Journal of Environmental Pathology, Toxicology and Oncology, 20(Suppl. 1):15–32 (2001)

Biological and Statistical Approaches to Predicting Human Lung Cancer Risk from Silica

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Chronic inflammation is a key step in the pathogenesis of particle-elicited fibrosis and lung cancer in rats, and possibly in humans. In this study, we compute the excess risk estimates for lung cancer in humans with occupational exposure to crystalline silica, using both rat and human data, and using both a threshold approach and linear models. From a toxicokinetic/dynamic model fit to lung burden and pulmonary response data from a subchronic inhalation study in rats, we estimated the minimum critical quartz lung burden (M_{crit}) associated with reduced pulmonary clearance and increased neutrophilic inflammation. A chronic study in rats was also used to predict the human excess risk of lung cancer at various quartz burdens, including mean M_{crit} (0.39 mg/g lung). We used a human kinetic lung model to link the equivalent lung burdens to external exposures in humans. We then computed the excess risk of lung cancer at these external exposures, using data of workers exposed to respirable crystalline silica and using Poisson regression and lifetable analyses. Finally, we compared the lung cancer excess risks estimated from male rat and human data. We found that the rat-based linear model estimates were approximately three times higher than those based on human data (e.g., 2.8% in rats vs. 0.9-1% in humans, at mean M_{crit} lung burden or associated mean working lifetime exposure of 0.036 mg/m^3). Accounting for variability and uncertainty resulted in 100–1000 times lower estimates of human critical lung burden and airborne exposure. This study illustrates that assumptions about the relevant biological mechanism, animal model, and statistical approach can all influence the magnitude of lung cancer risk estimates in humans exposed to crystalline silica.

KEY WORDS: crystalline silica, quartz, risk assessment, lung cancer, excess risk, chronic inflammation

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Acknowledgments: We would like to thank Dr. Mike Attfield and Ms. Faye Rice for use of their Poisson regression modeling results in this analysis. We would like to thank Mr. Randall Smith for assistance in S-plus programming and Mr. James Bena for preparing the figures.

0731-8898/01/$5.00

Introduction

Chronic inflammation is considered the hallmark of silica-induced lung injury.[1] It is a key step in the development of both non-neoplastic and neoplastic lung diseases in rats exposed to respirable particles. Chronic inflammation, which is characterized by persistent, increased numbers of polymorphonuclear leukocytes (PMNs) (also known as neutrophils) in the bronchoalveolar lavage fluid (BALF), is associated with the overloading of alveolar macrophage-mediated clearance in rats.[2-4] Particle surface area and number have been shown to be better metrics than mass in predicting overloading, neutrophilic inflammation, and lung tumors in rats exposed to various types of particles.[5-7] High levels of PMNs (e.g., 40% PMNs in BALF) have been associated with the development of lung tumors in rats exposed to respirable particles, including quartz.[8,9] In addition, interstitial inflammation (i.e., inflammatory cells in lung tissue) has been related to increased tumor incidence in rats exposed to quartz and other particles.[10] In humans, chronic inflammation has been associated with particle-related non-neoplastic lung diseases. For example, Rom[11] found a statistically significant increase in the percentage of PMNs in humans who had been occupationally exposed to asbestos, coal, or silica and who had respiratory impairment (4.5%, vs. 1.5% PMNs in controls). Elevated levels of PMNs have been observed in the BAL fluid of miners with simple coal workers' pneumoconiosis (31% of total BALF cells, vs. 3% in controls)[12] and in patients with acute silicosis (also a 10-fold increase over controls).[13,14] Lung diseases characterized by chronic inflammation and epithelial cell proliferation (e.g., idiopathic pulmonary fibrosis and scleroderma) have been associated with an increased risk of lung cancer[15]; however, chronic inflammation has not been etiologically linked to the development of particle-related lung cancer in humans.

Inhaled crystalline silica in the form of quartz or cristobalite in occupational settings has been classified as a human carcinogen,[16] but debate continues concerning the mechanism of action. It is not known whether crystalline silica reacts directly with DNA to cause mutation (i.e., a direct or primary genotoxin) or whether tumorigenesis follows inflammation, oxidative damage, and the development of silicosis (i.e., an indirect or secondary genotoxin). An indirect genotoxic mechanism for the quartz form of crystalline silica is suggested by a study in which the neutrophils and macrophages from the BAL fluid of quartz-instilled rats were found to be mutagenic to epithelial cells from rats not exposed to quartz.[17] This mutagenic response was dependent on the release of reactive oxygen species, and it was significantly greater with neutrophils than macrophages. The quartz particles themselves were not mutagenic in the same assay. Other evidence suggests that quartz may act through a direct genotoxic mechanism. Quartz particles were observed in the nuclei and mitotic spindles of fetal rat lung alveolar type II cells in culture, and oxygen radicals produced by quartz produced DNA strand breaks in vitro.[18] However, this mechanism has not been observed in vivo.

Although the carcinogenic mechanism of crystalline silica is uncertain, it is known that silica damages the lungs through a cycle of oxidant-induced cellular damage and scarring. These mechanisms may include (1) direct cytotoxicity by the silica particle, due to reactive species on the particle surface and lipid peroxidation of cell membranes, with release of intracellular enzymes causing additional tissue damage and scarring; (2) oxidant generation by activated alveolar macrophages, which can overwhelm the lung antioxidant defenses, causing further lipid peroxidation and cell damage; (3) altered expression of inflammatory cytokines and chemokines by alveolar macrophages and/or epithelial cells, leading to recruitment of PMNs and macrophages and more oxidant generation; and (4) secretion of fibrogenic factors from alveolar macrophages and/or epithelial cells, causing chemotaxis of fibroblasts to focal accumulations of silica particles and macrophages.[13,19,20] The pulmonary responses to silica, including the release of specific cytokines, appear to be similar in both humans and animals.[21]

Epidemiological studies have reported an increased risk of lung cancer in humans with occupational exposure to respirable crystalline silica, and exposure-response relationships have been demonstrated in several studies, including diatomaceous earth workers[22] and granite workers.[23] In studies of occupational exposure to respirable coal mine dust (containing approximately 5% quartz), no increased lung cancer has been observed.[24,25] It may be that

the quartz particles are coated with the clay or other substances in the dust, inactivating the reactive surfaces that are thought to be important in the pathogenicity of quartz.[26] IARC recognized that the carcinogenicity of crystalline silica may depend on either its inherent characteristics or external factors affecting its activity.[16]

It is generally recognized that the rat is more sensitive than other rodent species (mice, hamsters) to the effects of inhaled particles.[27,28] A sensitive animal model is usually considered to be beneficial for identifying substances that may be human health hazards. However, some have argued that the particle-induced lung cancer in rats is a species-specific response to the overloading of pulmonary clearance.[8] The rat response to long-term quartz exposure includes fibrosis, epithelial cell hyperplasia, and lung tumors, including malignant neoplasms; in contrast, mice develop fibrosis but no epithelial cell hyperplasia or lung tumors, and hamsters develop granulatomous ("silica-storage") lesions but no fibrosis, hyperplasia, or tumors.[27,29]

The rat neoplastic response resembles a human fibrosis-associated lung cancer known as scar cancer,[29] and the rat is the only rodent species that exhibits lung cancer as observed in some human studies. An International Life Sciences Institute (ILSI) workgroup[15] determined that there is insufficient information in humans to conclude that the rat response to particles is not relevant to humans. This workgroup also recommended that early responses or precursor lesions be used to extrapolate from high to low doses of inhaled particles in rats. Persistent neutrophilic inflammation and cell proliferation were suggested as appropriate precursors of neoplastic responses. The theory is that the precursor events are necessary steps (although not necessarily sufficient) in the development of the later responses, and that lung particle burdens lower than those causing these early events would not lead to the subsequent adverse responses (i.e., essentially a threshold approach).

In this study, we compute the excess risk estimates for lung cancer in humans with occupational exposure to respirable crystalline silica, using both rat and human data. We use biological and statistical approaches to account for species differences in particle clearance and retention kinetics and to evaluate different assumptions about the mechanism of carcinogenesis, including direct genotoxicity (low-dose linear model) and indirect genotoxicity (threshold dose estimated from early pulmonary responses). This approach illustrates the extent to which the risk predictions, and possibly subsequent determinations about safe human exposure levels, may be influenced by assumptions about the relevant biological mechanisms and risk models.

Materials and Methods

Most of the data described in this study are for the quartz form of respirable crystalline silica, but one human study includes the cristobalite form. The term *respirable* refers to particles of aerodynamic sizes likely to enter the gas-exchange (alveolar) region of the lungs following inhalation (<10 μm).

Rat Data

We used data from two studies of rats exposed by inhalation to 15 mg/m^3 of respirable quartz, for up to 6 months in the 1997 study and for up to 3 months in the 1999 study. Details of the design and results of both studies are reported elsewhere.[30-32] In the 1997 study, pathogen-free male Fischer 344 rats were exposed to an aerosol of crystalline silica for 6 hours per day, 5 days per week; and groups of 10 quartz-exposed and control rats were sacrificed after 1, 7, 14, 21, 28, 42, 56, 112, and 161 days of inhalation exposure. In the 1999 study, an equal number of control and quartz-exposed rats were sacrificed after 28, 56, and 84 days of inhalation exposure; additional groups of rats were removed from exposure for a 36-day recovery period. Quartz lung burdens were measured by inductively coupled plasma-atomic emission spectroscopy of acid-digested lung samples. In the 1997 study, lung tissue samples were used to obtain the quartz concentration in the lungs (mg quartz/g lung), whereas in the 1999 study, whole lungs and lung-associated lymph nodes were used to calculate the mg quartz per organ. The whole lung quartz burdens for the 1997 study rats were estimated by interpolating the lung weights from the 1999 study data. Numerous pulmonary responses were measured in both

studies, including cell counts of PMNs and alveolar macrophages; levels of superoxide dismutase, phospholipids, and lipid peroxidation in the BALF; and histopathological assessment of fibrosis and epithelial cell hypertrophy and hyperplasia.

The quartz dust used in the inhalation exposures was Min-U-Sil 5, and the same lot was used in both studies. The mass median aerodynamic diameter of the quartz in the aerosol was 1.62 μm (SD = 0.12) in the 1997 study. The mean specific surface area was 4.57 m^2/g (SD = 0.51) (Wallace, personal communication, June 9, 2000), determined by BET nitrogen gas absorption.[33]

Data from a study of Fischer 344 rats exposed to 1 mg/m^3 (74% respirable quartz, DQ-12 type, with MMAD of 1.4 μm and GSD of 1.8) for 6 hours per day, 5 days per week for up to 2 years,[9,34] were also used in this analysis to estimate excess lung cancer risks in humans exposed to quartz. These data included the lung and lymph node quartz burdens and the tumor response at the end of the study. All lung tumors (malignant and benign) were included as the response, because it is assumed that during the longer human lifetime, such benign tumors may become malignant. Among the 50 male and 50 female rats exposed to quartz, 6 males and 13 females developed tumors,[9] whereas among equal numbers of control rats, 2 male and 1 female developed tumors (Muhle, personal communication, April 6, 2001).

Human Data

Results from two recent analyses of lung cancer in humans with occupational exposure to respirable crystalline silica were also used to estimate excess risk. One study included 2342 Caucasian male diatomaceous earth workers (mining and processing) in California exposed primarily to cristobalite for a mean duration of 7.5 years and a mean intensity of 0.29 mg/m^3.[22] The other study included 5414 male Vermont granite shed workers exposed to quartz dust for a mean duration of 20 years and a mean intensity of 0.08 mg/m^3.[23]

Statistical Analyses

The approach used for this risk assessment is provided in Table 1. First, we used the rodent data of

TABLE 1. Biologically Based Risk Assessment Approach for Quartz

Rat data	
1	Compute NOAELs and LOAELs for early pulmonary responses in rats.
2	Determine minimum critical lung burden (M_{crit}) for reduced pulmonary clearance and neutrophilic inflammation from rat toxicokinetic/dynamic lung model.
3	Evaluate predictability of the short-term kinetic model to chronic quartz exposure.
4	Predict lung tumor response in rats at M_{crit}, using chronic data and quantal multistage model.
5	Express M_{crit} as species-independent metric, as mass (mg quartz/g lung tissue) and surface area (m^2 quartz/m^2 alveolar epithelium).
Human data	
6	Predict working lifetime quartz exposures associated with M_{crit} lung burden, using two separate human kinetic lung models.
7	Predict human lung cancer excess risk at working lifetime exposures associated with M_{crit}, using human data from two studies and linear relative rate model with accounting for competing risks.
Species comparison	
8	Compare human lung cancer risk estimates based on rat and human data, assuming either direct genotoxicity (using linear models in rats and humans) or indirect genotoxicity (threshold approach using M_{crit} from rat data).

early responses to quartz inhalation exposure to identify the lung burdens at which there was either no observed adverse effect level (NOAEL) or the lowest observed adverse effect level (LOAEL). The NOAEL was defined as the mean lung burden of the highest exposure group in which a given pulmonary response did not differ significantly from that of the control group and which immediately preceded the LOAEL; the LOAEL was defined as the mean lung burden of the first exposure group in which a given response *did* differ significantly from the control group.[35] Pairwise *t* tests, in which unequal variances were assumed, were used to compare the exposed and control groups. Bonferroni adjustment for multiple comparisons were used in determining statistical significance—that is, a test was judged statistically significant if the *p* value of a pairwise test (exposure and control) was smaller than $0.05/g$, where g is the number of tests.[36]

An alternative to identifying NOAELs or LOAELs might have been to estimate a benchmark dose,[37] which is a regression-based prediction of doses associated with a specified level of adverse response. However, to estimate these quantities, assumptions about the model form and variance structure would have been required. For these rat data, the variance appeared heterogeneous but without a natural pattern that would be easily accommodated in the standard regression methods (e.g., generalized linear models), although some weighted regression might have been considered. Thus, while the shortcomings of the NOAEL as a design-sensitive potency measure are recognized (e.g., influenced by sample size, spacing of exposure groups, etc.),[37] we felt that comparing exposed and control group responses in a pairwise manner for each time was appropriate for these experimental data.

Second, we compared these values (NOAEL or LOAEL) to the minimum critical lung burden (M_{crit}) identified in a recently developed toxicokinetic/toxicodynamic model of quartz particle clearance and retention and pulmonary response.[38] The model was fit to these rat data[30-32] using the nonlinear least-squares routine NLINFIT in Matlab.[39] M_{crit} is an estimate of the mean total lung burden (i.e., lung and lung-associated lymph nodes) at which the rate of alveolar macrophage-mediated clearance begins to decline and the number of PMNs begins to increase, as specified in the kinetic model.[38] In these rat data, it was not possible to determine a NOAEL for neutrophilic inflammation, because the PMN cell counts were significantly elevated at even the lowest measured lung burden. Thus, we used the kinetic model estimate of M_{crit} (both the mean value and the lower 95% lower confidence limit, or 95% LCL) as a threshold burden. M_{crit} is a LOAEL (not a NOAEL) since it represents the internal dose where quartz-elicited changes are beginning to occur.

Third, because an objective of this analysis was to use the early response data to predict the long-term pulmonary response to quartz, we investigated whether the kinetic model[38] developed using these subchronic rat data[30-32] could predict the quartz lung and lung-associated lymph node burdens in a long-term study in rats exposed to a lower concentration of quartz.[9,34] We also examined the results from several rat studies to determine whether the neutrophilic response at M_{crit} was likely to become persistent.

Fourth, we predicted the lung tumor risks at various lung burdens, including M_{crit}, by fitting a linear multistage model to the long-term dose-response data in rats,[9,34] using Global83[40]:

$$P(D) = 1 - \exp(-Q0 - Q1*D)$$

where $P(D)$ is the probability of lung cancer at a given dose, $Q0$ is the coefficient for the background tumor rate, $Q1$ is the coefficient for the dose, and D is the dose (quartz burden in the lungs and lymph nodes). The maximum likelihood estimates (MLE) and 95% upper confidence limits (95% UCL) of the risks were computed. In the chronic rat study,[9,34] only two concentrations were available (control and exposed); thus, the model fit through both points. Obviously, more dose groups would be desired in a dose-response study to facilitate description of any curvature in the dose-response. However, none of the published studies included multiple dose groups, and this study provides the best data available for lung cancer in rats exposed to quartz. The experimental conditions were well described, rats were chronically exposed by inhalation, an acceptable number of animals was used in exposed and control groups, and the lung and lymph node quartz burdens over time were provided. In addition, data for both sexes were available; however, we used only the male rat data because the rat study used in the toxicokinetic model to derive M_{crit}[31,32,38] and both human studies[23,23] included

males only (alternatively, the female rats, which had greater lung tumor response, could have been used as the more sensitive sex). In the fifth step, we extrapolated these results to humans by expressing dose as either mass (mg quartz/g lung tissue) or surface area (m^2/m^2 alveolar epithelial surface), and assuming equal responses to equivalent doses in each species.

In the sixth step, using human data, we predicted the quartz concentrations during a 45-year working lifetime that would result in the human-equivalent M_{crit} quartz burden. This was accomplished by using our previously developed human kinetic lung model for pulmonary clearance and retention,[41-43] which includes alveolar, interstitial/sequestration, and hilar (thoracic) lymph node compartments. The model was calibrated using data of coal dust exposures and lung burdens in US coal miners[44,45] and validated using coal dust data from UK coal miners.[46] The model was then modified to describe quartz clearance and retention kinetics in human lungs, using quartz data from coal miners in the UK.[46] For this study, we used the geometric mean parameter values from the UK data-derived quartz model to estimate the mean quartz lung burden in US coal miners. We also estimated the mean quartz lung burden using a second kinetic human lung model, which was developed using radionucleotide data.[47] That model includes three separate alveolar/interstitial compartments (with different particle deposition fractions and clearance rate coefficients assigned to each) and a thoracic lymph node compartment (we coded this model in Matlab[39]). We compared the predictions from each human model to the observed mean quartz lung burden in US coal miners.[44,45] In addition, we used both human models to estimate the lung and lymph node burdens associated with working lifetime exposure to respirable crystalline silica at the current US occupational exposure limits, including the permissible exposure limit (PEL)[48,49] (0.1 mg/m^3 for quartz; 0.05 mg/m^3 for cristobalite) and the recommended exposure limit (REL)[50] (0.05 mg/m^3 for each type). We assumed the same deposition and clearance kinetics for the quartz and cristobalite polymorphs.

In step 7, we predicted the silica-attributable (i.e., excess) risk of lung cancer at these target working lifetime exposures, using data from two recent human studies of workers exposed to silica.[22,23] Estimated coefficients describing the relationship between cumulative exposure to respirable crystalline silica and lung cancer, reported in those studies, were used in this analysis. Both studies used the linear relative rate form of the Poisson regression model with external adjustments (based on US death rates) and lags (10-year in Rice et al.[22] and 15-year in Attfield and Costello[23]). The MLE estimated coefficient associated with cumulative silica exposure, and the 95% UCL from those studies were, respectively, 0.1441 and 0.3107 (Rice et al.[22]), and 0.1900 and 0.3013 (Attfield and Costello[23]).

In the present study, we compared the lung cancer rates among silica-exposed workers to the background rate in persons without silica exposure, also using the linear relative rate form of the Poisson regression model:

$$R(X) = R0 * (1 + \beta * X)$$

where R is the relative rate at a given exposure, $R0$ is the background rate (at exposure equal to 0), β is the coefficient for cumulative exposure to crystalline silica (either cristobalite[22] or quartz[23]), and X is the cumulative exposure to silica (mg/m^3 × years), estimated in an internal cohort analysis. The lifetime excess risks were estimated using these $R(X)$ estimates in an actuarial (or lifetable) method that accounts for competing causes of death.[51] This method was implemented in SAS.[52] These lifetime excess risk estimates were computed for the selected exposure concentrations during a 45-year working lifetime from age 20 to 65, and the annual risks were accumulated up to age 75. The age-specific background rates for both lung cancer and competing causes were obtained from the 1992 US vital statistics for all males.[53]

Finally, in step 8, we provided the excess risk estimates for lung cancer in humans with occupational exposures to respirable crystalline silica, predicted from linear models using both rat and human data. This method is consistent with an assumption of direct genotoxicity by crystalline silica. These risk estimates were computed for several working lifetime average airborne concentrations (including current exposure limits) and for several internal doses (including mean and 95%LCL M_{crit}). Also, we adjusted the mean M_{crit}, a LOAEL for chronic inflammation derived from rat data, using standard factors to account for variability and uncertainty in order to estimate an internal dose and external ex-

posure below which lung cancer would not be expected to occur in humans, under an assumption of indirect genotoxicity.

Results

The estimated LOAELs and NOAELs for biochemical, cellular, and histopathological responses in the lungs of rats are presented in Table 2. Figure 1 illustrates the shape of the exposure-response relationships for these lung responses in exposed and control rats. In general, there is a consistency in the time course of the pathophysiological responses and the exposure duration and lung burdens. For example, the earliest expected response, neutrophilic inflammation, had the lowest LOAEL and no NOAEL (statistically significant increase in number of PMNs observed even at the lowest time point and lung burden). The next lowest LOAEL and NOAEL were for increased superoxide dismutase, the antioxidant enzyme that converts superoxide radical into hydrogen peroxide; this measure reflects the lung response to the increased reactive oxygen species from both the quartz particles and the elevated numbers of PMNs and alveolar macrophages. The increase in phospholipids and lipid peroxidation, which are associated with oxidative damage to the lung cell membranes, became statistically significant at days 21 and 56, respectively. The increase in alveolar macrophages did not become statistically significant until day 56, possibly due to the considerable variability in the cell counts of both the control and exposed groups (Fig. 1). Fibrosis developed relatively late, as expected, and was first observed by histopathological examination in exposed animals at day 112. Interestingly, alveolar epithelial cell hypertrophy and hyperplasia occurred relatively early (day 28) compared to fibrosis.

The mean minimum lung burden for increased neutrophilic inflammation (M_{crit}), essentially a threshold burden, was estimated from the rat toxicokinetic/toxicodynamic model[38] to be 0.39 mg quartz/g lung tissue. This value was computed by running the model and determining the internal dose (total lung and lymph node quartz burdens) associated with the critical phagocytized particle burden (M_{crit} of 0.208 mg/lung) at which macrophage-

TABLE 2. NOAELs and LOAELs for Early Responses in Rats Exposed to 15 mg/m^3 of Respirable Quartz, By Exposure Duration and Lung Dose

	No observed adverse effect level (NOAEL)[a]		Lowest observed adverse effect level (LOAEL)[b]	
Pulmonary response[30-32]	Exposure duration calendar days	Mean lung burden (SD) (mg/lung)	Exposure duration calendar days	Mean lung burden (SD) (mg/lung)
Polymorphonuclear leukocytes, PMN ($\times 10^6$ cells/lung)[a]	NA	NA	7	0.58 (0.18)
Superoxide dismutase (ng SOD/mL)[a]	7	0.58 (0.18)	14	0.85 (0.21)
Phospholipids (μg Pi/mL)[a]	14	0.85 (0.21)	21	1.28[c]
Lipid peroxidation (nmoles LPO/lung)[a]	28	1.48 (0.15)	56	3.20 (0.34)
Alveolar macrophages, AM ($\times 10^6$ cells/lung)[a]	42	2.28[c]	56	3.20 (0.34)
Fibrosis[b]	56	3.20 (0.34)	112	5.51 (0.78)
Alveolar epithelial hypertrophy and hyperplasia[b]	14	0.85 (0.21)	28	1.48 (0.15)

[a] Measured in bronchoalveolar lavage fluid.
[b] Measured by histopathologic examination, as the sum of the severity and distribution scores, for either fibrotic lesions or hypertrophied alveolar epithelial cells (which have been associated with alveolar type I cell damage and alveolar type II cell hyperplasia).[54]
[c] Estimated by interpolation of values on preceding and succeeding days.
Note: NA = not available; effect was observed even at lowest exposure group.

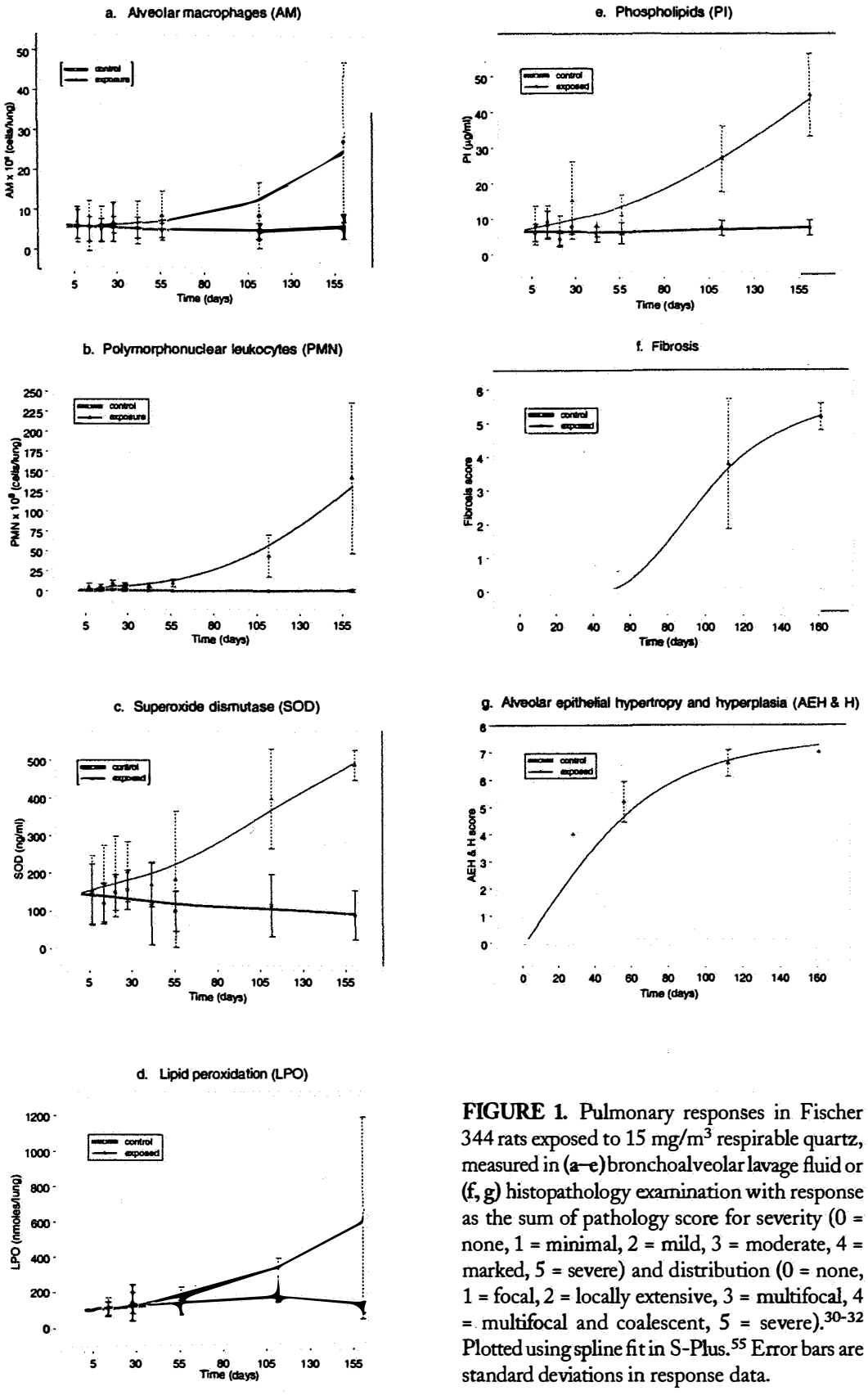

FIGURE 1. Pulmonary responses in Fischer 344 rats exposed to 15 mg/m^3 respirable quartz, measured in (a–e) bronchoalveolar lavage fluid or (f, g) histopathology examination with response as the sum of pathology score for severity (0 = none, 1 = minimal, 2 = mild, 3 = moderate, 4 = marked, 5 = severe) and distribution (0 = none, 1 = focal, 2 = locally extensive, 3 = multifocal, 4 = multifocal and coalescent, 5 = severe).[30-32] Plotted using spline fit in S-Plus.[55] Error bars are standard deviations in response data.

TABLE 3. Predicted Minimum Critical Lung Burden (M_{crit}) in Male Rats and Humans, Expressed as Mass and Surface Area

	Lung: organ values		Predicted M_{crit}[a]: mass		Predicted M_{crit}: surface area	
Species	Whole lung mass (g)	Alveolar epithelial surface area (m^2)[b]	Mass (mg) quartz per whole lungs	Mass (mg) quartz per mass (g) of lung tissue	Surface area (m^2) quartz[b] per whole lungs	Surface area (m^2) quartz per surface area (m^2) of lung
Rat	0.9[c]	0.406[56,57]	0.35[a]	0.39	1.60×10^{-3}	3.94×10^{-3}
Human	1000[58]	96[56,59]	390	0.39[d]	1.78	1.85×10^{-2}

[a] Lung burden associated with reduced pulmonary clearance and neutrophilic inflammation; estimated from rat kinetic lung model.[38]
[b] Mean specific surface area of quartz (Min-U-Sil 5): 4.57 m^2/g (SD = 0.51) (Wallace, personal communication, June 9, 2000), determined by BET nitrogen gas absorption.[33] Surface area of crystalline silica in human exposures assumed to be the same.
[c] Mean lung weight (g) of male control rats in 3-month study[30-32] used to calibrate kinetic model and derive M_{crit}.[38]
[d] Assuming equal sensitivity in humans and rats.

mediated clearance begins to decline and neutrophilic inflammation begins to increase.[38] The model-derived total internal dose (0.35 mg/lung) was then divided by the average lung weight of 0.9 g for the rats in that 3-month study[31,32] to obtain the mean M_{crit} of 0.39 mg/g. Similarly, the 95% lower confidence limit (95% LCL) M_{crit} was computed to be 0.049 mg/g lung (from the model-derived internal dose of 0.044 mg/lung associated with the 95% LCL M_{crit} of 0.0234 mg/lung, and assuming average lung weight). The mean M_{crit} value, expressed as either mass or surface area per unit of lung tissue, is then extrapolated to humans (Table 3). Because the rat data[9,34] and the kinetic models[38,42,46] used in this study are based on particle mass dose, we extrapolated to humans using the mass dose. However, when the rat mass dose (0.39 mg quartz/g lung) is converted to surface area dose (3.94×10^{-3} m^2 quartz/m^2 lung), the human-equivalent dose is 5 times greater than the rat dose (Table 3). These calculations assume the same surface area of silica to which the rats and humans were exposed.

Table 4 provides a comparison of the observed quartz burden in the lungs and lung-associated lymph nodes (LALN) in rats exposed to 0.74 mg/m^3 respirable quartz for 2 years[9,34] to those values predicted from the kinetic model used to estimate M_{crit}, using the mean parameter estimates from that model[38] to fit data from the subchronic rat study.[30-32] In general, the kinetic model, which was developed from the

TABLE 4. Observed and Predicted Quartz Lung Burden in Male Rats Exposed to 0.74 mg/m^3 Respirable Quartz for 2 Years

	Observed mass: mean mg/organ (SD)[34]			Predicted mass: mean mg/organ[38]		
Exposure duration (mos.)	Lungs	LALN	Total lungs and LALN	Lungs	LALN	Total lungs and LALN
3	0.25 (0.02)	NA	NA	0.172	0.0207	0.193
9	0.65 (0.05)	0.117	0.767	0.340	0.176	0.515
15	0.85 (0.05)	0.303	1.153	0.416	0.417	0.833
21	0.96 (0.05)	0.423	1.383	0.452	0.700	1.15
24	1.03 (0.09)	0.488	1.518	0.461	0.849	1.31

Note: NA = not available; not reported in article.[34]

shorter-term, higher-concentration data, underpredicts the observed lung burden and overpredicts the observed lymph node burden from the longer-term, lower-concentration study. However, the predictions for the total quartz burden in the lungs and lymph nodes are reasonably close to (although lower than) the observed values; the predicted total burden is 86% of the observed total burden at 24 months.

In humans, the time-weighted average (TWA) respirable quartz concentration over a full working lifetime that is estimated to be associated with the M_{crit} lung burden (0.39 mg/g lung tissue) is presented in Table 5. Also shown are the estimated quartz lung burdens and lymph node burdens associated with the current US occupational exposure limits for respirable quartz. These estimates are provided from two separate human kinetic lung models for respirable particle clearance and retention, developed using data from coal miners[41-43,46] and from humans exposed to radioactive particles.[47] The quartz concentration associated with M_{crit} was estimated to be 0.036 mg/m^3 using the Kuempel/Tran model and 0.45 mg/m^3 using the ICRP model. The predictions from these two kinetic models differ by more than an order of magnitude.

To evaluate which kinetic model is likely to better predict the relationship between occupational quartz exposure and lung burden, we compared the predictions from those models to available data on observed lung burden data from US coal miners. These miners were exposed to a mean concentration of respirable coal mine dust (with approximately 5% respirable quartz) for a mean of 36 years. At autopsy, the mean quartz lung burden was 0.38 g/whole lung, with an estimated 95% confidence interval of 0.096–3.7 g/whole lung (values calculated from mass of quartz in the dry lung, assuming dry/wet tissue weight ratio of 1/5 and whole lung weight of 1000 g).[45] Although the Kuempel/Tran model was developed for coal mine dust using US data, it was extended to describe quartz clearance and retention in the lungs using UK data. Thus, it is the UK-derived parameter values that were used to predict the US data. That model predicted a mean quartz lung burden of 0.66 g/whole lungs for US coal miners. This prediction is 1.7 times greater than the observed quartz lung burden, but within the confidence interval. The ICRP model predicted a mean quartz lung burden of 0.074 g/whole lungs in the US miners. This prediction is five times lower than the observed mean quartz lung burden and also below the 95% lower confidence limit. Thus, we used the Kuempel/Tran model to predict the human internal dose (lung and lymph node quartz burdens, at age 75 years, following a 45-year working lifetime, beginning at age 20 years, plus 10 years' retirement) associated with exposure to a given average airborne concentration of respirable crystalline silica (Table 6). This model was also used to predict the average quartz concentra-

TABLE 5. Average Respirable Quartz Exposure Over a 45-year Working Lifetime and Associated Lung and Lymph Node Quartz Burdens

	Kuempel/Tran model[41-43,46]		ICRP model[47]	
Airborne respirable quartz average concentration (mg/m^3)	Predicted mean lung burden (mg/g tissue)	Predicted mean lymph node burden (mg/g tissue)	Predicted mean lung burden (mg/g tissue)	Predicted mean lymph node burden (mg/g tissue)
0.036[a]	0.22	0.17	0.042	0.013
0.05[b]	0.31	0.23	0.033	0.010
0.1[c]	0.62	0.46	0.067	0.021
0.45[d]	3.57	2.68	0.30	0.094

[a] Predicted from Kuempel/Tran model to be associated with M_{crit} (i.e., 0.39 mg/g in the lungs and lung-associated lymph nodes), estimated from kinetic model in rats.[38]
[b] Recommended exposure limit for respirable crystalline silica 50 and cristobalite.[48,49]
[c] Permissible exposure limit for respirable quartz.[48,49]
[d] Predicted from ICRP model to be associated with M_{crit}.

TABLE 6. Human Lung Cancer Excess Risk Associated with Respirable Crystalline Silica Exposure and Retained Lung Burden, Predicted from Rat and Human Studies

Species and study	External exposure in humans (mg/m^3)	Internal dose in rats or humans (mg/g lung)	Excess risk (%) Maximum likelihood estimate	Excess risk (%) 95% upper confidence limit
Rat				
Muhle et al.[9]	NA	0.39[a]	2.8	6.5
	NA	0.54	3.9	8.9
	NA	1.08	7.7	16.8
Human				
Rice et al.[22]	0.036[b]	0.39	0.87	1.8
	0.05[c]	0.54[e]	1.2	2.6
	0.1[d]	1.08[f]	2.4	5
Attfield and Costello[23]	0.036	0.39	0.97	1.5
	0.05	0.54	1.3	2.1
	0.1	1.08	2.7	4.2

[a] M_{crit}: critical mass in the lungs and lymph nodes associated with reduced pulmonary clearance and neutrophilic inflammation; estimated from kinetic model in rats.[38]
[b] Average airborne concentration over 45-year working lifetime, predicted from human kinetic lung model[41-43,46] to be associated with M_{crit}.
[c] Recommended exposure limit (REL) for respirable crystalline silica 50 and cristobalite.[48,49]
[d] Permissible exposure limit (PEL) for respirable quartz.[48,49]
[e,f] Internal dose predicted from human kinetic lung model[41-43,46] to be associated with 45-year working lifetime exposure at either REL or PEL.

tion associated with a given internal dose, for example, M_{crit} (Table 6).

Finally, the human lung cancer excess risk estimates, from low-dose linear models fit to both rat and human data, are also presented in Table 6. The lung cancer excess risk estimates based on male rat data are approximately three times higher than those based on the male human data, for both MLE and 95% UCL. The risk estimates from the female rat data were 12 times higher than the risk estimates in human males (not included in Table 6). Using a threshold approach (e.g., M_{crit}), based on the concept of preventing early pulmonary responses (including chronic neutrophilic inflammation) that are prerequisites to disease, one might predict a zero excess risk of lung cancer at exposures and lung burdens below that level. The MLE excess risk estimates for lung cancer, assuming a 45-year working lifetime average exposure to respirable crystalline silica at 0.036 mg/m^3 (associated with an M_{crit} internal dose of 0.39 mg/g), were 0.87% and 0.97% from the two humans studies and 2.8% from the rat data (Table 6). However, M_{crit} is a mean value, and thus many of the rats would have had reduced pulmonary clearance and increased PMNs at a lower lung burden. To partially address this issue, we also estimated the excess lung cancer risks at the 95% LCL of M_{crit} (0.049 mg quartz/g lung). The MLE excess risk estimates for lung cancer at the 95% LCL M_{crit} were 0.36% based on the rat data[9,34] and 0.12% and 0.14% based on the human studies (Rice et al.[22] and Attfield and Costello,[23] respectively) (not included in Table 6). We also estimated the average respirable quartz concentration over a 45-year working lifetime in humans associated with the 95% LCL M_{crit} to be 0.005 mg/m^3. This estimated threshold exposure value considers only the variability in the rat data. Additional factors to account for variability and uncertainty are necessary when extrapolating rodent data to humans (discussed in the next section).

Discussion

In this study, we used early response data in rats to identify the exposures and crystalline silica lung burdens associated with the NOAELs and/or LOAELs for neutrophilic inflammation and other pulmonary responses. Neutrophilic inflammation was selected as the most sensitive and earliest measure of adverse pulmonary response to silica. We then investigated the extent to which the silica-attributable (i.e., excess) risks for lung cancer in humans, estimated using linear models fit to both human and rat data, would differ from the assumption of zero risk at lung burdens below those estimated to be associated with chronic neutrophilic inflammation. By computing these excess risk estimates using data from studies of both rats and humans with long-term exposures to respirable crystalline silica, we were also able to evaluate how well the rat-based estimates would predict human lung cancer risk.

As in any risk assessment, a number of assumptions were required in this study. One assumption in estimating M_{crit} as a threshold dose is that this lung burden will be retained in the rat and continue to elicit neutrophilic inflammation, and that further pathophysiological effects will develop, ultimately including lung cancer. This assumption is implicit in the concept of the continuum of exposure to disease,[60] but questions remain about the critical dose and the mechanism of silica-induced lung cancer. Assuming a nongenotoxic mechanism for silica-induced lung cancer, there is some evidence to support the use of M_{crit} as a threshold value. First, because the M_{crit} value represents the internal burden at which impaired lung clearance begins in rats, the associated increase in neutrophilic inflammation is likely to be persistent due to the reduced capacity of the lungs to clear the quartz particles. Second, the number of PMNs in the BAL fluid has been shown to remain elevated or to actually increase following cessation of exposure to quartz, both in the 1999 rat inhalation study used here[31,32] as well as in an earlier study of PVG rats exposed by inhalation (to either 10 or 50 mg/m^3 Sikron F600 quartz for 32 or 75 days) and then removed from exposure for an additional 2 months.[61] In another study, in which PVG rats were administered DQ12 quartz by instillation, BALF neutrophils remained elevated above control levels (0.5% PMNs in total BALF cells) at 21 days after instillation in rats receiving either 0.1 mg quartz (46.3% PMNs in total BALF cells) or 1 mg quartz (61.5% PMNs of total BALF cells).[62] In contrast, BALF neutrophils had returned to control levels in rats administered 0.01 mg quartz. Thus, a lung burden for persistent neutrophilic inflammation of 0.1–1 mg/lung suggested by Cullen and Li[62] is consistent with the mean M_{crit} of 0.35 mg/lungs predicted in this study (the mean lung weights of rats in this study and the Cullen and Li study were both approximately 0.9 g (Cullen, personal communication, February 16, 2001). Finally, the toxicokinetic/-toxicodynamic model[38] that we used to estimate M_{crit} provided reasonable predictions of the total quartz burden in the lungs and lymph nodes of rats in another study with chronic exposures.[9,34] The result of underpredicting the observed lung burden and overpredicting the observed lymph node burden is consistent with the expectation that the lungs would respond to the higher dose of toxic particles (in the rat study used to develop the model[31,32]) by clearing the particles to the lymph nodes at a faster rate. It is noteworthy that the predicted total burden was 86% of the observed total burden, despite the differences in the two rat studies (i.e., the study used to develop the toxicokinetic model[31,32] and the chronic study[9,34]). These studies used different types of quartz (Min-U-Sil vs. DQ-12) and different exposure conditions (6 months at 15 mg/m^3 vs. 24 months at 0.74 mg/m^3). It is also of interest to note that the estimated human working lifetime mean quartz concentration of 0.036 mg/m^3 (associated with mean M_{crit} quartz lung burden) and 0.005 mg/m^3 (associated with 95% LCL of M_{crit}) are similar to the NOAELs for silicosis of 0.007 to 0.1 mg/m^3 estimated from five separate human studies.[63]

A second assumption, initially, is that humans and rats will respond in the same way (i.e., be equally sensitive) to equivalent lung burdens. This assumption was evaluated in the comparison of the rat- and human-based risk estimates, and the findings suggest that the rat may be more sensitive. However, given the uncertainties inherent in interspecies extrapolations and the limitations in the data, these results could also be interpreted as showing that the risk estimates are reasonably similar in both species. The rat-based lung cancer risk estimates were based on only one study (the only chronic inhalation study for quartz in which lung burden data were reported[9,34]).

That study evaluated several types of particles, and (like other published rat studies) included only one exposure concentration for quartz. Another source of uncertainty about inter-species response is dose metric. Although we used the available particle mass dose data, recent studies have shown that particle surface area or number are better predictors of chronic inflammation and lung tumors in rats (i.e., the differences in toxicity observed per unit mass among various particle types were diminished when the mass dose was converted to surface area or number).[5-7]

It is not known which dose metric is a better predictor of human lung disease. However, by using particle mass as the dose metric, we are assuming that the surface area of the silica to which the rats and humans were exposed was the same. If these were different (e.g., due to different particle size distribution), then the effective dose (as surface area) may not be equivalent. Also, the differences in the ratios of the human/rat lung mass and surface area (Table 3) would also influence the surface area dose extrapolation.

The human data include uncertainties as well, including the estimation of crystalline silica exposures and possible confounding from smoking and other factors. The possibility that smoking would have had a significant influence on the excess risk estimates is diminished given that the US background rates for lung cancer and competing causes of death were used. The distribution of smoking habits of the exposed workers would have had to differ significantly from those of the general population, or there would have to have been an interaction between the effects of smoking and silica, to influence the excess risk estimates. Such interaction, if synergistic, would increase the excess risk estimates in the silica-exposed workers, yet the human-based risk estimates are actually lower than those derived from the rat data. Attenuation due to misclassification error in the human exposure estimates is a possible explanation for the lower human-based risk estimates (e.g., assigning higher or lower estimated exposure than the actual). Evidence that the human and rat responses to inhaled crystalline silica are qualitatively similar have been reported for both neoplastic and non-neoplastic lung diseases.[1,16,29] The findings of this study suggest that, quantitatively, the rat and human neoplastic response to crystalline silica is also reasonably similar.

Finally, a third assumption in these analyses is that cumulative exposure (i.e., intensity × duration) to respirable crystalline silica is the appropriate metric for estimating lung cancer risk. Cumulative exposure is the metric used in the human studies.[22,23] This metric implies that persons with equal cumulative exposures will have equal risks, whether they were exposed for shorter durations to higher concentrations or for longer durations to lower concentrations. This assumption, known as Haber's rule,[64,65] has been shown to be reasonable as a dose metric for humans exposed to respirable coal mine dust.[66] However, for crystalline silica, which is cytotoxic, there is some evidence that the intensity of exposure (as either average or peak concentration) is a better predictor of the risk of silicosis and lung cancer than is either cumulative exposure or duration of exposure.[23,67] This observation is consistent with the theory that quartz may overwhelm the lungs' antioxidant defenses, causing lung tissue injury and unleashing a cascade of events resulting in disease. Also, neither cumulative exposure, intensity of exposure, nor lung burden takes into account the effect of residence time of the quartz in the lungs. We believe residence time is important because of the progressive nature of silica-induced lung injury, e.g., silicosis, and the use of early response data to predict the risk of a progressive disease may, therefore, underestimate the long-term response. However, the use of these shorter-term, higher-concentration data may also overestimate the long-term response if the lung has a greater ability to handle that continuing exposure to a lower concentration (e.g., through antioxidant defenses).

Improving our understanding of the mechanism of action for particle-induced lung diseases would reduce uncertainty and improve our ability to accurately predict the risk of exposure to inhaled particles including silica. As discussed earlier, there is evidence from rat studies that crystalline silica may cause lung cancer through indirect genotoxic mechanisms involving oxidative damage and chronic inflammation,[13,17,19,68] although other evidence suggests that crystalline silica may be a direct genotoxin.[18] In this study, the finding that epithelial hypertrophy and hyperplasia occur earlier and at lower lung burdens than fibrosis (Table 2) suggests that fibrosis may not be a required precursor step for tumorigenesis, at least in the rat. It has been proposed that preventing fibro-

sis would also prevent silica-related lung cancer.[69] However, instead of neoplasia being linked to fibrosis, these findings suggest that either the quartz particles themselves, the lungs' inflammatory response to quartz, and/or damage to alveolar epithelial type I cells may have initiated the hyperplasia,[54] an early change associated with the development of particle-induced neoplasia.[15] These results thus indicate that cell proliferation, a precursor for neoplasia,[15] was occurring early in these rats, in conjunction with the inflammation. In humans, epidemiological studies have shown that silica-exposed workers have an increased risk of lung cancer, especially those who have developed silicosis (meta-analysis summary of relative risks: 2.3 and 1.3, among silicotics and non-silicotics, respectively).[70] These findings may indicate that silicosis is a "marker" for either high exposure or a susceptible population, or that fibrosis may be associated with neoplastic progression (e.g., high levels of TGF-β that promote fibrosis may lead to selection of cells that escape the epithelial growth controls of TGF-β, a common feature of respiratory epithelial neoplastic progression).[71] It cannot be concluded from current information whether crystalline silica can cause lung cancer in humans by a direct and/or indirect genotoxic mechanism, or whether the relative importance of the two paths may depend on the dose level, individual susceptibility, and other factors.

One approach for examining the evidence of a threshold dose-response (i.e., evidence of an indirect genotoxic mechanism) is to explicitly include a threshold parameter in a regression model. However, when these models have been fitted to human data, the large variability in the individual responses often results in the confidence intervals for a threshold parameter including zero, that is, suggesting no threshold is present.[35] If a LOAEL or NOAEL from an animal study is used to estimate a threshold dose for extrapolation to humans, several factors need to be considered. For example, if a LOAEL (e.g., M_{crit} in this study) is used, an adjustment is needed to account for the greater disease risk at a LOAEL than a NOAEL (dividing the LOAEL by a default factor of 10 is common). An additional factor of 10 is commonly applied to account for inter-individual variability. Interestingly, the 95% LCL M_{crit}, which reflects variability in the rat data, was nearly a factor of 10 below the MLE M_{crit}; however, animal studies often underestimate the variability in human populations.[72-74] Additional factors may be needed to account for uncertainty in the appropriate dose metric for particle lung burden (mass or surface area), and for limitations in the animal data. It could be argued that an uncertainty factor is not required for interspecies exposure-dose extrapolation since lung burden was available in rats and expressed as dose per unit of lung tissue (i.e., directly extrapolates across species, but assumes mass dose is correct metric). Also, because the rat-based risk estimates were higher than those from human data in this study, one could argue that an uncertainty factor is not needed for inter-species differences in dose-response or sensitivity (however, in most situations human data would not be available for comparison). Thus, if one were to conclude that there is adequate evidence of a threshold mechanism for lung cancer in humans exposed to respirable crystalline silica, then, using the default risk assessment approach just described to account for variability and uncertainty, the mean M_{crit} of 0.036 mg/m^3 would be reduced by a factor of 100–1000. This would result in a working lifetime average airborne concentration of respirable crystalline silica in the ng/m^3 range. Thus, whether a direct or indirect mechanism of carcinogenesis is assumed for respirable crystalline silica, the risk estimates indicate that workers exposed for a working lifetime at the current exposure limit have an excess risk of developing lung cancer. This finding is consistent using both linear modeling and a threshold approach, and using both rat and human data.

Conclusions

This article describes a multidisciplinary approach to risk assessment based on alternative assumptions about the mechanism of action for silica-induced lung cancer, utilizing both rat and human data. The findings show that the rat-based estimates of excess lung cancer risk in humans exposed to crystalline silica are reasonably similar to those based on two human occupational epidemiology studies. The findings also show that linear modeling and a threshold approach (reflecting assumptions of direct or indirect genotoxicity, respectively) both demonstrate an excess risk of lung cancer in humans

exposed for a working lifetime to respirable crystalline silica at the current exposure limit. Further research is needed to improve our understanding of the biologic mechanisms of particle-induced lung diseases in order to provide an improved basis for accurately estimating the risk of exposure to various types of inhaled particles.

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