ENVIRONMENTAL RESEARCH 30, 349–360 (1983)

Mount St. Helens' Volcanic Ash: Hemolytic Activity

VAL VALLYATHAN,[1] M. SHARON MENTNECH, LLOYD E. STETTLER, DONALD D. DOLLBERG, AND FRANCIS H. Y. GREEN

Pathology, Immunology, Chemistry, and Analytical Sections, National Institute of Occupational Safety and Health, Morgantown, West Virginia 26505 and Cincinnati, Ohio

Received November 9, 1981

Volcanic ash samples from four Mount St. Helens' volcanic eruptions were subjected to mineralogical, analytical, and hemolytic studies in order to evaluate their potential for cytotoxicity and fibrogenicity. Plagioclase minerals constituted the major component of the ash with free crystalline silica concentrations ranging from 1.5 to 7.2%. The *in vitro* hemolytic activity of the volcanic ash was compared to similar concentrations of cytotoxic and inert minerals. The ash was markedly hemolytic, exhibiting an activity similar to chrysotile asbestos, a known fibrogenic agent. The hemolysis of the different ash samples varied with particle size but not with crystalline silica concentration. The results of these studies taken in conjunction with the results of our animal studies indicate a fibrogenic potential of volcanic ash in heavily exposed humans.

INTRODUCTION

The recent volcanic eruptions of Mount St. Helens in Washington state have released large quantities of respirable ash particles into the atmosphere with resultant exposure to over a million people (Fruchter *et al.*, 1980). In the most severely affected areas the Environmental Protection Agency estimated that the level of total suspended particulates was as high as 30–35 mg/m^3 of air (Morbidity and Mortality Weekly Report, 1980). A continuing problem is the reaerosolization of settled dust by dry windy weather conditions and by the activities of man. Certain occupational groups such as loggers, farmers, highway maintenance crews, and others may be exposed to greater concentrations of ash than the general population due to reaerosolization of ash.

The long-term health implications of exposure to volcanic ash are difficult to assess as there are few precedents for this type of disaster. Previous reports deal only with acute irritation of the respiratory tract and eyes (Baxter *et al.*, 1981; Thorarinsson, 1979). There is a large body of knowledge on various types of mineral pneumoconiosis. Volcanic ash is reported to be composed predominantly of feldspar silicates with 5–10% free silica (Fruchter *et al.*, 1980; Green *et al.*, 1981; Dollberg *et al.*, 1981). Free silica is known to be highly cytotoxic and fibrogenic at low concentrations. However, less is known about the cytotoxic and fibrogenic properties of other silicates present in the volcanic ash although there is evidence that several similar silicates have a moderate potential to induce fibrosis

[1] To whom correspondence should be addressed: Pathology Section, ALOSH-NIOSH, 944 Chestnut Ridge Rd, Morgantown, W. Va. 26505.

0013-9351/83/020349-12$03.00/0

(Parkes, 1974; Sherwin *et al.*, 1979; Brambilla *et al.*, 1979). Any permanent pulmonary impairment resulting from the inhalation of volcanic ash is likely to take years or even decades to manifest itself. For these reasons it was considered important to analyze the volcanic ash mineralogically and to assess the toxicity with short term *in vitro* tests.

Hemolysis assay is considered a useful rapid test to determine the cytotoxicity of mineral dusts (Harington *et al.*, 1971; Stalder and Stöber, 1965; Schnitzer and Pundsack, 1970; Hefner and Gehring, 1975; Potts *et al.*, 1978). The degree of hemolysis correlates well with the known cytotoxic potential of several mineral dusts including free silica and chrysotile asbestos; however, there are some notable exceptions. One example is crocidolite asbestos; however these exceptions invariably give false negative results in the hemolytic assay. A correlation of hemolytic activity and the *in vivo* fibrogenicity of certain mineral dusts has also been reported (Harington *et al.*, 1971; Hefner and Gehring, 1975; Potts *et al.*, 1978). The specific purpose of this study was, therefore, to assess the hemolytic capacity of volcanic ash from different volcanic eruptions and to compare it to both biologically inert and toxic mineral dusts.

MATERIALS AND METHODS

Volcanic ash samples were initially collected as bulk, sedimented dry samples from geographic locations representing four different volcanic eruptions (Table 1). Ash samples from Moses Lake, representative of the first volcanic eruption, was fractionated in a particle sizer (Accucut Particle Classifier, Donaldson-Majal Division, St. Paul, Minn.) to $<5\ \mu m$. All the other samples were used in the hemolysis studies unfractionated, as there was insufficient material available for processing in the automatic particle sizer.

Crystalline silica in the <10-μm fraction of the bulk samples was determined using a modified X-ray powder diffraction method (Dollberg *et al.*, 1981). Each sample was chemically treated with hot phosphoric acid to remove the plagioclase minerals. Crystalline silica was then determined by both X-ray powder diffraction, using a Philips automated powder diffractometer, and the Talvite spectrophotometric method (Talvite, 1951).

The ash samples were also studied by scanning electron microscopy (SEM), X-ray energy spectrometry (XES), and automated image analysis to determine morphology, elemental composition, and particle size. For these studies the ash suspensions were made in deionized distilled water, deposited on Nuclepore filters, attached on carbon planchets, and examined in a scanning electron microscope at magnifications varying from 400 to 2000×. Particles selected at random at these magnifications were subjected to elemental analysis at point mode for 100 sec. Particle-size analyses for each sample, except chrysotile, were performed at 400× and either 1000× or 2000× using a Lemont B-10 image analysis system interfaced with the scanning electron microscope. All particles in a minimum of 10 randomly selected fields of view were analyzed. The particle diameters determined were the circular area equivalent diameters. Mass median and aerodynamic diameters were calculated for each of the nonfibrous samples using the circular

TABLE 1

VOLCANIC ASH: SOURCE, PARTICLE SIZE, TOTAL CRYSTALLINE SILICA, AND MAJOR POLYMORPHS

Volcanic ash source	Mean circular area equivalent diameter[a] (μm)	Mass median diameter[b] (μm)	Cristobalite (%)	Quartz (%)	Total crystalline silica (%)
Spokane	1.7	6.0	4.2	3.0	7.2
1st eruption	(0.11–10.7)				
Moses Lake	1.1	2.5	0.7	0.8	1.5
1st eruption	(0.18–6.1)				
Centralia	1.0	7.2	3.0	3.5	6.5
2nd eruption	(0.11–11.5)				
Gales Creek	0.9	6.0	3.3	1.4	4.7
3rd eruption	(0.11–8.9)				
Chewalah	0.3	16.7	3.2	0.5	3.7
4th eruption	(0.11–10.9)				

[a] Mean and range of circular area equivalent diameter measurements is based on 1000, 1040, 1005, 1013, and 1043 particles respectively in the five samples in a minimum of 10 fields of view at 1000 or 2000×.

[b] Mass median data are based on 1216, 1019, 1505, 1463, and 1041 particles respectively in five samples in a minimum of 10 fields of view at 400×.

area equivalent diameters determined in the 400× particle-size analyses, an assumed bulk density for each dust sample, and the further assumption that the individual dust particles are spherical. The calculated parameters, although they are rather crude approximations, are useful in determining the homogeneity of the analyzed samples. The chrysotile asbestos analyses were performed manually from photomicrographs taken in a transmission electron microscope (TEM) at a magnification of 10,000×. Particle sizing was also carried out by using an optical microscope interfaced with an automated image analysis system (Optomax, Image Analyser). For these studies the particles were prepared in distilled water with a drop of Tween 85 to assure dispersion. Samples were then placed on microscopic slides, covered with a cover glass, and magnified to 800× for image analysis. Images of dust particles in a field of view of 160 × 105 μm were measured by the automated program. All the particles in the field of view were measured by the longest horizontal chord, and 10 similar fields were measured for each dust studied.

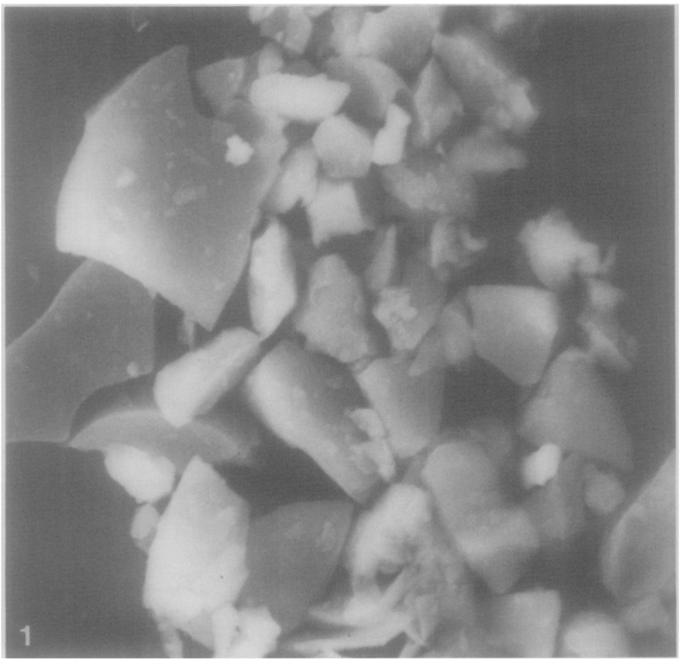
For comparative studies nine different mineral dusts were used selected on the basis of their known biological properties. The mineral samples used in this study were either obtained from commercial sources, from NIOSH standard reference samples, or from NIOSH collections. Particle sizing of these minerals was also made simultaneously with the volcanic ash samples using both the scanning and optical systems.

Hemolytic activity of the ash and other mineral samples were determined using a 2% sheep erythrocyte preparation according to the method of Harington *et al.* (1971). All tests were carried out in a total volume of 4 ml consisting of 2 ml of washed 2% sheep erythrocyte preparation in Veronal buffer of pH 7.2 and a suspension of sonicated volcanic ash or other minerals in 2 ml of buffer. Mineral dusts or volcanic ash suspensions were initially mixed by gentle inversion in test tubes and inverted every 10 min during the 50 min incubation at 37°C. At the end of the incubation period the samples were centrifuged at 1500 rpm for 5 min and the optical density of the supernatant solution was read at 541 nm against a Veronal buffer–saline blank using a Gilford spectrophotometer Model 300. Controls using only the reagents were always run simultaneously with the tests to check lysis of the preparations.

RESULTS

Morphologically, the majority of the ash particles had flat surfaces with angular edges. Lesser numbers of particles with rounded borders and porous surfaces were seen (Fig. 1). Asbestiform minerals were not identified in any of the ash preparations.

The results of the quantitative X-ray diffraction studies on all the volcanic ash samples are presented in Table 1. The data show that the concentration of total free crystalline silica varies considerably in the five volcanic ash samples collected from different locations and eruptions. Plagioclase minerals accounted for the remaining material in all the samples. Scanning electron microscopy with the aid of X-ray energy spectrometric analysis complemented the X-ray diffraction studies. X-ray spectrometric analysis of randomly selected particles indicated that



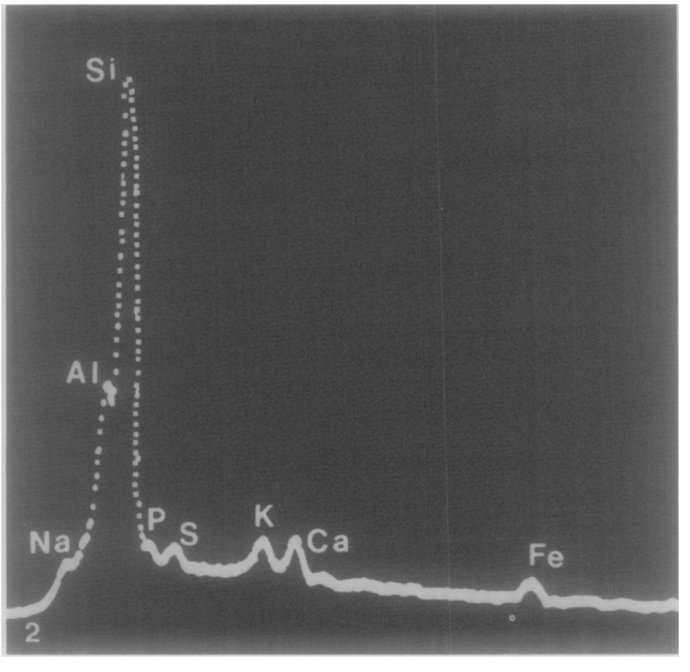


FIG. 1. Scanning electron micrograph of volcanic ash particles. Large number of particles show flat angular edges. (3000×.)

FIG. 2. X-ray energy analysis spectrum of a particle showing the major elemental composition of ash.

TABLE 2
HEMOLYTIC ACTIVITY OF VOLCANIC ASH COMPARED WITH OTHER TOXIC AND INERT MINERAL DUSTS

Mineral/ source	Median circular area equivalent[a] (μm)	Mass median diameter (μm)	Percentage hemolysis[b]
Spokane ash 1st eruption	1.7 (0.11–10.7)	6.0	64.3 + 1.1
Crystalline silica NIOSH Q-1	0.4 (0.14–14.2)	15.0	94.3 + 1.1
Kaolin/Baker	0.6 (0.09–14.6)	10.2	83.5 + 2.1
Chrysotile/NIOSH CH-29	0.05 (Median width)	0.9 (Median length) (0.1–30)	67.9 + 1.8
Talc/NIOSH TA-99	0.7 (0.17–14.5)	16.1	57.9 + 1.7
Coal/Pittsburgh-seam	0.8 (0.11–3.2)	6.6	30.1 + 1.1
Bentonite/Fisher Scientific	0.6 (0.14–4.3)	3.7	18.1 + 1.2
Amosite/NIOSH G-68	0.2 (Median width)	1.8 (Median length) (0.15–6.2)	13.2 + 0.7
Gypsum/NIOSH collection	0.4 (0.14–6.1)	31.4	5.2 + 0.9
Crocidolite/NIOSH CR-37	0.1 (Median width)	0.8 (Median length) (0.13–2.1)	0.5 + 0.2

[a] Mean and range of circular area equivalent diameter measurements. Data presented are based on the analyses of 1040 (volcanic ash), 1008 (silica), 1007 (kaolin), 537 (chrysotile), 1059 (talc), 1051 (coal), 1042 (bentonite), 1057 (amosite), 1037 (gypsum), and 1095 (crocidolite) particles in a minimum of 10 fields of view at 1000 or 2000×.

[b] Mean and standard error of eight replicate experiments.

the majority of the ash particles analyzed contained Na, Si, Al, K, and Ca, consistent with the plagioclase class of minerals (Fig. 2). Occasional particles with a single elemental peak for silicon were also present in the ash preparations.

Particle sizing on randomly selected fields of view of ash preparations revealed that on a number basis 99% of particles were within the respirable size range of less than 10 μm (Fig. 3). The data obtained by TEM and SEM with the aid of automated image analyses on particle size of all the volcanic ash samples and other minerals used in this study are presented in Tables 1 and 2. Optical microscopic studies with the aid of automated image analyses were consistent with the electron microscopic data. The large differences between median circular area equivalent diameters and the calculated mass median diameters for some of the samples in Tables 1 and 2 indicate that these samples are not very homogeneous. These samples contain a significant weight percentage of particles which are nonrespirable.

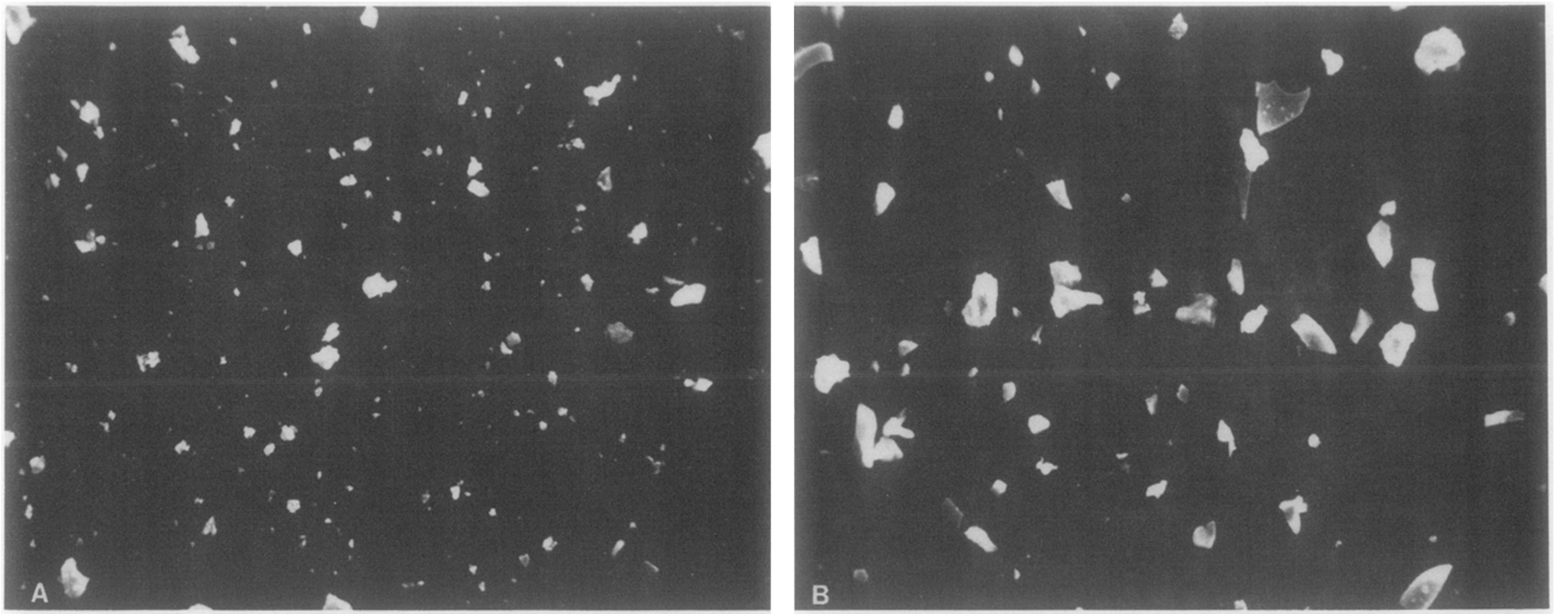


FIG. 3(A–F). Scanning electron micrographs of crystalline silica and volcanic ash samples used in the hemolysis study. (A) Crystalline silica, (B) Spokane sample from the first eruption, (C) Moses Lake sample from first eruption cut to 5 μm, (D) Centralia sample from second eruption, (E) Gales Creek sample from third eruption, and (F) Chewalah sample from fourth eruption. (1000×, bar = 10 μm.)

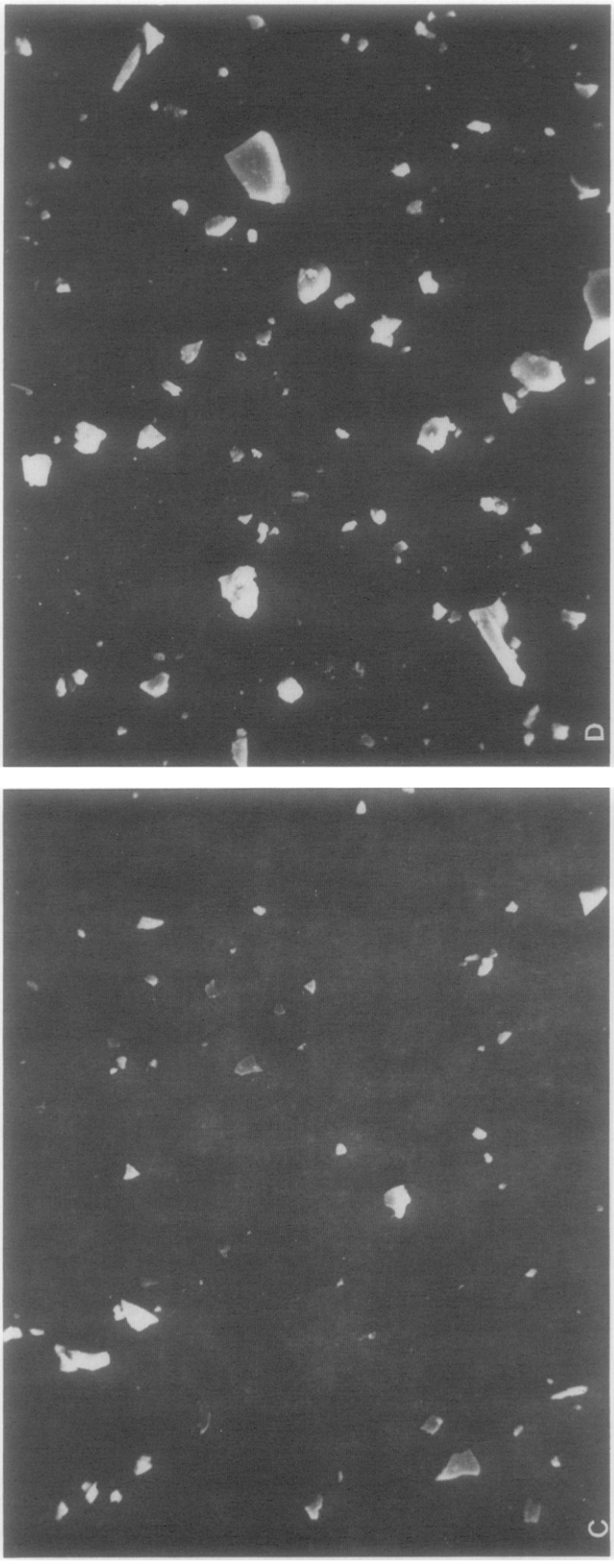


FIG. 3—*Continued.*

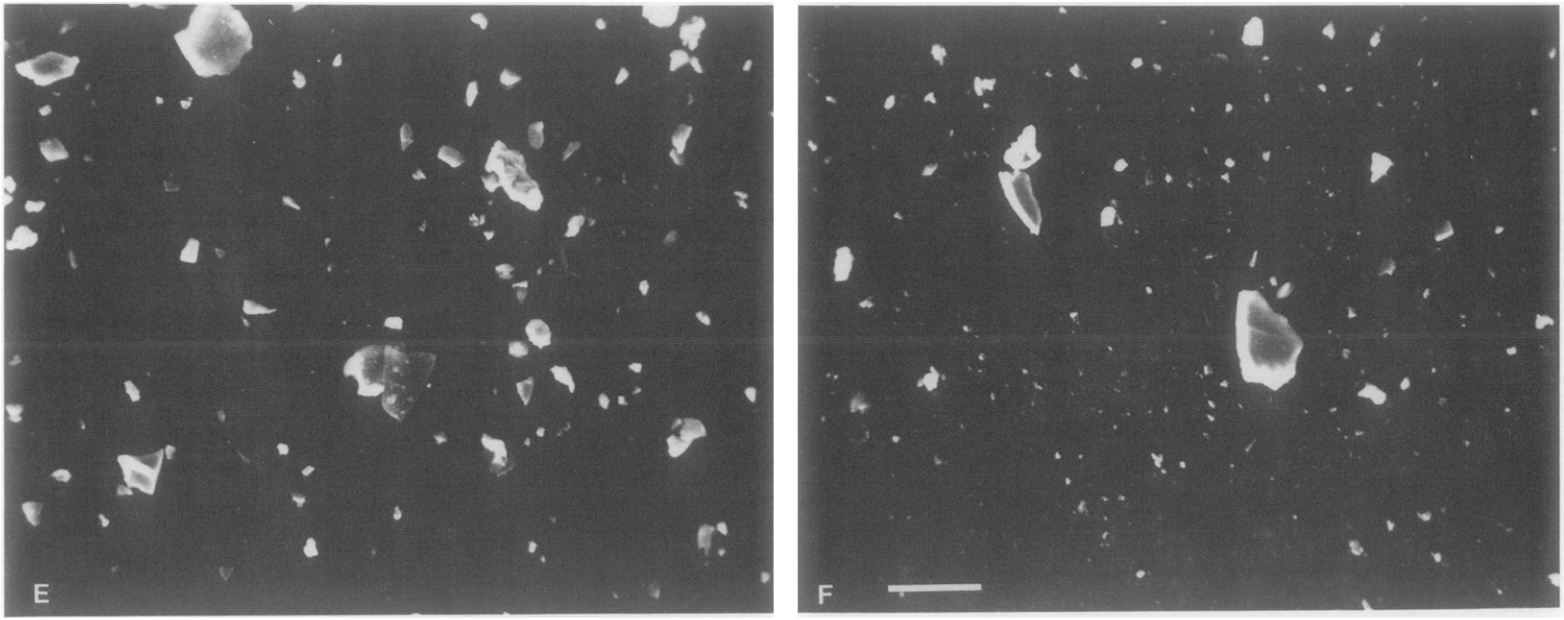


FIG. 3—*Continued.*

The degree of hemolysis produced by one of the volcanic ash samples was compared with hemolytic activity of other mineral dusts (Table 2). In order to compare the hemolytic activities of different mineral dusts independent of the effect of pH, temperature, and other experimental variables all dust samples were treated simultaneously using the same concentration and erythrocyte preparation. The standard concentration of 12.5 mg/ml volcanic ash induced a 64% hemolysis in comparison to that of crystalline silica with 94.3% hemolysis. This level of hemolysis also correlated well with other known fibrogenic minerals such as chrysotile (67.9%) and talc (57.9%).

We also investigated the differences in hemolytic activity among the five different volcanic ash samples collected from different locations representing four volcanic eruptions (Fig. 4). The data presented in Fig. 4 illustrate the variability in hemolysis of these ash samples. All the ash samples showed increasing hemolytic activity with increasing concentration. However, at the standard concentration of 12.5 mg/ml, hemolysis induced by ash samples collected from Centralia and Chewalah representative of the second and fourth volcanic eruptions were approximately 50% lower than the other samples.

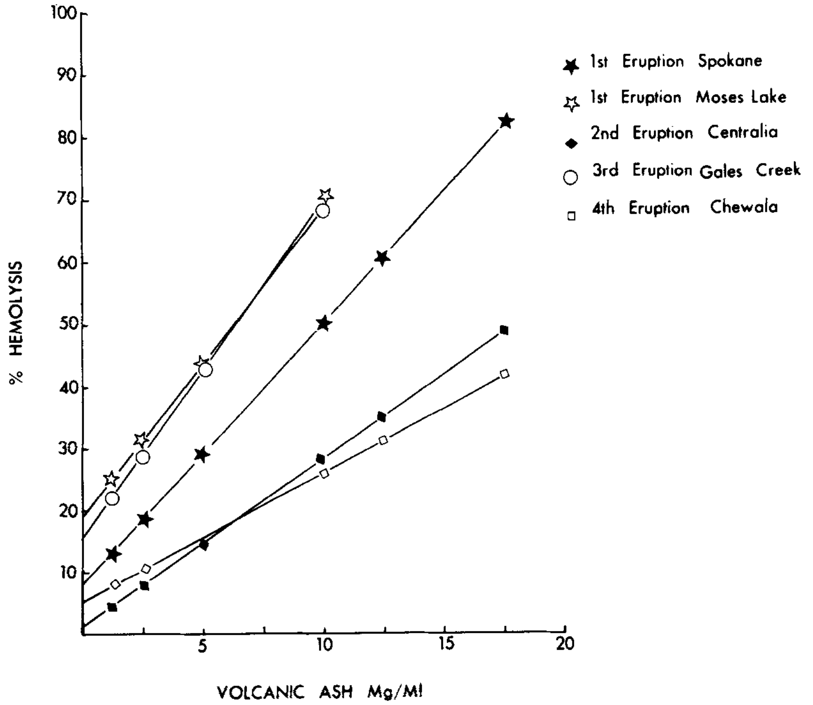


FIG. 4. Effect of volcanic ash in varying concentrations on the hemolysis of sheep blood erythrocytes. The slopes and points represent the results of linear regression analysis of triplicate experiments.

DISCUSSION

The results obtained in our assays using 12.5 mg/ml for chrysotile asbestos and silica are very close to those reported by other investigators (Harington *et al.*, 1971). A good correlation exists between *in vitro* hemolysis and *in vivo* fibrogenicity of several mineral dusts (Harington *et al.*, 1971; Stalder and Stöber, 1965; Schnitzer and Pundsack, 1970; Hefner and Gehring, 1975; Potts *et al.*, 1978). The results of the studies reported here do indicate that all the volcanic ash samples tested from different volcanic eruptions are hemolytic and are thereby cytotoxic. Hemolysis is influenced by a variety of factors other than the toxicity of minerals. Among the various factors the particle size and the concentration of crystalline silica are known to influence hemolysis significantly (Hefner and Gehring, 1975). We tried to investigate this effect by determining the concentrations of crystalline silica and particle size of the volcanic ash samples used in these tests. It was found from these studies that the variations in the concentrations of crystalline silica in the volcanic ash samples did not significantly alter the hemolytic activity. However, the mass median diameter of ash particles significantly influenced hemolysis. This is evident from the high rate of hemolysis resulting from the volcanic ash samples obtained from Moses Lake and Gales Creek with respective mass median diameters of 2.5 and 6.0 μm. In contrast to this the volcanic ash samples obtained from Centralia and Chewalah were the least hemolytic with mass median particle diameters of 16.7 and 7.2 μm, respectively. The ash sample from Spokane with a mass median diameter of 6.0 μm induced hemolysis intermediate to the rate of the other ash samples. The mass median diameters are an indirect measure of surface area, i.e., the larger the mass median diameter, the smaller the surface area of the particle. Data presented here indicate the particles with the highest surface area cause the greatest hemolysis.

The hemolysis studies reported here indicate the volcanic ash to be biologically cytotoxic and it should be considered a potential fibrogenic agent. This indication of cytotoxicity from the *in vitro* studies are supported by our recent *in vivo* animal experimental studies. Specific-pathogen-free rats intratracheally instilled with 1 and 10 mg volcanic ash developed pulmonary granulomas with collagen deposition after 1 month and the severity and extent of lesions had progressed in the lungs at 3 and 6 months (Green *et al.*, 1981). A moderate cytotoxic effect of the volcanic ash was also evident from the studies on the ash-induced enzyme release from the alveolar macrophages (unpublished observations).

In conclusion, four volcanic ash samples from different locations and eruptions exhibited hemolytic activity similar to that of chrysotile asbestos. The degree of hemolysis appeared to be more dependent on particle size than on free silica content. These results taken in conjunction with the animal studies indicate a fibrogenic potential in exposed humans. Attempts should be made to minimize exposure in high risk occupational groups.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Robert Bernstein, Mr. Jerry Clere, Ms. Alice Greife, Mr. Michael McCawley, and Mr. James Tucker for their assistance in sample collection and preparation. We are also grateful to Mr. Michael Moore for the preparation of illustrations, Ms. Kay Kennedy and Ms. Mitzie Martin for the manuscript preparation, and Ms. Molly Pickett-Fredlock for editorial assistance.

REFERENCES

Baxter, P. J., Ing, R., Falk, H., French, J., Stein, G. F., Bernstein, R. S., Merchant, J. A., and Allard, J. (1981). Mount St. Helens eruptions, May 18 to June 12, 1980. An overview of the acute health impact. *J. Amer. Med. Assoc.* **246**, 2585–2589.

Brambilla, C., Abraham, J., Brambilla, E., Berinschke, K., and Bloor, C. (1979). Comparative pathology of silicate pneumoconiosis. *Amer. J. Pathol.* **96**, 149–170.

Dollberg, D. D., Sweet, D. V., Bolyard, M., Carter, J. W., Stettler, L. E., and Geraci, C. L. (1981). Analysis of Mount St. Helens volcanic ash for crystalline silica. *In* "Fourth Annual Symposium on Electron Microscopy and X-Ray Applications to Environmental and Occupational Health Analysis." Ann Arbor Science Publ., Ann Arbor, Mich., in press.

Fruchter, J. S., Robertson, D. E., Evans, J. C., Olsen, K. B., Lepel, E. A., Laul, J. C., Abel, K. H., Sanders, R. W., Jackson, P. O., Wagman, N. S., Perkins, R. W., Van Tuyl, R. H., Beauchamp, R. H., Shade, J. W., Daniel, J. L., Erickson, R. L., Sehmel, G. A., Lee, R. N., Robinson, A. V., Moss, O. R., Briant, J. K., and Cannon, W. C. (1980). Mount St. Helens Ash from the May 18, 1980 eruption: Chemical, physical, mineralogical and biological properties. *Science* **209**, 1116–1125.

Green, F. H. Y., Bowman, L., Castranova, V., Dollberg, D. D., Elliot, J. A., Fedan, J. S., Hahon, N., Judy, D. J., Major, P. C., Mentnech, M. S., Miles, P. R., Mull, J., Olenchock, S., Ong, T., Pailes, W. M., Resnick, H., Stettler, L. E., Tucker, J. G., Vallyathan, V., and Wong, W. (1982). Health implications of Mount St. Helen's eruption: Laboratory investigations. *Ann. Occup. Hyg.* **26**, 921–933.

Green, F. H. Y., Vallyathan, V., Mentnech, M. S., Tucker, J. H., Merchant, J. A., Kiessling, P. J., Antonius, J. A., and Parshley, P. (1981). Is volcanic ash a pneumoconiosis risk? *Nature (London)* **293**, 216–217.

Harington, J. S., Miller, K., and Macnab, G. (1971). Hemolysis by asbestos. *Environ. Res.* **4**, 95–117.

Hefner, R. E., and Gehring, P. J. (1975). A comparison of the relative rates of hemolysis induced by various fibrogenic and non-fibrogenic particles with washed rat erythrocytes. *Amer. Ind. Hyg. Assoc. J.* **36**, 734–740.

Morbidity and Mortality Weekly Report (1980). *Center for Disease Control, Atlanta, Ga.*, Vol. **29**, No. 28, p. 338.

Parkes, R. W. (1974). "Occupational Lung Disorders," pp. 194–197. Butterworths, London.

Potts, W. J., Lederer, T. S., and Gehring, P. J. (1978). Hemolysis of washed rat erythrocytes *in vitro* by dust particles. *Amer. Ind. Hyg. Assoc. J.* **39**, 497–502.

Schnitzer, R. J., and Pundsack, F. L. (1970). Asbestos hemolysis. *Environ. Res.* **3**, 1–13.

Sherwin, R. P., Barman, M. L., and Abraham, J. L. (1979). Silicate pneumoconiosis of farm workers. *Lab. Invest.* **40**, 576–582.

Stalder, K., and Stöber, W. (1965). Hemolytic activity of suspensions of different silica modifications and inert dusts. *Nature (London)* **207**, 874–875.

Summerton, J., and Hoening, S. (1977). The mechanism of hemolysis by silica and its bearing on silicosis. *Exp. Mol. Pathol.* **26**, 113–128.

Talvite, N. A. (1951). Determination of quartz in the presence of silicates using phosphoric acid. *Anal. Chem.* **23**, 623–627.

Thorarinsson, S. (1979). *In* "Volcanic Activity and Human Ecology" (P. D. Sheets and D. K. Grayson, Eds.), pp. 125–159. Academic Press, New York.