CHAPTER 7

Evaluation of Physical Health Effects Due to Volcanic Hazards: The Use of Experimental Systems to Estimate the Pulmonary Toxicity of Volcanic Ash

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Introduction

When Mount St. Helens erupted violently on May 18, 1980, no precedents existed by which to predict the likely respiratory effects of exposure to volcanic ash. As experimental studies conducted in model systems *in vitro* and *in vivo* have been useful in the evaluation of the potential health effects of a variety of minerals, a number of laboratories adapted these techniques to the study of volcanic ash.[1,2] The result was the accumulation in a short period of time of information that could be used to compare the toxicity of volcanic ash with the toxicities of inert and fibrogenic reference compounds. Although these data have been accumulated in a variety of experimental systems, the results from a number of independent laboratories indicate that respirable volcanic ash is generally much less toxic to the lung than free crystalline silica, which is a known hazard in the workplace.

Major Experimental Issues and Questions

Three types of experimental approaches have been used to evaluate the biological effects of volcanic ash on the respiratory system: *in vitro* studies; intratracheal injection studies; and inhalation studies (Table 1). Studies conducted *in vitro* using tissue culture systems provide rapid approximations of the comparative effects of test and reference minerals on cells that are involved in the expression of toxicity *in vivo*. Alveolar macrophages, pneumocytes, and erythrocytes have been used as target cells. Alveolar macrophages are important mediators of the response of the lung to inhaled particulates (for review see [3,4]). Macrophage cytotoxicity *in vitro*, measured by vital dye exclusion or the release of cytoplasmic enzymes, generally parallels the toxicity of inhaled minerals in the lung.[5–8] Macrophages that ingest particulates such as asbestos and silica release mediators that stimulate fibroblast proliferation[9] and neutrophil migration.[10,11] The latter provides a mechanism whereby the alveolar macrophage can amplify inflammatory responses by recruiting bloodstream leukocytes to the lung. Fibrogenic minerals also lyse type II pneumocytes, the alveolar epithelial cells that are the source of surfactant and the progenitors of type I pneumocytes, which make up most of the surface area of the alveolar space.[12] Interaction between particulates and erythrocyte membranes *in vitro* leads to the rupture of erythrocytes, an affect that parallels the fibrogenicity of the particulates *in vivo*.[13,14]

Although the basis for these membrane damaging effects is not well understood, *in vitro* assays serve as useful first approximations of the fibrogenicity of a mineral *in vivo*. These *in vitro* assays are advantageous because they are rapid and relatively easy to perform in a laboratory that is equipped for tissue culture. The major disadvantages are that they are far removed from the whole organ system and in some cases manipulation of the *in vitro* system can influence the results. For example, the presence of proteins or lipids in tissue culture media minimizes the toxicity of α-quartz for pneumocytes and macrophages.[12,15] This raises the possibility that *in vivo* coating of particules by lipids or proteins in the airway might serve to limit the toxicity of a particulate in the lung.

Two approaches have been used to measure the pulmonary effects of volcanic ash *in vivo:* intratracheal injection of volcanic ash and inhalation studies (Table 1). Similar parameters are measured using both routes of mineral administration. For example, after either type of mineral exposure lungs can be fixed for light and electron microscopic examination, or they can be lavaged to recovery airway cells, surface active material, and enzymes.

Intratracheal injections are performed via orotracheal inoculation or direct tracheal puncture using lightly anesthetized animals. This technique permits the rapid administration of a measured dose of dust directly into the airway[16–18] and sophisticated exposure equipment is not required. In an experienced laboratory, a large number of animals can be inoculated in a short period of time and a variety of parameters can be measured, including qualitative and quantitative changes in lung histopathology, bronchoalveolar cell and protein profiles, and even collagen synthesis rates in lung slices. The intratracheal injection technique is useful as a rapid screening test to determine the relative toxicities of test and reference materials in the lung. Some evidence suggests that the same relative ranking of toxicity can be obtained from both intratracheal injection and inhalation of a test substance.[18,19] The major disadvantage of the intratracheal injection technique is that the particle distribution in the lung after a single bolus injection is less homogeneous than from that achieved by inhalation techniques.[16] Particles may deposit primarily in lower lobes, in a pattern that favors small airways and proximal alveoli.[17] Peripheral alveoli in the lower lobes and most of the upper lobes may be relatively spared. High local concentrations of dusts may occur in some areas with relatively low concentrations in other areas, resulting in non-uniform agent-to-target ratios. In some cases, this may result in more severe pathologic changes than is seen after inhalation exposure.[19,20] Further, the physical effects of the intratracheal injection technique on the mucociliary system, which is important in the removal of particles from the airways, have not been studied in great detail.[17]

Inhalation exposure, in which spontaneously breathing animals are exposed to well-characterized respirable aerosols, simulates human inhalation exposure more closely than the intratracheal injection of a mineral. However, inhalation studies require sophisticated aerosol chambers and monitoring equipment and are time consuming and expensive. Alveolar deposition of particles is more uniform than after intratracheal injection,[16,17] but the relatively low deposition fraction in spontaneously breathing animals may require long

NOTE: Author affiliations and addresses are listed on p vi.

TABLE 1—Results of Published Studies on Biological Effects of Volcanic Ash

Type of Study	System	Dose	Time	Parameters Measured	Results	Comments
In Vitro						
1. Robinson[22]	Rabbit AM	0.1–0.5 mg/ml; VA, Q T_iO_2, PVT basalt, feldspar	20–24 hr	AM viability	Q > VA = TiO_2	Effects of VA similar to an inert agent
2. Green[24]	Monkey kidneys, rat trachea, rabbit AM	Variable	Immediate	Mutagenicity (none), interferon induction (no suppression), AM toxicity (little), complement activation (none), RBC hemolysis (moderate)		Most studies examined only volcanic ash
3. Vallayathan[25]	Sheep RBC	1–20 mg/ml	50 min	RBC hemolysis	Q > VA = chrysotile, asbestos, gypsum	Well controlled study
4. Castranova[26]	Rabbit TII, rat AM, rat lung microsomes	5 mg/ml VA	1 hr	AM and TII O_2 consumption, peroxidation, AM superoxide production	No change in O_2 consumption or lipid peroxidation; reduced AM superoxide release	No inert or fibrogenic control; superoxide studies suggest that VA may increase infection risk
5. Martin[12]	Human AM, rat TII, A549 cells	0.1–1 mg/ml VA, Q	0–2 hr	Cell viability	Q >> VA; airway proteins reduced the cytotoxicity of quartz	No inert dust control
6. Dodson[32]	Human AM	0.1 mg/ml	5 min–18 hr	Cell morphology	VA caused no morphologic changes in AM	No inert or fibrogenic controls
7. Schiff[33]	Hamster trachea	1–100 μg/ml VA	2 hr–2 wks	Ciliary morphology, mucous secretion	VA increased mucous secretion	No inert or fibrogenic controls
In Vivo (Intratracheal)						
8. Beck[35]	Hamster	0.15–3.75 mg VA, Q, Al_2O_3	24 hr	Airway cells, proteins, cellular enzymes	Q > VA ≥ Al_2O_3	Well controlled studies; VA similar to inert dust Al_2O_3
9. Fedan[37]	Rat trachealis muscle	10 mg VA	1 hr	Response to smooth muscle agonists	VA reduces tracheal response to serotonin	No inert or fibrogenic controls
10. Sanders[38,39]	Rat	(1) 40 mg VA, Q, soil (2) 77 mg VA total in repeated weekly doses	3 mo	Pathology	Q > VA > soil	Well controlled, VA caused more fibrosis and lymph node hyperplasia than soils
11. Vallyathan[40,41]	Rat	10 mg VA	6 mo	Pathology	VA caused granulomas	No inert or fibrogenic controls
12. Akematsu[43]	Rat	17.5 and 52.5 mg VA	7 days	Pathology	VA was phagocytosed by epithelial cells and caused cell injury	No inert or fibrogenic controls
13. Kornbrust[46]	Rat	50 mg VA, Q	6 mo	Lung lipids	Q and VA increase lung lipids	No inert controls
14. Shirakawa[48]	Rat, rabbit	500 mg VA	4 mo	Pathology	Inflammation and emphysema with VA	Exceedingly high doses, no controls
In Vivo (Inhalation)						
15. Wiester[49]	Guinea pig	10 mg/m³ VA	2 hr	Airway response to histamine	VA reduced airway reactivity	No inert or fibrogenic controls
16. Raub*[42]	Rat	VA and Q	6 mo	Pathology, pulmonary function	No effect on pulmonary function; little effect on pathology	Studied VA with an added pollutant, SO_2
17. Martin[47]	Rat	100 mg/m³, VA Q air only	2 wk exposure, 9 mo. follow-up	Pathology, airway cells, lipids lung density, macrophage function	Q >> VA by all parameters	No inert dust controls
18. Wehner[51]	Rat	VA 50 mg/m³, 5 mg/m³, Q 50 mg/m³, air only	6 hr/d, 5 d/wk for up to 12 mo	Pathology	Q >> VA	No inert dust controls
19. Martin*[54]	Rat	20 mg/m³, VA, Q, Al_2O_3, air only	6 hr/d, 5 d/wk for 4 wk, 9 mo follow-up	Pathology, bacterial clearance	VA is similar to Al_2O_3; accelerated bacterial clearance in mineral-exposed groups	Well controlled; no evidence that VA impairs antibacterial defenses
20. Wehner[53]	Rat	135 mg/m³ of neutron-activated VA for 60 min	Months	Mineral clearance rate	Initial deposition = 6%; T-1/2 = 39 days	Clearance rate is similar to other mineral particles
21. Grose*[55]	Mouse, rat	9.4 mg/m³ VA ± 2.5 mg/m³ SO_2	2 hr/d × 5 d, 6 mo follow-up	Survival after exposure to streptococci and cytomegalovirus	VA did not affect bacterial or viral resistance	Studied VA with an added pollutant, SO_2

Abbreviations: AM = alveolar macrophage; TII = type II pneumocyte; VA = volcanic ash; Q = quartz; TiO_2 = titanium dioxide; PVT = polyvinyl toluene; Al_2O_3 = aluminum oxide; RBC = erythrocyte.

*These studies included intratracheal injections and inhalation exposures.

exposures to high aerosol concentrations in order to achieve high lung dust burdens. Furthermore, in the case of whole body exposures, particle deposition occurs on the fur of the animals, leading inevitably to ingestion of particles and the possibility of nonpulmonary biologic effects.

These three general types of animal studies represent a progression from the relatively inexpensive and rapid *in vitro* tests to the more prolonged and expensive *in vivo* tests that require longer follow-up periods to detect biologic effects. These experimental approaches provide a variety of ways to compare the biologic effects of an unknown compound, such as volcanic ash, with agents that are known to be fibrogenic or inert in animal and human systems. The objective in each type of study should be to compare the toxicity of a test compound with those of agents whose biologic effects are well defined. Therefore, one of the most important considerations in the evaluation of experimental studies is experimental design. In order to draw firm conclusions, it is important that simultaneous controls using inert and toxic reference compounds be included to represent a range of effects with which an unknown agent can be compared. Furthermore, it is important to test samples with similar particle size distributions, as particle size and surface area are important variables that may influence results. For example, small silica particles are more toxic to macrophages than larger ones.[13,21] Whenever possible with mixed samples such as volcanic ash, the composition of the agent should be measured to evaluate subfractions in the sample, such as free silica, that might account for any observed toxicity or differences in toxicities observed in different laboratories.

An important caveat regarding results from experimental systems is that although conclusions about the *comparative* biologic effects of a test compound may be valid in carefully designed experiments, it does not necessarily follow that the actual effects observed occur in the human lung. For example, to demonstrate macrophage cytotoxicity *in vitro,* it may be necessary to compare agents at concentrations of up to 1.0 mg/ml. This dose greatly exceeds that encountered in the human lung, as an individual exposed to a very high respirable particle mass concentration of 5 mg/m^3 would be exposed to 5×10^{-6} mg/ml of air at the nose and only 5×10^{-7} mg/ml of air would deposit at the level of the alveoli, assuming a 10 per cent deposition fraction. Similarly, a single 10 mg intratracheal injection of an agent might represent the total integrated dose after several weeks of inhalation exposure, depending on the ambient aerosol concentration, the ventilation rate, the nasal filtration efficiency, and the mucociliary clearance rate in the animal or human. Thus, results in animal systems permit conclusions about the relative toxicity of test and reference compounds, but they do not necessarily permit direct extrapolation of the results to man.

Results of Published Studies (Table 1)

In Vitro Experiments

The results of a variety of *in vitro* experiments with volcanic ash have been reported. In studies conducted immediately after the major eruption of Mount St. Helens, Robinson, *et al,*[22] used rabbit alveolar macrophages and compared the toxicities of a composite ash sample and individual samples from collecting sites 16, 260, and 580 km downwind from the volcano with the toxicities of quartz, basalt, feldspar, eastern Washington soil, titanium dioxide (TiO_2), and polyvinyl toluene (PVT) particles. Free crystalline silica was not detected in the ash samples, but the phosphoric acid digestion procedure recommended by the National Institute for Occupational Safety and Health (NIOSH) for the analysis of free silica was not used,[23] raising the possibility that existing free silica in the samples was not detected. The test dusts were added to macrophage monolayers, and cell viability was measured by trypan blue exclusion. The particle sizes in the specimens were not measured, although each dust was allowed to sediment to remove large particles. When macrophages were exposed for 20–24 hours to 0.5 mg/ml of each ash sample, macrophage viability was slightly reduced (about 10 per cent) compared with macrophages exposed to PVT. At lower mineral concentrations, the effects of volcanic ash were similar to those of TiO_2, PVT, and eastern Washington soil. Feldspar and basalt were not cytotoxic. As each of the samples was less toxic than quartz, these early studies provided the first evidence that volcanic ash was not likely to be as fibrogenic *in vivo* as quartz.

Investigators at the National Institute of Occupational Safety and Health surveyed the biologic effects of volcanic ash in a wide variety of assays that are used to screen mineral samples encountered in the workplace.[24–27] The ash sample consisted of 7.2 per cent free crystalline silica by weight and 99 per cent of the particles were < 10 μm in diameter. The ash sample was not mutagenic in the Ames Salmonella assay, which tests the ability of a sample to cause mutations in a standard bacterial strain. This suggests a low potential for the induction of malignancy. Volcanic ash also did not impair the release of interferon from monkey kidney cells stimulated with PR8 influenza virus, implying that it may not alter antiviral defenses. In this regard, it differs from asbestos and coal dust, which do depress interferon production.[28,29] The ash also failed to activate complement, indicating that it lacks the capacity to initiate inflammation via the complement cascade.[27]

Observations by Castranova, *et al,* raised concern that volcanic ash might impair antibacterial defenses in the lung.[26] In studies of macrophage superoxide anion production in response to volcanic ash, stimulated superoxide production was impaired after exposure to volcanic ash *in vitro* and *in vivo,* although resting superoxide anion production was normal. Because the production of toxic oxygen intermediates, such as superoxide anion and hydroxyl radical, is an important microbicidal mechanism in activated macrophages, as well as neutrophils,[30] the authors inferred that volcanic ash might pose an infection risk by compromising phagocyte antibacterial function. The effects of inert and fibrogenic control dusts were not studied in this system, so that the specificity of this finding for volcanic ash is uncertain. This study served to stimulate further investigations regarding lung antibacterial defenses in *in vivo* systems (discussed later).

Martin, *et al,* studied the comparative effects of quartz and volcanic ash on two types of cells derived from the alveolar region of the human lung, alveolar macrophages and A549 cells.[12] The A549 cells have many morphologic and synthetic characteristics of type II pneumocytes.[31] Volcanic ash and quartz were added to target cell monolayers in concentrations ranging from 1–10 mg/ml. Volcanic ash was less toxic than quartz for macrophages, as measured by trypan blue exclusion and the release of lactate dehydrogenase, a cytoplasmic enzyme. Volcanic ash also caused less lysis of the A549 cells, as measured by ^{51}Cr release. Similar results were obtained with actual type II pneumocytes freshly isolated from rat lungs. Quartz stimulated the release of a chemotactic factor for neutrophils from human alveolar

macrophages, but volcanic ash did not. This observation, together with the observation that volcanic ash does not activate complement,[27] supports the conclusion that volcanic ash is not a potent stimulus to inflammation in the lung. The addition of airway proteins such as IgG, IgA, and albumin to the type II pneumocyte assay system reduced the cytotoxicity seen with quartz, suggesting that airway proteins may play a protective role in mediating the effects of inhaled dusts on the lung. Surfactant also may have a similar effect.[15] A control non-fibrogenic dust was not studied in these systems so that the similarities between volcanic ash and an inert agent could not be evaluated.

Dodson, *et al,* found that human alveolar macrophages incubated with submicronic (< 0.6 μm) volcanic ash particles phagocytosed ash particles without morphologic evidence of cytotoxicity,[32] supporting the results of Martin, *et al.*[12] However, neither inert nor cytotoxic control dusts were used for comparison.

Schiff, *et al,* examined the effects of volcanic ash on hamster tracheal explants, and found that short exposures to respirable volcanic ash (two hours) reduced ciliary beat frequency and increased mucous production by the explants.[33] As comparisons with fibrogenic and inert agents were not made, the specificity of these findings for volcanic ash are uncertain, but the results do suggest that mucous hypersecretion might result from exposure to respirable volcanic ash.

Vallyathan, *et al,* published the only comparative studies of volcanic ash from three different volcanoes, Mount St. Helens, Galunggung, and El Chichón.[34] The free crystalline silica content of the respirable fractions ranged from 1.50–1.95 per cent. The ash specimens from the different volcanoes differed with respect to lysis of sheep erythrocytes and stimulation of alveolar macrophage enzyme release. Interestingly, the toxicities of the specimens correlated with the estimated surface areas of the particles in the samples—those with greater surface area had greater toxicity. The authors emphasized the need to study well characterized samples that are collected using similar techniques from well defined geographic locations.

Intratracheal Injection Studies

Eight groups have reported the results of intratracheal injection studies with volcanic ash. Beck, *et al,* injected 0.15 to 3.75 mg of respirable volcanic ash (particle size < 5.0 μm) intratracheally into Syrian golden hamsters[35] and used bronchopulmonary lavage to measure airway cells, proteins, and cellular enzymes such as lactic dehydrogenase and β-glucuronidase, which correlate with injury to the epithelial cells in the airway and alveoli. In all parameters measured, volcanic ash was similar in effect to aluminum oxide (α-Al_2O_3), which is inert in rats.[36] Quartz was more toxic than volcanic ash. These well controlled studies suggest that the acute effects of volcanic ash administered directly into the airspace are similar to those of inert dusts.

The effects of volcanic ash on airway reactivity may be important for patients with chronic airflow obstruction, who usually have increased airway reactivity. Fedan, *et al,* exposed rats to volcanic ash either intratracheally or via inhalation and then measured the response of excised trachealis muscles to smooth muscle agonists such as KCl, isoproterenol, acetylcholine, and 5-hydroxy-tryptamine (5-HT).[37] Both intratracheal injection (10 mg) and a one-hour inhalation exposure reduced the responsiveness of the trachealis muscle to 5-HT. The responses of the trachealis muscle to KCl, isoproterenol, and acetylcholine were unchanged. This suggested that volcanic ash exposure might reduce tracheal reactivity, but the cellular basis for this effect was not investigated. As the effects of other toxic and inert dusts were not studied, the specificity of these findings for volcanic ash is uncertain.

Sanders, *et al,* examined lung histopathology in rats followed for up to 400 days after the intratracheal injection of 40 mg of volcanic ash, quartz, eastern Washington soil, or saline only.[38,39] Additional rats received smaller injections of volcanic ash each week (total of 77 mg). Volcanic ash and soil caused minimal pulmonary fibrosis, but quartz caused alveolar proteinosis and intense fibrosis. These studies demonstrate that high doses of intratracheal volcanic ash can cause pulmonary fibrosis but that the pattern of injury is much less severe than that due to quartz. Longer-term follow-up of these animals indicated that volcanic ash caused more tissue fibrosis and reactive changes in pulmonary and mediastinal nodes, and suggested that ash may pose a pneumoconiosis risk for chronically exposed individuals (e.g., loggers or agricultural workers).[39]

Vallyathan, *et al,* studied lung histopathology in rats after the intratracheal injection of 10 mg of volcanic ash.[40,41] The animals were killed sequentially over six months. The animals developed distinct pulmonary granulomas in which mineral particles could be identified by polarized light. While the authors concluded that volcanic ash is not inert and is probably moderately fibrogenic, inert and fibrogenic control dusts were not used. Thus it was not clear how much more extensive the reaction might have been to a fibrogenic dust, or whether an inert agent (such as α-Al_2O_3) might also cause similar granulomas when administered intratracheally at that dose.

Raub, *et al,* compared the effects of two different quantities, of coarse (12.1 μm MMAD) volcanic ash or quartz in rats.[42] The animals received a single intratracheal injection of 50 mg or 0.3 mg of either volcanic ash or quartz. None of the volcanic ash treatments changed pulmonary function in exposed rats, but silica caused restrictive defects in pulmonary function and increased lung hydroxyproline, suggesting an increase in lung collagen content. Six months after the intratracheal injections, the lungs of rats exposed to the high dose of volcanic ash had areas of focal inflammation, whereas all of the animals exposed to the high dose of silica developed severe fibrosis.

Akematsu, *et al,* observed that following intratracheal injection, volcanic ash particles can be ingested by bronchiolar cells without morphologic evidence of cytotoxicity.[43] Both tracheal and alveolar epithelial cells phagocytosed mineral particles, which may be a mechanism whereby ash particles are translocated from the airspace to the interstitium of the lung. Similar observations have been made using asbestos fibers.[44,45]

The effects of volcanic ash and quartz on lung lipids have been examined after intratracheal injection and after inhalation of these minerals.[46,47] Kornbrust, *et al,* found that the intratracheal injection of quartz caused a marked (11-fold) increase in total airway phospholipids, most of which was due to an increase in phosphocholine.[46] Volcanic ash caused a two-fold increase that was maximal one month after administration. As phosphocholine is the major component of surfactant, the authors inferred that the findings reflected increased surfactant synthesis from alveolar type II cells. Martin, *et al,* also found similar results after inhalation exposure.[47] In these studies, the surface tension lowering

activity of the lipid fraction in airway lavage fluid was markedly increased, and electron microscopic examination of lung parenchyma showed that the alveoli of quartz-exposed animals were filled with lamellar bodies and tubular structures with the appearance of tubular myelin. These findings suggest that the excess lipid output after quartz exposure is functional, rather than inactive, surfactant.

Shirakawa, *et al,* studied the effects of ash from the Sakurajima volcano administered intratracheally or via inhalation.[48] The ash contained up to 10 per cent quartz, by X-ray diffraction analysis. No control positive or negative agents were studied. Rats received up to 500 mg of bulk ash intratracheally, or inhaled ash for four hours daily for up to 112 days. The intratracheal exposures produced severe pathologic changes, with diffuse parenchymal fibrosis and infiltration with lymphocytes, macrophages, and giant cells. Rabbits exposed by inhalation developed nodular fibrosis. The details of the inhaled dose and particle distribution profile were not given, so that direct comparison of these results with other studies is difficult. Very large doses of ash were used in these studies, and the absence of an inert control dust makes it difficult to conclude that the observed changes are specific for volcanic ash. Dose-response data were not provided, so that it is difficult to draw inferences about what responses might be likely in humans exposed to much lower doses of inhaled volcanic ash.

Inhalation Studies

Four laboratories have investigated the response of the rodent lung to inhaled volcanic ash. Weister, *et al,* studied airway reactivity in guinea pigs exposed to volcanic ash aerosol (10 mg/m^3) for two hours.[49] The animals were then exposed to progressively increasing doses of histamine, and dynamic compliance (C_{dyn}) of the lung was measured. After ash exposure, a larger concentration of histamine was required to cause the same decrease in C_{dyn} seen in animals exposed to clean air. Inert and fibrogenic control dusts were not studied in this system. Surprisingly, this work and the study by Fedan, *et al,*[37] suggest that volcanic ash exposures tend to reduce tracheal reactivity, in contrast with inert dusts which tend to increase airway resistance in humans.[50] The reason for this remains uncertain and its relevance for humans has not been established.

Raub, *et al,* found that a short-term inhalation exposure (two hours daily for five days) to a low concentration of volcanic ash (9.4 mg/m^3) caused no immediate or long-term effects on lung histology in normal rats or rats with elastase-induced emphysema.[42] Additional animals were exposed to SO_2 and volcanic ash simultaneously, and these animals also had no demonstrable histologic changes. This is the only study of the effects of volcanic ash in animals with pre-existing lung disease, and it suggests that the presence of emphysema does not potentiate the effects of volcanic ash. As elastase induces primarily parenchymal abnormalities, the effects of volcanic ash in animals with diseases of the airways remain uncertain.

Martin, *et al,* exposed rats to high levels (100 mg/m^3) of respirable volcanic ash (6.7 μm MMAD), quartz (7.5 μm MMAD), or clean air for two weeks and observed the animals for nine months thereafter.[47] Histopathology, airway cell populations, alveolar macrophage viability and chemotaxis, airspace lipids and surface-active material, and lung density by computed tomography were measured sequentially. Volcanic ash caused a mild acute lung injury, shown by ultrastructural evidence of type I pneumocyte damage. Quartz, however, caused acute silicoproteinosis immediately after the two-week exposure period. Volcanic ash, unlike quartz, did not increase lavage surface-active material or lung density. Six months after exposure, volcanic ash and quartz-exposed animals had parenchymal fibrosis evident by light and electron microscopy, but the fibrosis was much more pronounced in quartz-exposed animals. More than 90 per cent of the alveolar macrophages recovered from volcanic ash and quartz-exposed animals were viable, as measured by trypan blue exclusion, but the migration of macrophages from both groups was reduced. Thus volcanic ash inhaled in high concentrations caused lung injury and fibrosis, but to a lesser degree than quartz. An inert control dust was not included in these studies, so that the similarity between volcanic ash and an inert dust could not be evaluated.

Wehner, *et al,* conducted long-term inhalation studies, exposing rats to 5 mg/m^3 or 50 mg/m^3 of respirable volcanic ash, or to 50 mg/m^3 quartz, for six hours/day, five days/week, for up to 24 months.[51] The results showed dose-response and agent-response relationships. After 12 months, the quartz-exposed rats had diffuse alveolar proteinosis, and a pronounced histiocytic reaction around small airways and in mediastinal lymph nodes. Animals exposed to 50 mg/m^3 of volcanic ash had similar but less pronounced lesions. The lungs of animals exposed to 5 mg/m^3 volcanic ash had only minimal changes, consisting primarily of aggregates of dust-laden alveolar macrophages in alveolar regions. These findings indicate that volcanic ash, inhaled at high respirable concentrations for long periods of time, can damage the lung; however, the effects of inhaled volcanic ash at the lower concentration were minimal, and this concentration is within the allowable limit for nuisance dusts.[52] An inert control dust was not included in these studies, and it is possible that similar changes might be produced by any agent inhaled in such high concentrations for long periods of time. Alternatively, it might be that the low concentration of free crystalline silica in the ash caused the pathologic changes observed in the 50 mg/m^3 exposure group.

Wehner, *et al,* also measured pulmonary deposition and clearance of volcanic ash in normal rats exposed by nose only for 60 minutes to 135 mg/m^3 of neutron-activated respirable ash.[53] The initial alveolar deposition was 6 per cent of the inhaled quantity of ash, and the clearance time for 50 per cent of the volcanic ash was 39 days.

The effects of volcanic ash exposure on antibacterial defenses in the lung have been examined in two studies, and neither found that volcanic ash exposure impaired lung antibacterial defenses *in vivo*.[54,55] Martin, *et al,* measured the pulmonary clearance rate of aerosolized *S. aureus* in rats after inhalation or intratracheal exposure to volcanic ash, quartz, aluminum oxide (used as an inert control agent), or in sham-exposed animals.[54] After both routes of exposure, the mineral-exposed animals cleared *S. aureus* more rapidly than sham-exposed animals. Quartz exposure was associated with the most rapid elimination of *S. aureus,* whereas the clearance rates in ash-exposed animals were generally similar to those of the animals exposed to aluminum oxide. The acceleration in *S. aureus* clearance paralleled the degree of inflammatory response in the lung caused by each mineral exposure suggesting that the neutrophilic inflammatory response induced by the mineral exposures might augment antibacterial defenses, as neutrophils have more potent microbicidal activity than macrophages.[30]

Grose, *et al,* used mice and found that short inhalation exposures or intratracheal injection of small doses of ash did

not affect survival after exposure to aerosolized group C streptococci, or after intratracheal or intraperitoneal inoculation with murine cytomegalovirus.[55]

Unanswered Questions

Immediately after the first eruption of Mount St. Helens, many investigators rushed to obtain volcanic ash samples to use in available experimental systems. Although the residents of eastern Washington were shoveling ash from rooftops, no central facility existed to collect and distribute standardized ash specimens to interested groups. Thus the specimens tested in different laboratories were collected by various methods from different locations downwind of the volcano. Variations in free crystalline silica content, particle size distributions, and the presence of contaminating soil all emerged as variables that could affect experimental results.

In the rush to generate information after the 1980 eruptions, many of the studies were planned quickly and, as a result, most have a number of weaknesses. For example, the experimental doses of volcanic ash vary widely, and the controls used range from none in many studies to the inclusion of both inert and fibrogenic reference dusts (Table 1). Mineralogic analysis was not always conducted to characterize the free silica content of the sample, and careful control over particle size distributions in test and control specimens is lacking in many studies. Further, the histologic studies reported all suffer from a lack of careful morphometric quantitation, so the interpretation of the results of pathologic studies with similar designs, yet somewhat different tissue reactions, is difficult.[38,40,42,47,51] An important issue that has not been addressed in any of the studies is whether or not the observed low level of toxicity of volcanic ash is caused by the presence of small amounts of free crystalline silica. For example, studies of inert agents "spiked" with added free silica have not been conducted.

Despite these criticisms and the range of experimental approaches, the experimental results are remarkably consistent and indicate that volcanic ash is much less toxic to lung than quartz, an agent that is highly fibrogenic in humans and animals.[56] The relationship between volcanic ash and inert dusts is less clear. The studies by Beck, *et al,*[35] and Sanders, *et al,*[38] suggest that volcanic ash is closer to the inert end of the spectrum than to quartz, but longer-term studies of Sanders, *et al,*[39] and the chronic inhalation study by Wehner, *et al,*[51] suggest volcanic ash at very high concentrations may cause pathologic effects similar to those of quartz.

An important question which arose soon after the first eruption of Mount St. Helens was whether or not volcanic ash exposure increases the risk of lung infections. The studies by Martin, *et al,* and Grose, *et al,* provide evidence that antibacterial defenses against pyogenic organisms and viruses are not impaired by ash exposure.[54,55] Whether or not ash exposure affects cell-mediated immune functions that are important defenses against agents such as mycobacteria remains uncertain.

Despite the number of experimental studies, there is very little information about the likely effects of volcanic ash on airways. The most common use of animal studies has been to study the effects of volcanic ash on the lung parenchyma, e.g., to determine whether granulomas or fibrosis can result after exposure. Potential airway effects such as the induction of bronchospasm and mucous hypersecretion could worsen symptoms in patients with pre-existing chronic lung disease. Two studies have suggested that volcanic ash reduces airway reactivity in animals[37,49] in contrast to findings in humans exposed to inert dusts, that generally increase airway reactivity.[50] Further studies are necessary to better define the effects of ash inhalation on the airways of the lung.

Lastly, the studies summarized here for the most part have examined the effects of volcanic ash in normal animals. An important unanswered question concerns the effects of volcanic ash in animals and humans with pre-existing airway diseases, such as asthma and chronic bronchitis, that alter the deposition and clearance of inhaled particles.

In conclusion, Mount St. Helens has continued to erupt periodically, and major eruptions in Mexico and Indonesia prove that exposure to volcanic ash continues to be a significant worldwide problem. For the first time, a variety of investigations have been conducted to test the biologic effects of volcanic ash, although long-term studies in exposed humans will be necessary to verify data from animal systems. These studies represent a pleuralistic approach to the problem of estimating the pulmonary toxicity of volcanic ash, as investigators have used available methodologies and assay systems to approach the problem as rapidly as possible. An important lesson for future studies is that a careful approach using both inert and toxic control minerals, precise measurements, and a well-defined starting material (e.g., mineralogic analysis and particle sizing) is essential if the results of experimental studies are to be useful in the prediction of possible effects in humans.

The message that emerges from the published studies is that volcanic ash is much less toxic than free crystalline silica in normal lungs. Indeed, volcanic ash may be very similar to an "inert" agent. Humans without lung disease who are exposed to volcanic ash are probably at very little risk for pulmonary toxicity unless the exposure is extremely heavy and prolonged.

Summary

Shortly after Mount St. Helens erupted in 1980, a number of laboratories began to investigate the effects of volcanic ash in a variety of experimental systems in attempts to predict effects that might occur in the lungs of humans exposed to volcanic ash. The published results are remarkably consistent, despite the use of non-uniform ash samples and variability in the experimental approaches used. The data indicate that volcanic ash, even in high concentrations, causes little toxicity to lung cells *in vitro* and *in vivo,* as compared with effects of free crystalline silica, which is known to be highly fibrogenic. Volcanic ash does not appear to be entirely inert, however, possibly because of low concentrations of free crystalline silica in the ash. The published experimental studies suggest that inhaled volcanic ash is not likely to be harmful to the lungs of healthy humans, but the potential effects of volcanic ash in patients with pre-existing lung diseases are more difficult to ascertain from these studies.

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REFERENCES

1. Martin TR, Wehner AP, Butler JB: Pulmonary toxicity of Mount St. Helens volcanic ash. Am Rev Respir Dis 1983; 128:158–162.
2. Craighead JE, Adler KB, Butler GB, *et al:* Health effects of Mount St.

Helens volcanic dust. Lab Invest 1983; 48:5–12.
3. Green GM, Jakab GJ, Low RB, Davis GS: Defense mechanisms of the respiratory membrane. Am Rev Respir Dis 1977; 115:479–513.
4. Heppleston AG: The macrophage in fibrogenesis by quartz. *In:* Dunnom DD (ed): Health Effects of Synthetic Silica Particulates. ASTM STP 732. Philadelphia: American Society for Testing and Materials, 1981; 62–66.
5. Aranyi C, Gardner DE, Huisingh JL: Evaluation of potential inhalation hazard of particulate silicious compounds using *in vitro* rabbit alveolar macrophage tests. *In:* Dunnom DD (ed): Health Effects of Synthetic Silica Particles. ASTM STP 732. Philadelphia: American Society for Testing and Materials, 1981; 48–61.
6. Waters MD, Gardner DE, Aryani C, Coffin DL: Metal toxicity for rabbit alveolar macrophages *in vitro*. Environ Res 1975; 9:32–47.
7. Marks J, Nagleschmidt G: Study of the toxicity of dust with use of the *in vitro* dehydrogenase technique. Arch Int Health 1959; 20:37–43.
8. Allison AC, Harrington JC, Birbeck M: An examination of the cytotoxic effects of silica on macrophages. J Exp Med 1966; 124:141–149.
9. Bitterman PB, Rennard SI, Hunninghake GW, Crystal RG: Human alveolar macrophage growth factor for fibroblasts. J Clin Invest 1982; 70:806–822.
10. Hunninghake GW, Gadek JE, Fales HM, Crystal RG: Human alveolar macrophage-derived chemotactic factor for neutrophils. J Clin Invest 1980; 66:473–483.
11. Martin TR, Altman LC, Albert RK, Henderson WR: Leukotriene B_4 production from human alveolar macrophages: a potential mechanism for amplifying inflammation in the lung. Am Rev Respir Dis 1984; 129:106–111.
12. Martin TR, Ayars G, Butler J, Altman LC: The comparative toxicity of volcanic ash and quartz: effects on cells derived from the airspace of the human lung. Am Rev Respir Dis 1984; 130:778–782.
13. Nolan RP, Lauger AM, Harington JS, Oster G, Selikoff IJ: Quartz hemolysis as related to its surface functionalities. Environ Res 1981; 26:503–520.
14. Hefner RE, Gehring PJ: A comparison of the relative rates of hemolysis induced by various fibrogenic and non-fibrogenic particles with washed rat erythrocytes. Am Ind Hyg Assoc J 1975; 36:734–740.
15. Emerson RJ, Davis GS: Effect of alveolar lining material-coated silica on rat alveolar macrophages. Environ Health Perspect 1983; 51:81–84.
16. Pritchard JN, Holmes A, Evans JC, Evans N, Evans RJ, Morgan A: The distribution of dust in the rat lung following administration by inhalation and by single intratracheal instillation. Environ Res 1985; 36:268–297.
17. Brain JD, Knudson DE, Sorokin SP, Davis MA: Pulmonary distribution of particles given by intratracheal instillation or by aerosol inhalation. Environ Res 1976; 1:13–133.
18. Watson JS, Spritzer AA, Auld JA, Guethoff MA: Deposition and clearance following inhalation and intratracheal injection of particles. Arch Environ Health 1969; 19:51–58.
19. Henderson RF, Rebar AH, Denicola DB: Early damage indicators in the lungs. IV. biochemical and cytologic response of the lungs to lavage with metal salts. Toxicol Appl Pharmacol 1979; 51:129–135.
20. Henderson RF, Rebar AH, Pickrell JA, Newton GJ: Early damage indicators in the lung. III. biochemical and cytological response of the lung to inhaled metal salts. Toxicol Appl Pharmacol 1979; 50:123–126.
21. Kysela B, Jirakova D, Holusa R, Skoda V: The influence of the size of quartz dust particles on the reaction of lung tissue. Am Occup Hyg Assoc J 1973; 16:103–109.
22. Robinson AV, Schneider RP: *In vitro* toxicity of Mount St. Helens volcanic ash: Final report to NIEHS on Task 3, Health Effects of Mount St. Helens Volcano. Richland, WA: Battelle Pacific Northwest Laboratories, 1982.
23. Talivitie NA: Determination of quartz in the presence of silicates using phosphoric acid. Anal Chem 1951; 23:623–629.
24. Green FHY, Bowman L, Castranova V, *et al:* Health implications of the Mount St. Helens eruption: laboratory investigations. Ann Occup Hyg 1982; 26:921–933.
25. Vallyathan V, Mentneck MS, Stettler LE, Dollberg DD, Green FHY: Mount St. Helens' volcanic ash: hemolytic activity. Environ Res 1983; 30:349–360.
26. Castranova V, Bowman L, Shreve JM, *et al:* Volcanic ash: toxicity to isolated lung cells. J Toxicol Environ Health 1982; 9:317–325.
27. Olenchock SA, Mull JC: Volcanic ash and human complement. Life Sciences 1981; 28:791–794.
28. Hahon N, Booth JA, Sepulveda M: Effects of lignite fly ash particulates and soluble components on the interferon system. Environ Res 1983; 32:329–343.
29. Hahon N, Eckert HL: Depression of viral interferon-induction in cell monolayers by asbestos fibers. Environ Res 1976; 11:52–65.
30. Klebanoff SJ: Oxygen metabolism and the toxic properties of phagocytes. Ann Intern Med 1980; 93:480–489.
31. Lieber M, Smith B, Szakal A, *et al:* A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int J Cancer 1976; 17:62–70.
32. Dodson RF, Martin RR, O'Sullivan MF, Hurst GA: *In vitro* response of human pulmonary macrophages to volcanic ash: a morphological study. Exp Molec Pathol 1982; 37:406–412.
33. Schiff LJ, Byrne MM, Elliott SF, Moore SJ, Ketels KV, Graham JA: Response of hamster trachea in organ culture to Mount St. Helens volcano ash. Scan Elec Micro 1981; 11:169–178.
34. Vallyathan V, Robinson V: Comparative *in vitro* cytotoxicity of volcanic ashes from Mount St. Helens, El Chichón, and Galunggung. J Toxicol Environ Health 1984; 14:641–654.
35. Beck BD, Brain JD, Bohannon DE: The pulmonary toxicity of an ash sample from the Mount St. Helens volcano. Exp Lung Res 1982; 2:289–301.
36. Gross P, Harley RA, deTreville RTP: Pulmonary reactions to metallic aluminum powders. Arch Environ Health 1973; 26:227–236.
37. Fedan JS, Ma J, Fromelel G, Mentneck MS: Effect of Mount St. Helens ash on the response of rat trachea to smooth muscle agonists. Environ Res 1981; 26:497–502.
38. Sanders CL, Conklin AW, Adee RR, Rhoads K: Pulmonary toxicity of Mount St. Helens volcanic ash. Environ Res 1982; 27:118–135.
39. Sanders CL, Rhoads K, Mahaffey JA: Long-term reactivity of lung and mediastinal lymph nodes following intratracheal instillation of sandy loam soil or Mount St. Helens volcanic ash. Environ Res 1983; 32:188–198.
40. Vallyathan V, Mentneck MS, Tucker JH, Green FHY: Pulmonary response to Mount St. Helens volcanic ash. Environ Res 1983; 30:361–371.
41. Green FHY, Vallyathan V, Mentneck MS, *et al:* Is volcanic ash a pneumoconiosis risk? Nature 1981; 293:216–217.
42. Raub JA, Hatch GE, Mercer RR, Grady M, Hu PC: Inhalation studies of Mt. St. Helens volcanic ash in animals. II. lung function, biochemistry and histology. Environ Res 1985; 37:72–83.
43. Akematsu T, Dodson RF, Williams MG, Hurst GA: The short-term effects of volcanic ash on the small airways of the respiratory system. Environ Res 1982; 29:358–370.
44. Mossman BT, Kessler JB, Ley BW, Craighead JE: Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab Invest 1977; 36:131–139.
45. Brody AR, Hill LH, Adkins B, O'Connor RW: Chrysotile asbestos inhalation in rats: deposition pattern and reaction of alveolar epithelium and pulmonary macrophages. Am Rev Respir Dis 1981; 123:670–679.
46. Kornbrust DJ, Hatch GE: Effect of silica and volcanic ash on the content of lung alveolar and tissue phospholipids. Environ Res 1984; 35:140–153.
47. Martin TR, Chi EY, Covert DS, *et al:* Comparative effects of inhaled volcanic ash and quartz in rats. Am Rev Respir Dis 1983; 128:144–152.
48. Shirakawa M, Fukushima R, Kyushima K: Experimental studies on the effects of Mt. Sakurajima volcanic ashes on the respiratory organs. Japan J Ind Health 1984; 26:130–146.
49. Wiester MJ, Setzer CJ, Barry BE, Mercer RR, Grady MA: Inhalation studies of Mount St. Helens volcanic ash in animals: respiratory mechanics, airway reactivity and deposition. Environ Res 1985; 36:230–240.
50. Dubois AB, Dautrebande L: Acute effects of breathing inert dust particles and carbechol aerosol on the mechanical characteristics of the lungs in man. J Clin Invest 1958; 37:1746–1754.
51. Wehner AP, Dagle GE, Clark ML: Lung changes in rats inhaling volcanic ash for one year. Am Rev Respir Dis 1983; 128:926–932.
52. Documentation of the Threshold Limit Values for Substances in Workroom Air, 3rd Ed. Cincinnati: American Conference of Governmental Industrial Hygienists, 1971.
53. Wehner AP, Wilkerson CL, Stevens DL: Lung clearance of neutron-activated Mount St. Helens ash in the rat. Environ Res 1984; 35:211–217.
54. Martin TR, Chi EY, Covert DS, *et al:* Comparative effects of volcanic ash, quartz, and aluminum oxide on antibacterial defenses in rat lung. Am Rev Respir Dis 1984; 129:138 (abstract).
55. Grose EC, Grady MA, Illing JW, Daniels MJ, Selgrade MK, Hatch GE: Inhalation studies of Mount St. Helens volcanic ash in animals. III. host defense mechanisms. Environ Res 1985; 37:84–92.
56. Ziskind M, Jones RN, Weil H: Silicosis. Am Rev Respir Dis 1976; 113:643–665.