56 Development of crystallinity, surface reactivity and potential to transform mammalian cells of a heat-treated diatomaceous earth

Z. ELIAS, I. FENOGLIO*, O. POIROT, F. TURCI*, B. FUBINI*
Institut National de Recherche et de Sécurité, Avenue de Bourgogne, 54501 Vandoeuvre Cédex, France
* Department of Chemistry IFM and Interdepartmental Center "G. Scansetti" for Studies on Asbestos and other Toxic Particulates, University of Torino, Torino, Italy

Diatomaceous earth is the largest source of biogenic silica. This material, widely used by several industries for various applications (e.g. filters, insulators etc.), is the product of deposition over geological time of the siliceous skeleton (frustule) of unicellular algae called diatoms. The powdered silica is made up of small irregularly shaped particles, which still retain the structure of the original diatom skeletons. The raw material is amorphous, but the commercial dusts are partially crystallized into cristobalite as a consequence of flux calcination used industrially to separate the silica component from the associated organic matter. The partially ordered structure of the silicon tetrahedra in the original structure as well as the presence of residues of various salts are believed to facilitate the transformation into cristobalite, which takes place at temperatures much lower than the regular transition from trydimite to cristobalite. While the original material, fully amorphous, is regarded as non-toxic, the heated one (cristobalite) is fibrogenic and carcinogenic to humans (IARC 1997). It is not clear, however, at which stage the material becomes toxic and whether, before a bulk crystallization takes place, the surface is modified and converted into a reactive and potentially carcinogenic one. Previous collaborative work in the two laboratories has shown that several diatomaceous earth samples, irrespective from the actual crystalline content and origin, were all toxic and induced morphological transformation of Syrian hamster embryo (SHE) cells. We have therefore started up a systematic investigation of one commercial diatomaceous earth and its precursor in order to identify the physicochemical conditions for the onset of toxicity. The present paper reports data on the starting material (a diatomaceous earth), on the commercial material (partially crystallized into cristobalite) and on the fine-particle fraction of the latter, separated from the original product with a sedimentation method. The starting material was 2 fold more toxic than the commercial product (LC50 of 24 μg/cm² compared to 47 μg/cm², respectively) but no transforming. The commercial product induced a low but dose-dependent transformation frequency whereas the fine fraction was 3 folds more transforming and more toxic than the former. The commercial product turned out to release a substantial amount of ·OH radicals, measured by means of the spin- trapping technique, in aqueous suspension, when in contact with hydrogen peroxide. The fine fraction was the most active component of the dust, confirming the direct relationship between morphological transformation of cells and free- radical release by particles surface.

57 Apoptosis induction *in vitro* and *in vivo* by quartz and kaolin dusts

M. KEANE, N. GAO, W. WALLACE
National Institute for Occupational Safety and Health, Health Effects Laboratory Division, Morgantown, WV, USA

Respirable quartz and kaolin dusts were used to study induction of apoptosis in NR 8383 rat alveolar macrophages *in vitro* and *in vivo* by intratracheal instillation in male Sprague-Dawley rats. While quartz is a fibrogenic respirable mineral dust, kaolin, a silicate mineral similar to silica, is not generally considered fibrogenic, but has similar cytotoxic properties *in vitro*. Both dusts were <5 μm fractions. *In vitro* concentrations ranged from 50-400 μg/ml, and the instilled animals received 4 or 20 mg in 0.5 ml saline. *In vitro* apoptosis methods included ELISA assays for nucleosomes, terminal deoxynucleotidyl transferase-mediated dUTP-fluorescein nick-end labeling (TUNEL) assays, and DNA ladders for apoptotic fragments. *In vivo* methods include TUNEL assays for lung tissue and flow cytometry for cells in bronchial alveolar lavage (BAL).

In vitro results indicated that quartz induces apoptosis in a dose-dependent and time-dependent manner, while kaolin showed activity only a the highest (400 μg/ml) concentration in the TUNEL assay and slight activity at the 200 and 400 μg/ml points in the ELISA assay. Time course studies *in vitro* at 100 μg/ml showed apoptosis at 6h, 12h, and 1, 3, and 5 days for quartz, but only at 5 days for kaolin. The effect of serum on the dose-response is discussed.

In vivo results show a time-dependent increase of apoptosis in the ELISA assay at both 4 and 20 mg doses, at times ranging from 2 days to 90 days. Kaolin does not show a significant response at any time points for the ELISA. Apoptotic cells as determined using flow cytometry showed a time-and concentration-dependent increase for quartz and no significant response for kaolin at times ranging from 2 to 90 days.

58 Molecular biology study on the carcinogenesis of silica

B. LIU, X. ZHANG, P. HE, X. ZHANG, Q. MIAO, B. YOU, M. XU, N. KANG, R. GUAN, P. ZHOU
Institute of Occupational Medicine, Chinese Academy of Preventive Medicine, Beijing, China

Background: Pneumoconiosis are the most serious occupational disease in China. Through the nation-wide epidemiological survey of pneumoconiosis and the annual report of prevalence of pneumoconiosis in China, we found that as of 2001, the accumulated cases of pneumoconiosis numbered 560,000. In 1996, crystalline silica was upgraded by IARC (International Agency for Research on Cancer) to human carcinogen (Group 1). However, the mechanism of its carcinogenesis is not well revealed.

Methods: We examined the p53 and K-ras gene mutations in lung cancer in workers with silicosis (LCWS). DNA was extracted from paraffin-embedded tissues and examined by PCR-RFLP, PCR-SSCP, and DNA sequencing.

Results: The mutation frequencies of p53 gene were high, but the mutation distributions in exons and among the histological types of LCWS differed from those of common (i.e., not sili-

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