.m^{-3}$.

Analysis of data showed that even with brief periods (a few months) of average exposure (presumably 8-hour TWA) to concentrations > 2 $mg.m^{-3}$, the risk of developing silicosis rises steeply. This is likely due to an overwhelming of lung defense mechanisms, setting in train progressive and irreversible processes of fibrogenesis.

Another limitation with this study is the fact that there were few individuals with silicosis at low cumulative exposures, and therefore few data points near the beginning of the exposure-response curve. However, there is another study that appears quite reliable in terms of providing information on the risks of silicosis from low levels of cumulative exposure. This is the follow-up survey of retired Vermont granite shed workers. Among 350 retired workers hired after 1940, the prevalence of pulmonary opacities (1/0+) was 5.7% (n=20). However, only 3 of these retirees (0.85%) had scores of 2/1 (this is more appropriate for comparison with the coalminers study and for meeting HSE's definition of silicosis). The mean years worked in granite for these 350 retirees ranged from 20 to nearly 40 years. Average exposures for this workforce were lower than for the study in Scottish coal miners, and were said to have stabilised to about 0.06 $mg.m^{-3}$ respirable quartz after 1955. The study in Scottish coalminers predicts a risk of 0.5% with a 15 year exposure to 0.04 $mg.m^{-3}$; this looks broadly consistent with the observed situation in the Vermont workers ie a prevalence of opacities (2/1) of 0.85% with 20-40 years exposure to 0.06 $mg.m^{-3}$. The Vermont study refers to an industry where exposures to dust had been tightly controlled for many years and so, given that cumulative exposures to respirable quartz appeared uniformly low across the study population, this study is unable to inform on the risks at higher exposures, and given the cross-sectional design of the study, it cannot reliably inform on exposure-response relationships. Nonetheless, the low observed prevalence of silicosis in this workforce is considered "real", and not undermined by inaccuracies in exposure assessment or health outcome.

Studies with lower reported risks than in Scottish coal miners
There are six studies that suggest lower risks of developing silicosis than reported in the study of Scottish coal miners; one in Ontario hard rock miners, one in heavy clay workers, one in diatomaceous earth workers, one in Hong Kong granite workers, one in pottery workers, and one in iron foundry workers.

It may be significant that the first 3 of these studies did not include any follow-up of workers post-exposure, which is likely to account substantially for the lower apparent risks. The heavy clay industry study was a cross-sectional survey, and so is not suitable for an exposure-response analysis. However, 10% of contemporary exposures to respirable quartz were >0.4 mg.m^{-3}, and the overall prevalence of opacities (1/0+) was only 1.4% across the workforce. Tentatively, one might speculate that the low prevalence of silicosis observed in this study may have been due to the aluminium content of the clay and possibly the absence of significant exposures to freshly fractured surfaces of quartz, although the lack of follow-up and insufficient time for the development of silicosis may also explain at least partially the low observed prevalence. A low prevalence of pneumoconiosis was observed in pottery workers in a study with a longitudinal cohort design. There did not appear to be any follow-up of leavers in this study, insofar as the study report suggests all radiographs were taken in current workers. However, as there was an average of 30 years between starting work in the industry and the first radiograph showing an abnormality, this partly reduces the uncertainty surrounding the lack of follow-up. Although there are uncertainties surrounding the exposure estimates for this study, it seems reasonable to assume that the lack of exposure to aged surfaces of quartz, combined with the co-exposure to aluminium-containing clays used as raw materials, may have been factors which reduced the hazards of the quartz present compared to the conditions which prevailed in the Scottish coalminers study.

The study in Ontario hard rock miners found the lowest cumulative prevalence of silicosis compared to any other study, but included no follow-up of leavers, and the average duration of employment (<18 years) may have been insufficient for sillicosis development. The workers in this industry were known to have inhaled aluminium oxide as a prophylactic against silicosis, so as with the heavy clay workers, the co-exposure to aluminium may be a reason for the low reported risk of silicosis. However, very little documented information about the inhalation of aluminium oxide powder is available, and such information as is available is almost of anecdotal nature only. In the study in diatomaceous earth workers (exposures to cristobalite) there had been a thorough attempt to construct reliable cumulative exposure estimates for each worker. However, there were uncertainties in the exposure assessment, including data gaps for historical exposure. There was also no follow-up of leavers in this study such that the true eventual cumulative prevalence of silicosis was unknown. Overall, the point is that there are clear potential explanations for why the reported risks of silicosis were lower in these studies than in the study in Scottish coalminers. Therefore, the lower reported risks in these studies do not undermine those from the coalminers study.

As for the other studies, the Hong Kong granite workers study was cross-sectional in design, and therefore cannot reliably inform on exposure-response relationships. However, analysis of this study suggests it may well have overestimated the risks at low cumulative exposures (due to underestimates of historical exposure), and underestimated the risks at high exposures (due to a bias in the selection of the retired worker population). This might change the shape of the exposure-response relationship bringing it more in line with that from the Scottish coalminers' study. However, given the uncertainties in the historical exposures in this study this would be speculative. In relation to the study in iron foundry workers, 12% of those with more than 30 years employment had radiographic changes consistent with silicosis, suggesting a significant problem with silicosis in this workforce, but there was uncertainty about when the radiographs were taken, and given that it was a cross-sectional study, it is not known what level of cumulative exposure led to these cases of silicosis. Hence, it is very difficult to compare the quantitative risk estimates from these cross-sectional studies with the longitudinal cohort study in the Scottish coalminers.

Studies with similar or higher reported risks than in Scottish coal miners
There are 4 studies in this category; two in gold miners, one in tin miners, and one in Colorado hard rock miners.

The study in South African gold miners included follow-up of leavers and validation of the radiographic silicosis status at autopsy. However, the exposure assessment was weak, being based on a single survey of respirable dust exposure, and the silica content of the dust was not measured at this survey, but was assumed to be present at 30% by mass. As explained in section 3.4, exposures to respirable quartz were likely to have been underestimated, possibly by 2-3 fold, suggesting that the risk estimates were overestimated. If the risk estimates from this study were adjusted to take account of the likely underestimated exposures, then they might more closely approach those from the re-analysis of the Scottsh study. However, this is speculative given the substantial weaknesses in the exposure assessment for the South African study. In the study in US gold miners, the diagnosis of silicosis was partly judged on death certificate information, which as discussed in the main text (see section 3.4) was fraught with interpretational problems and very likely subject to bias. The exposure assessment for this study was weak and was based on a number of unverifiable assumptions; no confidence can be attached to the predicted risk estimates from this study.

The study in Chinese tin miners suffers from a large number of uncertainties in the exposure assessment. Dust exposures in this industry were determined as exposure to Chinese total dust, based on area sampling over 15-minute periods. Various conversions and assumptions had to be applied to convert the Chinese total dust data to 8-hour TWA estimates of respirable quartz. The level of uncertainty in the cumulative exposure indices for respirable quartz is high, and how the data relate to personal inhalation exposures for the workers is unknown.

While there was clearly a high rate of silicosis development in this workforce, the numerical risk estimates are very uncertain, and again no meaningful comparison with the Scottish study can be made.

Finally, the study in Colorado hard rock miners was a population-based survey, and the design of the study does not permit exposure-response relationships to be identified. Among the population of retired miners invited to take part in the study (only 100) some already had a physician's diagnosis of silicosis, so there may well have been some selection bias involved. The miners had worked in a number of different mines in the area (molybdenum, lead, gold and zinc), but exposure data were only available from the larger molybdenum mine and were assumed to be applicable to similar job titles in the other mines, an assumption which is at least questionable, and it would also seem a remarkable coincidence if the silica content in all of these different mines was the same. Overall, the predicted risk estimates from this study are considered to be very unreliable, and it would be meaningless to compare them to the study in Scottish coal miners.

Conclusions
The approach taken in this analysis has been to take the Scottish coalminers' study as a benchmark for comparison with other studies. This is because this study ranks more highly than any other in terms of the reliability of the exposure data and the identification of silicosis cases.

The study in Scottish coalminers suggests a relatively high risk of silicosis compared to six other studies, and a similar or lower pattern of risk compared to four, and for the remaining study, appears to yield a broadly similar estimate of risk for low cumulative exposures. To some extent, a slight pattern emerges in the analysis; those studies that report lower risks tend to either lack adequate follow-up of leavers and/or involved co-exposures to aluminium-containing minerals and/or exposure to aged rather than freshly cut surfaces. In other studies, inaccuracies in the exposure data and diagnosis of health outcome precluded a useful comparison with the Scottish study.

A clear threshold for silicosis development cannot be identified from the available studies. In the Vermont cohort of retired workers, there was one case of silicosis among the 69 workers hired after 1959 (a prevalence of 1.5%); these workers would have had about 20 years exposure to a concentration of 0.06 mg.m^{-3} respirable quartz. In the study in Scottish coalminers, a risk of 0.13% was predicted by the model for zero exposure, but it should be noted that there were no actual cases of silicosis with cumulative exposures less than ~1.15 mg.m^{-3}.years; this would be equivalent to 15 years exposure to 0.076 mg.m^{-3}.

As one moves up the exposure-response curve, a feature of the Scottish coalminers study and the South African gold miners study is that the risk is not linearly related to cumulative exposure, but seems to rise more steeply as absolute exposure concentrations increase. This was also noted in the study in diatomaceous earth workers, although less reliability attaches to this study. The re-analysis of the Scottish coalminers study showed that periods of exposure, even of just a few months, to high absolute concentrations >2 mg.m^{-3} are predicted to pose an extremely high risk of silicosis.

If the exposure-response information from the Scottish coalminers study is accepted as a benchmark, then it needs to be placed in context with other potential industrial exposures. In this study, exposures to quartz were generated by drilling into a sandstone seam; this would have produced freshly cut surfaces of quartz, likely to be reactive and inflammogenic in the lungs. Therefore, the risk predictions might be taken as representative of any workplace situation in which exposures to freshly cut surfaces of crystalline silica might occur. It is possible that where there is exposure to "aged" surfaces, and/or where there is exposure to aluminium-containing minerals, lower risks of silicosis might obtain than observed in the Scottish coalminers' study. This contention is given some support from the study in heavy clay workers and the study in pottery workers. Both studies showed relatively low prevalences of silicosis in relation to estimates of cumulative exposure, and both involved co-exposures to aluminium-containing clays and aged surfaces. However, there are caveats to this conclusion. There was no follow-up of leavers in the heavy clay workers study, and therefore the eventual burden of silicosis development in this cohort is unknown. Furthermore, animal evidence clearly shows that following cessation of exposure, the protective effect of aluminium compounds begins to wear off, and the toxicity of quartz retained in the lungs begins to be expressed. Therefore, for workers leaving industries with co-exposures to quartz and aluminium-containing minerals, it is possible that in the subsequent years post-exposure, there may be some delayed development of silicosis. This may be more of a problem for younger rather than older workers, as younger workers have more time available for eventual silicosis development.

HSE notes that the studies in the potteries and the heavy clay industry both involved exposures to "aged" surfaces and aluminium. Hence, it is not possible to dissect out the individual influences of these factors on the toxicity of quartz.

From the above analysis, it is conceivable that there might be situations where lower risks might obtain than those predicted from the Scottish coal miners, but what of the converse situation? Might there be occupational exposures to crystalline silica that might lead to even higher risks than those observed in the Scottish coal miners? This appears unlikely; the study in diatomaceous earth workers gave no indication of a greater risk of silicosis with cristobalite (although again caution is needed seeing as there was no follow-up of workers in this study). Also, the study in pottery workers showed no indication of a greater risk of silicosis those workers with the greatest potential for exposure to cristobalite. Unfortunately there are no cohort studies of silicosis in workers exposed to silica flour, which might help to inform on the importance of particle size.

If an attempt is to be made to construct an exposure-response relationship for the risk of developing silicosis, then the study in Scottish coalminers appears to provide the most reliable data set compared to other studies. Taken with supporting evidence from the follow-up study in retired Vermont granite workers, it would seem that a low risk of developing silicosis would attach to long-term average exposures to about 0.06 mg.m^{-3}. The numerical risk estimates derived from the Scottish coalminers' study are likely to be representative of the majority of industrial situations where occupational exposure to quartz occurs, encompassing activities which generate freshly cut surfaces of crystalline silica. From the re-analysis of the coalmine data, the following risk predictions are derived:-

Predicted risks of developing silicosis based on a study in Scottish coalminers

15 years exposure to crystalline silica (8-hr TWA) mg.m^{-3}	Equivalent cumulative exposures mg.m^{-3}.years	Risk of developing silicosis 15 years post-exposure as indicated by ILO score 2/1+
0.02	0.3	0.25%
0.04	0.6	0.5%
0.1	1.5	2.5%
0.3	4.5	20%

These risk predictions only apply when exposures do not exceed absolute concentrations of 2 mg.m^{-3}. If absolute exposures exceed this value, then the risk of developing silicosis is likely to rise very steeply.

It needs to be emphasised that the exposures to quartz in this workforce were not typical of most coalmining situations, where quartz is more closely admixed with coal dust. Rather, the quartz exposures derived from cutting into major sandstone seams (almost pure quartz), as opposed to the more typical coalmining situation where the quartz grains are found in dirt bands within the coal strata.

The above Table shows the predicted exposure-response relationship for the risk of developing silicosis in terms of Category 2/1+ profusion of opacities on the ILO (1980) scale. Category 2/1+ has been selected as the index of response because it is regarded as the most reliable basis for identifying true cases of silicosis in large-scale occupational studies. In most studies that have investigated exposure-response relationships for silicosis, lower radiographic scores, either Category 1/0 or Category 1/1, have been used as indicators of silicosis development. However, Category 1/0 represents only a minor radiographic abnormality, and is not necessarily indicative of the development of silicosis; nor would it be expected to be associated with any functional impairment. A key difficulty associated with the ILO score Category 1/1 relates to the subjective nature of the scoring process. There is a particularly high degree of inter-reader variability associated with Category 1/1 which makes it difficult to reach agreement on the number of X-ray films that should be assigned this score. Hence, reaching agreement on what should be regarded as the most reliable position on the exposure-response relationship based on Category 1/1 scores is correspondingly difficult and uncertain.

In contrast, the scoring of Category 2/1 is less subject to problems of reader variability. Furthermore, compared to Categories 1/0 and 1/1, the ILO score Category 2/1 is a more specific, though less sensitive indicator of the presence of silicosis in workers with a history of occupational exposure to crystalline silica.

However, the predicted risks of developing ILO Category 1/0+ based on the study in Scottish coal-miners are provided in order to allow a comparison with other studies. Statistical analysis of the results of this study suggest that 15 years exposure to respirable crystalline silica at 0.02, 0.04, 0.1 and 0.3 $mg.m^{-3}$ (8-hour TWA) would lead to risks of developing Category 1/0+ profusion of opacities of 16, 18, 25 and 54% respectively, 15 years after exposure ceased.

It needs to be considered to what extent the risk estimates presented above would be representative of silicosis risks in other industries? The risk estimates shown apply to conditions in which workers are exposed to freshly cut uncontaminated surfaces of quartz. Such conditions occur in many industries where abrasive processes take place (eg grinding, drilling). Hence, it is suggested that the scope of the risk estimates above should be extended, such that they can be considered to be well-founded approximations of the dose-response relationship for the development of silicosis (of different radiographic categories) in all such situations. However, in some industries, greater or lesser risks (at specified exposure levels) might pertain, depending on the presence of factors ("potency factors") that could influence the toxicity of crystalline silica.

Appendix 1

Comments on the International Labour Organisation (ILO (1980) scoring system for the:

International Classification of Radiographs of Pneumoconiosis

The scoring process involves assessment (usually by at least 3 separate medically trained readers) of the profusion of small opacities seen on chest radiographs according to a twelve-point scale of severity based on written definitions and comparison with standard radiographs.

Categories 0/- and 0/0, the first and second points on the scale, would be considered normal (small opacities would be absent or less profuse than the lower limit of Category 1). Category 0/1 (the third point) is on the borderline between normality and abnormality, and category 1/0, the fourth point, represents definite but slight abnormality. The latter score does not necessarily represent a diagnosis of pneumoconiosis, since ageing, smoking and other diseases can account for the appearance of this score. Category 2/1 or greater is usually understood to represent established pneumoconiosis, and the diagnostic uncertainty is small in dust-exposed workers. The classification score does not necessarily correlate with any clinical impairment in pulmonary function. The size and shape of the opacities is also recorded. The shape can be either round (pqr) or irregular (stu); round opacities are more typically associated with dust-induced fibrosis, irregular opacities can occur as a result of ageing and with cigarette smoking, but can also be a reflection of dust-induced fibrosis. The causes are not distinguishable from the chest radiograph appearance alone. Small and large opacities are usually associated with what is described as "simple" or "complicated" pneumoconiosis respectively.

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