ALTERATION OF RESPIRABLE QUARTZ PARTICLE CYTOTOXICITY BY THERMAL TREATMENT IN AQUEOUS MEDIA

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ABSTRACT

Respirable quartz cytotoxicity, as measured by erythrocyte hemolysis and pulmonary macrophage release of lactate dehydrogenase *in vitro*, is neutralized by boiling in water in glass test tubes for 10 to 40 minutes. The cytotoxicity is reduced to near zero by boiling 1 to 10 mg quartz per milliliter water. For greater concentrations of quartz in water the hemolytic potential after 40 minutes of boiling approaches that of native quartz. Replacing the medium with fresh water midway through boiling results in full detoxification through 20 mg quartz per milliliter water. Pre-boiling the medium with silica reduces the detoxification effect. Detoxification persists after mild drying at 110°C for 8 hours, and persists after three days of resuspension in water at room temperature.

INTRODUCTION

Reasearch underway to determine interactions of quartz and other mineral dust surfaces with pulmonary fluids and alveolar macrophages in culture led to the observation that when dusts were autoclaved in aqueous suspension, their cytotoxic effects on macrophages were suppressed, in some cases fully and even after several days incubation with the cells. This finding was in direct contradiction to earlier results from both short term macrophage lysosmal enzyme release assays, as well as longer term cytotoxicity assays from macrophages in culture; in those studies, dusts were steam autoclaved at 121°C with no liquid water but with steam present.[1,2] However detoxification under boiling conditions has been reported in other research.[3] It was decided to use the hemolysis assay to further investigate these findings, because of its sensitivity, simplicity and cost.

RESULTS AND DISCUSSION

Respirable quartz dust used in this study was taken from a stock of crystalline silica, Min-U-Sil, obtained from Pennsylvania Sand Glass Corporation, fractionated in air with a particle classifier. The small size fraction retained for use was 80% less than 5 micrometer particle diameter, with an area equivalent median diameter of 1.24 micrometers as estimated by automated image analysis. The silica was at least 98.5 mass percent silica as determined by X-ray energy spectrometric analysis; and the crystalline form was alpha-quartz as determined by X-ray diffraction. It's specific surface area was 3.97 square meters per gram as determined by nitrogen adsorption isotherm methods.[1]

To measure the erythrocyte hemolytic potential of treated and untreated dusts we use the method of Harington et al,[4] with minor modification.[1] Briefly, dusts suspended in buffer are mixed with an equal volume of 4% sheep red blood cells, and incubated 60 min. at 37°C with periodic mixing. Next the cells are spun down, and the absorbance of the released hemoglobin from any lysed cells read at 540 nm. Absorbance values are compared to positive controls (100% lysed cells) and negative controls (cells in buffer only).

Initial experiments involved bringing deionized water to a boil, adding the dry dust (12 mg), vortexing, and boiling for periods up to 60 min., without stirring. This was done in flint glass tubes for samples with dust concentrations of greater than 1 mg/ml, and in polycarbonate tubes for lower concentrations. After the boiling period was completed, sample tubes were spun down for 60 sec., the supernatant discarded, and the dust resuspended in phosphate buffered saline (PBS) and run in the hemolysis assay. Results indicated that the toxicity was reduced almost to zero at 1 mg/ml, and increased in a roughly linear fashion to approximately full (native dust) toxicity at 20 mg/ml dust concentration during boiling. (Figure 1).

When individual magnetic stirrers were used in each sample, results were similar, except the toxicity was reduced to virtually zero at concentrations to 10 mg/ml, and then increased in a linear fashion. Samples were also boiled for half the specified times, centrifuged, the medium changed to fresh water, and boiling continued for the rest of the period. The toxicity was reduced to very low levels through the highest

concentration tested. (Figure 2) As also shown, pre-boiling the water with a separate quartz sample before using the still hot supernatant to boil the test sample, somewhat diminished the detoxification phenonenon. We observed this diminution also in the case of pre-boiling the water with silica gel.

Experiments involving various boiling times showed a weak dependence of detoxification with time, except at 1 mg/ml, where detoxification progressed with boiling time. (Figure 3)

Limited tests of the persistance of the detoxification have been made and are continuing. One question was whether or not the passivation effect was due to some gel or other coating which might not withstand drying and resuspension. Samples were vacuum dried after boiling, and assayed the following day. Fully detoxified samples remained the same, and partially detoxified samples had slightly less toxicity after drying than replicate samples promptly assayed. (Figure 4) Fully detoxified samples boiled at 1 and 10 mg/ml which were decanted and placed in fresh distilled water or PBS did not retoxify over a 3 day period. (Figure 5) Other samples were left standing after boiling in the supernatant from the boiling water at room temperature. Fully detoxified samples boiled at 1 mg/ml remained at zero toxicity after 4 days. Samples at higher concentrations showed some increase with time; the sample boiled at 10 mg/ml was essentially fully retoxified. (Figure 6)

Certain samples in the assay yielded consistently anomolous results, and were difficult to reconcile with any simple physical model; specifically, samples boiled at 0.5 mg/ml were not detoxified. The only experimental difference in these samples was that they were boiled in plastic (polycarbonate) centrifuge tubes, since glass tubes were not available in an appropriate size. When quartz was boiled in flint glass, polycarbonate, and Tefzel tubes, only partial detoxification was seen. When boiled in polycarbonate tubes using water that had been boiled only in polycarbonate, no detoxification was seen at any concentration. (Figure 7)

Since the effect seemed clearly to be an effect of the glass containers, additional experiments were done to clarify the finding. Quartz dusts were boiled in water in polycarbonate tubes with varying amounts of 3 mm soda-lime glass beads. (Figure 8) There is a roughly proportional dependence of detoxification on the number of glass beads, and thus the glass surface area present. The effect was investigated also by using shards of glass cover slips in polycarbonate tubes during boiling. In general detoxification occurs with increasing glass content, but with a lessening of the effect seen at the highest glass content level. (Figure 9) The converse of this hypothesis, that polycarbonate somehow supressed the detoxification of quartz, was tested by boiling quartz in flint glass tubes with polycarbonate pieces in suspension; no significant effect of the polycarbonate was seen. (Figure 10)

An additional anomolous result occured when there was a failure in the reverse osmosis water purification cartridge in our laboratory building distilled water system, resulting in a higher impurity level than that present in tap water. Toxicity was partially supressed in all samples, even those boiled in polycarbonate; but detoxification was not complete for any treatment, even using flint glass tubes. (Figure 11) When the water system was restored to proper operation, the results

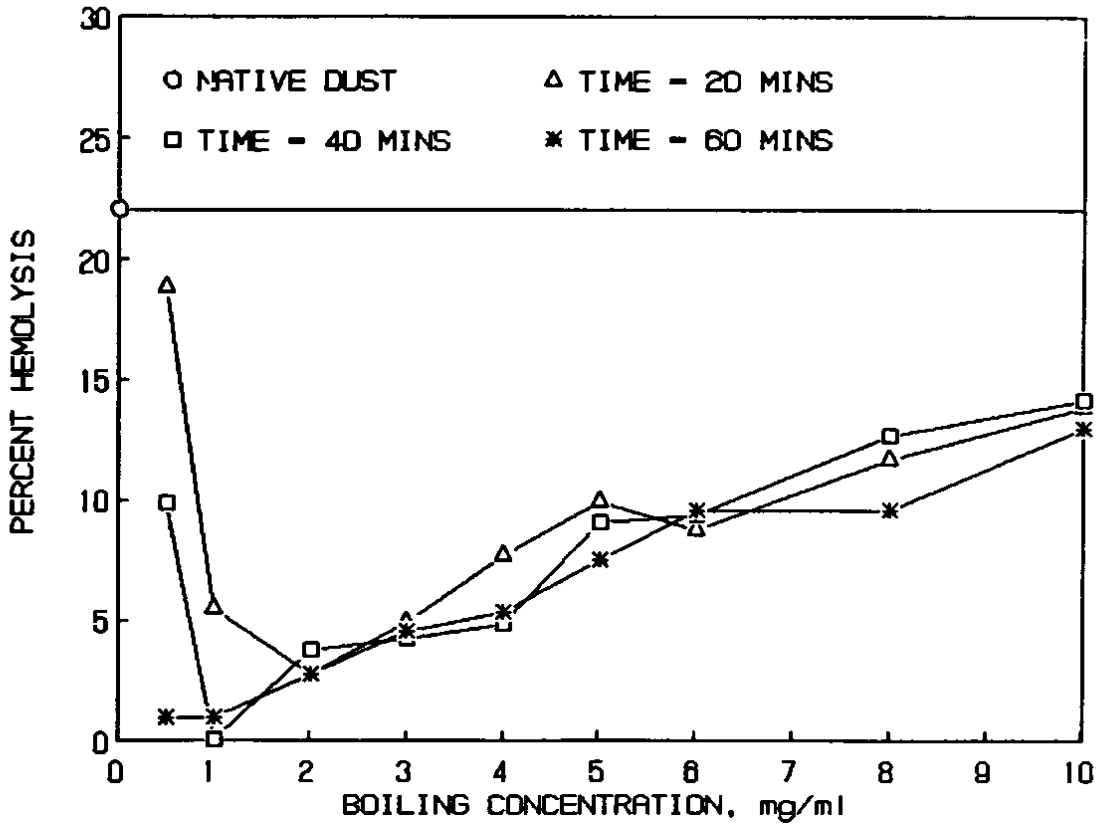

Figure 1. Silica cytotoxicity with boiling.

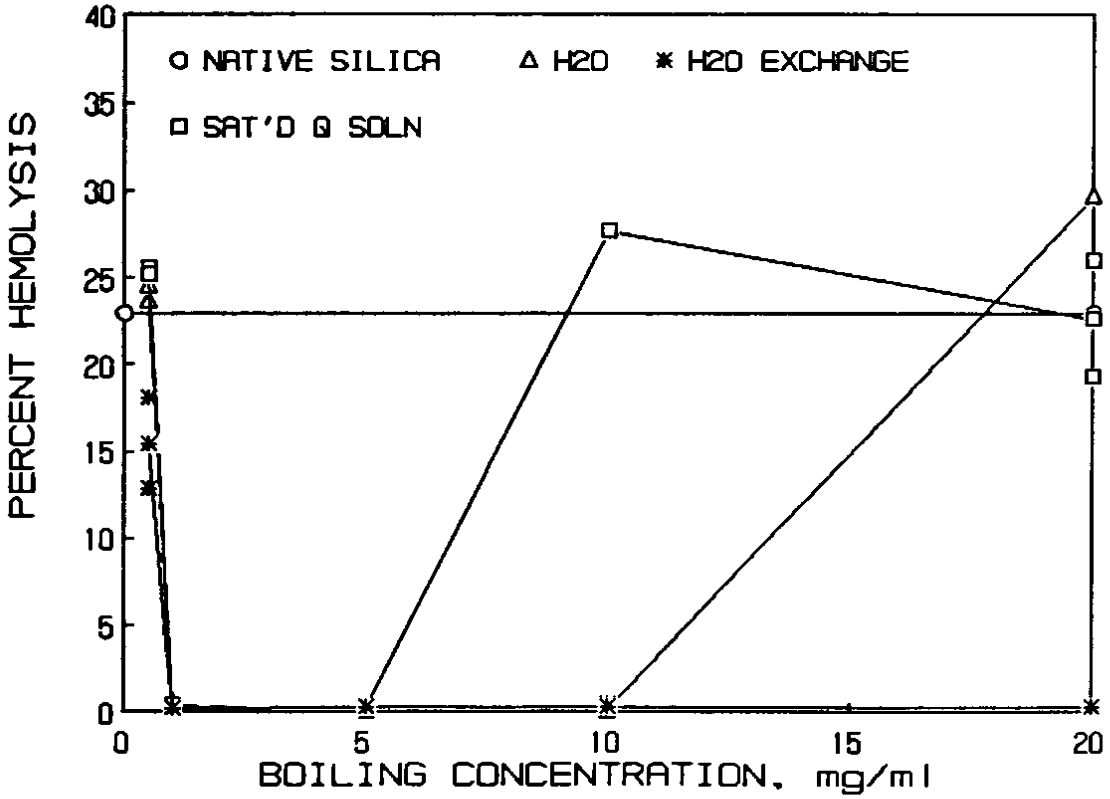

Figure 2. Quartz boiled 40° in various solutions.

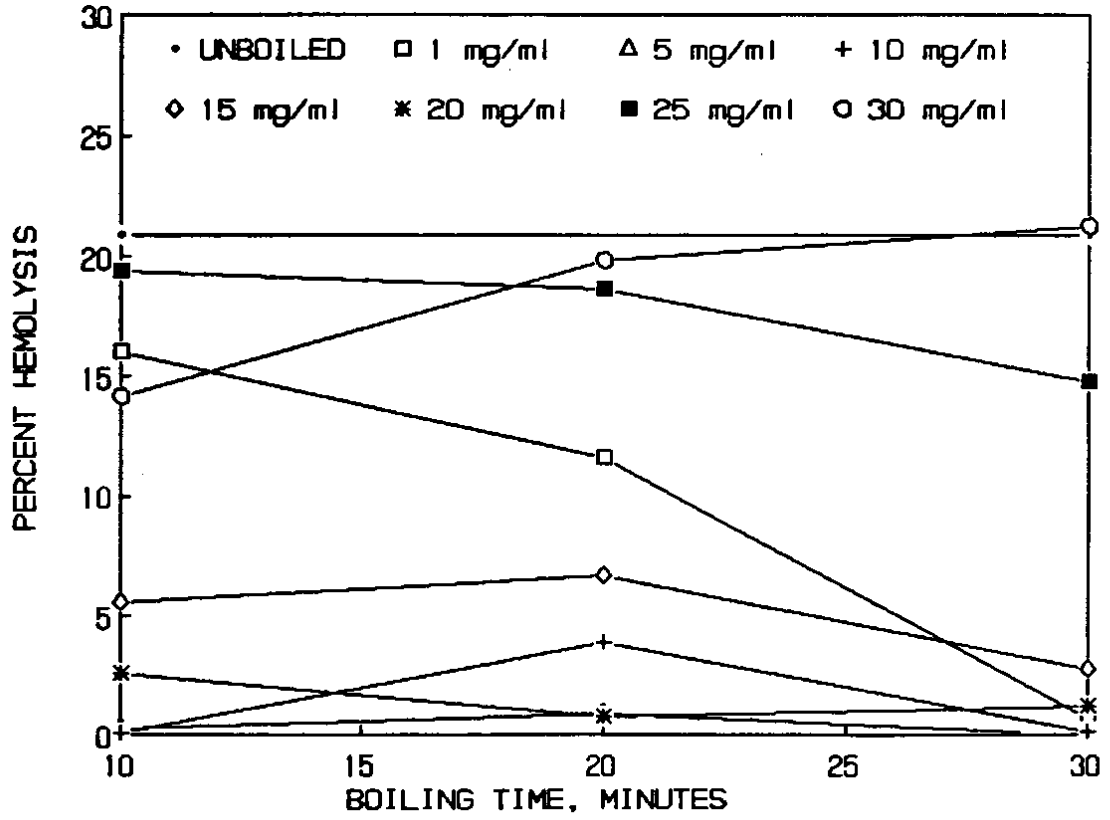

Figure 3. Hemolysis vs. boiling time.

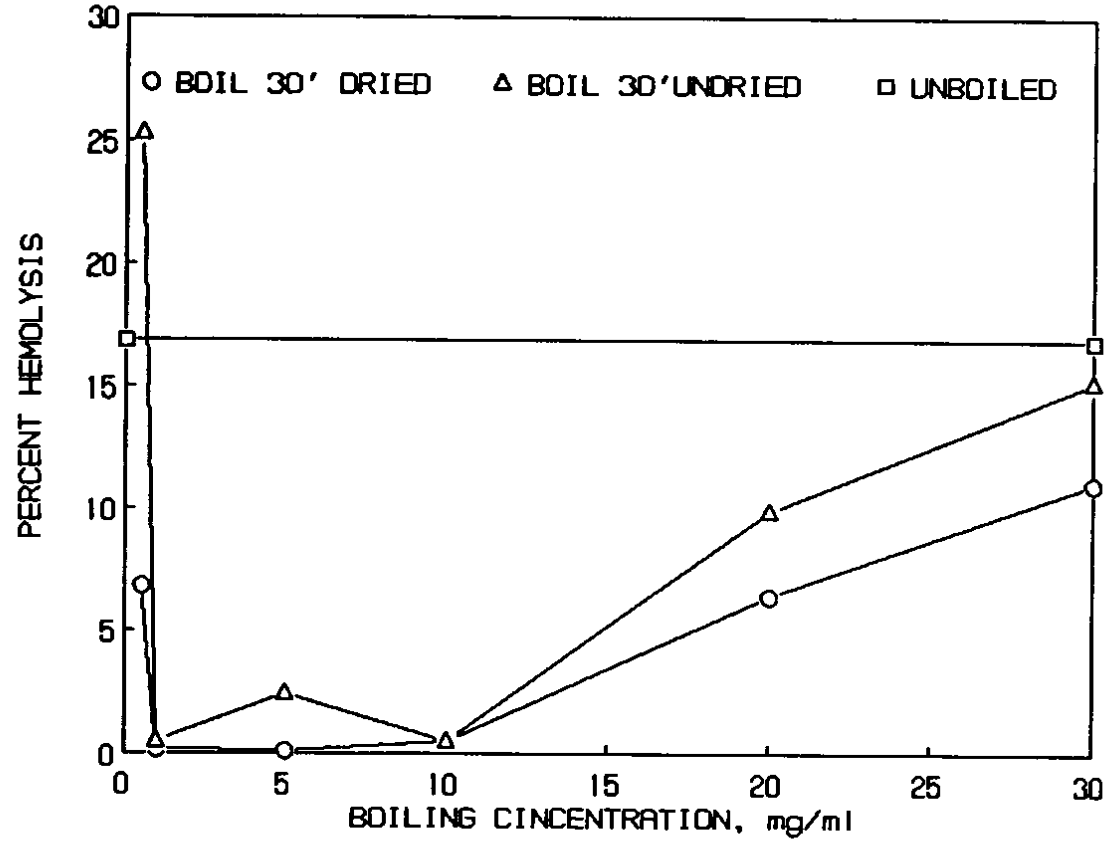

Figure 4. Hemolysis vs. post-boiling drying.

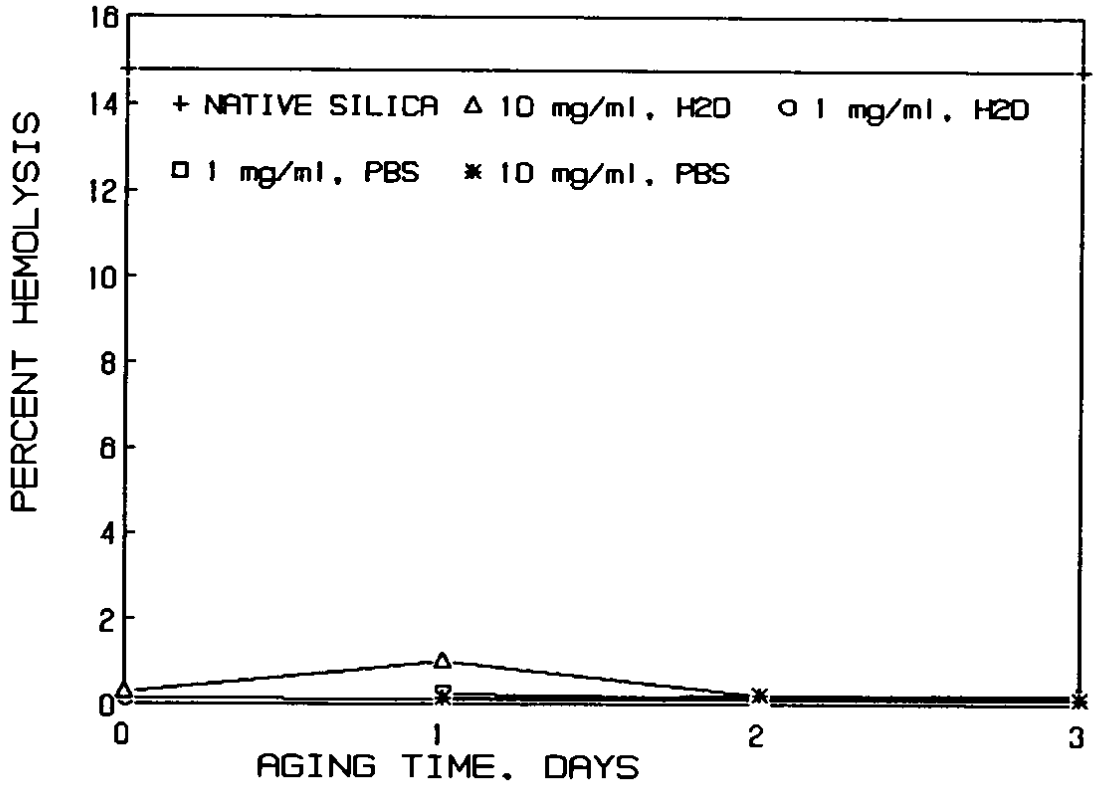

Figure 5. Quartz aged in H_2O and PBS.

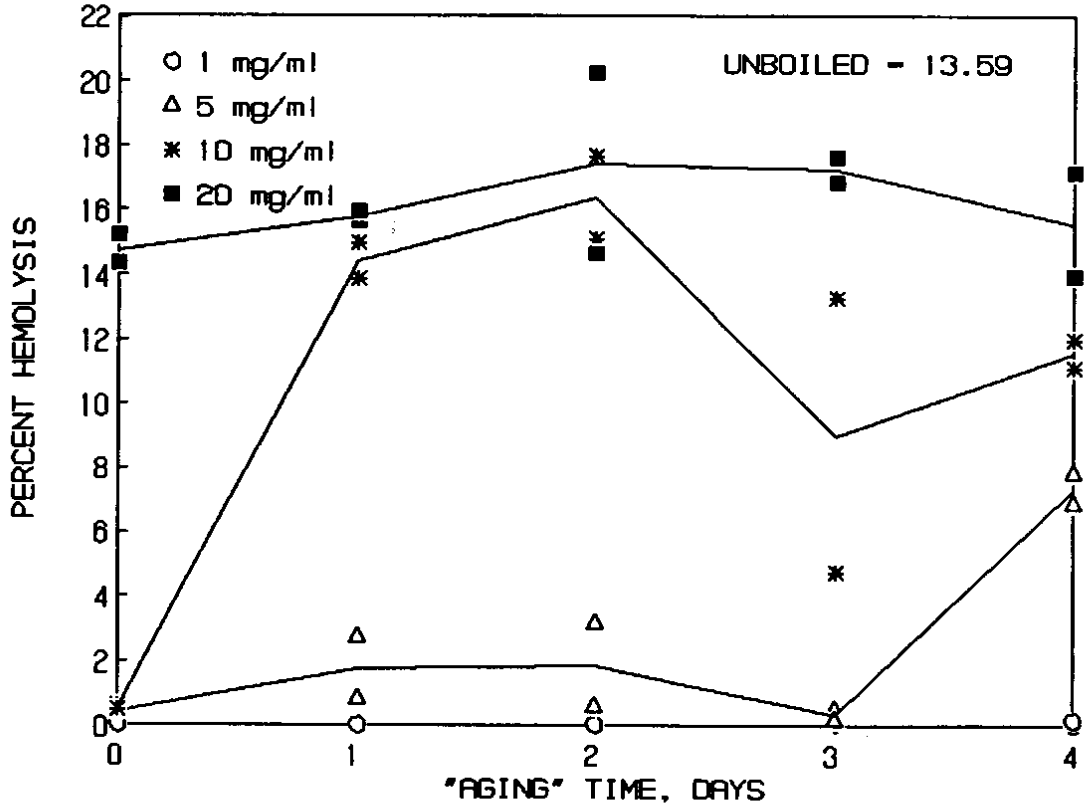

Figure 6. Hemolysis vs. time in supernatant after boiling.

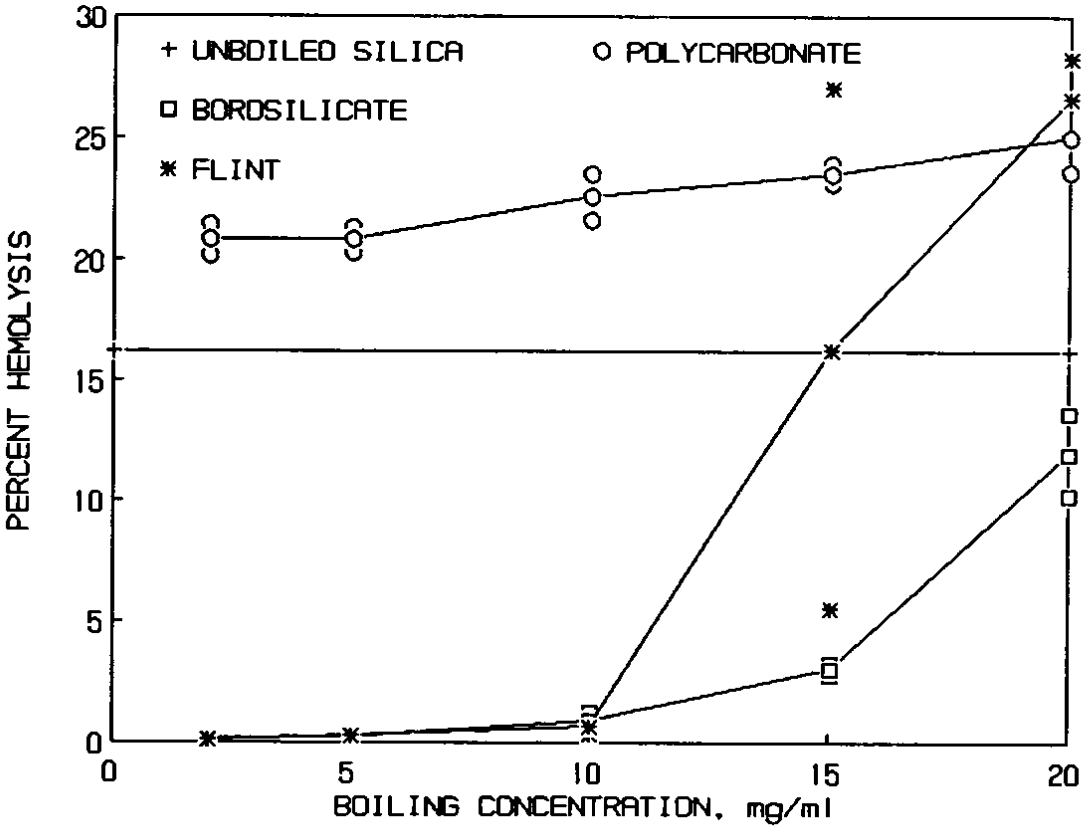

Figure 7. Hemolysis: quartz boiled in various tubes.

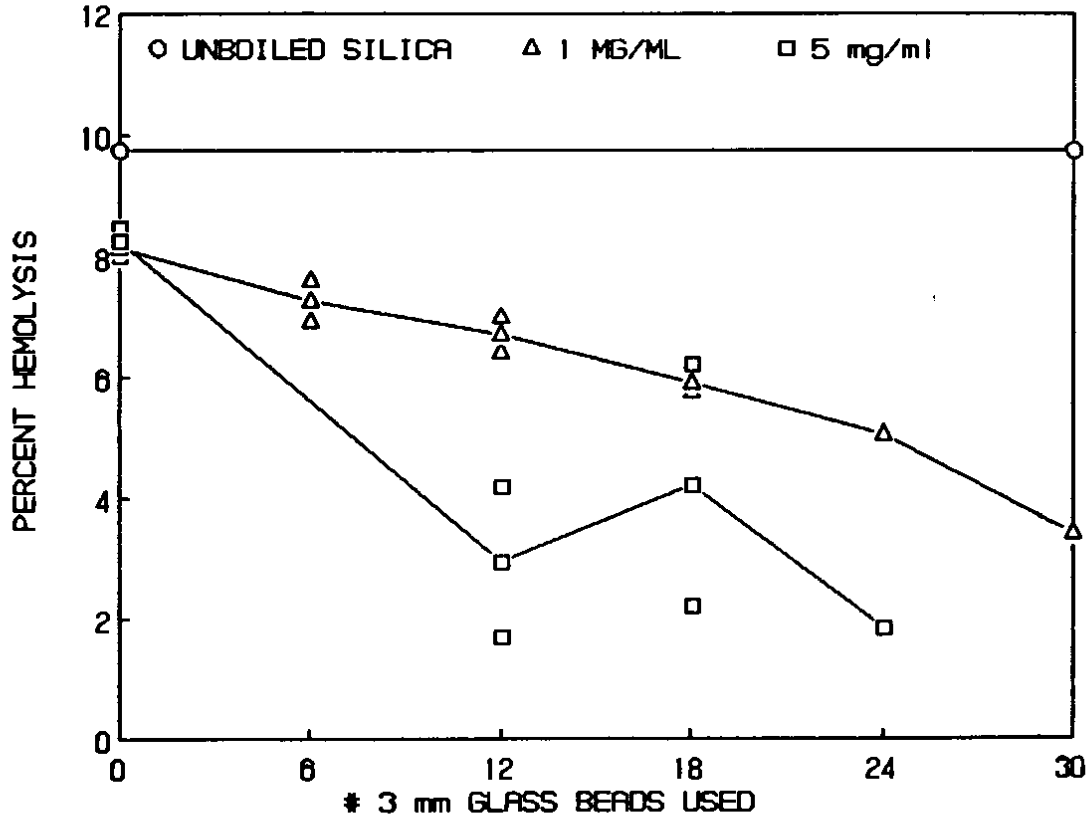

Figure 8. Quartz boiled 30° in polycarb W/glass beads.

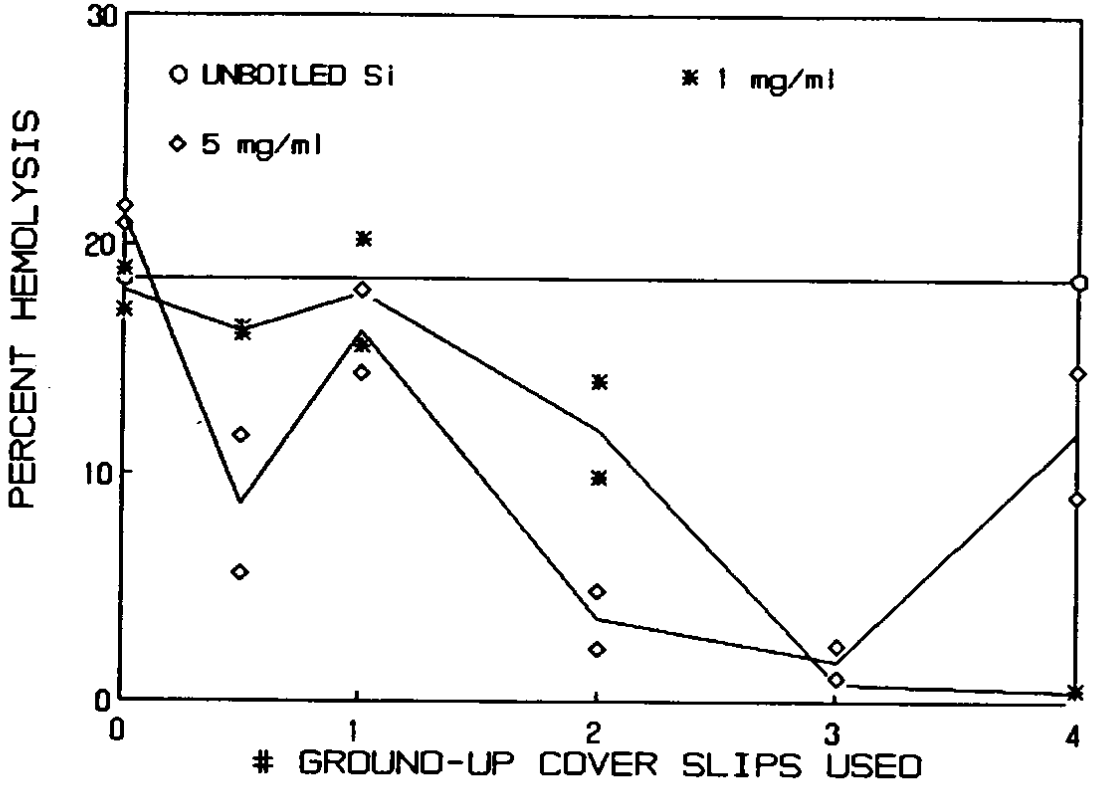

Figure 9. Quartz boiled 30° in polycarb W/glass pieces.

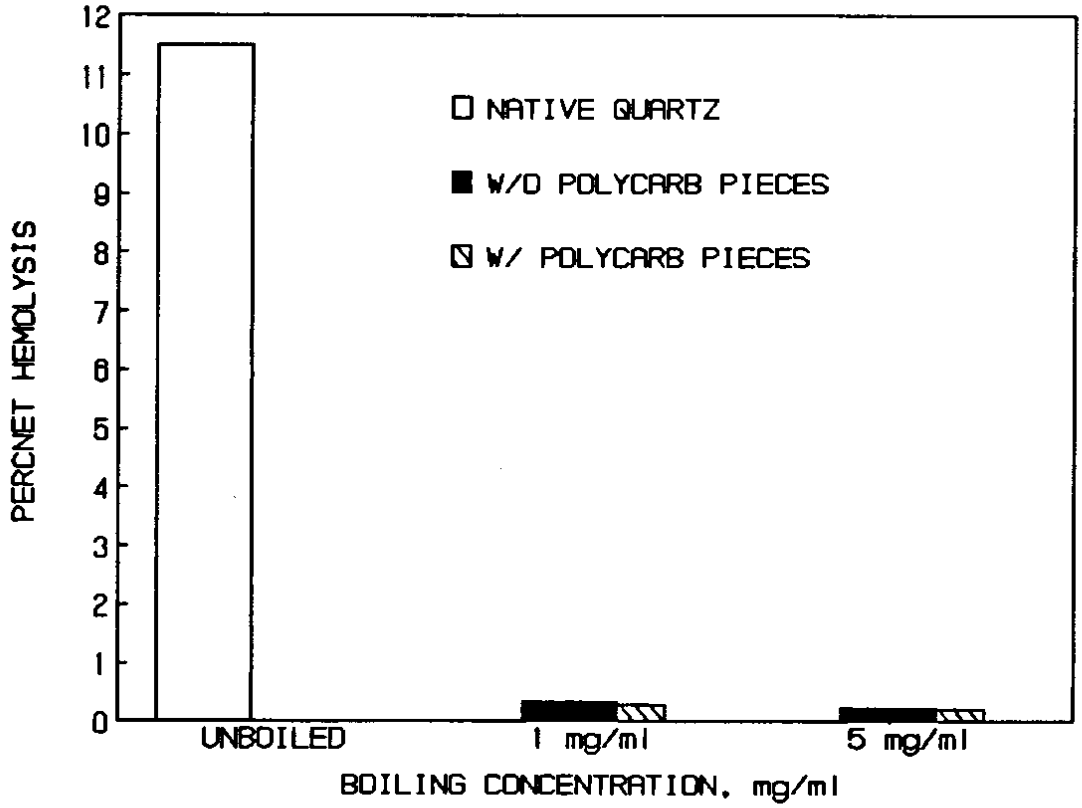

Figure 10. Hemolysis of quartz boiled in flint glass with and without polycarbonate pieces.

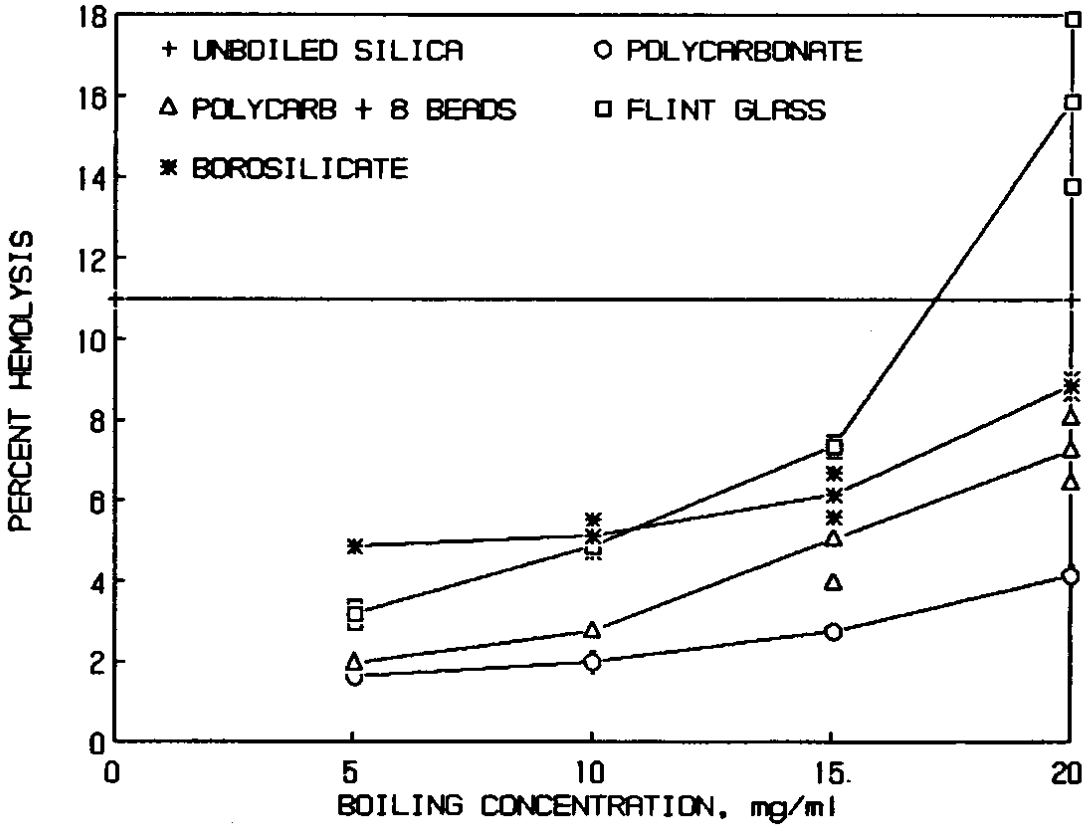

Figure 11. Hemolysis: quartz boiled in various tubes in contaminated water.

agreed with previous findings.In a limited investigation of this, quartz samples were boiled with sodium and calcium chloride solutions of several different concentrations; the effects were weak, slightly lessening the detoxification. (Figure 12)

Initial zeta potential measurements have been made on samples of unboiled quartz and on quartz boiled at 5 mg/ml in flint glass and in polycarbonate tubes. The zeta potentials for unboiled quartz and for quartz boiled in polycarbonate are essentially identical, while the samples boiled in flint glass show a less negative zeta potential. (Figure 13)

The boiling treatment was also applied to kaolin and alumina dusts. The kaolin dust, previously described,[1] was unaffected. A commercially obtained respirable sized alumina expressed hemolytic potential in its untreated state, and was detoxified upon boiling. (Figure 14) The untreated and treated alumina samples were subsequently analyzed by photoelectron spectroscopy, courtesy of the U.S. Department of Energy, Morgantown Energy Technology Center. The intention was to determine if the elemental composition of the alumina surface showed substantial levels of silicon in addition to aluminum after treatment. Results of the test showed, however, that the surface of the untreated alumina itself had a silicon-to-aluminum elemental ratio of about 4-to-1. This was reduced to about 1-to-1 after boiling. Studies using other dusts including asbestiform materials are ongoing.

CONCLUSIONS

At this point, several conclusions may be stated, and a partial working hypothesis formulated, namely:

- Quartz boiled in flint glass for times greater than ten minutes at concentrations between 1 and 10 mg/ml is partially to fully detoxified in the hemolysis assay.
- The effect is strongly concentration dependent between 10 and 30 mg/ml
- If a change is made to fresh boiling water midway in the process, then full detoxification occurs across the entire concentration range.
- The effect is present only in samples boiled in glass tubes, flint or borosilicate having been tested thus far; plastic tubes do not show the effect.
- The effect is only moderately time-dependent, tests having been limited to boiling times of 10 minutes or more thus far; at most concentrations the effect seems nearly complete at 10–15 minutes.
- The effect seems to persist on mild drying (overnight vacuum drying).
- Fully detoxified samples appear to show little or no cytotoxicity after soaking at room temperature in the supernatant from boiling for periods up to 4 days; partially detoxified samples show an increase with time.

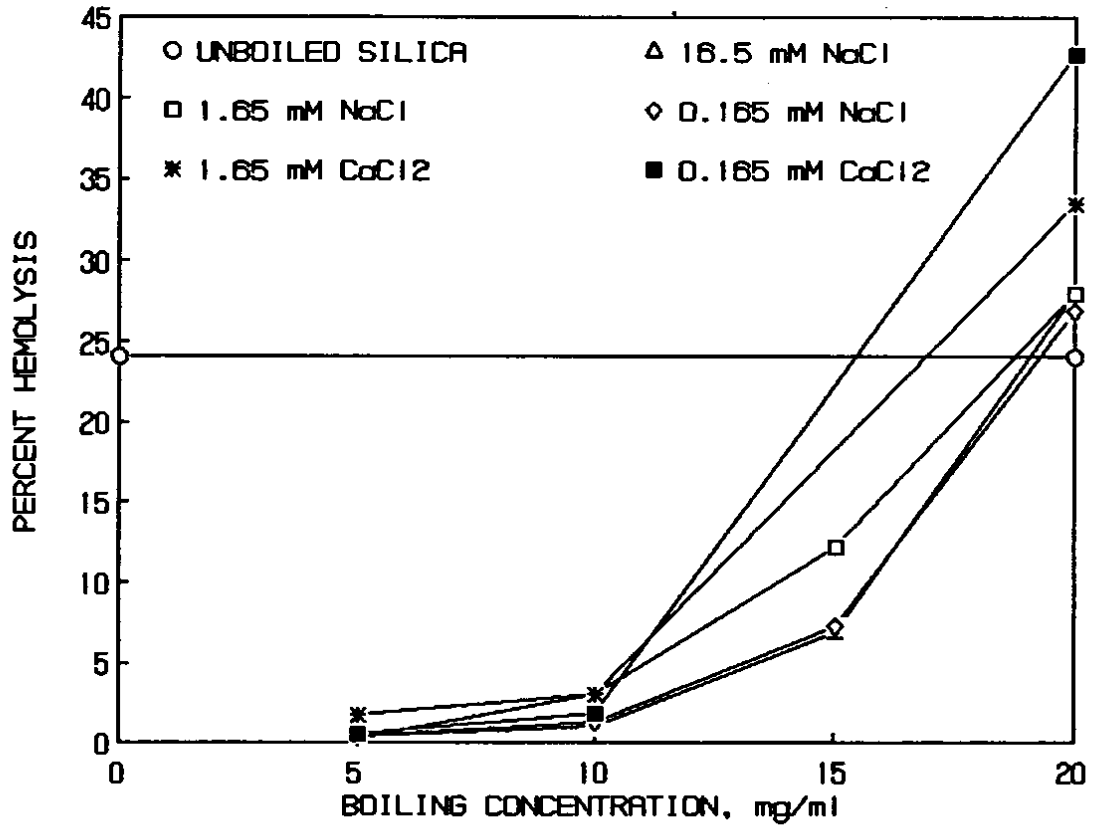

Figure 12. Hemolysis: silica boiled in NaCl and $CaCl_2$.

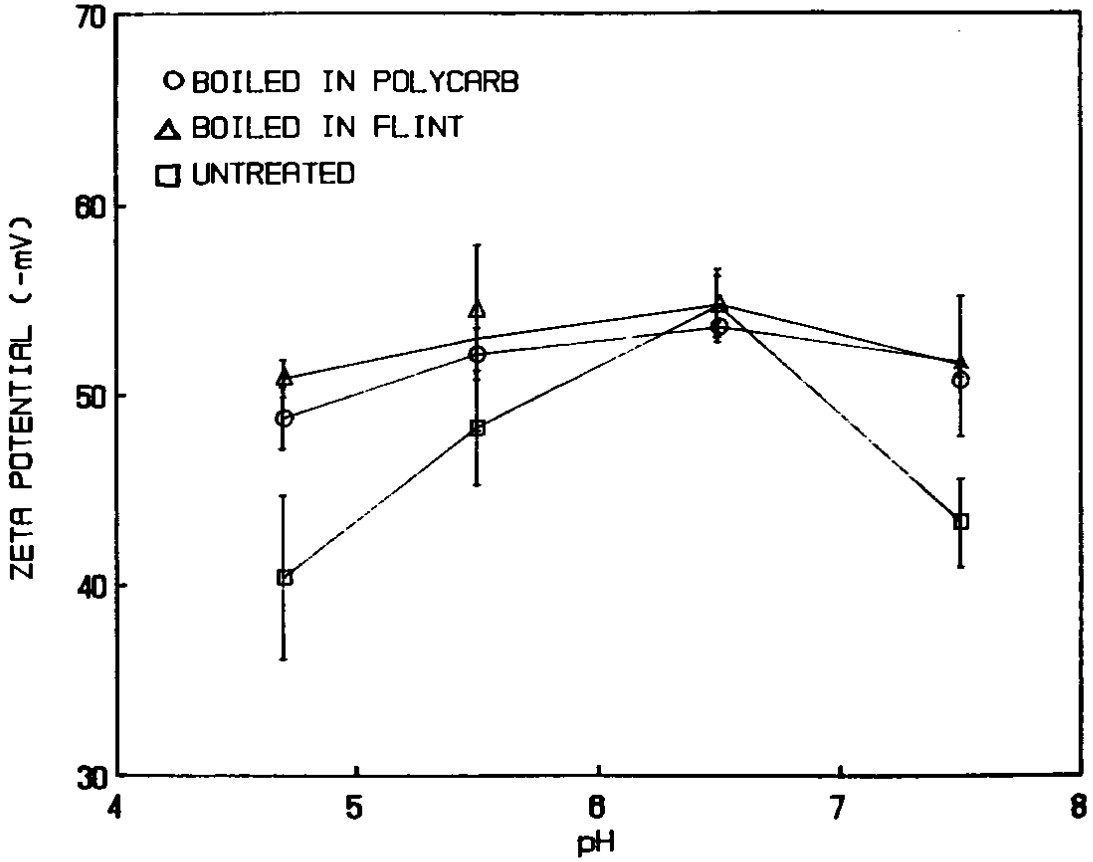

Figure 13. Zeta potential of quartz boiled in flint or polycarbonate.

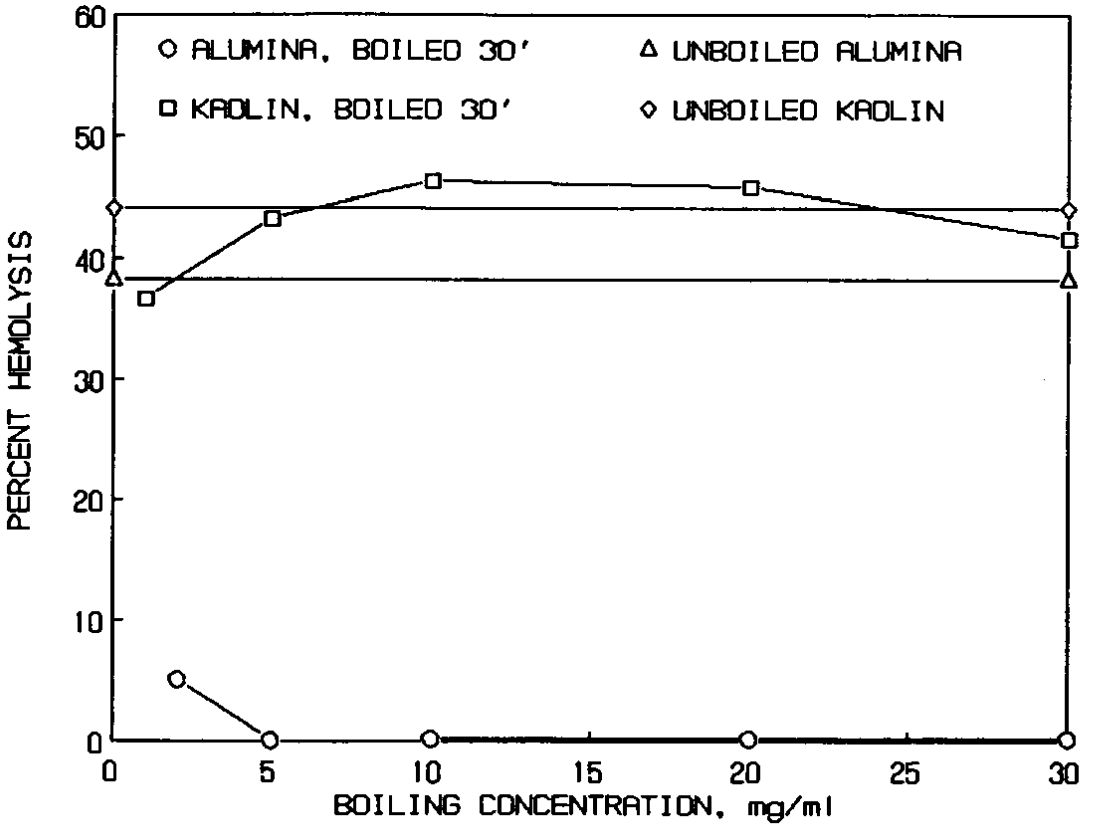

Figure 14. Hemolysis of boiled kaolin and alumina dusts.

- The detoxification shows some proportionality to available surface area of glass present during boiling.
- There is some indication that pre-boiling water with silica partly diminishes the detoxification effect for subsequently boiled quartz.

Further investigation is needed to more fully clarify the mechanism of quartz detoxification, but a partial hypothesis can be stated:

Boiling water releases a soluble or partially soluble factor, possibly silicic acid or sodium and/or calcium silicates or hydroxides, which, in monomeric or polymeric form, react or are physically adsorbed on the quartz surface, which fully or partially detoxify the mineral surfaces, as shown in *in vitro* cellular toxicity assays.

There is a significant amount of discussion in the literature concerning the dissolution of silica in water. Holt and King found that all sizes of quartz particles behave as if a soluble fraction of silica is leached from their surface, and that surface leached at pH 9 will rapidly adsorb the dissolved silica species.[5] Baumann measured the uptake of silicic acid by quartz from aqueous solution prepared by mixing silica gel in water.[6] In general, various silicates, including vitreous glass and quartz, are reported to have slight solubilities in aqueous media. The values found for quartz are on the order of one magnitude lower than the values obtained for glass under the same test conditions.[7] Iler states that the ability of quartz surface to hold water of hydration even after outgassing at 100C, in contrast to the behavior of amorphous silica, suggests a powerful hydrogen bonding capacity of the quartz surface silanol groups. He suggests this may be related to the peculiar power of quartz to adsorb multilayers of silicic acid as noted by Baumann.[8] This seems to favor a hypothesis that some soluble form of silica dissolves from both quartz particles and the glass container; that the "silicic acid" or a polymerized derivative re-adsorbs to the quartz; and this masks or otherwise passivates the quartz surface. Tests using pre-saturated medium raise the possibility that the quartz surface must undergo a desorption step or some conditioning before or in conjunction with adsorption of passivating species.

Suggested strategies for clarifying this would include radiolabel experiments to distinguish the source of surface silica groups after boiling treatment, and to determine if native quartz surface groups are exchanged with the medium in the passivation process; further investigation of the effect of treatment on the zeta potential of quartz; and the attempted use of surface spectroscopy methods, such as diffuse reflectance Fourier transform infrared spectrophotometry to identify surface structural changes following treatment. If acid-base reactions are involved, the pH dependence of detoxification should be looked at in detail.

The prime question raised here is under what moderate treatment conditions will quartz be surface modified so that it becomes biologically inert for cytotoxicity in cellular assays or for fibrogenicity *in vivo*. That is, what physical and chemical conditions are necessary and sufficient to passivate the quartz surface? This study has identified some parameters involved: the process proceeds in aqueous solution; glass surface must be present; there is a concentration dependence; details of the boiling procedure can significantly affect the results.

Another question is whether the passivation effect persists. The effect should be monitored in long term experiments involving physical and/or chemical methods, as well as *in vitro* assays, and possibly *in vivo* bioassays to determine the long term persistance in air and in physiological fluids.

The last question is whether this phenomenon is a feasible basis for prevention strategies. One major unknown here is the long term persistance of the effect under *in vivo* conditions.

In any event, the possibility exists for de-toxification of quartz by relatively mild treatment conditions. Seemingly innocuous preparation procedures used in biological assays of quartz could produce respirable dust surface property changes which are not readily detected by chemical or physical analysis, but which can confound interpretation of bioassay results. The possibility for such should be recognized in research protocols.

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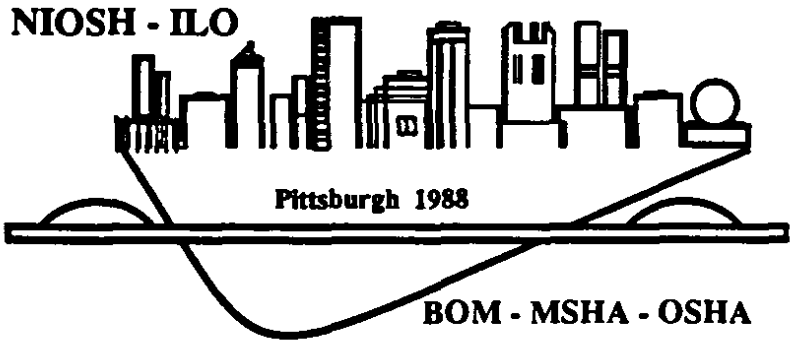

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