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► To cite this version:

Sylvie Honnons, Dominique Oberson, Agnès Wastiaux, S. Dzwigaj, H. Pezerat, et al.. Pulmonary responses to silica dust: the influence of crystalline structures and physico-chemical properties. 2. International Symposium on Silica Silicosis & Cancer, Oct 1993, San Francisco, United States. ineris-00971883

HAL Id: ineris-00971883

<https://ineris.hal.science/ineris-00971883>

Submitted on 3 Apr 2014

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PULMONARY RESPONSES TO SILICA DUST: THE INFLUENCE OF CRYSTALLINE STRUCTURES AND PHYSICO-CHEMICAL PROPERTIES

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ABSTRACT

The inhalation of silica crystalline polymorphs leads to the development of pulmonary nodules that vary in extent and in fibrotic content.

In this study, a single protocol was used to test different silica samples (i.e., 2 non-crystalline samples: one vitreous silica, one diatomaceous earth, 4 crystalline samples: one pure α -quartz ground in a wet atmosphere, one of the same quartz heated until it becomes α -cristobalite and 2 cristobalites obtained by heating diatomaceous earth).

Wistar rats received a single intra-tracheal injection of alveolar dust and were followed over a period of 3 months.

The results were exploited using macroscopic examination (wet lung weight), histological examination and biochemical dosing (hydroxyproline, lipid, and silica content).

The results showed that vitreous silica, diatomaceous earth, wet-ground quartz and both types of cristobalites are responsible to a greater or lesser extent for the pulmonary reaction typical of exposure to silica (i.e., nodules); with different cellular and fibrotic surface areas for each sample.

α -cristobalite (obtained from the wet-ground quartz heated to a very high temperature; 1,300 °C) leads to the formation of only small foreign-body granulomas.

All crystalline minerals tested caused alveolar lipoproteinosis. It was thus concluded that the crystalline structure plays a less important role than the surface state in the formation of silicotic nodules.

INTRODUCTION

The inhalation of dust containing crystalline silica has long been known to be a direct cause of a serious lung disease, silicosis [16]. The reactivity of α -quartz dust in in-vivo experimental studies was rapidly found to present considerable variations. The numerous studies aimed at individualizing the physico-chemical characteristics of different types of α -quartz have not been able to explain the observed variations in biological reactivity [2, 3, 7, 8, 9, 15, 18, 19]. What is more, the idea that the crystalline structure could condition the nocivity of inhaled silica dusts has been called into question [1, 4, 5, 6, 10, 11, 12, 20].

In this study, a single protocol was used to test different silica samples: 1 diatomaceous earth sample, 2 cristobalite samples obtained by heating diatomaceous earth, 1 pure α -quartz ground in a wet atmosphere and the same quartz heated to 1,300°C. Another sample of amorphous vitreous silica had been tested following the same protocol in a previous study.

MATERIALS AND METHODS

Groups of 6 female EOPS Wistar rats supplied by Iffa-Credo (France) received a single intratracheal instillation of 30 mg of alveolar dust suspended in saline. Three control animals received saline alone. All animals were housed in a controlled area for a period of three months.

Silica samples provided by H. Pezerat (CNRS, France) :

- 1 diatomaceous earth sample (Diat),
- 1 "cristobalite" (CR 1210) obtained by heating the same diatomaceous earth,
- 1 other "cristobalite" (CR 1212) obtained by heating different diatomaceous earth.

These three dust types were not subjected to grinding in our laboratories, and the alveolar fraction of less than 6 μ was obtained using an elutriator. The granulometry was carried out by MEB, as these dust particles partially retain the shape of fossilized diatoms, making it difficult to measure their aerodynamic diameter with precision.

Silica samples provided by B. Fubini (Univ. Torino, Italy):

- a very pure quartz sample ground in a wet atmosphere (Qtz). The alveolar fraction of less than 3μ was obtained after grinding by centrifugation,
- the same quartz was heated for four hours to $1,300^{\circ}\text{C}$ in an inert atmosphere (Qtz 1300). The heat changed the crystalline structure, transforming this sample into a cristobalite with different granulometric properties. The obtained granulometry remained within the limits of the alveolar fraction but decreased the number of instilled dust particles (i.e., some of the final particles presented greater volume than the original quartz).

Silica samples provided by CHERCHAR (France):

- a vitreous silica (VS) was obtained by grinding melted quartz. The alveolar fraction of less than 3μ was obtained using a cyclone.

Histological method

The animals are sacrificed following standard operational laboratory procedures three months after exposure. The lungs are then removed and weighed. The left lung is preserved after insufflation. For the histological examination, the samples are dyed using Hematein-Eosin -Saffron.

Biochemical method

After removing a sample from the left lobe for the histological examination, the right lung is again weighed and, in some cases, stored at -20°C until biochemical quantification is carried out. The samples are dried at 137°C (overnight). Individual quantification of lipids is obtained by three successive ethyl alcohol extractions; they are then weighed after evaporation. The hydroxyproline content of each lung is determined according to the method developed by Stegemann [17] by oxidation with chloramine T at pH 6, followed by a reaction with paradimethylaminobenzaldehyde in the presence of perchloric acid. The absorbency of the obtained pyrrole derivative is measured at 560 nm.

The silica is quantified using the method developed by King [13].

Statistical analysis

Results were expressed as the mean \pm standard deviation (sd), used as an index of variation. Experimental results were subjected to variance analysis (i.e., ANOVA test). When there was a significant F value for the effect of dust, the individual means were compared for significance using the t test.

RESULTS

The weight of wet lungs and the results for the different biochemical parameters (e.g., hydroxyproline, lipid and silica content) are presented in Figures 1, 2, 3 and 4.

The histological examinations gave the following results:

Diatomaceous earth: the alveolar lipoproteinosis is barely visible, in some cases nearly invisible. Granulomatous-like areas are scattered around the pulmonary parenchyma, located mainly around the small airways and alveolar walls. These cannot be considered true silicotic nodules, but rather coniotic collections, as the contour of such collections was angular, the cellularity intense and the collagenization weak.

Cristobalite (1210), cristobalite (1212) and α -quartz (Qtz): for these samples, the alveolar lipoproteinosis is intense, and is associated with foam cells. The silicotic nodules are characteristic, numerous and often located near the terminal bronchioles.

The histological analysis does however indicate structural differences: the nodules caused by the two cristobalites show weakly cellular cores, revealed by their yellow color under the saffron dye, and a peripheral crown that is highly cellular. What is more, for CR 1210, the centers are proportionally greater in volume than those for CR 1212. The nodules caused by the α -quartz did not however show this delimitation, the collagen bundles (dyed yellow) were mixed together with the cellular elements.

α -quartz heated to $1,300^{\circ}\text{C}$ (Qtz 1300): the alveolar lipoproteinosis is intense and is associated with foam cells. No silicotic nodules are observed and the few cellular collections that are observed are foreign-body granulomas surrounding the particles of greatest volume.

Vitreous silica (VS): the alveolar lipoproteinosis is discreet or nearly invisible. No silicotic nodules are observed, but there are some coniotic collections with angular contours formed essentially of cells with a small number of collagen bundles.

Biological grading: if the quantification used is that was previously proposed [3] with Intensity x Profusion, obtaining:

- Diat:	4
- CR 1210 — CR 1212 — Qtz:.....	9
- Qtz 1300.....	1
- VS:	4

For the different samples that were tested, the injuries described as successive to overloading [14] (e.g., thickening of the alveolar walls, cubic metaplasia...) were rarely observed.

DISCUSSION

In-vivo experimental studies of the comparative nocivity of silica particles pose the problem of which biological parameters should be taken into account. What is more, certain parameters such as the histological method cannot be easily quantified.

The most readily accessible measurement is certainly that of wet lung weight. This measurement may however encompass some rather diverse modification, since the increase linked to the formation of silicotic nodules and that linked to alveolar lipoproteinosis are indistinguishable. The silicotic nodules include numerous collagen bundles which can be quantified using the hydroxyproline method. These nodules are also formed of variable cell quantities. The quantity of lipids increases if there is a large number of cells since the cellular membranes are rich in lipids. Alveolar lipoproteinosis is also rich in lipid structures; it is thus impossible using biochemical quantification to distinguish between the lipid increase linked to cell quantity from that linked to lipoproteinosis. In this case, a histological examination must be carried out.

We can also take the example of samples CR 1212 and Qtz 1300; if we examine the wet lung weight, CR 1212 is the most reactive and Qtz 1300 is weakly reactive. If we examine the hydroxyproline quantification, the CR 1212 is found to be significantly less reactive than CR 1210 or Qtz. As for Qtz 1300, it presents nearly the same level as the control group. If we turn to the results for lipid quantification, CR 1212 once again appears to be the most reactive and Qtz 1300 is found to be more reactive than diatomaceous earth. The histological examination shows notable cellularity on the silicotic nodules for CR 1212, whereas Qtz 1300 shows significant alveolar lipoproteinosis.

If only the histological examination is used, the quantification of injuries becomes quite difficult. It must be noted that the proposed scores [3] do not take into account the influence of lipoproteinosis (hence the very low score for Qtz 1300). Nor do they quantify the percentage of cellular elements relative to the collagen bundles in the silicotic nodules. This will lead to discrepancies between biochemical and histological results for certain samples [18: previous study conducted in this laboratory]. Only an image analysis quantification technique based on selected cross-sections of lung tissue will be likely to offer reliable histological grading.

It thus seems that a combination of different biochemical and histological parameters is needed to provide an accurate evaluation of in-vivo nocivity of silica dust.

Using a combination of these biochemical and histological elements would thus allow us to determine what is actually modified, either by evaluating changes in the crystalline structure or by changes in the surface properties of the particles under study.

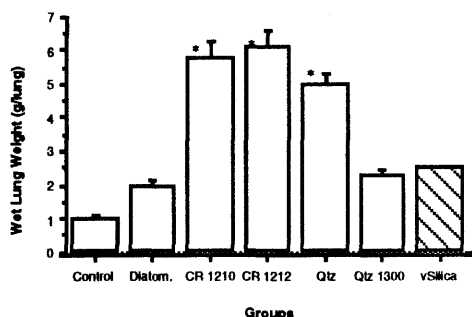
The two of the silica types considered as non-crystalline (i.e., diatomaceous earth and vitreous silica), therefore present relatively weak nocivity (perhaps linked to overloading). Such silica types provoke virtually no reaction that can be considered characteristic of silicosis (e.g., silicotic nodules or alveolar lipoproteinosis).

Both crystalline silica types (CR 1210 and CR 1212) obtained by heating diatomaceous earth, as well as the wet ground α -quartz (Qtz), gave rise to silicotic nodules and to alveolar lipoproteinosis. CR 1210 and CR 1210 did however present nodules with histological structures different from those found with Qtz. What is more, a larger quantity of collagen is found to form with CR 1210 than with CR 1212.

Lastly, Qtz 1300 (which presents the crystalline structure of a cristobalite) gives rise to alveolar lipoproteinosis of equal intensity to that found for CR 1210 and CR 1212 and Qtz samples, but no silicotic nodules are observed.

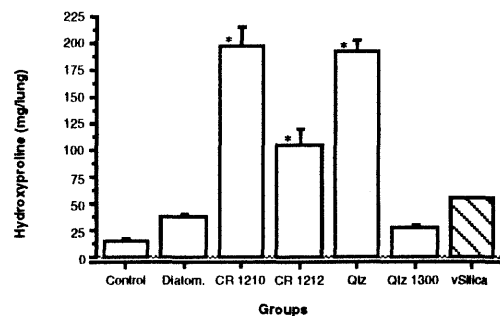
Quantifying the silica in the lungs after sacrifice may provide a representation of lung clearance. No clear correlation with the crystalline structure or with induced pathologies is observed since Qtz 1300 and CR 1210 seem to present better clearance than CR 1212 and Qtz.

The results for such biological parameters will require a more in-depth comparison with the physico-chemical properties of the samples used in this study (e.g., surface state...). The reactivity of the Qtz 1300 [5, 11] confirms the relatively limited influence of the crystalline structure on the formation of silicotic nodules (the characteristic of chronic silicosis).



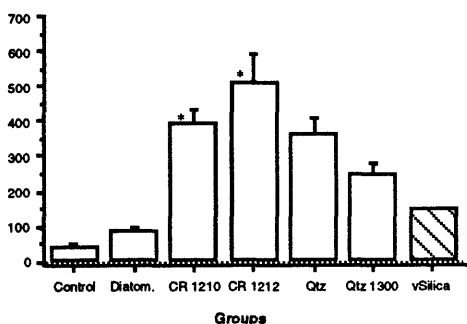
* significantly different compared to control group and to Diatom and Qtz 1300 groups.

Fig. 1 : Lung weight (g)



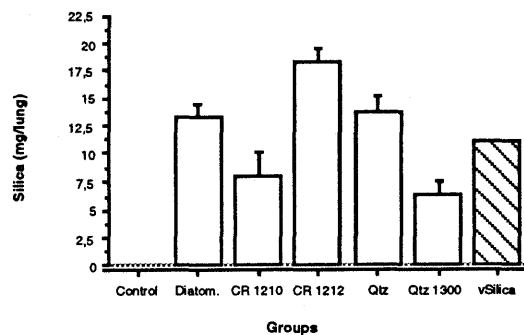
* significantly different compared to control group
CR1212 : significantly different compared to quartz 1300 and CR 1210

Fig. 2 : Hydroxyproline (mg/lung)



* significantly different compared to control group
CR1212 : significantly different compared to Diatom.

Fig. 3 : Lipids (g/lung)



CR1212 : significantly different compared to quartz 1300 and CR 1210

Fig. 4 : Silica (mg/lung)

ACKNOWLEDGMENTS

J.P. Lefèvre and A. Gaudillot for the in-vivo study, M. Fray, A. Lebrun, A.M. Oisel for the histological and biological data, B. Graham for the translation.

Financial support was gratefully received from CECA, Convention No 7280/03/006.

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