Final report[☼] on a study of the feasibility of research on pathologic types of lung cancer in silica- and radon-exposed miners

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SUMMARY

Background A large collection of histology slides from autopsies of some 29,000 former residents in uranium mining districts of east Germany is currently being catalogued and studied at the German Center for Cancer Research in Heidelberg, Germany. We have explored systematically and in detail whether and how we might gain access to this material, and whether we could use it to test certain specific hypotheses about how exposure to silica dust increases risks of lung cancer.

Main research hypothesis The underlying idea is that silica dusts, or their fibrotic effects in the lung and lymph nodes, might interact biologically with other carcinogenic agents, including ionizing radiation, in a way that potentiates lung cancer risks. The postulated bio-mechanisms would be expected to result in unusual distributions of lung cancer cell types. Those distributions of cell types can be identified only by appropriately designed histo-pathologic (rather than death-certification-based epidemiologic) studies.

Methods With the collaboration of the German authorities responsible for maintenance of the materials and data concerned, we identified a sample of 302 former east German uranium miners included in the autopsy archive. The sample was selected from all those potentially available so as to generate as wide a range as possible of exposures to silica dust and to ionizing radiation.

Estimates of cumulative exposures to silica dust, to ionizing radiation, to asbestos, and to arsenic were obtained by arranging for computerization of very detailed manuscript occupational histories of the individuals sampled, and by integrating these data with assessments, by three individuals familiar with underground conditions at the mines over the years, of mine-, job- and calendar-time-specific concentrations of the occupational pollutants.

More than 2,700 histology slides, referring to 292 of the 302 miners sampled, were retrieved from the archive and were examined independently by four pathologists in the USA. The laboratory examinations were made according to a protocol based on World Health Organization criteria for lung cancer typing.

Results The distribution of time worked in uranium mines of those sampled ranged from a few weeks to 41 y (median 15.7 y). Estimates of cumulative exposures to silica and to radon daughters were correlated but not collinear ($r = 0.73$). Correlations between average intensity of exposure to radiation (WLM per exposure year) and cumulative exposures to arsenic, to asbestos, and to silica were low ($r = 0.20, -0.20$ and 0.23 respectively.) The patterns of joint distributions of occupational exposures suggest that appropriate further sampling from the autopsy archive can provide data that could be used to test the underlying research hypothesis - provided that adequate histologic material is available.

Information on smoking habits was obtained for only 52% of those sampled; most (92%) of them had smoked at one time or another. It is unlikely that more complete data on the smoking habits of the miners of interest can be established from the available records.

All four pathologists were able to distinguish between different pathological types of primary lung cancer in about two thirds of the sampled cases. Concordance between any two of the pathologists in their designations of five lung cancer cell types ranged from 60% to 76%. Most (93%) of the sets of slides included some presenting lymph nodes. About 86% of the cases provided sets of slides that were interpretable regarding the presence or absence of fibrosis at different anatomical sites. The quality of these slides was sufficient for all four pathologists to distinguish between cases that indicated the presence of lymph node silicosis only, parenchymal silicosis only, and the presence of both types of silicosis; but there were some marked differences between the pathologists about which particular cases exhibited the features concerned. Pre-neoplastic lesions were identified on slides for between 10% and 20% of the cases. Levels of agreement between the pathologists regarding which cases were affected was low, ranging from 13% to 32% of cases where either one of two of them recorded the presence of a pre-neoplastic lesion.

Conclusions Access to histologic material that would be required for the research envisaged has been verified in practice and is assured for the future. Laboratory examinations of the material have demonstrated that it can be used for the kind of studies required, but further discussion and standardization of pathologic criteria are necessary before continuing with the work.

Data on occupational exposures of the uranium miners considered, to silica dust and to various occupational carcinogens, are retrievable. The sampling strategy adopted for the feasibility study has succeeded in generating distributions of estimates of exposures that, in principle, would permit rigorous testing of the main research hypothesis. Some adjustment to that strategy should be considered, to increase the proportion of subjects for whom smoking data are likely to be available. The results indicate that a further sample of about 600 individuals from the autopsy archive would provide sufficient statistical power ($\geq$ 80%) to discriminate, at least at a 5% significance level, between the underlying research hypothesis and its negation. We recommend that such studies should be pursued.

ZUSAMMENFASSUNG

Hintergrund Eine große Sammlung von histologischen Schnitten aus Obduktionen von ungefähr 29.000 ehemaligen Bewohnern der Uranbergbaubezirke Ostdeutschlands wird zur Zeit im Deutschen Krebsforschungszentrum in Heidelberg katalogisiert und untersucht. Wir haben systematisch exploriert, ob und wie wir Zugang zu diesem Material gewinnen können und ob wir es nützen könnten, um spezifische Hypothesen zur Frage, wie die Quarzstaubexposition Lungenkrebsrisiken erhöht, zu testen.

Hauptforschungshypothese Die zugrunde liegende Idee ist, daß Quarzstäube oder ihre fibrotischen Effekte in der Lunge und Lymphknoten biologisch mit anderen karzinogenen Agenzien einschließlich ionisierender Strahlung auf eine Weise interagieren könnten, so daß Lungenkrebsrisiken potenziert werden. Die postulierten Biomechanismen sollten zu einer ungewöhnlichen Verteilung von Lungenkrebszelltypen führen. Diese Verteilungen von Zelltypen können nur durch angemessen gestaltete histo-pathologische Studien identifiziert werden (statt durch epidemiologische Studien, die sich auf Todesbescheinigungen stützen).

Methoden In Zusammenarbeit mit den deutschen Behörden, die für die Unterhaltung der betreffenden Materialien und Daten verantwortlich sind, identifzierten wir eine Stichprobe von 302 ehemaligen ostdeutschen Uranerzbergleuten, die in dem Obduktionsarchiv eingeschlossen sind. Die Stichprobe wurde aus all denen gezogen, die potentiell verfügbar waren, um eine größtmögliche Variation von Expositionen gegenüber Quarzstaub und gegenüber ionisierender Stahlung zu erreichen.

Schätzungen der kumulativen Expositionen gegenüber Quarzstaub, ionisierender Strahlung, Asbest und Arsen wurden erreicht, indem sehr detaillierte Berufsanamnesen der einbezogenen Personen computerisiert wurden, und indem diese Daten mit Abschätzungen zu Bergwerks-, Tätigkeits- und Kalenderzeit-spezifischen Konzentrationen der Arbeitsplatzschadstoffe integriert wurden. Die Konzentrationsabschätzungen wurden durch drei Personen vorgenommen, die mit den Untertagebedingungen der Bergwerke während dieser Jahre vertraut waren.

Mehr als 2700 Dünnschnitte, die zu 292 der 302 eingeschlossenen Bergleute gehören, wurden aus dem Archiv gewonnen und wurden unabhängig durch 4 Pathologen in den USA untersucht. Die Laboruntersuchungen wurden entsprechend eines Protokolls durchgeführt, das auf Kriterien für Lungenkrebstypisierungen der WHO basierte.

Ergebnisse Die Verteilung der Zeit, die die Bergleute der Stichprobe in Uranbergwerken gearbeitet hatten, reichte von wenigen Wochen bis zu 41 Jahren (Median 15,7 Jahre). Schätzungen von kumulativen Expositionen gegenüber Quarzstaub und Radontöchter waren korreliert aber nicht collinear ($r = 0,73$). Korrelationen zwischen der durchschnittlichen Expositionsintensität gegenüber Strahlung (WLM pro Expositionsjahr) und kumulativen Expositionen gegenüber Arsen, Asbest und Quarzstaub waren schwach ($r = 0,20$, $-0,20$, bzw. $0,23$). Die Muster von verknüpften Verteilungen von Arbeitsplatzexpositionen weisen darauf hin, daß das angemessene weitere Sampling aus dem

Obduktionsarchiv Daten liefern kann, die zur Überprüfung der zugrunde liegenden Forschungshypothese genutzt werden können - vorausgesetzt, daß adäquates histologisches Material verfügbar ist.

Informationen über Rauchgewohnheiten wurden für nur 52 % der Stichprobe erhalten; die meisten (92 %) von diesen hatten zu irgendeinem Zeitpunkt geraucht. Es ist unwahrscheinlich, daß vollständigere Daten über die Rauchgewohnheiten der Bergleute aus den verfügbaren Unterlagen gewonnen werden können.

Alle 4 Pathologen konnten zwischen verschiedenen pathologischen Typen von primärem Lungenkrebs in ungefähr 2/3 der eingeschlossenen Fälle unterscheiden. Übereinstimmungen zwischen jeweils 2 Pathologen bezüglich ihrer Kennzeichnung von 5 Lungenkrebszelltypen reichten von 60 bis 76 %. Die meisten Dünnschnittsätze (93 %) schlossen einige ein, die Lymphknoten zeigten. Ungefähr 86 % der Fälle boten Schnittsätze, die bezüglich des Vorliegens oder Nichtvorliegens von Fibrose an verschiedenen anatomischen Orten interpretierbar waren. Die Qualität dieser Dünnschnitte war ausreichend für alle vier Pathologen, um zwischen Fällen zu unterscheiden, in denen ausschließlich Lymphknotensilikose, ausschließlich Parenchymsilikose und in denen beide Silikosetypen vorlagen; aber es gab einige ausgeprägte Unterschiede zwischen den Pathologen bezüglich der Tatsache, welche Fälle diese Merkmale zeigten. Präneoplastische Veränderungen wurden auf den Dünnschnitten bei 10 bis 20 Prozent der Fälle identifiziert. Der Grad der Übereinstimmung zwischen den Pathologen bezüglich der betroffenen Fälle war niedrig und reichte von 13 bis 32 Prozent der Fälle in denen jeder der beiden das Vorliegen von präneoplastischen Veränderungen feststellte.

Schlußfolgerungen Der Zugang zu histologischem Material, das für die vorgestellte Forschung erforderlich wäre, wurde verifiziert und ist für die Zukunft gesichert. Laboruntersuchungen des Materials haben gezeigt, daß es für die Art der notwendigen Studien benutzt werden kann, aber weitere Diskussion und Standardisierung der Pathologiekriterien sind notwendig bevor die Arbeit fortgesetzt wird.

Daten bezüglich der Arbeitsplatzexpositionen der betrachteten Uranerzbergleute - gegenüber Quarzstaub und gegenüber verschiedenen Arbeitsplatzkarzinogenen - können gewonnen werden. Die Strategie zur Stichprobenziehung, die für die Machbarkeitsstudie gewählt wurde, war erfolgreich, da Verteilungen von Expositionsschätzungen generiert wurden, die im Prinzip ein rigoroses Testen der Hauptforschungsfrage erlauben würde. Ein gewisses Anpassen dieser Strategie sollte in Erwägung gezogen werden, um den Anteil von Personen, für die wahrscheinlich Daten über Rauchgewohnheiten verfügbar sind, zu erhöhen. Die Ergebnisse zeigen, daß eine weitere Stichprobe von ungefähr 600 Personen aus dem Obduktionsarchiv ausreichende statistische Power ($\geq$ 80 %) bieten würde, um, mindestens auf dem 5 % Signifikanzniveau, zwischen der zugrunde liegenden Forschungshypothese und ihrer Verneinung zu diskriminieren. Wir empfehlen, daß solche Studien durchgeführt werden sollten.

1. INTRODUCTION

This report describes the design and results from a one-year feasibility study. The aim was to determine whether *post mortem* materials stored in a large German autopsy archive could be used for research on how exposure to silica dust, or the presence of silicosis, influences pathologic characteristics of lung cancer in humans.[1]

1.1 The underlying research issue

The question of silica carcinogenicity in humans has been debated at length during the last two decades.[2,3,4,5] Experimental animal studies have shown that silica induces lung cancer in rats, but not in other rodent species, and that the carcinogenicity may be related to the fibrous lesions of silicosis.[6] The latter have been shown to be associated with pre-neoplastic lesions including Type II pneumocyte hyperplasia, adenomas, and adenomatoid lesions. Lung carcinomas in silica-exposed rats tend to be located in the most peripheral airspaces or terminal acini, and the most prevalent lung cancer cell type is adenocarcinoma, in particular bronchioloalveolar carcinomas. Type II pneumocyte hyperplasia and atypical alveolar hyperplasia have been noted in human lung tissue,[7] and there are some (anecdotal) reports that they are associated with bronchioloalveolar carcinomas. We hypothesised, therefore, that peripheral adenocarcinomas might be more prevalent in silica-exposed, or silicotic, individuals than in those without such exposure or silicotic lesions.

A majority of experts advising the International Agency for Research on Cancer (IARC) in 1997 concluded that sufficient evidence had accumulated to justify classification of silica as a human carcinogen.[8] But interpretation of the available epidemiologic evidence remains controversial.[9] In particular, it is not clear whether the association between silicosis and lung cancer reported in some studies should be interpreted as implicating only the silicotic lesions as such, or whether it may be concluded that exposure to silica dust, even in the absence of silicosis, is also associated with increased risk of lung cancer. Epidemiologic studies of these questions in occupationally defined groups are faced with the problem of having to distinguish between the effects of several carcinogens to which workers may have been exposed (for instance, tobacco smoke, radon progeny, asbestos, polycyclic aromatic hydrocarbons) from the postulated effects of silica or of silicosis.[10] The degree to which different studies have been able to resolve these issues convincingly has varied.[11] A further difficulty is that most of the relevant epidemiologic studies have had to rely on death certification data as evidence of lung cancer. Such certification does not normally distinguish between different pathological types of lung cancer. Yet at least one study[12] has suggested that excess lung cancer incidence may be associated specifically with silicosis of the lymph nodes, rather than with other pathologically distinguishable types of silicosis.

The underlying idea motivating the present study was the possibility that silica dust, or silicotic lesions, might exert a carcinogenic effect in the lungs by interacting biologically with other carcinogens.[1]

1.2 Two research hypotheses

Different plausible pathologic mechanisms that are consistent with this general idea may be

expressed as formal hypotheses that are testable in principle, provided that appropriate data are obtainable. We have postulated two such mechanisms and formulated corresponding hypotheses[1] (see also Appendix 1 to the Annex with this report). The hypotheses may be summarized as follows:

> (1) inhaled silica or parenchymal silicosis induces proliferation of pulmonary epithelial cells and thus increases the risk of lung cancer; and
>
> (2) silicosis of hilar lymph nodes impedes clearance of known carcinogens and thus increases their residence time.

Both these formulations imply that silica, or its fibrotic sequella (or both) interact with known carcinogenic agents. Any attempt to test these hypotheses using data on humans would therefore require that the individuals studied are known to have been exposed to known carcinogenic aerosols (other than the suspect silica dust), and that such exposures are quantifiable. Those exposures would be essential covariables in the corresponding analyses of results (rather than nuisance variables or 'confounders'.)

The mechanisms implicit in these hypotheses suggest also that the presence of silicosis, or high exposure to silica, would result in unusual distributions of histologically differentiable types of lung cancer. Variables reflecting these differences in distributions of types of lung cancer can therefore be regarded as response variables in analyses that are intended to test the hypotheses.

1.3 The *'Wismut'* autopsy archive and associated data

The feasibility study was planned in 1994 on the initiative of scientists working with the US National Institute for Occupational Safety and Health (NIOSH). The initiative was stimulated by the publication of an article[13] which suggested that, as a result of the re-unification of Germany, data and materials relevant to the research questions outlined above might be available there. Our further inquiries and discussions with colleagues in Germany established the following more detailed background information relevant to the contemplated research.

> (i) A histo-pathological autopsy archive, referring to some 29,000 individuals who had died in east Germany in the period 1957-1992, was being held in trust at the German Center for Cancer Research (DKFZ) in Heidelberg, Germany.[14] In 1994, work had just started to catalogue that material.
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> (ii) The archive was said to include autopsy reports and histologic slides from at least 5,000 miners who had been employed in east German *('Wismut')* uranium mines up to 1992 and who had died from lung cancer. Some paraffin blocks of lung specimens for those who had died after 1985 had also been preserved.
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> (iii) Very detailed manuscript occupational history records existed for all those who worked in the *Wismut* uranium mining complex from 1946 to 1990. The *Hauptverband der gewerblichen Berufsgenossenschaften (HVBG* - the official

Federal German organization responsible, among other things, for compensating workers for occupational accidents and disease) had access to these confidential records.

(iv) The *HVBG* had established a system for transferring the manuscript records of occupational histories to computer. That system had been used successfully to establish a computer file of occupational histories for several hundreds of thousands of former *Wismut* employees who were still alive in 1992. (That information was being used to help assess eligibility for compensation for occupational diseases in a large-scale and continuing program of medical examinations of surviving former *Wismut* employees.)[15,16] However, manuscript occupational history records for those no longer alive in 1992 (including, by definition, all those for whom *post mortem* material existed in the Heidelberg archive) had not been processed. There were no immediate plans to computerize those data at that time.

(v) The *HVBG* had access to confidential manuscript records of periodic medical examinations of former *Wismut* employees. Those records were said to include some information on individuals' smoking habits - but only for those who were examined medically after 1970.

(vi) Increasingly comprehensive industrial hygiene measurements had been made underground from 1954 onwards, and a variety of manuscript records had been maintained. These included some records of radiation levels, and of konimetric assessments of underground dust concentrations and their quartz content, for the years 1960-1972. Estimates of personal exposures to ionizing radiation and to silica dust were said to have been recorded for all underground miners from 1972 onwards, and for all employed at the mines from 1985. There was no quantitative information about dust and radiation levels during the initial post-World War II period (1946-54), when the concentrations of both these pollutants were known to have been exceptionally high. However, some *HVBG*-sponsored simulation studies, based on re-creation of early working conditions and mining methods, had begun, in an effort to estimate the corresponding dust and radiation levels.

It appeared to us that, collectively, the information and material potentially available might provide a sound basis for testing the research hypotheses outlined above. But it was clear also that, in practice, accessing appropriate pathologic materials and the associated data would present formidable logistic and administrative problems. We therefore arranged with the *HVBG* to conduct a joint feasibility study. The aim was to determine whether the materials and data required for the research envisaged are accessible in practice, and whether the quality and extent of this material would be sufficient to test the hypotheses.

2. THE FEASIBILITY STUDY

2.1 Objectives

Achievement of the broad aim defined above required answers to three types of questions.

(1) Would it be possible to use at least some of the archived histology slides for the kind of laboratory studies that we wished to pursue? That is, would we be able to identify and gain access to a sufficient number of histology slides from individuals who had worked underground in the uranium mines? And would laboratory examination of those slides permit distinctions to be made (a) between peripheral, centrally located, and other types of lung cancer; and (b) between parenchymal and lymph node silicosis?

(2) Will it be possible to use the occupational hygiene data, the individual occupational histories, and the medical records to estimate, at least on ordinal scales, individuals' exposures to silica dust and to known carcinogens (e.g., tobacco smoke, ionizing radiation, arsenic)?

(3) If the answers to questions (1) and (2) are "yes", what kind of sampling strategy and sample sizes would be required to test the research hypotheses?

2.2 Design

An attempt to answer question (1) implied systematic exploration of the material stored in the archive and an effort to access the sub-set of histology slides that referred to an appropriate sample of uranium miners who had died with lung cancer.

To answer question (2) it was necessary to retrieve occupational histories of selected individuals, and to estimate their exposures to silica dust, ionizing radiation, and other carcinogens.

Answers to question (3) required information on the joint distributions of (and hence correlation between) exposures to silica and to ionizing radiation among individuals included in the autopsy archive. The aim was to devise a sampling strategy that could generate a sample that would include informative numbers of individuals with high exposures to one of these agents and low exposures to the other. Thus the initial sample, for the feasibility study, had to cover as wide a range as possible of estimated joint exposures to silica and to radiation.

The original plan (April 1994; see Annex) was to sample some to 200 to 250 individuals from the close to 30,000 for whom manuscript records of autopsy reports were included in the archive. Sampling was to be constrained so as to include only former *Wismut* employees who died after 1970, in an effort to maximize chances of accessing information on smoking habits. The sample was to be stratified according to year of birth and year of death, so as to generate as wide a range as possible of both intensities and durations of exposures underground. (We were told that concentrations of dust and radiation had been reduced dramatically during the years 1946 to 1990, with very high

levels during the first 8-year period, followed by systematic reductions in subsequent years, and unremarkable, relatively low, levels from the early 1970s onwards.) At that time (1994) it was anticipated that sampling would have to be conducted by labor intensive decision-rule-based scrutiny of (manuscript) summary records that identified individuals who were included in the autopsy archive.

The plan was to then try to obtain access to occupational exposure information and smoking data for those sampled, and for several pathologists to examine the histology slides independently and according to a standard protocol based on World Health Organisation (WHO) guidelines for classifying lung cancer cell types. The specific tasks required to implement this plan were listed in section 3 of the proposal (see Annex).

2.3 Methods

Work on the feasibility study began in November 1995, 19 months after the proposal had been prepared. In the meantime, the *HVBG* had made significant further progress on developing data systems that referred to all former *Wismut* employees, including those who were no longer alive in 1992.[17] These developments promised to greatly simplify the sampling procedure that had been envisaged originally, since it would allow identification and sampling of individuals directly from the newly created computer files (rather than from the manuscript records with the autopsy archive.) But the computer file then available, with indicators identifying whether or not individuals had died from lung cancer, included less than one third of the approximately 30,000 individuals who were thought at that time to be included in the autopsy archive. We decided, therefore, to proceed with sampling in two stages: an initial sample (December 1995) based on the incomplete listing of individuals potentially available, to be followed by a second sample when the *HVBG* had completed identification of the remainder of those eligible.

By mid-January 1996, it had become clear that although the *HVBG* would be in a position very soon to list all individuals included in the autopsy archive, the then ongoing work to identify those who had been employed at the mines, and entry of indicators of pathological evidence regarding cause of death, would not be completed in a time-scale consistent with the feasibility study schedule. A further modification to the second stage of the sampling procedure was therefore adopted as follows.

(1) A sub-set of approximately 500 individuals possibly eligible for inclusion in the feasibility sample (but for whom information regarding employment at the mines and cause of death had not necessarily been established) was selected from the available list of all those included in the autopsy archive, on the basis of known ranges of dates of birth and of death that were of interest.

(2) The *HVBG* then gave priority to providing missing information for this sub-set regarding employment at *Wismut* mines and cause of death.

(3) This sub-set of the population of interest was then used to select a second sample of individuals for inclusion in the feasibility study.

The plan was to explore in detail the amount and quality of the information regarding exposures to various carcinogenic materials for all those sampled. Here too, the approach envisioned originally (paragraph 3.6 in the Annex) had been overtaken by progress in *HVBG* efforts to document and organize information relating to former *Wismut* workers. An *HVBG*-commissioned project, to establish a job-radiation exposure matrix for *Wismut* miners, was well advanced.[18] A similar project to establish a matrix referring to exposures to silica-containing dusts had also started in 1996.[19] We were assured that we would be able to use results from both these job-exposure matrices for the purposes of the contemplated research, but work on the job-dust exposure matrix would not be completed within the 12-month period contracted for the feasibility study. We therefore arranged an alternative approach to estimate exposures to silica dust *ad interim*, as follows.

Records of sampled individuals' occupational histories in the manuscript records were identified and transferred onto a computer-readable file, as planned originally. Following successful completion of a small-scale pilot trial, the computer-generated records of sampled individuals' detailed job histories were inspected and annotated by three experts. All three had been employed at the *Wismut* mines for many years and were familiar with the changes in mining methods and working conditions that had occurred. The three experts assessed likely levels of (konimetrically determined) concentrations of respirable dusts, the quartz contents of the corresponding total (ambient) dust clouds, as well as likely exposure to arsenic- and asbestos-containing dusts, for calendar-time periods, mine and job-specific entries in the sampled occupational histories. The assessments were made on ordinal scales, as follows.

Respirable dust:

1: up to 250 ppcc
2: in the range 250 to 500 ppcc
3: in the range 500 to 2,000 ppcc
4: more than 2,000 ppcc.

Quartz in total dust:

1: less than 1%
2: 1% to 5%
3: 5% to 20%
4: more than 20%

Exposure to asbestos:

0: no (occupational) contact with asbestos-containing materials
1: low exposure (1 fibre per cc or less for up to 10 h per day)
2: moderate exposure (1 fibre per cc or less for more than 10h per day)
3: higher exposure (more than 1 fibre per cc)

Exposure to arsenic:

0: no verifiable contact with arsenic-containing minerals
1: possible contact with arsenic-containing minerals and dusts.

Notional indices of individuals' cumulative exposures to dust were calculated as follows. Segments of time worked in distinct mine-, job- and calendar-time-specific periods, as recorded in the occupational histories, were multiplied by arbitrary attributions of concentrations of dust to the ordinal categories as 250 ppcc, 375 ppcc, 1,250 ppcc, and 2,000 ppcc for categories coded 1 to 4, respectively. These products were then summed to provide what we interpreted as at least relative measures of individuals' cumulative exposures to dust. Percentages of quartz in the dusts were approximated as 1%, 3%, 12.5% and 20% for categories coded 1 to 4, respectively. Each individual's cumulative exposure to quartz was estimated by multiplying each of his mine-, job and calendar-time-specific dust exposure segments by the corresponding approximations to percentages of quartz, and then summing those products. The nominal exposure units, for dust and for quartz dust, are therefore "ppcc.working-year", where the "working-year" was calculated separately for each individual sampled on the basis of the recorded number of days worked in distinct calendar-year periods.

Indices of exposures to asbestos and to arsenic were calculated as weighted sums of the number of years (and fractions of years) exposed, with weights defined arbitrarily as the corresponding ordinal codes (0, 1, 2, and 3 for asbestos, and 0 and 1 for arsenic). Thus the arsenic exposure index is simply the number of years worked that involved possible contact with arsenic-containing minerals and dusts.

All available histology slides for the individuals sampled were extracted from the autopsy archive and were transported by secure means to the USA. There they were reviewed by five pathologists working independently and according to a protocol outlined in the original proposal (see Appendix 2 with the Annex to this report.) The histologic parameters recorded included the presence, location, and type of lung cancer; the presence, location and degree of silicosis; and the presence, type, and location of any pre-neoplastic lesions. Photo-copies of pathology reports referring to the individuals sampled, suitably edited by *HVBG* staff to remove personal identification information, were provided subsequently to the US-based pathologists.

The *HVBG* arranged for searches of medical records of those sampled, extracted information on smoking habits, and made this information available as a computer file. Estimates of exposures to radon progeny, expressed in Working Level Months (WLM), were made by *HVBG* staff using results available to them from the then unpublished work on the job-(radiation) exposure matrix. (A draft of the final report[20] on that project became available in August 1997.)

All manuscript and computer-generated records referring to individuals included in this study were provided by the *HVBG* in a form that ensured anonymity of the individuals concerned, in compliance with German law regarding confidentiality of medical and other personal information.

3. RESULTS

3.1 Summary of progress

Table 1 summarizes progress in the work chronologically. Identification of the first sample of individuals included in the autopsy archive ($n_1 = 152$), and extraction of their occupational history and smoking data, was accomplished within the first five Project Months. The corresponding histology slides were available for review in the USA in Month 7, and estimates of the exposures to dust for that first sample were produced by Month 8. Similar information referring to the second sample ($n_2 = 150$), was obtained in Month 11. It was clear by that time that there would be further delays in acquiring data to estimate exposures to ionizing radiation. Moreover, there were unexpected and serious gaps in the smoking data that had been provided, and this had to be investigated. A "no-cost-extension" to the 12 month period allocated originally was therefore agreed between the contracting parties (the *HVBG* and Battelle Memorial Institute, acting on behalf of NIOSH). Preliminary results from laboratory examinations of the first sample of histology slides were described at a *HVBG* research review Colloquium in December 1996.[21] Statistical analysis of all the data began in Project Month 21 (July 1997) and continues. Production of this report is more than two years later than was intended originally. The delay is due in part to modifications to the originally envisaged plan (as outlined above), some unanticipated data processing problems, unavoidable delays in accessing data on exposures to ionizing radiation, and to constraints on the availability of resources for analyzing the results.

3.2 Sampling

The (incomplete) data file available for sampling in November 1995 consisted of 8,517 records that referred to former *Wismut* workers for whom autopsy reports existed in the archive. Each record consisted of a unique study-specific identification code, a gender indicator, year of birth, year of death, and an indicator of whether or not lung cancer had been recorded at autopsy. (The latter indicator was missing for 1,611 individuals, all of whom had died before 1971.) Only a few (132) of the 8,517 records referred to women.

For the initial feasibility study sample, we restricted attention to 6,261 men who died in 1971 or later and who were born after 1906. A total of 152 identification codes were selected from this sub-group by stratified random sampling: 131 from 2,223 records that referred to lung cancer, and 21 from the 4,600 with no reference to lung cancer. Stratification within both these groups was by year of birth and year of death. This was arranged so as to generate approximately uniform distributions of lung cancer cases with respect to year of birth from 1907 through 1934, and with respect to year of death from 1971 through 1985. Higher proportions were sampled from those who were born after 1934 and who died after 1985.

A file with records referring to an additional 17,996 individuals included in the autopsy archive, but with no indication (at that time) regarding either the presence of lung cancer or whether the individual had been employed by *Wismut*, became available subsequently. The available information on year of birth and of death was used to identify 464 records referring to individuals on that file who were born after 1925 and who had died after 1970. The *HVBG* then gave priority to determining

whether or not those 464 had ever been employed at the *Wismut* mines, and on whether there were references to lung cancer or silicosis in the corresponding autopsy reports. Up-dated information for this sub-group revealed that 277 of the 464 individuals had been employed by *Wismut* and that

60 of those 277 were indexed as having lung cancer;
37 as having silicosis;
14 as having lung cancer and silicosis;
156 as having 'no lung cancer'; and there were
10 with no cause of death recorded.

The second sample for the feasibility study was then defined as: all (74) with some reference to lung cancer, plus all (37) with silicosis (but no lung cancer), plus a sample (39) from the 156 records indexed as having no lung cancer, stratified by year of birth. The total number of former *Wismut* workers considered for the feasibility study was thus 302; 205 of them had lung cancer (Table 2), and at least 17% were recorded as having pathologically diagnosed silicosis at autopsy. We arranged for the sample to include miners with and without lung cancer, and with and without silicosis, because we wanted to see whether the available data, and the sampling strategy that we were contemplating, could generate material that would allow us to examine the idea that pre-neoplastic lesions would be present in miners with silicosis but not in those without, and whether this would be true both for those with lung cancer and for those without (see Appendix 1 with the Annex to this report).

3.3 Exposure assessments

Each worker's occupational history record (based on manuscript wages records) consisted of a series of dates of start and finish of one of more than 750 coded jobs associated with a specified shaft and mine within the *Wismut* complex. These basic data were supplemented by coded summary information classifying jobs within each calendar time period according to the degree to which they involved work underground (more than 50%, up to 50%, none). Separate codes were provided for absences due to sickness and for missing data.

Preliminary analysis of these records for the 152 individuals in the first sample confirmed that the sampling strategy (stratification by year of birth and year of death) had generated a potentially informative distribution of days in employment at *Wismut* mines with respect to calendar periods that were said to be associated with essentially different intensities of exposures to dust and to radiation. Eighteen per cent of the aggregate number of days employed occurred before January 1955 (when environmental conditions at the mines were known to have been extraordinarily severe); 33% in the subsequent decade (when increasing efforts were being made to control exposure levels); 17% from the start of 1965 through 1970 (when conditions were reported to have improved steadily); and 32% from January 1971 (when exposures to dust and to radiation were reported to have been unremarkable in comparison with similar mining operations in other countries.)

A small pilot trial verified that it would be possible to estimate dust exposures usefully *ad interim* even in the absence of the awaited job-exposure matrix. Each of three ex-*Wismut* employees in our team annotated the detailed occupational history records for 10 of the 152 individuals in the first

sample. These 10 records generated 84 distinct work-place and job-associated calendar periods for which intensities of exposures had to be assessed on defined ordinal scales. The results indicated sufficiently high concordance between the three experts to justify continuing along these general lines for the purposes of the feasibility study. (The three sets of classifications with respect to concentrations of respirable dust were identical on the 4-category ordinal scale for 38 of the 84 time periods considered (45%), and two of them agreed precisely in their classifications of the other 46 intervals. The estimates of the likely range of percentage quartz in the dusts were more variable, but even here, at least two agreed precisely for 81 of the 84 time periods.) However, the experts involved recommended that more secure assessments were likely to be forthcoming if they worked collectively, rather than separately. Therefore, this was the procedure adopted subsequently to classify all (302) sets of occupational histories. The definitions of the various ordinal categories were clarified and modified slightly on the basis of the pilot trial experience.

Results from integrating the intensity of exposure estimates with durations of exposure are summarized in Figure 1. These histograms refer to 299 of the 302 men sampled. (Information on work histories for the other three men was insufficient for any intensity of exposure estimates.) Summary statistics, and some pair-wise correlations between the exposure indices, are shown in Table 3. Exposures to silica and to ionizing radiation are expressed there in two ways. First, as cumulative exposures (E_{silica} and E_{radon}), where E_{silica} is the estimate of cumulative exposure as defined above (§2.3) and E_{radon} is measured in Working Level Months (WLM). Second, each of those sets of 299 estimates of cumulative exposures were divided by the sums of the exposure periods (T) that contributed to the cumulative exposure estimates. These quotients, $C_{silica} = (E_{silica}/T)$, represent time-weighted <u>average concentrations</u> of (or intensities of exposure to) silica. C_{radon} is a time-weighted average intensity of exposure to radiation, in WLM/y.

Figure 1a confirms that the distribution of years worked is wide (up to 40 years) and close to uniform up to 30 years. The distributions of cumulative exposures to dust and to silica (Figures 1b and c) are positively skewed but nevertheless informative over the wide ranges covered, while that showing the average silica concentrations to which individuals were exposed (Figure 1d) is nearly uniform over the range 60 to 400 ppcc. Estimates of exposures to ionizing radiation (Figure 1g) ranged from less than 200 WLM for 121 individuals, to 1400 WLM or higher for another 13% in the sample. Twenty-five men (8%) had been exposed to 1600 WLM or more. The time weighted concentrations of ionizing radiation (Figure 1h) have a very similar positively skewed distribution.

The lower section of the first column in Table 3 shows that five of the seven correlations between exposure variables and the number of years worked in *Wismut* mines (T) are positive. This is as expected, since those five exposure variables are weighted sums of those same number of years worked, with weights reflecting the varying <u>intensities</u> of exposure. The highest of these five correlations, that with exposure to respirable dust, is 0.65. This confirms that, as intended, the sampling strategy identified some individuals with short periods of relatively high intensities of exposure, and others with long periods of relatively low intensities of exposure.

The correlation between years worked and the re-parameterized silica exposure variable (C_{silica}) is also moderate, and negative (r = -0.45). In consequence, the correlation between C_{silica} and E_{dust} is also low in this sample of men (r = 0.18). This indicates that although high cumulative exposures to respirable dust were associated with high exposures to silica (r = 0.93), high average intensity of exposure to silica did not necessarily imply high exposure to respirable dust.

Of particular interest in the context of this feasibility study is the fairly low correlation between the two measures of exposure to silica, E_{silica} and C_{silica} (r = 0.40), and their separate correlations with exposure to radon daughters (r = 0.73 and 0.45, respectively.) Figure 2 shows that despite the fairly high correlation between exposures to silica dust and radon daughters (r = 0.73), there is a substantial scatter of individual points, particularly in the upper-right quadrant defined by the medians of the two plotted variables. This is attributable to the fact that the sampling strategy has identified some individuals in the autopsy archive who experienced relatively low intensities of exposure to silica (C_{silica}) but fairly high levels of exposures to ionizing radiation (E_{radon}), and *vice versa* (Figure 3). The upper-left quadrant in Figure 3, representing relatively high exposures to radon daughters with relatively low intensities of exposures to silica dust, includes 34 of the 299 individuals sampled (11%). The bottom-right quadrant embraces another 11% of the plotted points. Note again the considerable scatter of points in the upper-right quadrant.

Figure 4 illustrates the joint distributions of the time-weighted average radiation burdens experienced (C_{radon}) and the cumulative exposures to silica (E_{silica}; r = 0.23). Analogous median-defined top-left and bottom-right quadrants in Figure 4 embrace, between them [(2x48)/299 =] 32% of the points plotted.

Figure 1e shows that about 14% of those sampled are likely to have had non-trivial occupational exposures to asbestos. The number possibly exposed to dusts containing arsenic is higher (130/299 = 43%; Figure 1f). Correlations between the intensity of silica exposure ($C_{siliica}$) and exposures to these two known carcinogens are both low (r = -0.24 and 0.27, respectively), as is the correlation between them (r = -0.19). The correlation between exposures to ionizing radiation and to arsenic is higher (r = 0.58), but not so high as to preclude attempts to estimate their separate effects given an appropriate sample size.

3.4 Smoking habits

As noted above (§2.2) sampling from the archive was restricted deliberately to individuals who died after 1970, because we knew that no information about smoking habits would be available from *Wismut* medical records for those who died earlier. In the event, however, the *HVBG* were able to locate medical files with information on smoking habits for only 157 (52%) of the 302 men selected. These individuals are identified by crosses (X) in Figures 2, 3 and 4.

It has not been possible thus far to locate the missing medical files, and it seems unlikely now that they will be found. About 8% of those for whom some information on smoking habits was available were recorded as having never smoked. Most (125/157 = 80%) were cigarette smokers after 1970, or had smoked cigarettes at an earlier time (7%; Table 4a). The smoking habit characterizations

used in Table 4a are based on extracts from varying numbers of (post-1970) records of medical examinations of any one individual. Information from any one such record was provided in the form of (7) numerical codes defined as:

non-smoker; non-smoker for at least one year; occasional smoker; pipe or cigar only; cigarette smoker for less than 5 years OR less than 10 cigarettes per day; cigarette smoker for at least 5 years OR 10 or more cigarettes per day; no data available.

On average, the 157 miners for whom smoking data were found had worked more than 6 years longer in the mines than the 145 for whom the *HVBG* were not able to trace records of smoking habits. In consequence, estimates of mean cumulative exposures to dust, to silica, to radiation and to arsenic also differed systematically between the two groups (Table 4b). Estimates of exposure to asbestos were similar, however; and the average intensities of exposures, to silica (C_{silica}), and to radiation (C_{radon}), did not differ significantly depending on the availability of smoking data. Correlations between the various measures of exposure were also similar in the two groups, and the distributions of the different symbols (squares and crosses) in Figures 2, 3 and 4 indicate that the general patterns illustrated there are not attributable to data from just one or the other of the two groups of men.

3.5 Histology

Histologic material was obtained from autopsies of 292 of the 302 individuals sampled. The slides were examined independently by five pathologists, of whom four completed work on all cases. The following results refer to only those four sets of complete data.

The pro-forma for recording results from inspections of the 292 sets of slides made provision for recording 18 items of information. Blanks on some of these record sheets were interpreted as missing information. There were between 10 and 15 such blanks on the four pathologists' forms regarding the presence (and number) of slides from lung sections. All four recorded that 96% of the sets of slides seen included at least one from the lung. The average number of such slides per case was 7 (SD = 3).

Between 185 and 202 of the sets of slides were noted as showing a primary lung cancer (Table 5). Agreement between pairs of pathologists about these diagnoses exceeded 90% of the cases where both recognized that a diagnosis was possible (Table 6).

The pathologists' opinions about lung cancer cell types that they saw are summarized in Table 7. More than two thirds of the types recognized were small cell or squamous, and between 20% and 35% were classified as adenocarcinomas or large cell carcinomas. Table 8 shows the levels of agreement between the pathologists regarding these judgements. Concordance between any two of them in their designations of four distinct cell-types and a fifth ('Other') category ranged from 60% to 76% (section (a) of Table 8). Re-arrangement of these multiple judgements, as dichotomies that distinguish between just one of the cell types and all others grouped together, shows that concordance between pairs of pathologists was at least 86% for small cell carcinoma, 80% for

squamous cell carcinoma, 82% for adenocarcinoma, and 75% for large cell carcinoma (sections (b) to (e) of Table 8). Pathologists #s 1 and 4 recognized cell types indicative of peripheral lung cancer in 39 and 65 cases, respectively (Table 7). Agreement between them regarding these designations in individual cases was (at least) 74% (section (f) of Table 8). Agreement in this respect between pathologists #1 and #2 reached 89%.

On average, the four pathologists noted that 271 of the 292 sets of slides (93%) included some presenting lymph nodes, with up to three such slides for 80% of the cases. Their judgements regarding the percentage of cases that provided slides from sections of both the lung parenchyma and lymph nodes ranged from 84% to 90%, and averaged 86%. Table 9 (bottom line) shows that, on average, 36% of such cases were characterized as demonstrating silicosis at both sites; 19% were recorded as showing the lesions in the lymph nodes but not in the parenchyma, and 7% of the records indicated parenchymal silicosis in the absence of lymph node silicosis. Some of the differences between the pathologists in these judgements are substantial. In particular, pathologist #4 diagnosed parenchymal silicosis much more frequently than the others; and pathologists #3 recorded lymph node silicosis in a relatively small proportion (31/162 = 19%) of cases with no parenchymal silicosis. (The corresponding aggregate result from the other three pathologists was 38%.)

The four pathologists recorded the presence of what they considered to be pre-neoplastic lesions for between 10% and 20% of cases [Table 10(a)]. The levels of agreement between them, regarding which particular cases exhibited these lesions, was low, ranging from 13% to 32% of those cases where either one of any two of them recorded that such a lesion was present [Table 10 (b)].

4. DISCUSSION

This discussion is arranged under main headings that correspond to the three sets of questions posed above under Objectives (§2.1).

4.1 Accessibility and suitability of the histologic material

4.1.1 Accessibility

The administrative and logistic problems that had to be tackled before laboratory studies could begin were, as anticipated, not trivial. In the event, given collaboration with, and consistent support from, the *HVBG*, we were able to gain access to more than 2,700 histology slides that referred to nearly 300 individuals in the population of interest. We have been assured, moreover, that similar histologic material, and the associated data, for up to 5,000 individuals in the archive could be made available for continuing research. These positive outcomes were by no means certain when the feasibility study was first conceived, and we therefore draw attention to them now explicitly.

4.1.2 Suitability

We needed also to assure ourselves that it would be possible to use the available histologic material to distinguish between (a) centrally located, peripheral and other lung cancers, and (b) parenchymal and lymph node silicosis. Tables 7 and 9 show that these distinctions are possible. The degree of concordance between the four pathologists in these assessments has been quantified and is

reasonably high with respect to the types of lung cancers observed (Table 8).

4.1.3 Observer variability

Agreement between the pathologists with regard to different types of silicosis was less impressive (Table 9). Appendix 2 with the original proposal (see Annex) specified that the extent of silicosis was to be graded according to protocols currently used by NIOSH scientists in their National Coal Workers' Autopsy Study. These rules require that a pathologic diagnosis of silicosis must be based on "the presence within the lung of discrete, rounded, whorled, hyalinized, fibrous nodules".[22] However, the Notes that accompanied the laboratory record sheets used in the feasibility study did not quote this definition (they referred only to "nodules".) Discussion between the pathologists involved, after the laboratory examinations had been completed, established that one of them (# 4) who was unfamiliar with the NIOSH autopsy study conventions, had diagnosed silicosis on the basis of fibrosis with dust particles even in the absence of concentric nodularity. This difference in interpretation of the protocol will have contributed to the variability between pathologists evident in Table 9. Nevertheless, the results in Table 9 show that all four pathologists were able to differentiate between what they considered to be parenchymal silicosis and lymph node silicosis. This verifies that the quality of the material is such that these distinctions are possible. Therefore we think that clarification of the criteria that define the different types of silicosis is likely to generate useful data for the purposes of the contemplated research.

The low level of agreement between the pathologists about what constitutes a pre-neoplastic lesion [Table 10 (b)] was disappointing. It is clear that if further studies of the available material are to include examination of hypotheses about the significance of these lesions then different pathologists' views about what constitutes such a lesion would first have to be defined, or at least described. If results are to be generalizable then a definition acceptable to all those involved would have to be formulated.

Factors that are likely to have contributed to variability in laboratory results include differences between the pathologists in their experience with human (as distinct from experimental animal) autopsy slides, and our decision that, for the purposes of this feasibility study, we would try to make finer distinctions between pathologic types of lung cancer than those usually necessary for prognostic or therapeutic purposes. Our experience confirms the value of including tests of the proposed laboratory procedures as part of the feasibility study, and it emphasizes the importance of arranging for multiple and independent assessments of the histologic material in research studies. (Without such independent replications the problems would not have been identified.) We are confident that inter-observer variability can be reduced prior to any continuing research, by more discussion between the pathologists to agree on definitions and criteria, and by some further adjustments to the lay-out of the recording sheet.

4.2 Occupational history and exposure data

We have verified that the manuscript occupational history records for former *Wismut* workers included in the autopsy archive are accessible, and that these records can, in practice, be transferred satisfactorily to computer files. We are satisfied also that the semi-quantitative estimates of

occupational exposures that we derived (to silica dust, to asbestos and to arsenic) provide informative pictures of the extent to which individuals' cumulative exposures varied, and of the degree of correlation between the different exposures (Table 3 and Figures 1 to 4). The feasibility study was designed to investigate precisely these specific aspects of the structure of the data (rather than the underlying research hypotheses), and this objective has been accomplished.

Individuals' cumulative exposures to silica, to radon (and indeed to any other occupational pollutant) depend to a large extent on the lengths of time that individuals were exposed underground, that is, on the durations of exposures (T). Therefore, if the intensities of exposures to any one pollutant had not varied much, or if they were highly correlated with variations in concentrations to other pollutants, or if they were strongly associated with the varying time periods during which individuals were exposed, then it would not be possible to use the data to distinguish between effects associated with different types of exposures. Table 3 and Figures 1 to 4 demonstrate that the material that we have extracted for this feasibility study do not exhibit patterns that would rule them out for further study on these grounds. A sampling strategy similar to that which we used (but with an appropriately larger total number of individuals sampled) would be expected to generate the kind of data needed to answer the underlying research questions. Factors possibly influencing variations in the occurrence of different types of lung cancer could be studied along the lines contemplated originally.[1] For instance, a logistic transformation of the proportions with defined types of lung cancer could be used as a response variable. Potential explanatory variables in subsequent analyses would include indices reflecting the presence or absence of different types of histologically verified silicosis, as well as different levels or intensities of exposures to silica dust, to radon daughters, to arsenic and to asbestos. A variety of mathematical expressions might be considered to reflect different formulations of combinations of the hypothesized explanatory variables. Such analyses could, for instance, distinguish between effects of exposure to high concentrations of ionizing radiation on the one hand, high cumulative exposures to silica on the other, and also the hypothesized interaction between these two circumstances. Since these analyses would be specific to (or "controlled for") defined types of silicosis, they would have the potential to contribute to answering the vexed question whether the presence of silicosis is a "necessary co-condition"[23] for silica related lung carcinogenesis or whether it is merely "an unrelated consequence" of high exposure.

The final report on a separate *HVBG*-sponsored project, concerned with the development of a job-(dust) exposure matrix for *Wismut* miners,[19] is expected during 1999. Another report, detailing work on the job-exposure matrix for ionizing radiation, is already available.[20] Both these matrices could be used in any continuing research. They would provide refined, and more reliable, estimates of individuals' exposures than the approximations summarized here in Table 3. We are confident, therefore, that in this respect, further research along the lines envisaged is possible and likely to be useful.

The duration of exposure variable that we have used (T) refers only to time worked in the *Wismut* uranium mining complex. Additional information exists concerning the number of years worked in coal-mining prior to recruitment into the *Wismut* complex. That information is currently recorded

only as part of confidential manuscript records associated with serial chest radiographic examinations. We did not attempt to access those data during this feasibility study but we have been assured that they could be made available for any continuing research.

4.3 The sampling strategy and sample sizes

4.3.1 Sampling strategy

The underlying research hypothesis is that exposure to silica dusts, and/or the presence of silicotic lesions, interact biologically with the effects of exposure to known lung carcinogens, and that they thus potentiate risks of lung cancer. As noted above, Figures 1 to 4, and Table 3, suggest that the sampling strategy adopted for this feasibility study is capable of generating data that could be used to test the hypothesis. In particular, Figure 4 indicates that, in principle, the hypothesized interaction between exposure to silica dust on the one hand, and to high concentrations of ionizing radiation on the other, should be detectable in the data, provided that the size of the sample examined is large enough.

Our failure to find information on smoking habits for about half of those sampled was very clearly associated with shorter durations of exposure in the mines among those men (Table 4b). This implies that difficulty in accessing information on smoking habits for some men was not just a random occurrence. To date, no explanation has been found for the systematic difference. It is reassuring, therefore, that the bias appears not to be associated with the intensities of the exposures experienced (that is, the averages of concentrations of silica dust and of radiation that were encountered by individuals in the mines over the years). It is reassuring also that the patterns of correlations between the exposure variables illustrated in Figures 2, 3 and 4 are not simply artifacts associated with the bias.

However, if the feasibility sampling strategy is retained unaltered in continuing research, then the likely gaps in the information about smoking habits would make it more difficult to identify a possible interaction between this important lung carcinogen and exposure to silica (or silicosis). Moreover, it is possible (though perhaps unlikely, given the low percentage of non-smokers among those for whom smoking data were found) that absence of information on smoking habits would interfere with attempts to examine the hypothesized interactions between biological effects of inhaled silica dust on the one hand, and exposure to other occupational lung carcinogens on the other. This possibility could be investigated in any continuing study by considering results from sub-sets of data where smoking habits are known, and then comparing them with results derived from the remainder, where smoking habits are not known. Such an analysis would be expected to indicate the extent to which the absence of information on smoking habits might have distorted results. Additionally, the sampling strategy might be adjusted so as to include higher proportions of miners for whom the *HVBG* are now able to confirm, in advance, that smoking data are available. Practical implementation of such an adjustment to the sampling procedure would require further discussion with the *HVBG*.

4.3.2 Sample sizes

The number of miners that would have to be studied to provide convincing information in any

continuing study is considered below in the context of predictions that follow from the two research hypotheses mentioned above (§1.2; see also Appendix 1 to the Annex with this report). The first prediction is that among miners with both lung cancer and histologically confirmed parenchymal silicosis, the proportion with peripherally located lung cancers will be greater than in those who have lung cancer but no histological evidence of any kind of silicosis.

Now, the pooled results from four pathologists, recorded in Table 7, suggest that, on average, the proportion of *Wismut* miners included in the archive with peripherally located, as distinct from other types, of lung cancer is about 27%. Figures 5a to 5c then illustrate the order of magnitude of the total sample sizes (N) that would be required to provide plausible evidence of the hypothesized interaction if it were to result in those with parenchymal silicosis having, say, 5%, 10%, or 15% higher than the average percentage of peripheral lung cancers. For instance, Figure 5a shows that the statistical power ($1-\beta$) to detect an increase in the percentage from 27% to 32% (implying a relative risk = 1.18) would only reach 0.8 with sample sizes greater than about 2,000, if statistical significance at the 10% level is regarded as the minimum acceptable evidence against the null hypothesis that there is no increase in the relative risk amounting to 1.18. (Note that the words "relative risk" as used here do not refer to risks of having lung cancer as such. The "risk" concerned is that of having a peripherally located lung cancer, as distinct from lung cancer at some other anatomical site. The "relative" risk is then the above defined risk among those with parenchymal silicosis relative to the risk of a peripherally located lung cancer among those with lung cancer but no silicosis.)

Figure 5c, however, shows that if the hypothesized increase in the percentage of peripherally located lung cancers is at least 15%, i.e., a relative risk of 1.56 or more, then the minimum sample size would be reduced substantially. For instance, the probability of failing to detect a relative risk equal to 1.56, with a ($100\alpha =$) 5% significance level or better, would not exceed $\beta = 0.1$, provided that the sample size (N) is at least 446. The calculations contributing to the patterns shown in Figure 5 are based on assumed normally distributed test statistics, corrected for continuity, for differences between the proportions of interest.[24] The ratio of the number of *Wismut* miners with pathologically verifiable parenchymal silicosis to those with no silicosis of any kind was taken as [(67+365)/390 =] 1.11 (from Table 9).

A further prediction that follows from the same hypothesis ("P2|H1", using the notation in Appendix 1 with the Annex) is that pre-neoplastic lesions will occur more frequently in the lungs of miners with pathological evidence of silicosis than in those with no such silicotic lesions. Calculation of sample sizes that would be required to test this prediction, using the pooled average results from Table 10 to estimate the base-line proportion of cases with pre-neoplastic lesions, would yield values of N in relation to power that are close to (a little lower than) those for the corresponding predicted values of excess risk shown in Figure 5. For instance, Figure 6 compares the values of N for both predictions that would be consistent with 15% excess risks and a 5% significance level. For 90% power, the minimum sample sizes shown there are 446 for "P1|H1" (as in Figure 5c) and 370 for "P2|H1". For 95% power the corresponding values of N are 545 and 448. However, in view of the ambiguity about what the pathologists meant by 'pre-neoplastic lesions' [Table 10(b)], calculations

of this kind for P2|H1 are almost meaningless; clearer definition of what a constitutes a pre-neoplastic lesion must be a pre-requisite for any sensible further research on this topic.

Figures 7a to 7c illustrate a spectrum of sample sizes that would be required to examine a prediction from the other hypothesis: that miners with lung cancer and lymph node silicosis will have a higher proportion of centrally located (small cell and squamous cell) carcinomas than those with no lymph node silicosis. For these calculations we assumed, again from Table 9, that the ratio of *Wismut* miners with lymph node silicosis to those with no lymph node silicosis is [(188+365)/(390+67)], i.e., 1.21, and that, on average, the proportion with centrally located lung cancers among those with lung cancer is [(354+170)/769], i.e, 68% (Table 7). Again, 5%, 10% and 15% increases in the baseline average proportion (68%), corresponding in this case to relative risks equal to 1.07, 1.15 and 1.22, respectively, would require very different total numbers in the sample. The numbers range from about 250 to 400 to detect a 1.22 relative risk convincingly (Figure 7c), to at least 2,500 for relative risks as low as 1.07 (Figure 7a).

Our use of the pooled data from Table 7 to estimate 'base-line' proportions under the null hypotheses will have overstated the numbers required for future research (and will have understated the corresponding estimates of relative risks) if, in fact, those null hypotheses are false. But we did not want to prejudge that issue on the basis of the limited data available from the feasibility study. We therefore used the average figures from Table 7 to estimate the base-line proportions, and we also accepted the average results from Table 9 as estimates of the proportions of miners with pathologically distinguishable types of silicosis. However, we satisfied ourselves that the estimates of the orders of magnitude of minimum sample sizes required for effective research, as depicted in Figures 5 and 7, are not sensitive to small variations in the estimates of the base-line proportions and types of silicosis distributions. This is illustrated, by way of example, in Figure 8, which compares the estimates of N shown in Figure 5b (where a 10% excess risk is assumed to represent a relative risk = 1.37) with estimates based on the realized (pooled) bivariate distribution of types of lung cancer and types of silicosis (not tabulated here). The latter figures imply that a 10% excess risk would represent a relative risk = 1.43; and the ratio of numbers with parenchymal silicosis to those with no silicosis would be 1.34 (rather than 1.11 as calculated from Table 9.) The revised estimates of N (dashed lines in Figure 8) are only a little lower than the corresponding more cautious estimates (continuous lines).

Collectively, the estimates depicted in Figures 5 and 7 suggest that histologic and occupational exposure data from a total of some 600 to 800 former *Wismut* miners would be sufficient to provide sensibly interpretable information relevant to the two hypotheses under consideration. Such a sample would be expected to detect the interaction-related relative risks if they are in the order of 1.2 to 1.4, with at least 80% statistical power and 5% or better levels of statistical significance. This suggested range for the size of the total sample refers to former *Wismut* miners who died with a primary lung cancer. Slides from about 190 such individuals have already been examined as part of this feasibility study (Table 5). Therefore, if the work is to be continued, it would be prudent to aim for at least an additional 600 such individuals.

Omission of individuals with no lung cancer from an additional sample would weaken the rigor with which the prediction of silica associated preneoplastic lesions could be tested. Any focused exploration of this particular aspect of the data, which pre-supposes clarification of what different pathologists recognize as preneoplastic lesions (see §4.1.3, above) would therefore be strengthened by extending the sample further to include more individuals with no lung cancer.

4.4 Other possible laboratory studies

When planning the feasibility study, in 1994, we hoped also to test laboratory procedures that might be used for immunohistochemical and molecular pathology studies. We had in mind analyses of samples from preserved paraffin blocks for selected cases who had died after 1985 (see Appendix 2 with the Annex). However, we were unable to secure access to these blocks during the early stages of the work and we therefore concentrated on studies of the readily available histology slides. The assurances that we have been given more recently concerning access to *post-mortem* materials and data that refer to up to 5,000 individuals in the archive (§4.1.1 above) includes access in principle to existing paraffin blocks for those sampled, subject to negotiated agreement with German scientists responsible for these materials. Thus some studies of this kind remain possible for the future. Detailed administrative arrangements would first have to be agreed with the *HVBG*, and the relevant laboratory methods remain to be tested.

5. CONCLUSIONS

This feasibility study has achieved its objectives. Access to histologic material that would be required for the research envisaged has been verified in practice and is assured for the future. Laboratory examinations of the material have demonstrated that it can be used for the kind of studies required, but further discussion and standardization of pathologic criteria are necessary before continuing with the work.

Data on occupational exposures of the uranium miners considered, to silica dust and to various occupational carcinogens, are retrievable. The sampling strategy adopted for the feasibility study has succeeded in generating distributions of estimates of exposures that, in principle, would permit rigorous testing of the main research hypothesis. This is that exposure to silica dust (and/or the presence of silicosis) increases risks of lung cancer by interacting biologically with carcinogenic effects of other materials.

Many detailed administrative, logistic and methodological problems arose during the course of this work . We have been able to resolve them successfully for the most part. On the basis of this experience, and of the results described in this report, we are satisfied that studies of this unique material should be pursued. Such research is likely to lead to a better understanding of silica-related biomechanisms that may enhance lung cancer risks. The knowledge gained would also help the authorities, in different countries, who are responsible for determining the extent to which exposure to silica dust, or the presence of silicosis, increases risks of lung cancer. It appears that useful new information relevant to these concerns could be obtained by retrieving from the archives additional

histology slides, and associated occupational exposure data, for another 600 miners. Some adjustment to the feasibility study sampling strategy would probably be helpful, and we recommend accordingly.

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Table 1 Chronological summary of progress

Study month	**Date**	
1	11/95	Start of feasibility study; first data file received (8,517 valid records).
3	1/96	Sample 1 selected ($n_1 = 152$).
4	2/96	Second data file provided (17,996 additional records with incomplete information).
5	3/96	Occupational history and smoking data for Sample 1 provided.
6	4/96	Sample 2 ($n_2 = 150$) selected and some smoking data extracted.
7	5/96	Slides for Sample 1 extracted from archive and transported to the US.
8	6/96	Newly computerized occupational history records for Sample 1 supplemented with estimates of mine-, job- and calendar-time-specific intensities of exposures to dust, asbestos and arsenic.
9	7/96	Examination of histology slides for Sample 1 completed independently by 5 pathologists; slides returned to archive; slides for Sample 2 extracted and transported to the US.
10	8/96	Occupational history data for Sample 2 extracted + some further smoking data.
11	9/96	Intensity of exposure estimates for Sample 2 completed.
14	12/96	Second set of slides returned to archive; no-cost extension of study contract arranged; preliminary report[19] on laboratory examinations of slides from Sample 1 presented at the *HVBG* Research Review Colloquium, Dresden.
17	3/97	Exposure to ionizing radiation for study sample estimated by *HVBG*.
19	5/97	Completion of histology slide review by US pathologists.
20	6/97	Data entry to computer completed.
21	7/97	Data analysis started.
23	9/97	Report on ionizing radiation job-exposure matrix released to NIOSH. Meeting in Washington DC (NIOSH, NCI; *HVBG*) to discuss preliminary results and consider feasibility of continuing research

Table 2 The feasibility study sample of records referring to deceased *Wismut* workers included in the autopsy archive

	Sample 1	Sample 2	Both samples
With lung cancer	131	74	205
No lung cancer	21	76	97
Total	152	150	302

Table 3 Descriptive statistics for the distributions of estimates of exposure variables, and their correlations; n = 299 of the 302 individuals sampled for the feasibility study.

	Years worked	**Dust exposure**	**Silica exposure**		**Asbestos exposure**	**Arsenic exposure**	**Radon exposure**	
	T	$\mathbf{E_{dust}}$	$\mathbf{E_{silica}}$	$\mathbf{C_{silica}}$	$\mathbf{E_{asbestos}}$	$\mathbf{E_{arsenic}}$	$\mathbf{E_{radon}}$	$\mathbf{C_{radon}}$
Nominal units	y	y.ppcc	y.ppcc	ppcc	y	y	WLM	WLM/y
Median	15.7	9806	1398	115	0	0. 6	398	33
Range	[0.1, 40.6]	[50, 40 884]	[6, 6653]	[2, 400]	[0, 77]	[0, 33]	[0, 2963]	[0, 302]
Mean	16.7	12191	1818	143	3	4	596	53
SD	11	8705	1558	117	8	6	657	64
Correlations								
$\mathbf{E_{dust}}$	0.6529	1						
$\mathbf{E_{silica}}$	0.4438	0.9340	1					
$\mathbf{C_{silica}}$	-0.4539	0.1798	0.3965	1				
$\mathbf{E_{asbestos}}$	0.2362	0.0494	-0.0957	-0.2389	1			
$\mathbf{E_{arsenic}}$	0.3061	0.6213	0.7098	0.2710	-0.1922	1		
$\mathbf{E_{radon}}$	0.1714	0.6226	0.7332	0.4470	-0.1565	0.5750	1	
$\mathbf{C_{radon}}$	-0.4142	0.0567	0.2339	0.7349	-0.2024	0.1982	0.6134	1

Table 4a Information on smoking habits among individuals sampled

	Number of men sampled	Number of men sampled as a percentage of those for whom some information on smoking habits was found: (b+c+d+e = 157)
(a) No smoking data found	145	
(b) Cigarette smoker	125	80 %
(c) Ex-cigarette smoker	11	7 %
(d) Pipe or cigar smoker	8	5 %
(e) "Never smoked"	13	8 %
Total (a+b+c+d+e)	302	

Table 4b Mean values of exposure variables by whether or not smoking data were found

	SMOKING DATA		DIFFERENCES (f - nf) and *(standard errors)*
	-found (f)	-not found (nf)	
Number of miners	155	144	
Years worked - T (y)	198	135	6.3 *((1.2)*
Dust exposure - E_{dust} (y.ppcc)	14357	9860	4497 *(975)*
Silica exposure - E_{silica} (y.ppcc)	2186	1421	765 *(175)*
- C_{silica} (ppcc)	139	148	-9 *(14)*
Asbestos exposure - $E_{asbestos}$ (y)	256	294	-0.38 *(0.93)*
Arsenic exposure - $E_{arsenic}$ (y)	54	25	2.9 *(0.7)*
Radon exposure - E_{radon} (WLM)	700	485	215 *(74)*
- C_{radon} (WLM/y)	499	564	-6.5 *(7.4)*

Table 5. Four pathologists' assessments of 292 sets of histology slides with regard to the presence or absence of primary lung cancer

Pathologist	No slides of lung sections available, or no diagnosis	Primary lung cancer	No primary lung cancer
1	8	197	87
2	7	202	83
3	12	188	92
4	23	185	84

Table 6 Percentage agreement between pairs of four pathologists concerning the presence or absence of primary lung cancer in sub-sets of 292 sets of slides where both agreed that a diagnosis was possible *(n)*

Pathologist	2	3	4
1	96% *(n = 283)*	92% *(n = 283)*	95% *(n = 269)*
2		91% *(n = 279)*	94% *(n = 268)*
3			92% *(n = 269)*

Table 7 Four pathologists' characterizations of primary lung cancer cell type for cases where at least one* cell type was distinguishable

Pathologist	CELL TYPE					Total number of primary lung cancer cases where at least one* cell type was distinguishable [f] = [a+b+c+d+e]	Centrally located lung cancers (%) 100[a+b]/[f]	Peripheral lung cancers (%) 100[c+d]/[f]
	[a] Small cell	[b] Squamous	[c] Adenocarcinoma	[d] Large cell	[e] Other			
1	82	62	28	11	12	195	74%	20%
2	82	55	49	4	13	203	67%	26%
3	94	29	21	28	14	186	66%	26%
4	96	24	26	39	0	185	65%	35%

* The record sheet used by the pathologists made provision for recording an additional cell type ("cell type number 2") in those cases where mixed cell types, or two separate primaries were detected. This facility was used variously by the pathologists, on 9, 23, 23 and 29 occasions respectively. The figures tabulated above are based only on entries recorded under the heading "cell type number 1". They therefore understate slightly the number of occasions when the different cell types were identified.

Table 8 **Percentage agreement** between pairs of four pathologists regarding primary lung cancer cell type* in sub-sets of cases where they agreed that cell type could be determined *(n)*

a: overall agreement regarding four distinct cell types and a fifth 'Other' category
b: agreement regarding presence of small cell carcinoma
c: agreement regarding presence of squamous cell carcinoma
d: agreement regarding presence of adenocarcinoma
e: agreement regarding presence large cell carcinoma
f: agreement regarding presence of adeno- OR large cell carcinoma

	Pathologist	2	3	4
(a) overall agreement	1	**76** *(n = 190)*	**66** *(n = 176)*	**60** *(n = 179)*
	2		**62** *(n = 180)*	**60** *(n = 181)*
	3			**69** *(n = 170)*
(b) small cell	1	**91**	**89**	**86**
	2		**92**	**87**
	3			**89**
(c) squamous	1	**87**	**83**	**80**
	2		**84**	**84**
	3			**89**
(d) adeno-	1	**86**	**90**	**88**
	2		**89**	**82**
	3			**94**
(e) large cell	1	**94**	**85**	**75**
	2		**84**	**78**
	3			**78**
(f) (adeno-) OR (large cell)	1	**89**	**82**	**74**
	2		**81**	**77**
	3			**76**

*"cell type number 1" only; see footnote with Table 7. The percentages tabulated above are therefore conservative estimates of the levels of agreement about cell types.

Table 9 Four pathologists' views about the number of cases with parenchymal silicosis or lymph node silicosis or both, in sub-sets of cases judged as providing one or more slides of sections at both sites (m). *[Row percentages in parentheses.]*

Parenchymal silicosis:	No		Yes		All cases (m)
Lymph node silicosis:	No	Yes	No	Yes	
Pathologist # 1	95 *[37%]*	68 *[26%]*	13 *[5%]*	81 *[32%]*	257 *[100%]*
2	101 *[41%]*	58 *[24%]*	13 *[5%]*	72 *[30%]*	244 *[100%]*
3	131 *[50%]*	31 *[12%]*	18 *[7%]*	82 *[31%]*	262 *[100%]*
4	63 *[26%]*	31 *[13%]*	23 *[9%]*	130 *[52%]*	247 *[100%]*
All pathologists	390 *[39%]*	188 *[19%]*	67 *[7%]*	365 *[36%]*	1010 *[100%]*

Table 10 Pre-neoplastic lesions (PNL)

(a) 4 pathologists' views about the presence or absence of PNL in subsets of cases where they judged that this was determinable;

(b) numbers of cases where both pathologists in a pair identified a PNL, as a fraction *[%]* of the number where either one or the other or both recorded the presence of a PNL.

(a) Presence of PNL:		Total	PATHOLOGIST	(b) Agreement about the presence of PNL: - second pathologist in a pair		
no	yes			# 2	# 3	# 4
207	53	260	# 1	15/75 *[20%]*	16/73 *[22%]*	18/57 *[32%]*
239	41	280	# 2		10/71 *[14%]*	7/55 *[13%]*
233	40	273	# 3			8/51 *[16%]*
219	25	244	# 4			

Figure 1 Distributions of years worked, and estimated exposures to noxious agents, for 299 of 302 individuals sampled for the feasibility study

Figure 1(a) Years worked, T (y)

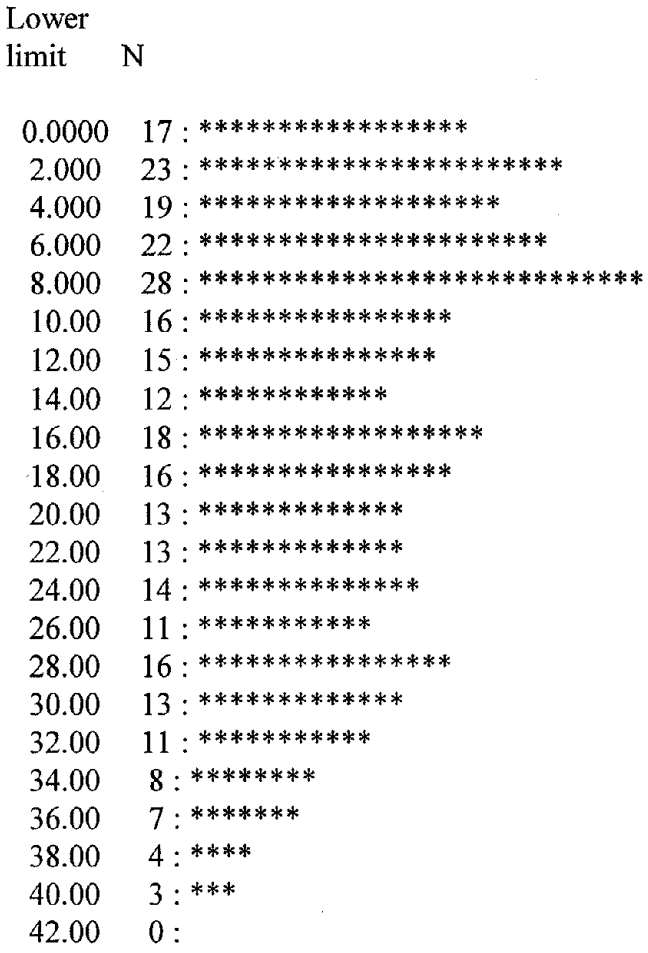

Figure 1(b) Dust exposure, E_{dust} (y.ppcc)

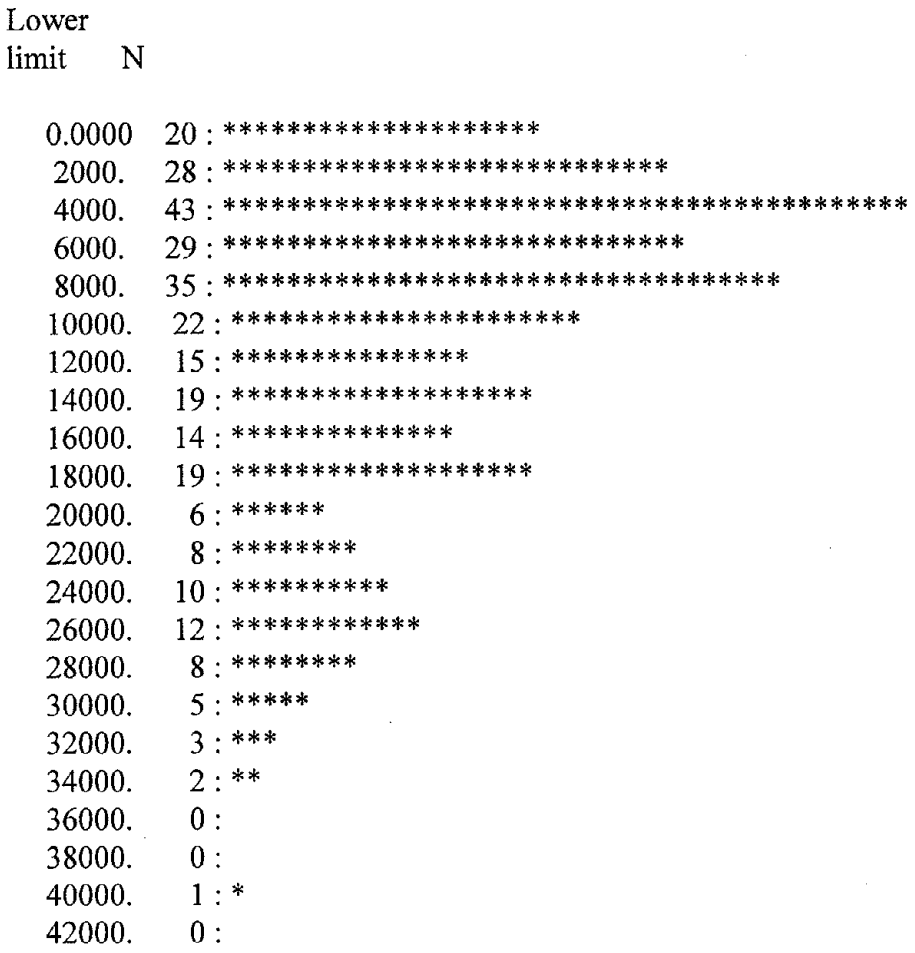

Figure 1(c) Silica exposure, E_{silica} (y.ppcc)

Each * = up to 2 cases

Lower limit	N	
0.0000	56	**********
400.0	45	**********
800.0	35	**********
1200.	31	**********
1600.	29	**********
2000.	18	*********
2400.	15	********
2800.	7	****
3200.	10	*****
3600.	16	********
4000.	9	*****
4400.	11	******
4800.	7	****
5200.	4	**
5600.	3	**
6000.	2	*
6400.	1	*
6800.	0	

Figure 1(d) Silica exposure, C_{silica} (ppcc)

Each * = up to 2 cases

Lower limit	N	
0.0000	28	**********
20.00	28	**********
40.00	54	**********
60.00	16	********
80.00	13	*******
100.0	19	**********
120.0	13	*******
140.0	17	*********
160.0	10	*****
180.0	12	******
200.0	13	*******
220.0	7	****
240.0	13	*******
260.0	7	****
280.0	9	*****
300.0	9	*****
320.0	5	***
340.0	4	**
360.0	7	****
380.0	0	
400.0	15	********
420.0	0	

Figure 1(e) Asbestos exposure, $E_{asbestos}$ (y)

Each * = up to 6 cases

Lower limit	N	
0.0000	256	: **********
5.000	14	: ***
10.00	9	: **
15.00	8	: **
20.00	2	: *
25.00	5	: *
30.00	1	: *
35.00	1	: *
40.00	2	: *
45.00	0	:
50.00	0	:
55.00	0	:
60.00	0	:
65.00	0	:
70.00	0	:
75.00	1	: *
80.00	0	:

Figure 1(f) Arsenic exposure, $E_{arsenic}$ (y)

Each * = up to 4 cases

Lower limit	N	
0.0000	169	**********
2.000	38	**********
4.000	17	*****
6.000	15	****
8.000	14	****
10.00	6	**
12.00	12	***
14.00	6	**
16.00	8	**
18.00	5	**
20.00	2	*
22.00	4	*
24.00	1	*
26.00	1	*
28.00	0	
30.00	0	
32.00	1	*
34.00	0	

Figure 1(g) Radon exposure, E_{radon} (WLM)

Each * = up to 3 cases

Lower limit	N	
0.0000	121	: **********
200.0	29	: *********
400.0	34	: **********
600.0	23	: ********
800.0	19	: *******
1000.	19	: *******
1200.	15	: *****
1400.	14	: *****
1600.	8	: ***
1800.	4	: **
2000.	2	: *
2200.	4	: **
2400.	2	: *
2600.	4	: **
2800.	1	: *
3000.	0	:

Figure 1(h) Radon exposure, C_{radon} (WLM/y)

Each * = up to 3 cases

Lower limit	N	
0.0000	134	**********
20.00	30	**********
40.00	39	**********
60.00	22	********
80.00	20	*******
100.0	12	****
120.0	13	*****
140.0	6	**
160.0	4	**
180.0	4	**
200.0	5	**
220.0	1	*
240.0	5	**
260.0	2	*
280.0	1	*
300.0	1	*
320.0	0	

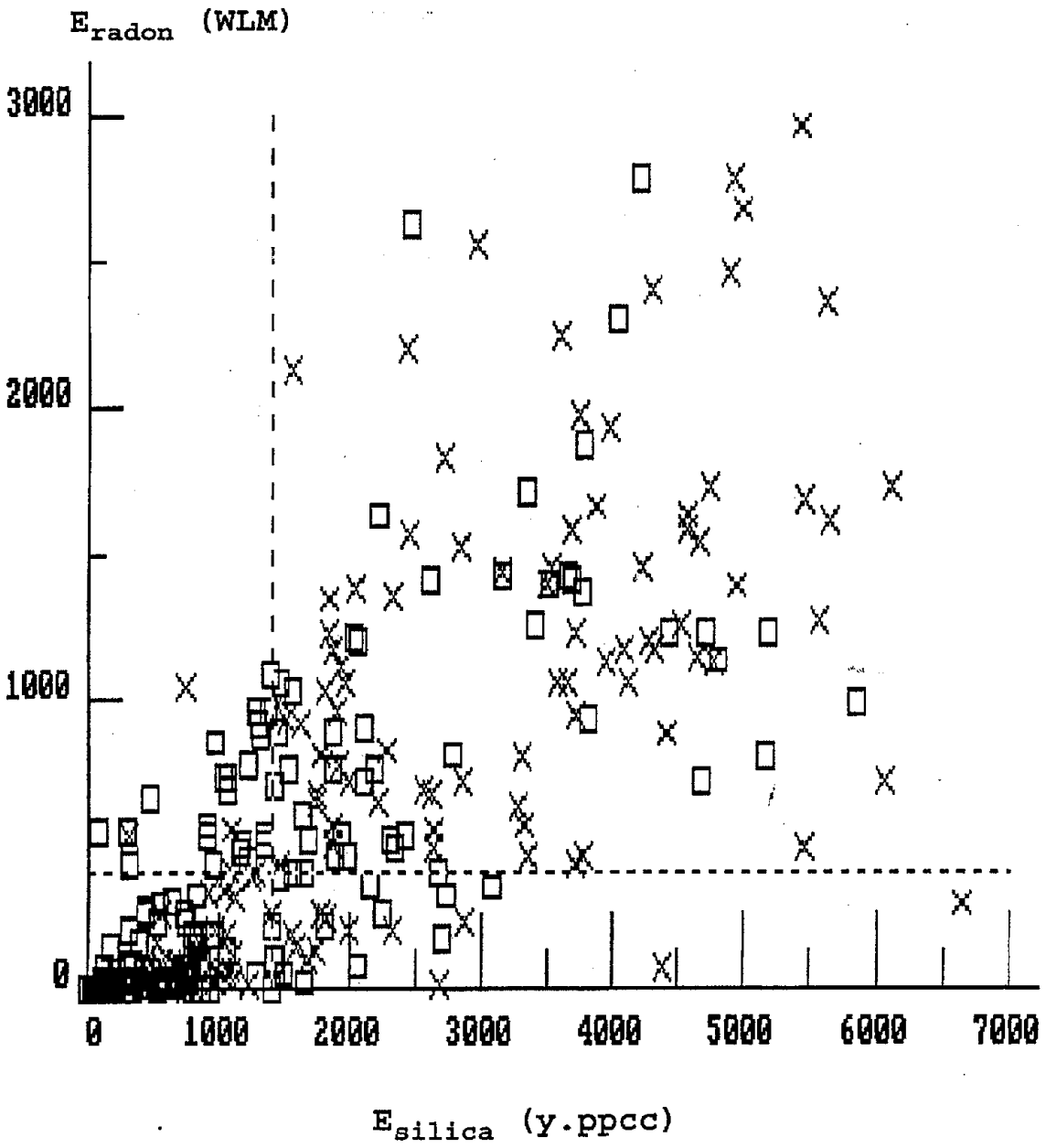

Figure 2

Relationship between estimates of exposures to ionizing radiation (E_{radon}, median = 398 WLM) and silica (E_{silica}, median = 1398 y.ppcc); r = 0.7332; n = 299.

□ NO smoking data available, r = 0.7237; n = 144
X YES smoking data available, r = 0.7253; n = 155

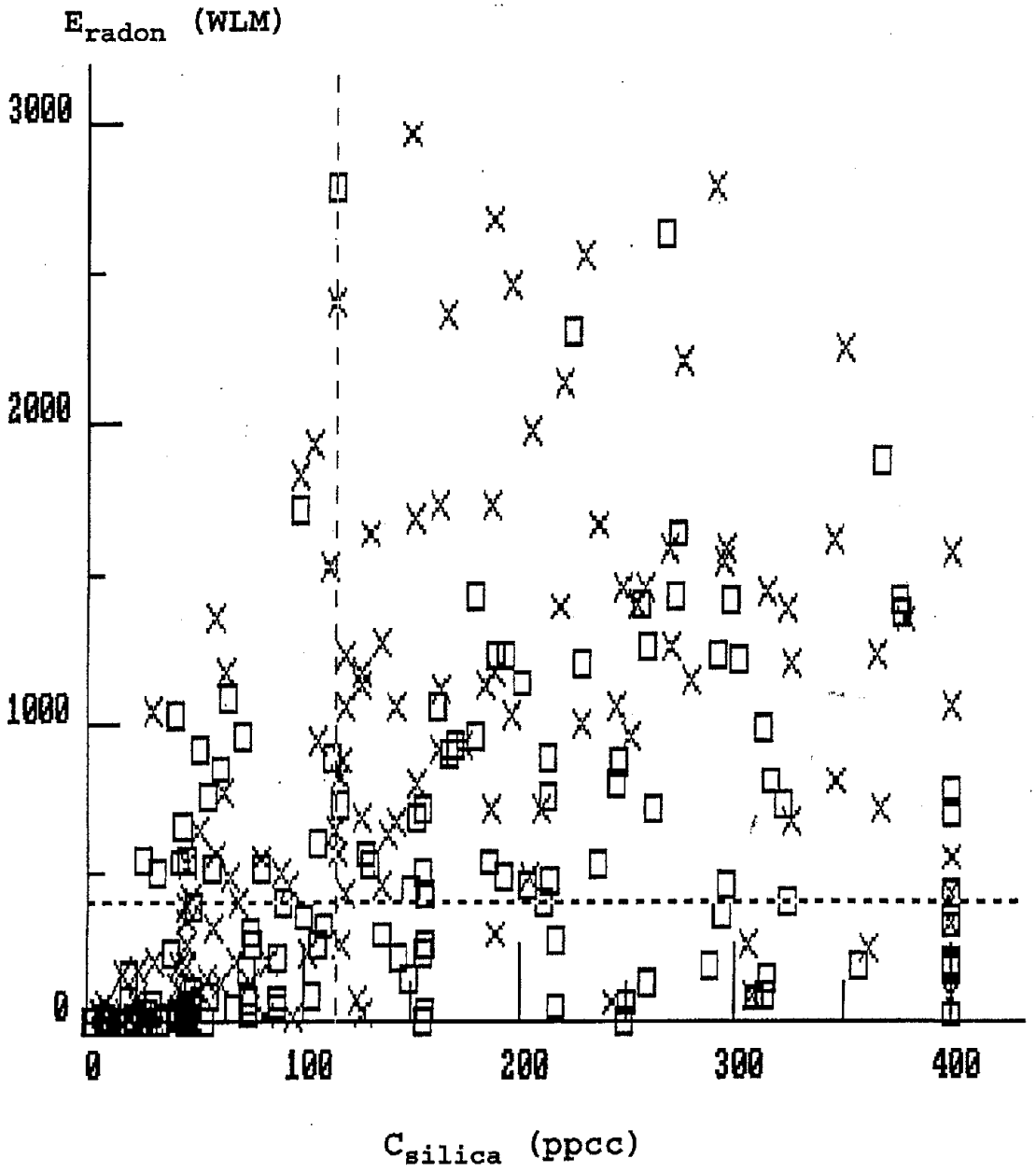

Figure 3

Relationship between estimates of exposure to ionizing radiation (E_{radon}, median = 398 WLM) and intensity of exposure to silica (C_{silica}, median = 115 ppcc); r = 0.4470; n = 299

□ NO smoking data available, r = 0.4061; n = 144
X YES smoking data available, r = 0.5098; n = 155

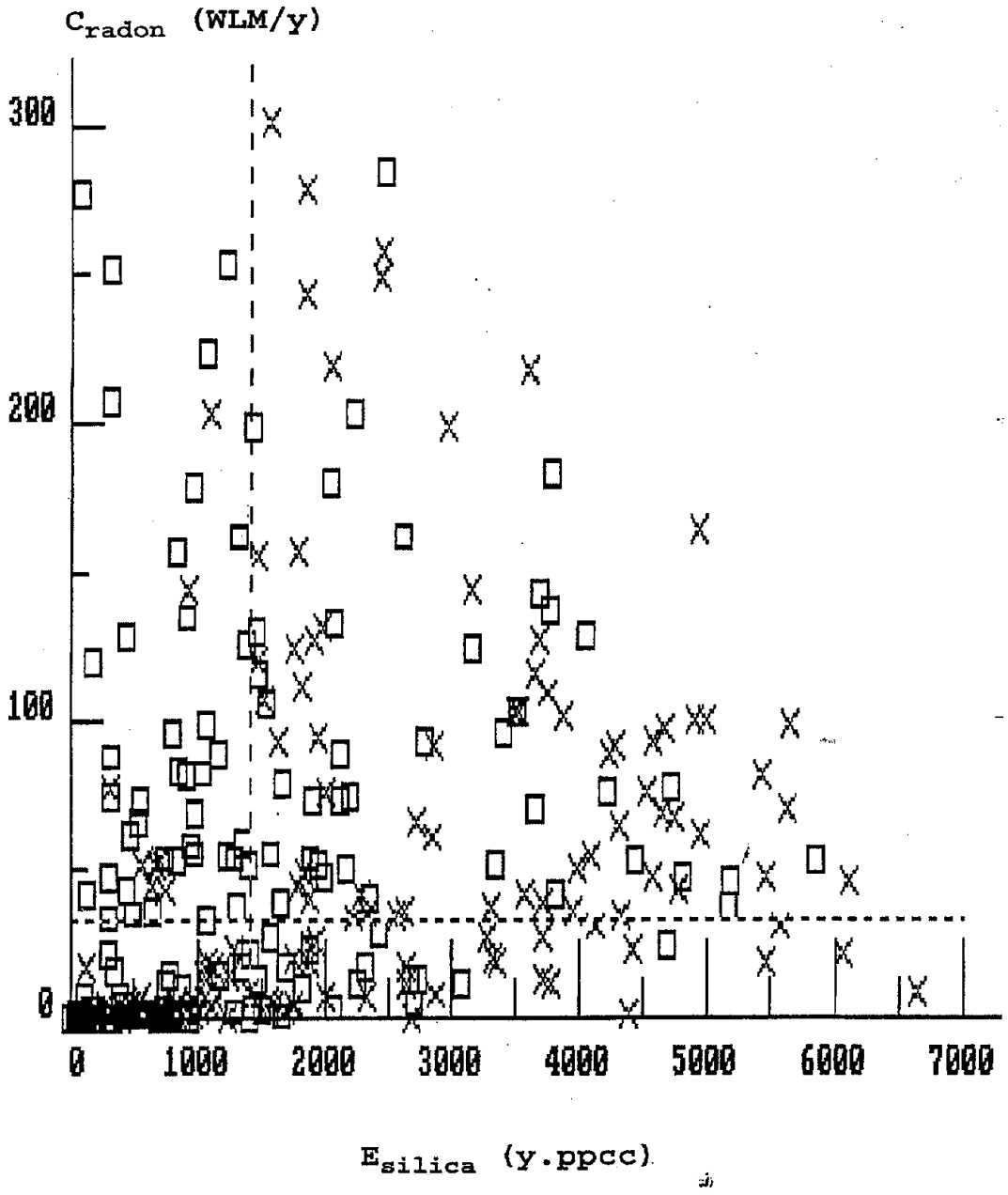

Figure 4

Relationship between estimates of intensity of exposure to ionizing radiation (C_{radon}, median = 33 WLM/y) and cumulative exposure to silica (E_{silica}, median = 1398 y.ppcc); r = 0.2339; n=299

□ NO smoking data available, r = 0.2171; n = 144
X YES smoking data available, r = 0.2859; n = 155

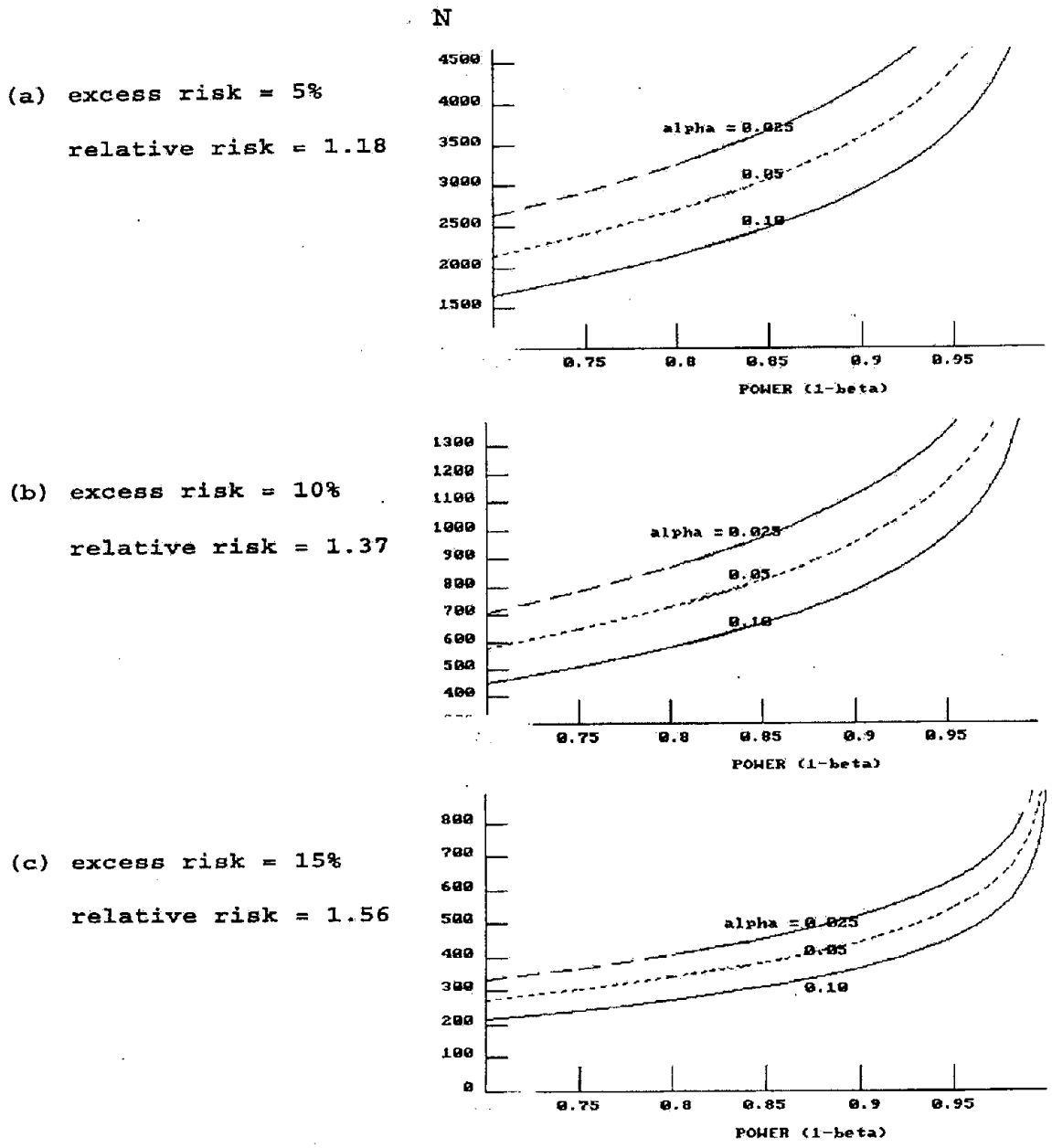

Figure 5

Sample sizes (N) consistent with verifying predictions that the proportion of peripherally located lung cancers among miners with parenchymal silicosis exceeds that occurring among miners with no silicosis, as a function of statistical power (1-beta) and three levels of statistical significance (alpha). Baseline proportion among those with no silicosis taken as 27%.

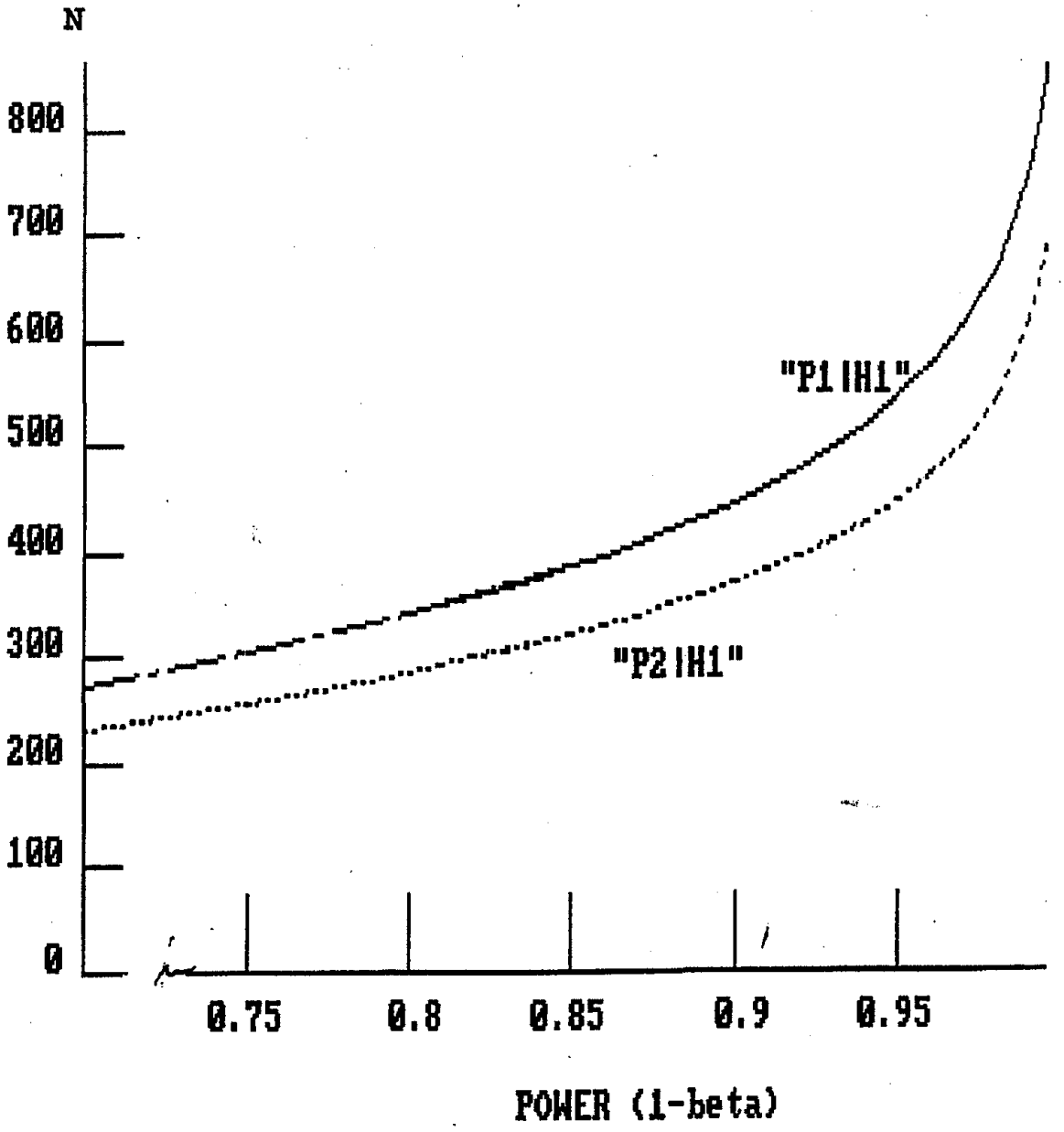

Figure 6

Sample sizes (N) and statistical power (i-beta) consistent with verifying two predictions (P1 and P2) arising from the same hypothesis (H1) with at least a 5% significance level - see text. In both cases the predicted excess risks are 15%; the corresponding relative risks are 1.56 and 2.00. Base-line proportions under the null hypotheses are 0.27 for P1/H1 and 0.15 for P2/H1 (from Tables 7 and 10, respectively). For P1/H1, the ratio of those with parenchymal silicosis to those with no silicosis (parenchymal or lymph node) was assumed to be 1.11 (from Table 9). For P2/H1, the ratio of those with and without any silicosis was assumed to be 1.59 (also from Table 9).

(a) excess risk = 5%

relative risk = 1.07

(b) excess risk = 10%

relative risk = 1.15

(c) excess risk = 15%

relative risk = 1.22

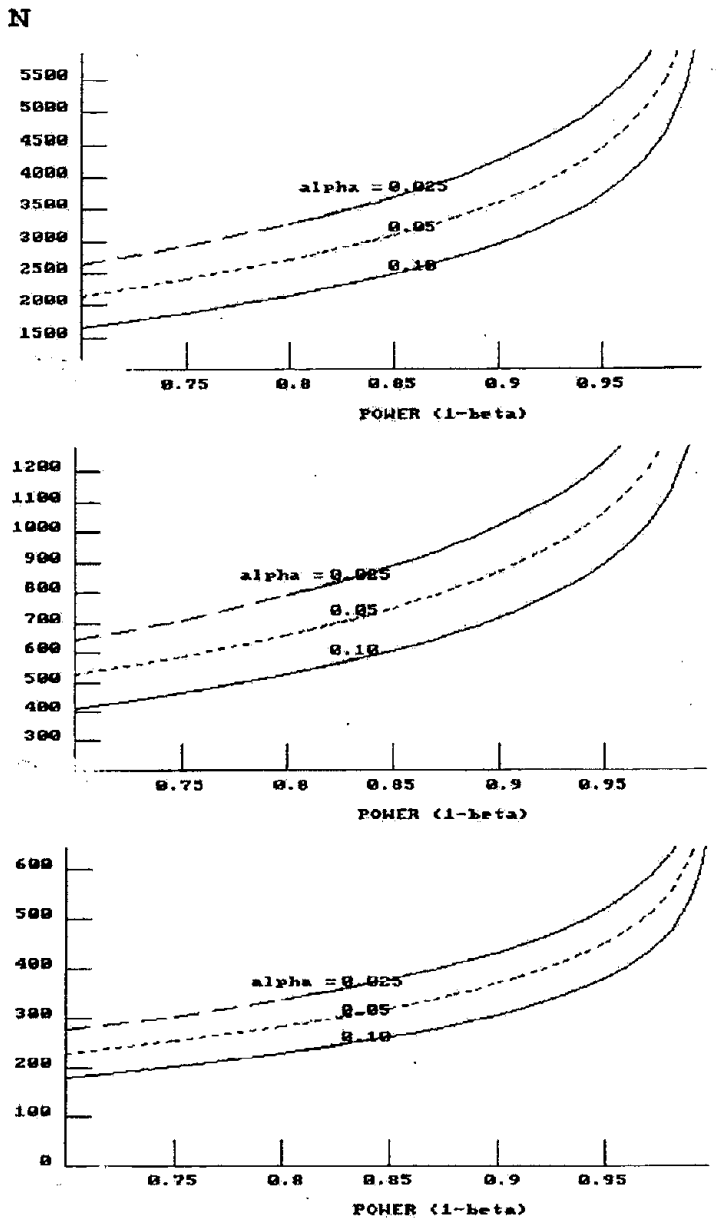

Figure 7

Sample sizes (N) consistent with verifying predictions that the proportion of centrally located lung cancers among miners with lymph node silicosis exceeds that occurring among miners with no lymph node silicosis, as a function of statistical power (1-beta) and three levels of statistical significance (alpha). Baseline proportion among those with no lymph node silicosis taken as 68%.

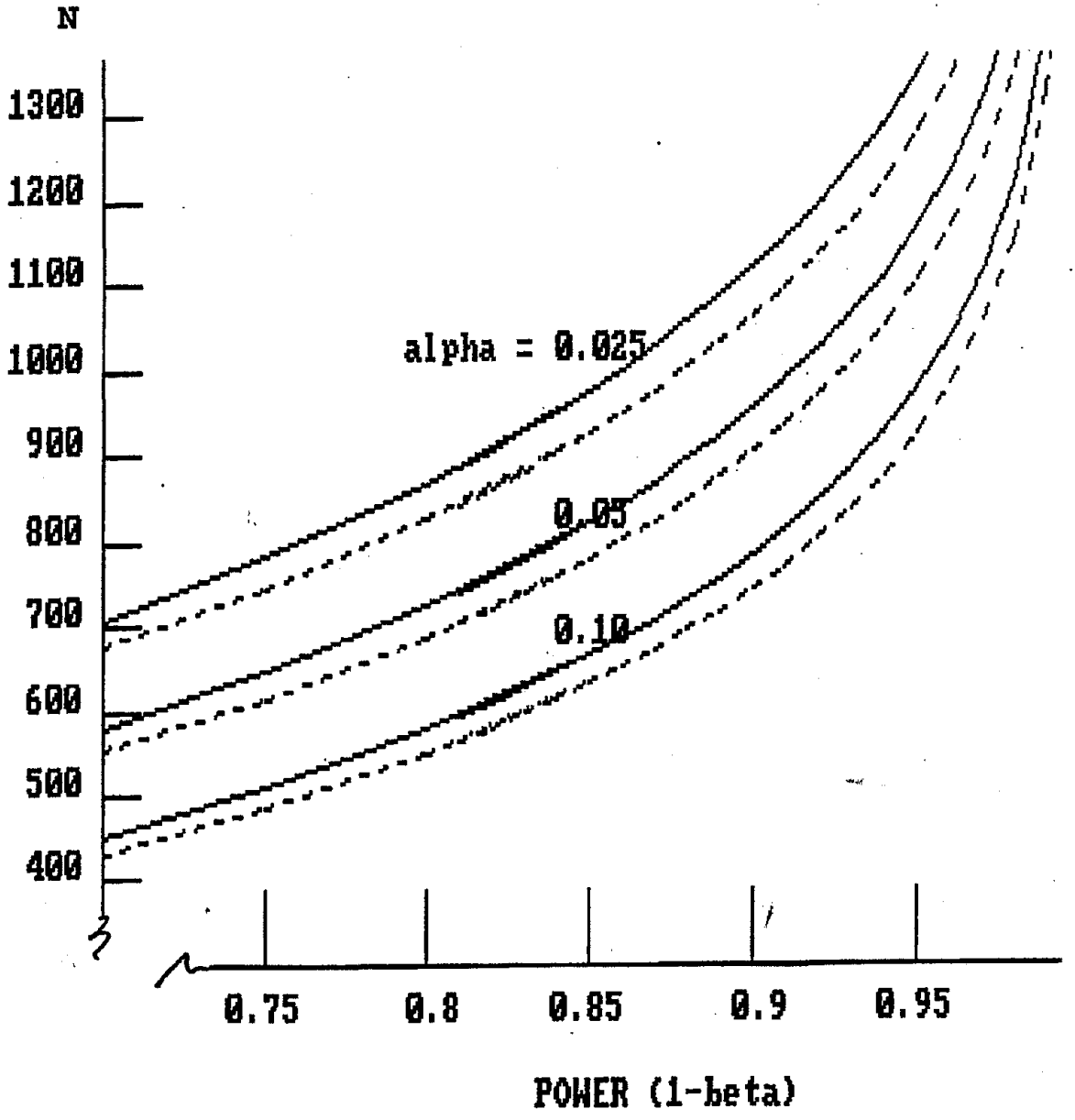

Figure 8

Comparison of sample sizes (N) consistent with verifying the prediction that the proportion of peripherally located lung cancers among miners with parenchymal silicosis exceeds that occurring among miners with no silicosis by at least 10%: _____ using univariate distributions of pooled results from Tables 7 and 9, as in figure 5b (relative risk = 1.37); - - - using realized bivariate distributions of pooled results (relative risk = 1.43).

PATHOLOGIC CHARACTERISTICS AND MECHANISMS OF LUNG CANCER IN SILICA- AND RADON-EXPOSED URANIUM MINERS - A PROPOSAL FOR A PILOT STUDY

1. Purpose

The purpose of this one-year pilot study is to determine whether it will be feasible to use archived lung tissue material from deceased miners, and from other workers who were employed in the uranium mining industry of the former German Democratic Republic, in research aimed at clarifying an hypothesized carcinogenic effect on the lungs of inhaled silica dust.

2. Rationale

2.1 Animal studies have shown that exposure to silica dust induces lung cancer in one species (rats) but not in others (hamsters, mice). Results from various epidemiological studies are inconclusive. Some suggest an association between silica-induced fibrotic disease (silicosis) and lung cancer, but other studies have failed to confirm those observations. The International Agency for Research on Cancer has therefore concluded that there is only "limited evidence" to support the contention that crystalline silica is carcinogenic in humans.

2.2 Resolution of the ambiguity is important from the public health point of view because of the large number of workers exposed to silica dust. In particular, the US Government's National Institute for Occupational Safety and Health (NIOSH) is anxious to pursue relevant research, so that its recommendations on occupational hygiene standards for silica dust are as soundly based as possible. With this in mind, NIOSH's Division of Respiratory Disease Studies, in Morgantown (West Virginia) and researchers at the National Cancer Institute (NCI) in Rockville (Maryland), wish to explore an idea which, if substantiated, might explain some of the equivocal epidemiological findings regarding lung cancer in silica-exposed workers. The idea, which appeals to plausible disease mechanisms that are consistent with observations in laboratory animals, is that silica induces only *specific, histologically distinguishable types* of lung cancer. Rigorous testing of a number of predictions that follow from elaborations of this basic hypothesis requires access to lung tissue material, for laboratory studies, from persons who have been exposed to a wide range of at least approximately quantifiable levels of silica dust and of known carcinogenic agents such as tobacco smoke or radon-daughters*. The proposed pilot study is intended to establish whether these requirements can be met realistically by using preserved lung tissue material and data relating to former *Wismut SDAG* employees and other former residents in the areas of east Germany concerned.

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*See Appendix 1 for an elaboration of the underlying idea and hypotheses.

3. Objectives

Specific objectives for the work proposed in this pilot study are as follows.

3.1 Identify a sample of about 200 to 250 individuals for whom autopsy records and lung tissue material have been preserved, according to a year-of birth and year-of death-stratified sampling scheme.

3.2 Locate the pathology reports ("protocols") for as many as possible of the identified sample, and copy (or extract) specified information required for the research from those reports.

3.3 Locate, and copy information on, the smoking habits of as many as possible of the sample.

3.4 Locate and copy all information on occupational histories of those sampled, particularly those who were ever employed by *Wismut SDAG.*

3.5 Document the extent to which occupational hygiene information relevant to the work histories among those sampled exists, and assess the degree of effort that would be required to make such data accessible for detailed study.

3.6 Construct a detailed job-exposure matrix for at least one former uranium mine at which sampled individuals have worked; assess the degree of effort and resources that would be required to construct similar matrices for other mines; and attempt to apply the compiled job-exposure matrix to a few (*ca.* 20-25) of the occupational histories compiled under (3.4) in a pilot effort to estimate individuals' occupational exposures to silica dust and to carcinogenic materials (e.g., radon, asbestos.)

3.7 Extract lung tissue material relating to approximately 50 individuals identified under step 3.1 from the archive, and transport this material to NIOSH's Morgantown laboratory for up to seven days to allow for intensive testing of the pathology laboratory methods and protocols that are contemplated.

3.8 Assess the results of the pilot study, and report with recommendations regarding the feasibility of continuing research.

4. Methods

4.1 NIOSH will contract with the *Hauptverband der Gewerblichen Berufsgenossenschaften (HVBG)* for access to all the materials and data required for the pilot study. The *HVBG* will, in turn, arrange for co-ordination of all study activities in Germany, and technical day-to-day liaison with the project team in Morgantown, through an academic university department of occupational

medicine in Germany with appropriate expertise in research on mining-related health problems. All data acquired for the purpose of this study will be transmitted to the project center in Morgantown in a form that ensures the anonymity of individuals to whom the data refer and which complies with German law regarding confidentiality of medical and personal information.

4.2 The *HVBG* will arrange for NIOSH access to lung tissue material and associated information as specified under 3.1, 3.2 and 3.7, above.

4.3 The individuals who will be included in the sample (3.1) will be determined using specifications of combinations of dates of birth and dates of death that are designed to include as wide a range as possible of calendar-time-related variations in levels of likely silica and radon daughter exposures. The sample will be restricted to deaths occurring after 1971 because no information on smoking habits of former *Wismut* employees is available for those examined medically only in earlier years.

4.4 Smoking histories will be extracted from *HVBG*-controlled medical records of former *Wismut SDAG* employees. The likely reliability of individual records involved will be assessed with the assistance of persons familiar with procedures used by medical staff of the former *Wismut SDAG* Health and Work Protection division.

4.5 Occupational histories will be compiled by the *HVBG* data processing department using methods and programs that were developed and applied successfully for surviving former *Wismut* employees.

4.6 The *HVBG* will arrange for access to historical records of dust, silica, radon daughter and other pollutant levels at work sites formerly controlled by *Wismut SDAG*. The potential value of this material for the purpose of estimating individual workers' exposures to those pollutants will be assessed jointly by NIOSH occupational hygienists and German scientists familiar with environmental conditions underground in the Erzgebirge during the years 1946-1991.

4.7 The laboratory methods to be tested (3.7) are outlined in Appendix 2.

5. Additional remarks

The report on results from this pilot study will discuss whether it will be practical to pursue the kind of research required to test the ideas outlined in Appendix 1. Any recommendations to that effect would include estimates, based on results from the pilot study, of the sample sizes that would be required to provide different levels of statistical power to detect pathologically significant effects convincingly. Note that such research could not provide direct evidence to implicate or exonerate silica as a carcinogen. However, if it transpires that, as hypothesized, only specific types of lung cancer are associated with silicosis, then this conclusion is likely to influence quite profoundly the direction and design of future epidemiological research on the topic.

6. Staff

The NIOSH Project Officer for this study is Dr Barbara S Ducatman. Dr Umberto Saffiottti is Project Officer for the NCI. The project team currently assembled comprises Drs. Mustafa Dosemeci (NCI), Ann Hubbs, Michael Jacobsen, Michael McCawley, William Miller (all at NIOSH), Peter Morfeld (Institut für Arbeitswissenschaften der Ruhrkohle AG, Dortmund), Tong-man Ong (NIOSH), Professor Claus Piekarski (Institut für Arbeits- und Sozialmedizin der Universität zu Köln), and Drs Nathaniel Rothman (NCI) and Val Vallyathan (NIOSH).

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APPENDIX 1

THE ROLE OF INHALED SILICA IN LUNG CANCER: SOME HYPOTHESES

Background

This appendix outlines the rationale for proposed research on hypothesized bio-mechanisms that might result in silica-induced lung cancer in humans. The question remains controversial despite decades of research. Some epidemiological studies have been interpreted as suggesting that there is an association, albeit weak, between silicosis and lung cancer. Other surveys, however, have not confirmed these observations, and debates continue about possible reasons for the apparently inconsistent results. A major problem with the epidemiological data is the difficulty of ensuring that adequate account has been taken of the effects of simultaneous exposure to known carcinogens such as tobacco smoke and, in underground miners, their exposures to naturally occurring radon daughters. Animal studies, on the other hand, have shown that silica exposure induces lung cancer - in rats, but not in hamsters or mice. Yet rats and mice both exhibit the fibrogenic effects of inhaled silica; hamsters do not.

Lung tissue material from a large series of autopsies of former uranium miners has been preserved in Germany, together with the associated autopsy reports, medical records, occupational hygiene and employment data. The existence of this material provides an unusual opportunity for new research on pathologic and molecular aspects of disease mechanisms that might explain some of the epidemiologic observations regarding lung cancer in persons exposed to silica dust.

Possible mechanisms

There are several plausible pathways that might explain the hypothesized increased risks of lung cancer in humans who have inhaled significant quantities of silica dust.

1. **Silica may act by inducing proliferation of epithelial cells.** In this case

    1.1 silica may be functioning as a "promoter", or

    1.2 silica-induced cell proliferation may be the initial event in carcinogenesis.

Mechanism 1.1 would imply that the excess epithelial cells might have experienced previous DNA damage by exposure to carcinogens such as radon daughters or tobacco smoke, or that they would be more vulnerable to such damage. In the classic initiation-promotion theory, an agent other than silica would be the inducer, causing irreversible damage (mutation) to DNA of lung epithelial cells. According to this model, silica would then induce proliferation of these cells, acting as a promoter, leading to clonal expansion of the transformed cells and thus, tumorigenesis.

If silicosis causes lung cancer by inducing proliferation of epithelial cells, then the cancers would be expected to follow the distribution of silicotic lesions, these being primarily peripheral and in the upper lung zones. Such cases are predicted to be adenocarcinomas, most likely "scar carcinomas", or bronchioloalveolar carcinomas. The absence of any reports in the literature attesting to an excess presence of these types of cancers in silica-exposed individuals appears to weigh against the idea. However, there are only a few studies that have considered different lung cancer cell types in silica-exposed individuals, and they suffer from small numbers and lack of pathologic information apart from cell type (including site of origin, presence of pre-neoplastic precursors and careful analysis of presence and extent of silicosis). Further research seems to be warranted, therefore, before rejecting the suggestion.

According to an alternative proliferation theory (1.2), silica could induce proliferation of alveolar epithelial cells, possibly due to the actions of cytokines. Cells are more susceptible to mutagenesis during mitotic division and such mutational events are passed onto successive generations. Thus the proliferating cells are more susceptible to initiating agents that mutate DNA, including radon. In these circumstances, pre-neoplastic lesions (i.e, type II alveolar cell hyperplasia, adenomas, adenomatoid lesions) or immunohistochemical staining results for cytokines in lungs of miners with silicosis, with or without lung cancer, would be expected to be similar to those observed in the rat model. The proliferative lesions might be either adjacent to or apart from the cancer, but they would be distributed peripherally. (There have been some anecdotal reports of proliferation of type II alveolar cells, but not adenomas and adenomatoid proliferations, in human lungs with silicosis and lung cancer. U. Saffiotti, Personal Communication, 1993.)

2. Silica may act as a carcinogen indirectly, by impeding clearance of other agents that are carcinogenic.

Mechanism 2 would imply that the presence of silicotic lesions in lymph nodes impedes the clearance, *via* the lymphatic system, of inhaled particles including carcinogens such as radon daughters or tobacco smoke. The resulting increase in residence time in the lung effectively increases the time available for induction of DNA damage and cellular changes that lead to cancer.

Some observations consistent with this idea come from an autopsy study of South African gold miners. An increased prevalence of lung cancer was found in miners with silicotic lesions in the lymph nodes, but not in the parenchyma.

The percentage of centrally located, small cell carcinomas, and to a lesser extent squamous cell carcinomas is reportedly increased in uranium miners exposed to radon daughters. However, studies of American uranium miners have not examined the presence and/or extent of silicotic lesions. Squamous cell carcinomas, and particularly small cell carcinomas are found with increased frequency among cigarette smokers. If, as postulated here, inhaled carcinogens have an increased chance of acting to damage the lung among those who have experienced high exposures to silica, then one would expect that the damage concerned would occur in the central airways where such cancers classically arise.

It is possible also that

3. silica is an "initiator" of the carcinogenic process, perhaps by generating free oxygen radical groups in close proximity to DNA molecules.

Recent *in-vitro* studies have suggested that silica can bind to the phosphate backbone of the DNA molecule, placing oxygen free radicals in close proximity to DNA. It is hypothesized that these free radicals then act to irreversibly damage DNA. If silica acted as an initiator, then mutations of genes that control cellular proliferation and differentiation would be expected. Those genes may be classified broadly into two categories: oncogenes and tumor suppressor genes. Mutations in the latent form, or proto-oncogenes, may lead to the activated form (oncogenes). On the other hand, mutations that inactivate the wild form of tumor suppressor genes, such as p53, may lead to accumulation of the mutant protein products, and loss of regulation of cellular proliferation.

Specific mutations of both proto-oncogenes and tumor suppressor genes have been associated with occupational and environmental carcinogens such as cigarette smoking and radon exposure. Thus, it is possible that particular carcinogens may have a characteristic "molecular signatures". If silica were an initiator, then it might induce a unique and specific mutation. The most likely genes of interest include p53, ras, and myc.

Hypotheses and predictions

The general cell-proliferation mechanism postulated above (1) can be formulated as an hypothesis that is testable in principle given appropriate material and data.

H1: Silica-induced proliferation of human lung epithelial cells increases lung cancer risks.

If this hypothesis is valid then we predict:

P1|H1: lung cancer in uranium miners with histo-pathological evidence of parenchymal silicosis will include a higher proportion of peripherally located adenocarcinomas than will be found in the lungs of other, similarly exposed, miners with no histo-pathological evidence of silicosis.

P2|H1: lungs of miners with histo-pathological evidence of silicosis will exhibit pre-neoplastic lesions (of the kind reported in rats) more frequently than lungs of similar miners with no *post-mortem* evidence of silicosis, *irrespective* of whether the lungs concerned are affected by cancer.

The suggested mechanism 2, above, may be re-phrased as an hypothesis as follows.

H2: Silicosis of lymph nodes reduces lymphatic drainage. This reduces the clearance rate from the lung of inhaled carcinogens (e.g radon daughters or tobacco smoke) and thus increases risks of lung cancer.

This hypothesis leads to

P3|H2: lungs of miners who were exposed to moderate or high levels of known carcinogens (e.g., tobacco smoke. radon daughters) and for whom there is evidence of silicotic lesions in the lymph nodes will have more centrally located small cell and squamous cell carcinomas than similarly exposed miners with no silicotic lesions in the lymph nodes.

Finally, if silica itself is an initiator of the carcinogenic process by acting directly on DNA *via* mechanism 3, then it *may* be that such activity is associated with one or more new, silica-specific, mutations of proto-oncogenes and/or tumor suppressor genes. Identification of such phenomena among miners with relatively high exposures to silica dust (but not in others) would lend some support to the theory. However, failure to identify such unique mutations could not be interpreted as evidence against the theory. In other words, no logically coherent predictions can be made regarding the occurrence of unique mutations of the genes concerned simply by hypothesizing that silica acts directly as an initiator of carcinogenesis.

On the other hand, testing predictions P1, P2 and P3 could lead to some relevant and useful conclusions. Consider the following eight possible outcomes from investigations designed to test those predictions.

	P1 TRUE		**P1 FALSE**	
	P2 TRUE	**P2 FALSE**	**P2 TRUE**	**P2 FALSE**
P3 TRUE	A	B	C	D
P3 FALSE	E	F	G	H

Given adequate data, satisfactory statistical power, and appropriate levels of statistical significance, these outcomes would be interpreted as follows.

A: representing confirmation of all three predictions, would support both hypotheses.

H: provides no evidence of carcinogenic activity by silica-induced proliferation of epithelial cells or by an indirect route associated with lymph-node-specific silica-induced fibrosis.

D: indicates results consistent with H2 but not H1, and

E: indicates results consistent with H1 but not H2.

B or F could both be interpreted as consistent with the idea that silica acts as a carcinogen by

inducing proliferation of epithelial cells (H1) although the hypothesized mechanism involving pre-neoplastic lesions of the kind observed in the rat model would not be supported.

However, the absence of evidence to support P1, in situations C and G, would be inconsistent with the hypothesized cell proliferation idea (H1), even though the lungs show some pre-neoplastic lesions of the kind observed in silica-dosed rats.
Outcomes C and G would therefore have to be interpreted as evidence against H1.

APPENDIX 2

PATHOLOGIC AND MOLECULAR TOXICOLOGY STUDIES CONTEMPLATED

1. Pathologic analysis

All lung tissue sections will be examined by a panel of expert NIOSH and NCI pathologists. The locations of tumors, and tumor types, will be specified according to WHO guidelines, as will the presence and types of pre-neoplastic precursors. These include squamous (metaplasia, dysplasia, and carcinoma in-situ) as well as alveolar (type II alveolar cell hyperplasia, adenomas, and adenomatoid lesions). The presence, location (parenchymal or nodal), and extent of silicosis will be graded according to protocols currently used for NIOSH's National Coal Workers' Autopsy Study.

2. Immunohistochemical analysis

Based on the histology, selected blocks will be examined for the presence of various cytokines which potentially play a role in fibrosis and cancer, including the interleukins, and transforming growth factor β.

3. Molecular pathology

Selected cases will be stained by immunohistochemical methods for p53 protein (monoclonal antibody D07) and p21 ras protein. A small subset of cases will be used for PCR amplification of K-ras and p53 DNA sequences with subsequent DNA sequencing via automated DNA sequencers to look for mutations in hot-spot codons according to published protocols.