CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Overview. Throughout this section, the term silica refers to all types of silica particles. Information that is specific to c-silica or a-silica is indicated as such. The following provides a general overview of the toxicokinetics of silica compounds. Additional information on the toxicokinetics of synthetic a-silica compounds, including results of unpublished studies, was reviewed by the European Centre for Ecotoxicology and Toxicology of Chemicals Joint Assessment of Commodity Chemicals report (ECETOC 2006).

- Absorption:
    - Respiratory tract: Absorption of silica compounds from the respiratory tract is the most studied absorption pathway.
        - No quantitative estimates of absorption of silica compounds from the respiratory tract are available. However, detection of silica in the urine of exposed workers indicates that c-silica undergoes absorption following inhalation exposure.
        - Respiratory particles of silica are cleared from the pulmonary region primarily by lymph drainage, macrophage phagocytosis and migration, and upward mucociliary flow.
        - Due to limited solubility, dissolution of c-silica, followed by absorption from the respiratory tract is not a predominant pathway for absorption. Dissolution of a-silica compounds may be a more important absorption pathway for a-silica.
        - Inhaled c-silica compounds may be retained within the lungs for years after cessation of exposure
    - Gastrointestinal tract: Limited information from animal studies indicates that absorption of silica compounds following oral exposure is negligible.
    - Dermal: No studies examining dermal absorption of silica compounds were identified, although it is anticipated that absorption through the skin would be negligible.

- Distribution: Studies in humans and animals show that inhaled c-silica is distributed to the kidneys, lymph nodes, blood, liver, and spleen. No information on distribution of silica compounds following oral or dermal exposure was identified.

- Metabolism: Absorbed silica compounds do not undergo metabolism.

- Excretion: Silica has been detected in urine of workers exposed to c-silica. Ingested silica compounds are excreted in the feces. No information on excretion of silica compounds following dermal exposure was identified.

3.1.1 Absorption

Inhalation Exposure. Inhaled silica particles that deposit in the respiratory tract are subject to three general distribution processes: (1) bronchial and tracheal mucociliary transport to the gastrointestinal tract; (2) transport to thoracic lymph nodes (e.g., lung, tracheobronchial, mediastinal); or (3) absorption by blood and/or lymph and transfer to other tissues (e.g., peripheral lymph tissues, kidney). The above processes apply to all forms of deposited silica, although the relative contributions of each pathway and rates associated with each pathway vary with the physical characteristics (e.g., particle size) and biological reactivity (e.g., macrophage recruitment, activation, and cytotoxicity).

Particles having diameters >5 μm deposit in the upper airways (extrathoracic, tracheobronchial regions) and are cleared from the respiratory tract primarily by mucociliary transport to the gastrointestinal tract (Bailey et al. 2007; ICRP 1994). Smaller particles (≤5 μm) are deposited primarily in the pulmonary region (terminal bronchioles and alveoli). Particles are cleared from the pulmonary region primarily by lymph drainage, macrophage phagocytosis and migration, and upward mucociliary flow. Dissolution, which contributes to absorptive clearance of some types of particles, is negligible for c-silica because of the low solubility of c-silica particles. Dissolution may play a larger role in clearance of a-silica, and may contribute to its faster pulmonary clearance compared to c-silica (Davis 1986; Kelly and Lee 1990; Reuzel et al. 1991; Schepers 1981).

The various processes that contribute to the clearance of silica from the respiratory tract give rise to multi-phasic lung retention kinetics (Katsnelson e al. 1992; Stober et al. 1999; Vacek et al. 1991). In most studies of lung retention, at least two kinetic components are evident. The faster phase is likely due to relatively rapid mechanical clearance mechanisms (e.g., mucociliary transport) and, for more soluble forms (e.g., a-silica), absorption to blood of soluble or relatively rapidly dissolved insoluble material deposited in the lung. The slower phase is likely due to physical transformation and dissolution and/or mechanical clearance of highly insoluble particles by phagocytosis and macrophage migration.

Rates for slow-phase clearance vary with the type of silica particle inhaled, inhaled dosage, and animal species (Kreyling 1990). In humans, slow-phase clearance of highly insoluble particles occurs with half-lives of several years (Bailey et al. 2007; ICRP 1994). The slow phase of clearance of silica particles explains the accumulation of particles in the human lung that can occur with repeated exposures to airborne silica as well as its detection in lung tissue years after cessation of exposure (Borm and Tran 2002; Case et al. 1995; Dobreva et al. 1975; Dufresne et al. 1998; Loosereewanich et al. 1995).

Studies conducted in rodents found that clearance of c-silica (quartz) was >10 times slower than a-silica (Davis 1986; Kelly and Lee 1990; Reuzel et al. 1991; Schepers 1981). A contributing factor to the slower clearance of c-silica may be its greater cytotoxic potency, related to its surface structure. In rats, clearance following inhalation of an aerosol of pure cristobalite was slower than following inhalation of aerosols of quartz, and rats showed a more pronounced lung inflammatory response to cristobalite compared to quartz (Hemenway et al. 1990). Macrophages play an important role in the mechanical clearance of silica particles (Absher et al. 1992; Brody et al.1982). A more intense inflammatory response to macrophage cytotoxicity induced by c-silica results in slow particle clearance (Donaldson and Borm 1998; Fenoglio et al. 2000; Warheit et al. 2007). In general, mechanical clearance of deposited particles appears to have a limited capacity. Macrophage-mediated clearance of respirable particles is inhibited at high particle loads. This phenomenon has been referred to as *particle overload* (Mauderly et al. 1990; Morrow 1992). The inhaled dose required to achieve particle overload is not the same in all animal species and may be lower in small mammals (Snipes 1996). Rats exhibit lower particle overload thresholds than hamsters (Saffiotti et al. 1993). Above the particle overload threshold, differences in clearance between c-silica and a-silica become less pronounced (Pratt 1983). Particle overload is an important consideration in low-dose extrapolation of dose-response relationships and in extrapolation across animal species because it may result in a nonlinear relationship between the inhaled dosages and particle burden in the lung (Lippmann and Timbrell 1990; McClellan 1990). Particle overload may also render the respiratory tract more vulnerable to other airborne particulates as a result of depressed particle clearance (Morrow 1992).

Oral Exposure. Little information regarding the gastrointestinal absorption of silica was identified. In rats, six gavage doses of 50 mg c-silica did not result in detectable silica particles in gastrointestinal submucosa or region lymph nodes, suggesting little or no transfer out of the gastrointestinal tract lumen (Gonzalez Huergo and Rojo Ortega 1991).

Dermal Exposure. Studies of dermal absorption of silica have not been reported and, given the solubility of silica dusts, dermal exposure is likely to be a minor pathway of absorption of silica. Skin samples collected from patients with progressive systemic scleroderma (PSS) and who were also exposed to c-silica (quartz dusts) showed evidence of quartz crystals in chorionic fibers, blood vessel walls, corneas, epidermal keratinocytes, and collagen fiber, based on detection of birefringent particles (Mehlhorn et al. 1990). This finding could indicate dermal absorption or dermal deposition of inhaled or ingested silica. Quartz crystals were not observed in skin tissue of patients who did not have PSS and were exposed to quartz dust, including silicosis patients.

3.1.2 Distribution

Inhalation Exposure. Few studies of distribution of silica outside of the respiratory tract have been reported (Absher et al. 1992). Evidence for associations between exposure to c-silica dusts and renal disease suggests that c-silica particles may distribute to the kidney (see Section 2.10, Inhalation, Systemic Effects). Silica has been detected in kidney tissue and urine of workers who have been exposed to c-silica, suggesting that systemic distribution can occur in humans following inhalation exposure (Giles et al. 1978; Hauglustaine et al. 1980; Ibrahim et al. 2011; Saldanha et al. 1975). Inhalation exposure of rats to c-silica shows distribution primarily to mediastinal lymph nodes and thymus; silica particles were detected in negligible amounts in the blood, kidney, liver, and spleen (Absher et al. 1992). These studies suggest that lymph may provide a mechanism for systemic distribution of silica particles (Vacek et al. 1991).

Oral Exposure. Studies of the systemic distribution of silica following oral exposures have not been reported.

Dermal Exposure. Studies of the systemic distribution of silica following dermal exposures have not been reported.

3.1.3 Metabolism

Absorbed silica is not metabolized. Although c-silica particles are highly insoluble, *in vitro* studies have found that silica particles dissolved from slate dust can bind to serum albumin (Singh et al. 1984).

3.1.4 Excretion

Inhalation Exposure. Silica has been detected in urine of ceramic factory workers exposed to c-silica, suggesting that systemic distribution can occur in humans following inhalation exposure (Ibrahim et al. 2011). Urine is an excretory pathway for silica absorbed from the respiratory tract.

Oral Exposure. Ingested silica is excreted in the feces. Absorbed silica, if absorption were to occur, may be excreted in urine; however, no studies of excretion of silica following absorption from the gastrointestinal tract have been reported.

Dermal Exposure. Studies of excretion of silica following dermal exposures have not been reported.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

No PBPK models for c-silica or a-silica were identified.

3.1.6 Animal-to-Human Extrapolations

Numerous animal studies examining effects of inhaled c-silica have been conducted, and have been particularly useful in investigating pulmonary clearance of particles and mechanisms of toxicity (Cox 2011; EPA 1996; NIOSH 2002). However, results of animal studies may be difficult to extrapolate to humans due to species differences in macrophage overloading, which can affect pulmonary clearance and toxicity (EPA 1996). Rats appear to be more sensitive than hamsters to macrophage overload (Saffiotti et al. 1993). Furthermore, it has been proposed that overload of lung macrophages in rats may not be relevant to humans (Snipes 1996). Regarding use of animal models to investigate the carcinogenic effects

of c-silica, c-silica is carcinogenic in rats exposed by inhalation or intratracheal instillation, but not in mice or hamsters (IARC 2012). Thus, not all experimental animals appear to be appropriate for use in extrapolation to humans.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to silica are discussed in Section 5.7, Populations with Potentially High Exposures.

Crystalline Silica.

Children. No information regarding susceptibility of children to c-silica or a-silica has been identified. Silicosis is considered to be an occupational disease that typically occurs with prolonged (years) exposure. The same adverse effects observed in adult workers would be expected to occur in children if sufficiently exposed. However, non-occupational exposure of children to c-silica could occur in rare circumstances (Bhagia 2012; Grobbelaar and Bateman 1991a, 1991b; Norboo et al. 1991a, 1991b; Ranavanya et al. 1992; Rees and Murphy 2007). For example, a mixed-etiology pneumoconiosis (combined exposure to c-silica, heavy dust, and heavy domestic smoke) has been reported in adults engaging in domestic maize hand-grinding activities using quartz rocks in South Africa (Grobbelaar and Bateman 1991a, 1991b). Unique geographical locations and environmental conditions may also result in elevated exposure leading to silicosis. In addition, radiographic evidence consistent with silicosis has been reported in older individuals in agricultural villages in the northwest Himalayas in India (Norboo et

al. 1991a, 1991b; Ranavanya et al. 1992). This area has frequent dust storms, producing silicotingen rock dust with high c-silica content. However, non-occupational exposure to elevated levels of c-silica that produce silicosis is very rare.

Underlying diseases. Progression of silicosis can cause serious decrements in lung function that may result in death due to respiratory failure (see Section 2.4, Inhalation, Systemic Effects, Respiratory). Thus, individuals with underlying lung and health conditions, such as asthma, emphysema, tuberculosis, and infection with human immunodeficiency virus, may be more susceptible to adverse respiratory effects of inhaled c-silica. Workers with underlying renal diseases also may be more susceptible to adverse renal effects of inhaled c-silica.

Smoking. As discussed in Section 2.19 (Inhalation, Cancer), results of recent studies show that the risk of lung cancer due to c-silica is higher in smokers than in nonsmokers. Results of a retrospective study in China examining lung cancer risk in smoking and nonsmoking c-silica workers showed a consistent increase (2.75–4.38-fold) in lung cancer risk in smokers versus nonsmokers over stratified exposure quartiles (Liu et al. 2013). The study authors stated that 'the joint effect of *[c-]*silica and smoking was more than additive and close to multiplicative."

Polymorphisms. Information in this section is from the following reviews: Ding et al. 2002; Gomez-Puerta et al. 2013; Iannello et al. 2002; IARC 2012; NIOSH 2002; Parks et al. 1999; Yucesoy et al. 2002.

Specific growth factors and cytokines have been identified as playing a crucial role in the pathogenesis of silicosis, particularly TNF-α or IL-1 (see Section 2.20.2 Mechanisms of Toxicity for more details). Evidence from human studies indicates that certain polymorphisms for TNF-α or IL-1 are associated with increased incidence and/or severity of silicosis following occupational exposure to c-silica. For example, in silicotic patients, the risk of developing severe fibrosis was associated with the HLA-Aw19-B18 TNF-α haplotype in the Caucasian population and the HLA-Bw54 TNF-α haplotype in the Japanese population. In a case-control study, the TNF-α variant (-238) was significantly associated with severe silicosis and the TNF-α (-308), IL-1RA (+2018), and IL-1RA (-208) variants were significantly associated with moderate and severe silicosis.

Allelic variants of TNF-α or IL-1 have also been associated with autoimmune and inflammatory diseases. For example, individuals with the HLA-DR3 TNF-α haplotype or a minor variant of the IL-1RA VNTR in linkage disequilibrium have a genetic predisposition to SLE. Therefore, individuals with

polymorphisms in these genes may also have increased susceptibility to autoimmune effects associated with occupational exposure to c-silica. In addition, individuals with other known genetic predispositions to autoimmune disease may have an increased risk of autoimmune dysfunction with occupational exposure to c-silica (e.g., genetic alterations in the major histocompatibility complex). For example, only specific strains of mice (NZB and MRL/MpJ) develop autoimmune pathology resembling SLE following exposure to c-silica dust.

A case-control study of workers with silicosis evaluated interactions between c-silica exposure and polymorphisms for genes encoding for components of the Napl3 inflammasome (Nalp3, caspase-1, IL-1β) (Weng et al. 2015). The Napl3 inflammasome is a multiprotein oligomer that activates inflammatory responses. The study population included 179 Chinese iron miners with silicosis and 201 controls. Results indicate that polymorphisms in Nalp3 and caspase-1 may be involved in individual susceptibility in workers with silicosis, and that there are interactions between polymorphisms and cumulative exposure, age, and smoking status.

Altitude. In a recent review, Vearrier and Greenberg (2011) concluded that workers at high altitude are at risk for more rapid development and progression of silicosis.

Amorphous Silica. No specific information regarding susceptible populations for a-silica was identified. Animal studies identify the respiratory tract as the primary target for inhaled a-silica; therefore, individuals with underlying respiratory disease may be more susceptible to the adverse respiratory effects of a-silica.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to silica are discussed in Section 3.3.1. The National Report on Human Exposure to

Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for silica from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by silica are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure to Silica

Crystalline Silica and Amorphous Silica. Silica has been detected in urine of ceramic factory workers exposed to c-silica, indicating that systemic distribution occurs in humans following inhalation exposure (Ibrahim et al. 2011). Thus, the presence of silica in the urine indicates that exposure has taken place. a-Silica is excreted in the feces. The source of fecal silica is most likely from unabsorbed particles of inhaled silica that are deposited in the oral cavity and swallowed or are cleared from the airway by muco-ciliary clearance and subsequently swallowed. However, the quantitative relationship between urinary silica and cumulative exposure is unknown. Thus, no biomarkers of exposure to c- or a-silica have been identified.

3.3.2 Biomarkers of Effect Caused by Silica

No biomarkers have been identified to characterize effects caused by c-silica or a-silica. Several studies have examined the association between biomarkers of oxidative stress and inflammation in blood and urine in small numbers of silica-exposed workers and in laboratory animals. Markers examined include lactate dehydrogenase, alkaline phosphatase, tumor necrosis factors, interleukins, Clara cell proteins, and numerous proinflammatory cytokines (Aggarwal 2014; Altindag et al. 2003; Braz et al. 2014; Deb et al. 2012; Jiang et al. 2015; Sauni et al. 2012; Sellamuthu et al. 2011; Slavov et al. 2010; Wang et al. 2007). Although associations have been observed, the biomarkers examined are not specific for exposure to silica or as markers of silicosis or pre-silicosis. Elevation of these markers also may be caused by exposure to many other chemicals and by diseases involving inflammatory processes or oxidative stress. Therefore, at this time, no reliable biomarkers for silica effects or for early detection of silica exposure-induced toxicity have been established.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Crystalline Silica. As discussed in Section 2.19 (Inhalation, Cancer), results of recent studies show that the risk of lung cancer due to c-silica is higher in smokers than in nonsmokers. Results of a retrospective study in China examining lung cancer risk in smoking and nonsmoking c-silica-exposed workers showed a consistent increase (2.75–4.38-fold) in lung cancer risk in smokers versus nonsmokers over stratified exposure quartiles (Liu et al. 2013). The study authors stated that ‘the joint effect of [c-]silica and smoking was more than additive and close to multiplicative.” In addition, different c-silica industries may involve co-exposures with other chemicals (e.g., radon, metals, trace elements, asbestos, formaldehyde, benz[a]pyrene) that could potentially increase the toxicity of inhaled c-silica.

Amorphous silica. No studies on interactions of a-silica with other chemicals were identified.