USE OF CHEMILUMINESCENCE ASSAYS TO MONITOR THE SURFACE CHARACTERISTICS AND BIOLOGICAL REACTIVITY OF FRESHLY FRACTURED VS AGED SILICA. V. Castranova[1], V. Vallyathan[1], K. Van Dyke[2], and N. S. Dalal[3]. [1]Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, and the Departments of [2]Pharmacology and Toxicology and [3] Chemistry, West Virginia University, Morgantown, WV 26505.

Evidence suggests that pulmonary disease which results from exposure to crystalline silica may be related to the direct cytotoxic interaction of silica with lung tissue as well as tissue damage caused by reactive products secreted from alveolar macrophages exposed to silica. Previous studies from our laboratory have used electron spin resonance spectroscopy to demonstrate that mechanical crushing or grinding of crystalline silica in air could produce significant amounts of silica-based radicals on the particulate surface and that these silicon-oxygen radicals could react with aqueous media to generate hydroxyl radicals. Our previous studies have also related these radicals to the cytotoxicity of silica and the ability of silica to activate alveolar macrophages. The current study describes the use of a simple chemiluminescence technique to monitor the generation of surface radicals on silica after crushing, the silica-induced generation of radicals in aqueous media, and the activation of secretion of reactive species from alveolar macrophages exposed to freshly ground silica. Chemiluminescence was monitored with a Berthold Luminometer. Crushing crystalline silica with an agate ball mill for 30 min results in the generation of surface silicon-based radicals which can be detected in air by measurement of chemiluminescence. Furthermore, chemiluminescence is also detectable when freshly ground silica is suspended in HEPES-buffered medium. Chemiluminescence of freshly ground silica decays with time both in air and suspension and is not detectable using uncut or aged silica. In vitro exposure of rat alveolar macrophages to freshly ground silica results in the generation of chemiluminescence at substantially greater levels than produced by silica alone. Although aged silica can activate chemiluminescence from alveolar macrophages, the degree of activation is far less than with freshly ground silica. These studies indicate that chemiluminescence represents a useful assay to monitor the surface properties and biological activity of silica and to evaluate the use of coatings or drug treatments in deactivating silica or preventing the activation of alveolar macrophages by silica. (BOM-G1135142 and BOM-5423)

PRODUCTION OF FREE RADICALS BY NON FIBROUS MATERIALS IN A CELL-FREE BUFFER MEDIUM
Costa D., Guignard J. and Pezerat H.
Laboratoire de Réactivité de Surface et Structure, Université P. & M. Curie, 4 place Jussieu, 75252 PARIS Cédex 05, France.

Because of the excess of lung pathologies (cancers, fibrosis, bronchitis ...) observed in many metallic mines and also in some industrial sites, it is necessary to search for the possible mechanisms of toxicity related to the nature and the surface activity of mineral dusts. In order to detect if the materials, when immersed in a cell-free potassium phosphate buffer medium, are capable of producing oxy-radicals by reduction of oxygen occuring on reducing surface sites of the particles, a spin trapping agent is used to capture free radicals, which are than measurable by ESR spectroscopy.

Different families of minerals are active in the production of oxy-radicals : they are divalent iron containing phyllosilicates (such as biotite, some chlorites and berthierines...) and carbonates, some nickel compounds (such as nickel arsenides and Ni_3S_2), copper arsenide, and some iron sulphides powders after a long time of exposure to air.Oxides and oxyhydroxides of Fe^{3+} and arsenosulphides are inactive. Some other compounds are found in intermediary categories of activity. The activity towards oxygen is due to reducing surface sites linked to cations in low valence state (such as Fe^{2+}, Cu^{+}, Ni^{+}). The activities observed are in good agreement with the redox potential corresponding to Cu^{+}, Ni^{+} and Fe^{2+} containing minerals, and can involve, in biological medium, some toxic phenomenous related to the oxidative stress.

In order to determine if the materials studied here are capable of a genotoxic activity, in-vitro and in-vivo tests will be necessary, wherein the processes of surface activation and passivation will be taken into account.

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