An Exposure-Response Threshold for Lung Diseases and Lung Cancer Caused by Crystalline Silica

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ABSTRACT

Whether crystalline silica (CS) exposure increases risk of lung cancer risk in humans without silicosis, and, if so, whether the exposure-response relation has a threshold, have been much debated. Epidemiological evidence is ambiguous and conflicting. Experimental data show that high levels of CS cause lung cancer in rats, although not in other species, including mice, guinea pigs, or hamsters; but the relevance of such animal data to humans has been uncertain. This paper applies recent insights into the toxicology of lung diseases caused by poorly soluble particles (PSPs), and by CS in particular, to model the exposure-response relation between CS and risk of lung pathologies such as chronic inflammation, silicosis, fibrosis and lung cancer. An inflammatory mode of action is described, having substantial empirical support, in which exposure increases alveolar macrophages and neutrophils in the alveolar epithelium, leading to increased reactive oxygen species (ROS) and nitrogen species (RNS), pro-inflammatory mediators such as TNF-alpha, and eventual damage to lung tissue and epithelial hyperplasia, resulting in fibrosis and increased lung cancer risk among silicotics. This mode of action involves several positive feedback loops. Exposures that increase the gain factors around such loops can create a disease state with elevated levels of ROS, TNF-alpha, TGF-beta, alveolar macrophages, and neutrophils. This mechanism implies a "tipping point" threshold for the exposure-response relation. Applying this new model to epidemiological data, we conclude that current permissible exposure levels, on the order of 0.1 mg/m^3, are probably below the threshold for triggering lung diseases in humans.

KEY WORDS: Crystalline silica, exposure-response, dose-response model, silicosis, lung cancer risk, mathematical model

1. Introduction: Is Crystalline Silica Hazardous at Currently Permitted Levels?

Crystalline silica (CS) is one of the most studied, yet most controversial, of substances currently classified as known human carcinogens (IARC, 1997). Like other poorly soluble particles, it has been associated with a variety of possible lung diseases. In addition to silicosis, non-specific responses such as chronic inflammation, fibrosis, lung cancer (Azad et al. 2008, American Thoracic Society 1997), and possibly chronic obstructive pulmonary disease (COPD) (Rushton 2007) have been suggested as possible consequences of high levels of exposure to CS and/or other dusts and respiratory irritants, including cigarette smoke.

Whether CS *at currently permitted exposure levels* (such as OSHA's PEL-equivalent of 0.1 mg/m^3 of respirable CS, or NIOSH's currently recommended exposure limit of 0.05 mg/m^3 for up to a 10-hour workday) creates an excess risk of lung disease has been much debated, but without clear resolution. For decades, scientists, regulators, and occupational health and safety risk managers have wrestled with the following three key questions about human health risks from CS exposures:

1. *Do the causal exposure-response relations between CS exposure and exposure-associated lung diseases have thresholds?*
2. *If so, are the exposure levels that cause increased risks of such diseases above or below currently permitted exposure levels?*
3. *Are risks of some diseases (such as lung cancer) elevated only at exposures that cause other diseases (e.g., silicosis)?*

Expert opinions on all three questions have been sharply divided. Epidemiology, risk assessment, and toxicological research have done much to illuminate the difficulty of answering them decisively (e.g., Soutar et al 2000, Erren et al. 2009), but have so far produced few unequivocal answers.

This report examines the causes and exposure-response relations for CS-associated lung diseases, drawing on recent advances in the biology of lung diseases caused by poorly soluble particles (PSPs), which include CS as a special case. For PSPs, chronic inflammation of the lung plays a crucial role in causing lung diseases such as asbestosis, silicosis, fibrosis, COPD, and lung cancer (Mossman 2000, Oberdorster 2002, Gulumian et al. 2006, Blanco et al. 2007, Haux 2007, Azad et al. 2008, Cox 2010). We seek to shed new light on the exposure-response relation for CS-associated lung diseases by applying recent insights into this inflammatory mode of action to model the relation between exposure concentrations and durations and the resulting cascade of changes in the lung environment that can hasten the onset and progression of lung diseases.

2. CS Epidemiology Is Ambiguous

A number of epidemiological studies have reported that lung cancer risk is elevated among patients with silicosis, especially among those who smoke (Kurihara and Wada 2004, Ulm et al. 2004, Amabile et al. 2009). Others find no such association (Hessel et al. 1990; Chan et al. 2000; Carta et al. 2001; Chen & Chen 2002; Yu et al. 2007), and a recent meta-analysis concluded that the association disappears when confounders (such as smoking or occupational co-exposures) are correctly adjusted for (Erren et al. 2009). Influential investigators have stated that risks of lung cancer appear

to them to be elevated even at exposure levels below current standards (e.g., Steenland et al. 2000, Stayner 2007). However, we believe that failure to correctly account for exposure measurement errors invalidates this interpretation of the data, as explained below (see Figure 1). Risk of COPD and reduced lung function appear to be elevated at estimated occupational exposures above 0.1 to 0.2 mg/m^3 of silica dust for at least 30-40 years, independent of silicosis (Rushton 2007), but a recent study of Vermont granite workers found no evidence of increased lung cancer risk due to silica exposure in occupational cohorts, even at the high exposure levels where mortalities due to silicosis and other non-malignant respiratory illnesses were elevated (Vacek et al. 2010). The apparent paradox of *reduced* risk of lung cancer in some workplaces with relatively high levels of silica exposure has also been noted (Brown 2009), further complicating any conjectured causal relation between silica exposure and lung cancer. One possible explanation for these differences among studies might be the different (and often highly uncertain) compositions of the dusts in different studies (e.g., Dahmann et al. 2008). For example, the toxicity of quartz particles depends on detailed properties of the fracture surfaces, with freshly fractured silica typically being more potent than aged silica in eliciting various cellular responses, including production of reactive oxygen species by alveolar macrophages (e.g., Porter et al. 2002). Differences in dust composition and ages might therefore create heterogeneous exposure-response relations, perhaps triggering different response mechanisms. In this case, biologically effective doses could be very uncertain, even if respired quantities of dust were measured accurately.

Whether or not silicosis increases lung cancer risk, epidemiological studies have not yet revealed whether silicosis is a *necessary* precondition for increased risk of lung

cancer due to CS exposure (Pelucchi et al. 2006, Erren et al. 2009). Yet, the answer is vital for current practical regulatory risk management decisions: "If silicosis were the necessary step leading to lung cancer, enforcing the current silica standards would protect workers against lung cancer risk as well. Alternatively, a direct silica-lung cancer association that has been suggested implies that regulatory standards should be revised accordingly" (Brown 2009).

Somewhat reassuringly, the increased risk of lung cancer among CS-exposed workers is most apparent "when the cumulative exposure to silica is well beyond that resulting from exposure to the recommended limit concentration for a prolonged period of time" (Lacasse et al. 2009), suggesting that enforcing current standards would protect workers from CS-associated lung cancer risks. However, other researchers have cautioned that, "The hypothesis of a silicosis-mediated pathway [for lung cancer], although more consistent from an epidemiological perspective, and reassuring in terms of the effectiveness of current standards in preventing lung cancer risk among silica exposed workers, does not seem to explain elevated risks at low silica exposure levels" (Cocco et al. 2007). Thus, the relation between silicosis and lung cancer has remained uncertain, based on various published interpretations of epidemiological evidence. There is no clear evidence that lung cancer risk is elevated in the absence of silicosis, but the question is unsettled. The following statement (Pelucchi et al. 2006) succinctly captures the present state-of-the-art: "A recent meta-analysis of 30 studies found a pooled RR [relative risk] of lung cancer of 1.32 (95% CI, 1.23–1.41) in subjects exposed to crystalline silica. In the same investigation, the pooled RR was 2.37 (95% CI, 1.98–2.84) in silicotics only (based on 16 studies), whereas no increase in risk emerged in non-silicotics (pooled RR = 0.96,

95% CI, 0.81–1.15, based on eight studies). The authors concluded that silica may induce lung cancer indirectly, probably through silicosis." Such evidence, although not conclusive, favors the hypothesis that lung cancer risk is elevated among silicotics, but not among non-silicotics.

We believe no credible epidemiological evidence actually shows that crystalline silica increases lung cancer risk at exposure levels that do not also cause silicosis. Rather, the foregoing observation by Cocco et al. 2007, that the "hypothesis of a silicosis-mediated pathway… does not seem to explain elevated risks at low silica exposure levels," as well as published reports of elevated risk of lung cancer at exposures below those that cause silicosis (e.g., Steenland et al. 2001), misinterpret the available epidemiological evidence. They do so by mistakenly interpreting exposure-response relations *estimated* from epidemiological studies (all of which have missing and highly uncertain and variable (usually, "reconstructed") exposure data) as providing valid evidence of "elevated risks [of lung cancer] at low silica exposure levels." But they do not. At most, such studies provide evidence of elevated lung cancer risks at low *estimated* levels of silica exposure. These are entirely different propositions, as explained next. When uncertainties in exposures are accounted for in the risk models, there is no evidence that risks *are* elevated at low levels of silica exposure (specifically, at or below those allowed by current standards). Studies that conclude that relatively low exposures to silica (below currently permitted levels, and below levels that cause silicosis) increase lung cancer risk, studies that conclude that they do so are undermined – without exception, as far as we know – by important upward biases in their low-exposure risk estimates. These biases result from imperfect control of potential confounders, ignored

model specification errors and uncertainties, and unmodeled errors and uncertainties in exposure estimates. Each of these limitations is briefly discussed next.

Imperfectly Controlled Confounding

Perhaps the most familiar threat to valid inference from epidemiological studies of CS is confounding, especially by cigarette smoking and by occupational co-exposures. For example, a recent study (Chen et al., 2007) reported that, "In a crude analysis adjusted for smoking only, a significant trend of increasing risk of lung cancer with exposure to silica was found for tin, iron/copper miners, and pottery workers. But after adjustment for relevant occupational confounders [arsenic and polycyclic aromatic hydrocarbons], no relationship between silica and lung cancer can be observed."

The possibility of such confounding has been well recognized and much discussed in the epidemiological literature on CS, but inability to rigorously and fully control for plausible confounders in most past studies continues to limit the validity of the exposure-response relations inferred from these studies (Erren et al. 2009). Attempts to adjust for possible confounding by smoking, based on subjective estimates of smoking habits and their effects (and an assumed bias model), have modestly reduced the estimated relation (standardized mortality ratio) for silica exposure and lung cancer (from 1.6 to 1.43) (Steenland and Greenland, 2004). Other assumptions and models might lead to further reductions. Currently proposed methods to account for most of the bias due to confounding by smoking, using differences between COPD and lung cancer rates to estimate bias effects (Richardson 2010), have not yet been applied to CS, leaving open

the question of how much of the apparent relation between CS exposure and lung cancer risk would be eliminated by fully controlling for smoking effects. Similarly, it remains unknown whether fully controlling for occupational co-exposures would fully eliminate the apparent associations between silica exposure and lung cancer risk (in other data sets as well as the one for Chinese miners and pottery workers), since most other studies have not provided the needed co-exposure data (Chen et al., 2007).

Unmodeled Errors and Uncertainties in Exposure Estimates Can Inflate Low-Exposure Risk Estimates and Hide True Thresholds

Perhaps the single most important limitation in CS epidemiology is that *true individual exposures to CS of various types and toxicities are unknown.* Therefore, guesses about exposures are used instead, typically based on reconstructions of exposure histories from estimated job exposure matrices, together with simplifying (and inaccurate) assumptions, such as that all silica dust has the same average toxicity or carcinogenic potency value. Exposure-response relations are then fit to the guessed-at exposures and observed responses. Although there is a sophisticated statistical literature on how to use such uncertain predictors in regression models (e.g., Carroll et al., 2010), these appropriate "errors-in-variables," measurement error, and missing data methods have typically *not* been used in the CS epidemiology literature. Instead, reconstructed exposure estimates are often treated as if they were true (error-free) data, for purposes of fitting statistical models. Then, unwarranted conclusions are drawn that fail to explicitly model and correct for the effects of errors in exposure estimates (e.g., Cassidy et al.

2007). This can create large, unpredictable biases in multivariate regression coefficients and other measures of exposure-response association (Veierød, Laake 2001).

If the true exposure-response relation is a threshold function, then failing to explicitly model errors and uncertainties in exposure estimates can smear out the threshold in the estimated exposure-response models, giving a misleading appearance of a smooth, s-shaped exposure-response function, complete with an apparent (but not real) smooth biological gradient (i.e., higher probabilities of response at higher estimated exposure levels) and elevated risks at estimated exposure levels well below the true threshold. Such incorrect modeling will over-estimate excess risks at exposures below the threshold, and under-estimate risks at exposures greater than the threshold.

To illustrate how a smoothly increasing estimated exposure-response relation arises from a true threshold relation when there are unmodeled errors in the exposure estimates, consider the following simple hypothetical example. Suppose that true individual exposure rates are uniformly distributed between 0 and 20 mg/m^3-years (for 40-year exposure durations), and that the true exposure-response relation has a threshold at 15 mg/m^3-years, so that the true risk of lung cancer is 0 for exposures of 15 mg/m^3-years or less, and 1 for exposures above 15 mg/m^3-years. Suppose that estimates of individual exposures are unbiased, but with some variance around their means, representing estimation errors. For simplicity, assume that the ratio of the estimated exposure to the true exposure, for each individual, is uniformly distributed between 0 and 2, with a mean value of 1 (i.e., *Estimated exposure = k*True exposure*, where *k* is a random variable, $k \sim U[0, 2]$, with $E(k) = 1$.) Table 1 shows true and estimated exposures

for 10 individuals, based on this simple model of errors in exposure estimates. Figure 1 shows the estimated exposure-response relation based on 10,000 individuals.

	True exposure ~ U[0, 20]	Random multiplier k ~ U[0, 2], E(k) = 1	Estimated exposure = k*True exposure	Response threshold	Response
1	0.14	1.4	0.19	15	0
2	6.07	0.7	4.30	15	0
3	18.54	0.0	0.75	15	1
4	7.54	1.6	11.99	15	0
5	19.85	0.6	11.31	15	1
6	17.89	0.4	7.52	15	1
7	9.20	1.6	14.74	15	0
8	7.72	1.0	7.77	15	0
9	5.41	1.2	6.75	15	0
10	15.13	0.1	1.81	15	1

Table 1: Hypothetical data for true and estimated exposures and resulting responses

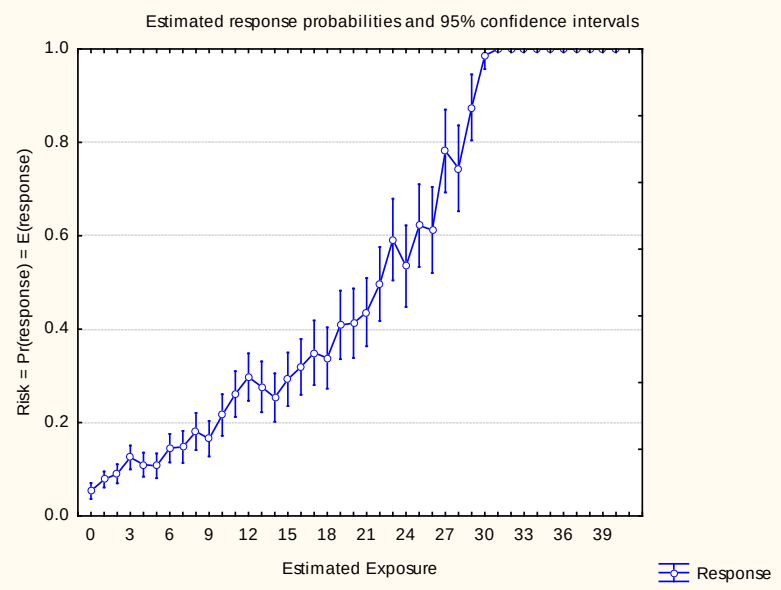

Figure 1: Estimated exposure-response relation for the simulated data in Table 1 (using 10,000 individuals instead of 10). The correct relation has a threshold at 15: Risk= 0 for exposure ≤ 15; risk = 1 for exposure > 15.

(For plotting purposes, each estimated exposure is rounded to the nearest integer, from 0 to 40.) The estimated exposure-response relation suggests that risk increases with exposure over the entire range of exposure values, and that it is slightly but significantly elevated even at relatively low exposure levels (e.g., 3 mg/m^3-years), even though we know that, in this example, the true exposure-response relation has no increase in risk at exposure rates below 15 mg/m^3-years. This same conceptual point holds for real data, provided that estimated exposures contain errors. However, for real data, we do not know what the correct exposure-response relation is. The use of estimated individual exposures tends to smear out the true but unknown exposure-response relation (e.g., turning a sharp threshold into a gradually increasing curve, as in Figure 1, or turning a narrow distribution of individual thresholds into a wider one). Recovering the correct exposure-response relation requires additional analysis to correct for this smearing effect by explicitly modeling the relation between true and estimated exposures (Carroll et al., 2010, Cheng et al. 2010, Lu and Lyles 2008). Estimated exposure-response relations for CS in the epidemiological literature have not made such corrections, and therefore they do not provide useful information about possible true exposure-response thresholds or trustworthy evidence that risks at low exposures are truly elevated.

Model Specification Errors and Uncertainties Can Obscure Threshold Relationships

Many CS epidemiology studies fit parametric statistical models to estimated exposure-response data, and then interpret the estimated model parameters (e.g., odds ratios or regression coefficients) as providing evidence of a positive effect at all exposure

levels. This procedure is not justified if different models hold at different exposure levels, as could be the case if there is an exposure threshold, with no increase in risk below the threshold and some increase above it.

The assumptions built into a statistical model can drive its conclusions, even if these disagree with the data used to fit the model. As an extreme, hypothetical, example, fitting the regression model $Risk = \beta * Exposure$ to data that are correctly described by $Risk = 1/Exposure$ would produce a positive estimate for β, which might be misinterpreted as a *positive* unit risk factor or potency for the effect of exposure on risk, even though the true relation $Risk = 1/Exposure$ shows that risk actually *decreases* with increasing exposure. This illustrates how a misspecified statistical model can override data, and produce a conclusion that risk is increased at low exposure levels, even if the data imply nothing of the sort.

To avoid such model specification errors and biases, it is useful to fit nonparametric models to exposure-response data. Figure 2 presents an example: a spline curve fit to estimated exposure-response data in the influential IARC pooled analysis study of Steenland et al. 2001. The authors interpreted this model as "support[ing] the decision by the IARC to classify inhaled silica in occupational settings as a carcinogen, and suggest[ing] that the current exposure limits in many countries may be inadequate."

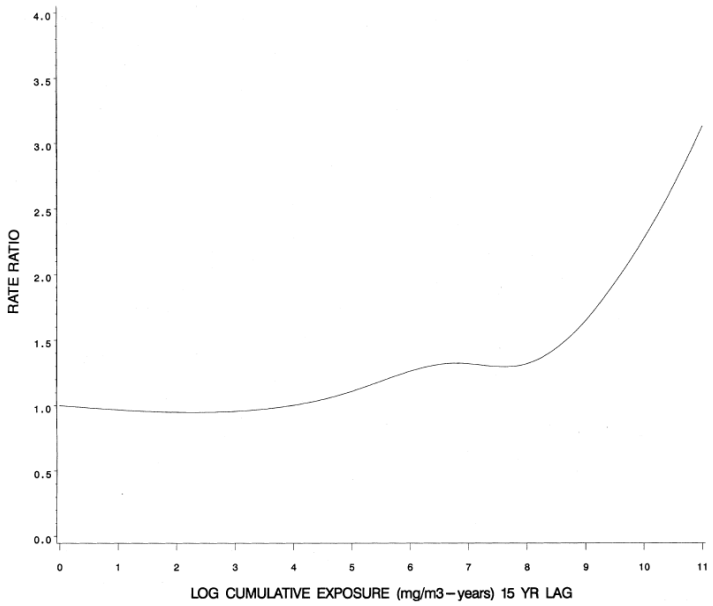

Figure 2: A Spline Curve Fit to Pooled Analysis Data Suggests a Threshold (*Source:* Figure from Steenland et al. 2001)

(The horizontal axis is admittedly somewhat mysterious, as it seems to show a value of 0 for the log of cumulative exposure (lagged by 15 years), which is impossible for a logarithm of a positive exposure variable. The y axis shows estimated relative risk of lung cancer, with 1 corresponding to no effect.) The authors interpreted Figure 2 as follows: "Analyses using a spline curve also showed a monotonic increase in risk with increasing exposure." However, a more accurate description is that *Figure 2 shows clear evidence of a threshold,* with no increase (and, if anything, a slight decrease) in risk at low exposure levels.

This finding of an apparent threshold can be buried, and converted to a reported finding of a "monotonic increase in risk," by fitting a parametric statistical model (such as $Risk = \beta * Exposure$, having parameter β, in the above example) which *guarantees* a positive estimate of β (as long as *Risk* and *Exposure* values are positive), and hence a monotonic increase in estimated risk even at low exposures, no matter what the data say.

(The slope parameter β is necessarily positive when both *Risk* and *Exposure* are positive, since the line *Risk* = β**Exposure* necessarily goes through the origin at its lower left, and slopes upward through the positive scatter plot.) The IARC team interpreted the data behind Figure 2 this way. They fit a similar parametric model (*log relative risk* = β**Exposure*) to data with positive values of *Exposure* and *log relative risk*, and therefore (necessarily) concluded that risks were increased at low exposure levels – a finding that they interpreted as supporting classification of CS as a known human carcinogen that might need tighter regulation. Figure 2 suggests that a less assumption-laden process could have produced a very different conclusion, *i.e.*, that the data do not indicate any increase in risk at low exposures.

In summary, epidemiological evidence on CS and lung cancer have often been interpreted as suggesting a causal relation between CS exposure and increased risk of lung cancer (Stayner 2007), even at relatively low exposure levels that do not cause silicosis. Our review of CS epidemiology indicates that this interpretation is unjustified. CS epidemiological studies and meta-analyses have not corrected for errors in individual exposure estimates, have not applied appropriate methods to estimate and fully control for confounding, and have not accepted and interpreted at face value the results of non-parametric analyses that provide clear, model-free, evidence of an exposure-response threshold. As a result, past epidemiological studies do not provide trustworthy information about the presence or absence of thresholds in exposure-response relations, or about the shape of individual or population exposure-response functions. To obtain more insight, it is necessary to turn to biological information about how and under what conditions CS increases risks of lung diseases.

3. CS Mode of Action

Over the past decade, molecular biologists and toxicologists have dramatically improved understanding of how PSPs in general, and CS in particular, cause lung diseases. The following steps, reviewed in more detail in Cox 2010 for COPD, are important in the development of many PSP exposure-related lung diseases.

1. *Sufficient exposure activates alveolar macrophages (AMs) and changes their phenotypes.* Intense and prolonged exposure to many PSPs permanently shifts alveolar macrophage (AM) populations toward more cytotoxic phenotypes with reduced phagocytic capacity and reduced ability to clear apoptotic cells via efferocytosis (e.g., Gulumian et al, 2006). For CS, AMs are activated via the MARCO receptor, which plays a crucial role in CS particle recognition and uptake (Haux 2007, Thakur et al. 2009). A shift in AM phenotypes and reduced AM phagocytic capacity has been documented for silica-exposed monkeys (Hildemann et al. 1992), as well as for rodents (Thakur et al. 2009).
2. *The altered AMs produce increased levels of reactive oxygen species (ROS), reactive nitrogen species (RNS), and pro-inflammatory cytokines, including TNF-α.* Exposure to PSPs increases AM production of ROS. Although increases in ROS production may initially be counterbalanced by compensating increases in antioxidants (AOX) (Janssen et al. 1992 for silica, Comhair and Erzurum 2002 more generally), sufficient exposure overwhelms and down-regulates AOX in rats, shifting the oxidant:antioxidant balance in the lung toward abnormally high ROS levels and

generating oxidative stress (Azad et al. 2008). Mechanisms of antioxidant reduction in human bronchiolar epithelial cells have started to be elucidated *in vitro* (e.g., Antognelli et al. 2009), although more remains to be done (e.g., to clarify the role of the Nrf-2 "master switch" for many antioxidants, and its pathways, such as the Nrf-2-ERK-MAP kinase-heme oxygenase (an antioxidant) pathway) (Eom and Choi 2009; see also Guo and Ward 2007.).

3. *A high-ROS environment, in turn, induces AMs (and, to a lesser extent, other lung cell populations, such as bronchiolar epithelial cells) to secrete more pro-inflammatory mediators – most notably, tumor necrosis factor alpha (TNF-α), as* well as IL-1β, TGF-β1, and other pro-inflammatory cytokines (e.g., Rimal et al. 2005, for CS). For CS, specifically, exposure increases AM production of both ROS and RNS in rats (Fubini and Hubbard 2003) and activates signaling pathways (including NF-kappaB and AP-1) that promote expression of pro-inflammatory mediators, oncogenes, and growth factors important in lung fibrosis and cancer (e.g., Castranova 2004, van Berlo et al. 2010). Increased ROS stimulates increased secretion of TNF-α by AMs, as observed *in vivo* in silica-exposed rats (Gossart et al. 1996) and *in vitro* in silica-exposed lung cell lines, in which ROS activates a specific transcription factor (nuclear factor of activated T cells (NFAT)) that increases TNF-α (Ke et al. 2006).

In humans, ROS markers such as 8-isoprostane remain elevated, or increase, in patients with silicosis (Pelclová et al. 2008) or COPD (Cox 2010) even long after exposure stops, suggesting that exposure "switches on" a self-sustaining process (e.g., a positive feedback loop) that keeps ROS permanently elevated. The increase in ROS levels and oxidative stress in the lung environment is considered crucial in causing

subsequent exposure-associated lung injury and in increasing risk of lung diseases, including fibrosis (Fubini and Hubbard 2003), silicosis, and lung cancer (Azad et al, 2008, Ding et al. 2000, Haux 2007, Shi et al 1998, Schins and Knaapen 2007).

4. *Increased TNF-α and ROS stimulate an influx of neutrophils to the lung.* Some specific causal pathways by which TNF-α and ROS attract neutrophils into the lung have been partially elucidated, as follows.

   - TNFα up-regulates interleukin 8 (IL-8) expression (Smart and Casale 1994). IL-8 (also called CXCL8 ligand) is a potent chemoattractant for neutrophils. It recruits additional neutrophils to the lung, via chemotaxis, and activates them (by binding with high affinity to the two chemokine receptors, CXCR1 and 2, on the neutrophil cell surface, stimulating their degranulation) (Pease and Sabroe, 2002). The lungs contain a large reservoir of marginated neutrophils, sequestered within the tiny capillaries of the pulmonary microcirculation and adhering to the capillary lining (endothelium). In response to IL-8, they squeeze across the alveolar-capillary membrane and into the interstitial air spaces. (How quickly this happens depends on the deformability of the neutrophils, which depends on oxidant-antioxidant balance (MacNee 2005)). IL-8 also increases the cellular adhesion of neutrophils (specifically, to fibrinogen and ICAM-1, via the β2-integrin cell surface adhesion molecule, Mac-1, i.e., CD11b/CD18 (Takami et al. 2002).) Thus, IL-8 increases the local concentration of activated lung neutrophils, both by attracting and by retaining them. This may be diagrammed as: $IL\text{-}8 \rightarrow N$ (where the arrow indicates that an increase in the quantity on its left (tail) increases the quantity on its right (head).)
   - ROS increases the release of IL-8 from cultured macrophages. Specifically, the lipid peroxidation product 8-isoprostane (which is elevated in COPD patients, as well as in the plasma and urine of atherosclerosis patients) increases IL-8 expression in human macrophages *in vitro* (via a pathway that involves both ERK 1/2 and p38 MAPK, but not NF-kappaB.) (Scholz et al. 2003).
   - ROS also increases IL-8 via the following ROS-EGFR pathway (Cox 2010): $ROS \rightarrow TGF\text{-}\alpha \rightarrow EGFR\ phosphorylation \rightarrow IL\text{-}8,\ VEGF,\ MUC5AC,\ MUC5B$ (where, again, each arrow indicates that an increase in the quantity on the left (tail) increases the quantity on the right (head) of the arrow). This pathway also increases mucus production in airways, via increased expression of the mucin genes *MUC5AC* and *MUC5B.* IL-8 is

produced by bronchiolar epithelial cells (BECs), dendritic cells, and other lung cell populations, following EGFR activation.

- TNF-α and ROS may also stimulate release of the ligand CXCL2 (C-X-C motif ligand 2, also called macrophage inflammatory protein 2-alpha (MIP2-α)), growth-regulated protein beta (Gro-beta) and Gro oncogene-2 by dendritic cells (DCs), monocytes and macrophages. CXCL2 is chemotactic for neutrophils, enhancing their influx into the airways (Mortaz et al. 2009, for murine cells in *vitro*; Thatcher et al. 2005 for CXCR2 effects on emphysema in smoke-exposed mice *in vivo*).

In rats exposed to CS, the initial influx of AMs and neutrophils leads to elevated levels of both that persist many months after exposure ceases (Absher et al. 1989).

5. *The increased neutrophils and AMs in the lung generate increased ROS levels and oxidative stress,* due in part to their respiratory bursts; in part to the release of neutrophil elastase (NE) from neutrophils; and in part to greatly increased numbers of apoptotic cells (primarily neutrophils, but also AMs and epithelial cells). This completes a positive feedback loop: $ROS \rightarrow TNF\text{-}\alpha \text{ from AMs} \rightarrow IL\text{-}8 \rightarrow neutrophils \rightarrow ROS$. NE also further activates the EGFR pathway (by cleaving pro-TGF-α, which stimulates release of mature TGF-α that binds to and phosphorylates EGFR), and potently stimulates goblet cell degranulation, contributing to mucus hypersecretion into the airways (Kim and Nadel 2004). This creates the following positive feedback loop: $TGF\text{-}\alpha \rightarrow EGFR \text{ phosphorylation} \rightarrow IL\text{-}8 \rightarrow neutrophils \rightarrow NE \rightarrow TGF\text{-}\alpha$. Activated neutrophils further amplify the EGFR pathway and inflammation by releasing TNF-α, which increases expression of EGFR on airway epithelial cells (Kim and Nadel 2004). Increases in NE can shift an entire protease-antiprotease network toward a new, high-protease state in which the excess proteases

digest lung tissue and cause emphysema and COPD, as well as increasing apoptosis of endothelial and epithelial cells (Cox 2010).

6. *High ROS and oxidative stress increase apoptosis of AMs, neutrophils, and alveolar epithelial cells, leading to lung tissue damage and destruction.* Apoptosis of alveolar epithelial cells, together with damage to the extracellular matrix (ECM) and alveolar wall from increased proteases, can eventually lead to tissue destruction and remodeling of the extracellular matrix, including deposition of collagen leading to scarring and fibrosis (Delgado et al. 2006 for human silicosis; Cox 2010 for human COPD). Experiments with silica-exposed knockout mice have confirmed that both IL-1β and inducible nitrogen oxide synthase (iNOS) are involved in apoptosis and inflammation during murine silicosis (Srivastava et al 2002). Increased ROS leading to increased apoptosis of alveolar cells and neutrophils has been observed in CS-exposed rats (Leigh et al. 1997, Zhang et al. 2002). Damaged and dying alveolar epithelial cells (especially Type II alveolar cells) cause the lung parenchyma to secrete, activate, and release transforming growth factor beta-1 (TGF-β1), as well as more TNF-α (thus completing still further positive feedback loops: $ROS \rightarrow TNF\text{-}\alpha \rightarrow IL\text{-}8 \rightarrow neutrophils \rightarrow ROS \rightarrow apoptotic\ cells \rightarrow TNF\text{-}\alpha$). Apoptotic cells (and, even more, necrotic cells, which form if apoptotic cells are not promptly and safely removed) also release high levels of ROS into the lung environment. TGF-β1 activates fibrogenic cells and powerfully attracts alveolar macrophages (which release more TGF-β1) and other inflammatory cells (neutrophils and lymphocytes) into parenchymal tissues (Kisseleva and Brenner 2008). ROS and TGF-β1 stimulate production of new extracellular matrix (ECM) by myofibroblasts, the fibrotic lung's

major collagen-producing cell population (*ibid*). High oxidative stress also decreases the ability of AMs to identify and remove apoptotic cells, further increasing their concentration, and hence the concentration of ROS and TGF-β1 in the lung environment.

7. *In rats, damage to lung tissue and altered apoptosis result in epithelial hyperplasia, clonal expansion of preneoplastic cells that would ordinarily be removed via apoptosis, and increased risk of lung cancer.* Oxidative stress from a high-ROS lung environment can both reduce apoptosis among some cells (thereby increasing lung cancer risk, if pre-neoplastic cells are less likely to be detected and removed via apoptosis) and stimulate proliferation and transformation of cells that contribute to increased lung cancer risk (Azad et al. 2008). For CS, specifically, exposure causes hyperplasia of epithelial cells and fibroblasts in rats, but CS does not induce similar hyperplasia (or lung cancer) in mice and primates (Mossman et al. 2000). CS induces hyperplasia of both neuroendocrine lung cells (Elizegi et al. 2001) and Type II alveolar cells in rats, although not in mice or hamsters (William et al. 1996, Saffiotti 2005). In rats (but, again, not in mice or hamsters, which do not show elevated lung cancer risk in response to CS exposure), TGF-β1 precursor is localized in hyperplastic alveolar type II cells and ECM next to granulomas (and adenomas, if any) (Williams et al. 1995, 1996). This suggests a close link between locations of alveolar cell death and attempted repair of ECM (both of which are associated with TGF-β1) and areas of increased hyperplasia/adenomas. Such usefully detailed biomolecular information links the process of silicosis (e.g., TGF-β1-mediated collagen production, ECM remodeling, epithelial–mesenchymal transition (Corvol et

al. 2009), and fibrosis) directly to epithelial cell proliferation and increased lung cancer risk (due to increased hyperplasia/adenoma of damaged lung tissue) – the crucial link that epidemiological data alone could not yet provide.

Studies of silica-induced lung cancer in rats – the only species in which CS exposure is known to cause lung cancer – indicate that CS does not act through classical mutational (e.g., *KRAS* or *EGFR* mutation) pathways for lung cancer, but rather promotes lung carcinogenesis through indirect epigenetic processes associated with increased proliferative stress and hypermethylation of the promoter region of tumor suppressor genes (TSGs), specifically including p16 (Blanco et al. 2007). In humans, aberrant promoter methylation of TSGs is more frequent in serum DNA from silicosis patients with lung cancer than in silicosis patients without lung cancer (Umemura et al. 2008), suggesting that epigenetic gene silencing of TSGs by this mechanism may be relevant in silicosis-associated lung cancers in humans, as well as in rats. The p16 gene normally participates in checking and regulating cell division (as part of the p16INK4a-Cyclin D1-CDK4-RB cell cycle control axis) (Cox 2009a). Disruption of p16 gene expression allows damaged cells that would normally be removed via apoptosis to undergo mitotic replication instead, increasing the prevalence of damaged (potentially preneoplastic) cells in lung bronchiolar epithelial tissue. Epigenetic silencing of p16 by CS-induced hypermethylation of its promoter region thus presumably increases survival and entry of altered (initiated) cells into a clonal expansion phase, thereby promoting expansion of preneoplastic cell populations and increasing the risk of lung tumors (e.g., Kuilman et al. 2008).

In summary, CS exposure stimulates production of ROS/RNS, down-regulates counter-balancing antioxidants, and activates immune cells, including alveolar macrophages (AMs) (as well as mast cells, and B-lymphocytes) (Haux 2007). Activated immune cells release more ROS, creating a positive feedback loop (Mossman 2000, Azad et al. 2008). The resulting high-ROS, chronically inflamed lung environment disrupts normal apoptosis and repair of epithelial and endothelial cells, increases epithelial cell proliferation and lung cancer risk, inhibits normal repair of damaged epithelial tissue, and promotes excess secretion of collagen and other proteins in the extracellular matrix. In rats, and probably in silicosis patients, these changes promote expansion of preneoplastic clonal patches and increase risk of lung cancer, probably in part by epigenetic silencing of tumor suppressor genes, such as p16. These general features of lung disease processes hold for many PSPs and mineral dusts and fibers, and for CS in particular, as documented in the cited references, although important biochemical details (such as the specific antioxidants generated in response to initial ROS increases) differ for different compounds (e.g., Janssen et el. 1992).

4. Exposure-Response Modeling

Although the inflammatory mode of action is complex, one of its main features is obvious: the key quantities and the regulatory relations among them form a network with multiple positive feedback loops. Figure 3 shows examples. In each loop (i.e., each directed cycle among a set of variables, with arrows entering and leaving each variable in it), an increase in one element stimulates an increase in its successor, so that eventually

all variables around the loop increase. (Figure 3 is not intended to be complete, e.g., it does not show the direct contribution of CS fragments to ROS, the shift in AM phenotypes toward less effective phagocytosis, the production of collagen by fibroblasts, or many other biological effects previously discussed. It simply illustrates some major positive feedback loops involved in CS-associated (and other PSP-associated) lung pathologies.)

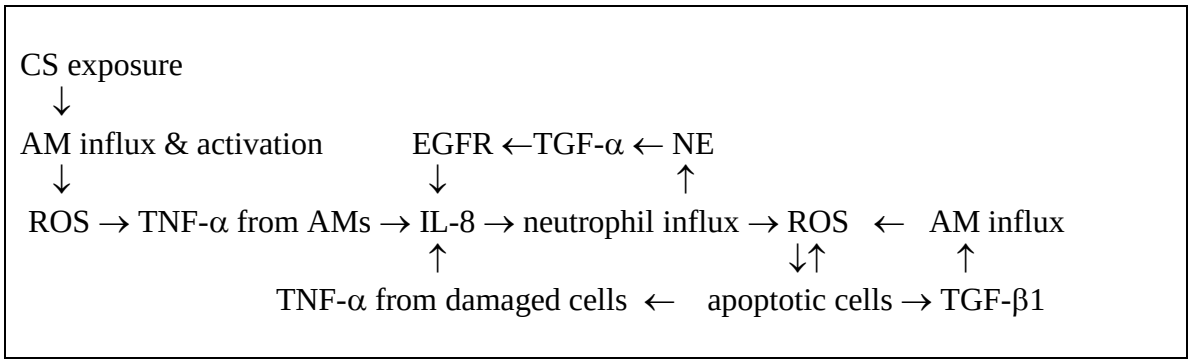

Figure 3: Examples of Positive Feedback Loops in a Silica Disease Causal Network

If specific quantitative formulas linking the rates of changes of different variables were known, then the dynamic response of such a network to changes in its exogenous inputs (such as CS exposure, in Figure 3) could be simulated. Even without such detailed quantitative information, however, the method of comparative statics analysis (Cox 2009b) can be used to study how equilibrium levels of variables change in response to exposure. The basic idea is to compute how equilibrium points change, even though the details of the adjustment process may be (and, for CS, still are) largely unknown. To do this, we focus on some variable, such as ROS, that appears in one or more loops. Let's call the selected variable *X*. Now, consider the following artificial adjustment process, which is constructed so that it will lead to the same equilibrium levels of *X* as the real but

unknown adjustment process. [Throughout, we assume, realistically, that all modeled variables are bounded, and that they adjust to their new equilibrium levels (or quasi-equilibrium levels, for slowly changing variables), in response to any change in inputs, relatively quickly – well within the lifetime of the exposed individual. These assumptions hold for the variables in more detailed models of COPD (Cox, 2010).] The artificial adjustment process is iterative. Each iteration consists of the following two steps:

(i) Hold X fixed at a specified level, denoted by X_t at iteration t. Let all other variables adjust until they are in equilibrium with X_t.

(ii) Next, hold all other variables fixed at their new levels, and let X adjust until it is in equilibrium with them. Denote by X_{t+1} this new value of X.

If the system were understood in enough detail to allow a full, explicit, dynamic simulation model to be constructed, then the mapping from each value of X_t to the corresponding value of X_{t+1} could be evaluated numerically. Even without such complete knowledge, we can denote this mapping by some (unknown) function, f, and consider its qualitative properties. By construction, equilibrium values of X (defined as values such that $X_{t+1} = X_t$) in the dynamic system are also fixed points of the artificial adjustment process represented by f. The model

$$X_{t+1} = f(X_t)$$

corresponds to a curve, which we call a *model curve*, in a graph that plots X_{t+1} against X_t, as shown in Figure 4.

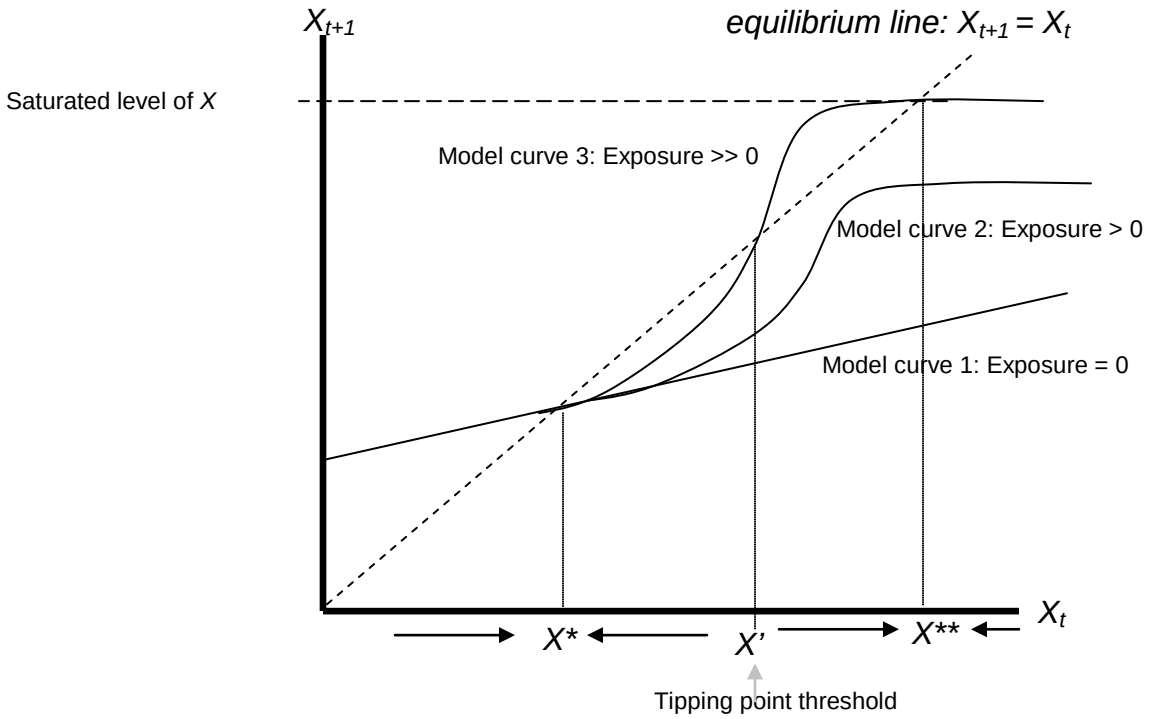

Figure 4: Exposures high enough to destabilize a feedback-control loop create an alternative equilibrium (potential disease) state (X^{**}) and a threshold (X')

Figure 4 actually shows three different model curves, 1-3, corresponding to successively greater exposure levels and/or sensitivities of exposed individuals. For model curves 1 and 2, there is a unique, globally stable equilibrium value of X, denoted by X^*, where the model curve intersects the equilibrium line (defined by the 45 degree line $X_{t+1} = X_t$) from above and to the left. This equilibrium is stable because $X_{t+1} > X_t$ to its left and $X_{t+1} < X_t$ to its right. In other words, if X_t differs from X^*, then the levels of other variables that are affected by X_t will not adjust to levels that sustain X_t, but instead will reach levels that, in turn, cause X_t to move closer to X^*. Such a globally stable equilibrium represents the normal, homeostatic equilibrium for the system when no

disease is present. Model curve 2 differs from Model curve 1 by showing saturation of *X* at its right end, i.e., a maximum possible level of *X*. Even a high level of exposure will not lead to an infinite level of *X*, but will, at most, saturate the response of the feedback loop(s) containing *X*, sending the affected variables to their maximum levels.

Model curve 3 shows a qualitatively different possibility for an exposed individual for whom the saturated level of *X* is high enough to intersect the equilibrium line from above and to the left. For such an individual, there are two alternative equilibria: the normal homeostatic equilibrium at *X**, and an alternative, locally stable equilibrium *X***, with *X* at its saturated level. In between them, for any continuous model curve, there must be a threshold or "tipping point," denoted by *X'* in Figure 4, such that *X* will adjust toward *X** from any starting point to the left of *X'*, but will adjust toward *X*** from any starting level to the right of *X'*. That is, *X'* is an *unstable* equilibrium separating the two basins of attraction for the "healthy equilibrium" *X** and the potential "disease equilibrium" *X***. (Topologically, such a threshold must exist whenever two alternative stable equilibria exist, for any continuous model curve; it is unique if the model curve is s-shaped.) As explained in detail by Cox (2010) for a specific parametric model of COPD (consisting of a system of ordinary differential equations and algebraic equations with estimated parameter values), exposure that increases a model curve enough to produce a saturated equilibrium (such as *X*** in Figure 4) does so by *destabilizing the positive feedback loop(s)* containing *X*, causing its variables to escalate until saturation is reached.

For a biological interpretation, suppose that *X* represents ROS, and that the mechanism by which long-term exposure increases the model curve is to shift cell

populations (such as AMs) toward phenotypes that produce higher levels of ROS (and/or higher levels of the causal drivers of increased ROS in Figure 3). Then X^{**} represents a *high-ROS equilibrium*, in which ROS and all the other variables in Figure 3 (which participate in positive feedback loops with ROS) have increased levels. If long-term exposures produce a model curve with two alternative equilibria (such as model curve 3), and if short-term exposure transients can then temporarily increase the level of X, then any exposure history that increases X past its tipping-point threshold will trigger a self-sustaining escalation in levels of X (and of all other variables that participate in a positive feedback loop with X, including all variables shown in Figure 3) until the high-ROS (saturated-equilibrium) state is reached. If defensive and repair resources are insufficient to counter the damage done in this high-ROS state, then tissue destruction and other clinical manifestations of lung disease may result. The threshold model in Figure 4 predicts that progression to the high-ROS potential disease state will occur, even in the absence of further exposure, once the tipping point has been passed.

The preceding threshold model is motivated by current understanding of the biology of lung responses to PSP exposures in general, and to CS exposures in particular, but it does not require detailed knowledge of the biological mechanisms involved, many of which remain uncertain. For example, with sufficient knowledge and data, each of the links between variables in Figure 3 could be further elucidated, perhaps expanding into an entire sub-network showing molecular-level details of how an increase in the variable at the tail of an arrow propagates through signaling pathways and other mechanisms to cause an increase in the variable at the arrow's head. But such a detailed description would not change the basic topology of the network, nor its properties derived from the

fact that multiple positive feedback loops dominate its qualitative behavior. The exposure-response threshold in Figure 4 does not depend on such details, and hence is robust to uncertainties about them. Although further biological information may eventually allow more detailed simulation and prediction of the time courses of lung disease initiation and progression, it should leave intact the insights that comparative statics analysis, of the type performed in this section, provides today.

Confirmatory Data: How Well Does the Theory Match Observations?

The analysis of alternative equilibria in Figure 4 implies the existence of an exposure threshold, below which lung damage is largely reversible (although the homeostatic equilibrium X^* can be shifted rightward if exposure shifts the whole model curve up), and above which escalation of ROS, and of the other variables in Figure 3, to permanently elevated levels will progress, even without further exposure. It is useful to compare this theoretical prediction to available data, which come largely from a series of studies in rats, undertaken by NIOSH. Porter et al. (2004) found experimentally that "the time course of rat pulmonary responses to silica inhalation as biphasic, [with] the initial phase characterized by increased but controlled pulmonary inflammation and damage. However, after a threshold lung burden was exceeded, rapid progression of silica-induced pulmonary disease occurred." They reported that "During the first 41 days of silica exposure, we observed elevated but relatively constant levels of inflammation and damage, with no fibrosis. Subsequently, from 41 to 116 days of exposure, rapidly increasing pulmonary inflammation and damage with concomitant development of

fibrosis occurred. This suggested that pulmonary defense mechanisms were initially able to compensate and control silica-induced pulmonary inflammation and damage, but after a certain threshold lung burden was exceeded, these control mechanisms no longer were adequate to prevent the progression of silica-induced pulmonary disease." In terms of Figure 4, these data could be interpreted as indicating that exposure initially moves the model curve upward, thus moving the homeostatic equilibrium rightward (yielding the reported controlled, reversible increases in levels of loop variables). Continued exposure moves the model curve further upward (e.g., because it selects for macrophages that produce higher levels of ROS for the same exposure), eventually creating a tipping point threshold and an irreversible disease state (saturated equilibrium), yielding the reported rapid progression of pulmonary disease.

Such a coincidence between qualitative predictions and experimental observations in rats while perhaps encouraging, does not prove that our conceptual model is correct. To test the specific biological interpretation (suggested by Figure 3) that a high-ROS equilibrium accounts for silica-induced lung diseases, it would be necessary to assess the levels of ROS in conjunction with the initiation and progression of silica-induced lung diseases. Fortunately, such experiments have been done. Porter et al. (2006) examined the mechanism by which injury progresses in rat lungs even after exposure ceases, and found that it is indeed mediated by a continuing increase production of ROS (and also reactive nitrogen species). They reported that "even after silica exposure has ended, and despite declining silica lung burden, silica-induced pulmonary NO [nitrogen oxide] and ROS production increases, thus producing a more severe oxidative stress. …iNOS and NO-mediated damage are associated anatomically with silica-induced pathological

lesions." This is fully consistent with the prediction (from Figure 4) that, once the tipping point threshold has been passed, the system will be in the basin of attraction for a high-ROS equilibrium, to which it will move (thus increasing the levels of all the loop variables positively linked to ROS) even after silica exposure has ended. A similar tipping-point threshold between two basins of attraction has been reported in an explicit dynamic simulation model of COPD (Cox 2010). Thus, this key feature of our theoretical analysis appears to be consistent with some limited available data.

Of course, rats are not people, and the relevance of experimental findings in rats to disease processes in people can be questioned. However, Porter et al. (2004) note that in human occupational populations, too, "Human epidemiologic studies have found that silicosis may develop or progress even after occupational exposure has ended, suggesting that there is a threshold lung burden above which silica-induced pulmonary disease progresses without further exposure." Thus, we believe there is empirical support for the inference that CS, like other PSPs that cause lung diseases following chronic inflammation (Azad et al. 2008), induces a *high-ROS state* as a possible alternative equilibrium to the usual, lower-ROS, homeostatic equilibrium – at least in susceptible individuals (defined as those in whom exposure shifts the model curve up enough to create the alternative stable equilibrium state, X^{**}). Exposures that push the dynamic system of interacting variables in the lung (see Figure 3) into the basin of attraction of this high-ROS state then trigger progression to the high-ROS state, even if no further exposure occurs. Depending on an individual's capacity to repair the multiple types of damage caused by the high-ROS state (see Figure 3), a variety of lung diseases, from silicosis to lung cancer, can result. We propose this as a unifying conceptual model for

understanding the induction and progression of inflammation-mediated lung diseases caused by inhalation of PSPs.

5. Discussion: Using the Model to Address Policy-Relevant Questions

Epidemiological investigations that do not include careful, well-validated modeling of exposure estimation errors may not yet be capable of delivering convincing answers to the policy-relevant questions raised in the introduction: whether exposure-related diseases occur together; whether crystalline silica has an exposure-response threshold for causing lung diseases; and, if so, whether currently permissible exposure limits lie above or below the threshold. However, combining available, imperfect epidemiological evidence with recent advances in understanding of lung responses to poorly soluble particulates (PSPs) in general, and crystalline silica (CS) in particular, as outlined in the previous two sections, allows us to shed new light on each of these practical questions.

Existence of an Exposure-Response Threshold

There are strong empirical, as well as theoretical, grounds for expecting a threshold in the exposure-response relation. In theory, knowledge that CS acts through positive feedback loops (Figure 3) suggests the presence of an exposure-response tipping point threshold (such as *X'* in Figure 4). Empirically, relatively low exposures have been observed to induce largely self-limiting and reversible effects in rats (consistent with a homeostatic equilibrium, *X**), while high exposures have been observed to trigger a self-

sustaining escalation to a permanent high-ROS state (consistent with an alternative equilibrium *X***) (Porter et al. 2004, 2006). Our review of CS epidemiology in Section 2 suggests that existing epidemiology is fully consistent with the biologically-based understanding of PSP mode of action and the two alternative-equilibria theory in Figures 3 and 4, and with their implied exposure-response threshold for exposure-related increases in lung disease risks (as observed for many PSPs in rats (Oberdörster 2002)), once a clear distinction is drawn between exposure-response curves for *estimated* exposures and exposure-response curves for *true* but unknown exposures. The former may lack a threshold, even if the latter have one (Figure 1).

Quantitative Estimation of the Exposure-Response Threshold: ≥ 0.4 mg/m^3

A potentially useful quantitative contribution from CS epidemiology is the observation that lung function appears to be diminished in some studies at estimated occupational exposure concentrations in excess of 0.1 to 0.2 mg/m^3 of respirable silica dust for durations of at least 30 to 40 years, in the presence of other occupational dust exposures (Rushton 2007). If this finding is confirmed, and if confounding by cigarette smoking and occupational co-exposures is eventually ruled out as an explanation (perhaps by building on new methods such as those in Richardson 2010), then 0.1 to 0.2 mg/m^3 of silica dust for 30 to 40 years might be accepted as a useful point of departure for estimating the exposure threshold that must be exceeded to create a disease state.

As in other epidemiological studies, there is large uncertainty in this review about true exposures, implying that any real exposure-response threshold is likely to be significantly greater (perhaps by several-fold) than the level at which the estimated

exposure-response threshold shows elevated risks (see Figure 1). To obtain a clear estimated concentration threshold between 0.1 and 0.2 mg/m^3, it is necessary to modify the example in Table 1. For example, Figure 5 shows a simulated exposure-response

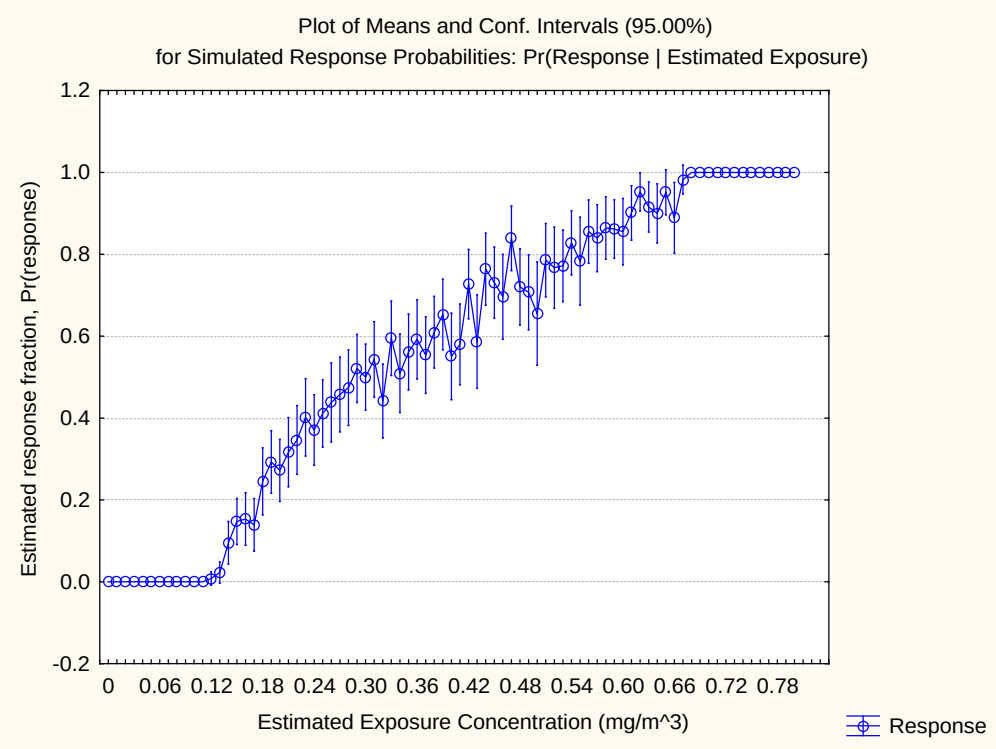

Figure 5. A True Threshold at 0.4 mg/m^3 Produces an Estimated Threshold Between 0.1 and 0.2 mg/m^3. (N = 10,000 samples; k ~ U[0.3, 1.7]; true exposure ~ U[0, 1] mg/m^3.)

curve when the true exposure is uniformly distributed between 0 and 1 mg/m^3 and there is a true response threshold at 0.4 mg/m^3 (with the true probability of response, i.e., exposure-induced illness, being 0 for concentrations below this threshold and 1 above it. In reality, of course, different individuals might have different thresholds, reflecting their own model curves and *X'* values, but it remains true that unmodeled error, even in unbiased exposure estimates, smears out and decreases the apparent threshold level of exposure at which excess population risks start to occur.) In the absence of detailed study

of real-world exposure estimation errors, such hypothetical examples suggest that an estimated exposure concentration threshold between 0.1 and 0.2 mg/m^3 might correspond to a true threshold value of about 0.4 mg/m^3 for the concentration threshold that must be exceeded before adverse health effects occur among susceptible workers.

However, this rough estimate of 0.4 mg/m^3 is contingent on as-yet unproved assumptions, including that the adverse health effects in Rushton (2007) were caused by CS, rather than by other exposures. We have assumed only a rather modest degree of variability in estimated exposures around the corresponding true values (namely, a uniform distribution around the mean, $k \sim U[0.3, 1.7]$, with no outliers or heavy tails). The true threshold could be substantially higher than 0.4 mg/m^3 if exposure estimates have greater variability than this. (As an extreme example, the true threshold could be as high as 2 mg/m^3 and still give an estimated threshold of 0.1 mg/m^3 if (a) Each individual with an estimated exposure of 0.1 has a 5% probability of having been exposed to 2 mg/m^3 and a 95% probability of having been exposed to 0 mg/m^3, for an average exposure of $0.05*2 + 0.95*0 = 0.1$ mg/m^3; and (b) The power of the study is such that at least 5% of individuals in an exposure group must respond in order for an excess risk to be detected.) Thus, to better estimate the true level at which adverse health effects associated with the high-ROS state are induced, it will be essential for future studies to more carefully characterize the error distribution of estimated exposures around true exposure levels, perhaps using more detailed simulations of workplace daily exposure distribution means and variances.

Meanwhile, it appears plausible that currently permitted exposure levels of 0.1 mg/m^3 of respirable CS could be well below (possibly by a factor of 2 to 10, based on the

hypothetical examples just described) the levels that might increase risks of adverse health effects. This conclusion becomes more robust if, instead of there being different thresholds for different CS-induced lung diseases, there is one large dichotomy, as illustrated in Figure 4, between a low-ROS homeostatic equilibrium and a high-ROS disease state equilibrium (which can then produce different ROS-mediated diseases in susceptible individuals, based on different vulnerabilities in their defensive and repair resources for responding to oxidative stress injuries). We now consider further the implications of such a dichotomy.

Is Increased Risk of Silicosis Necessary for Increased Risk of Lung Cancer?

The study of Rushton (2007) examines estimated concentrations for *longitudinal* effects, so that even long-delayed health effects can eventually be counted. This is very useful when the alternative-equilibria theory in Figure 4 is combined with an assumption that the high-ROS equilibrium is necessary (although perhaps not sufficient, if defensive and repair capabilities are sufficiently strong) to cause increased risk of ROS-mediated lung diseases. Together, these assumptions imply that if increased rates of ROS-mediated lung diseases do eventually occur in an exposed occupational population, then exposure must have been sufficient to create the high-ROS state in susceptible individuals – and, therefore, high enough to have increased risks of several different diseases associated with the high-ROS state among individuals susceptible to each type (e.g., due to limited capacity for alveolar epithelial tissue repair, for emphysema; or ECM repair, for fibrosis; or apoptosis of pre-malignant cells, for lung cancer, and so forth). Conversely, this understanding of the disease process implies that protecting against any of the high-ROS

diseases, by keeping exposures below the levels that induce a high-ROS state in an individual or species, will protect against all of them, from silicosis to inflammation-mediated lung cancer. This makes it plausible that exposures that are too low to cause increased risk of silicosis (even among susceptible individuals) will also not cause increased risk of lung cancer, even if silicosis is not a necessary precondition for CS-induced lung cancer: failure to create the high-ROS alternative equilibrium protects against both. According to this logic, increased risk of silicosis (and other indicators of the high-ROS state) in susceptible individuals should be expected as a necessary accompaniment to increased risk of other high-ROS diseases (such as inflammation-mediated lung cancer caused by CS (Blanco et al. 2007; Azad et al. 2008)), whether or not silicosis causally contributes to CS-induced lung cancer.

6. Conclusions

Postulating an exposure-response threshold for lung diseases (including lung cancer) associated with exposure to CS and other PSPs is not new. It has long been discussed for CS, with rat data, human data, and mechanistic information being cited in support of thresholds (e.g., Oberdörster 2002). For example, in 1995, researchers from California's Department of Toxic Substances Control (Klein and Christopher 1995) reviewed the then-available evidence on the carcinogenicity of crystalline silica, and concluded that "The weight of evidence for both rats and humans indicates that fibrotic and silicotic lesions in the lung result from inhalation exposure to crystalline silica and that lung cancer is secondary to those lesions in the lung. Thus crystalline silica should be

considered to have a threshold for causing cancer. The critical exposure criterion is that exposure level which does not produce a fibrogenic or silicotic response; thus it is necessary to determine the no observed adverse effect level (NOAEL) for fibrogenesis."

Our analysis supports these earlier conclusions. To do harm, exposures to PSPs such as CS must be large enough and last long enough to trigger the chronic inflammatory responses and progression to a high-ROS state that can eventually lead to diseases. *In vitro* evidence in cell cultures, as well as *in vivo* experiments in rats, indicate exposure thresholds for inflammation (Donaldson et al. 2008), oxidative stress, and resulting diseases, including lung cancer (Oberdörster 2002). Moreover, normal lung cell populations interact via homeostatic (negative) feedback loops that stabilize and maintain oxidant-antioxidant balance (Liu et al. 2008, D'Autréaux and Toledano 2007) and other (e.g., proteinase/anti-proteinase) equilibria (Cox 2010). Disease risk is not increased by exposures while homeostasis is maintained. Disrupting normal homeostasis requires activating positive feedback loops (Figure 3) capable of damaging tissue (respiratory epithelium) and overwhelming normal repair processes. Both rat data (Oberdörster 2002) and mathematical modeling of inflammation-mediated lung diseases (Figure 4) indicate that these responses to PSPs have exposure-response thresholds. Of course, these data and models are limited, and much remains to be learned about the details of the biological inputs and feed-back loops that they describe, as well as others that may yet be discovered. Thus, we cannot completely exclude the possibility that a threshold does not exist. But our model-based analysis may add to previous weight-of-evidence conclusions by suggesting how exposure-response thresholds naturally arise between alternative basins of attraction in positive feedback loop systems.

For CS and many other PSPs, sufficient exposure triggers AM activation and phenotype change, release of ROS and RNS, attraction of monocytes, AMs, and neutrophils to inflamed areas, damage and destruction of alveolar epithelial tissue and extracellular matrix, disruption of normal apoptosis and epithelial tissue repair and ECM repair, sustained epithelial proliferation and hyperplasia, and possible promotion of lung cancer. These disease processes may be modeled as networks of damaging positive feedback loops that are either "switched on" (meaning that the loop is attracted to a new, stable equilibrium with increased values of its variables, such as X^{**} in Figure 4) or "switched off" (meaning that the loop remains in the basin of attraction of the healthy equilibrium, X^{*} in Figure 4). Excess risk of inflammatory lung diseases and lung cancer arises only at exposure intensities and durations that are large enough to switch on these disease processes. For crystalline silica, these trigger levels may be on the order of 0.4 mg/m^3 or more of silica dust, depending on the distribution of exposure estimation errors around true values. Such levels significantly exceed currently permissible levels (e.g., 0.05 to 0.1 mg/m^3), implying that further reductions in permitted exposure levels – if permitted levels are enforced – should not be expected to produce further reductions in human health risks.

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