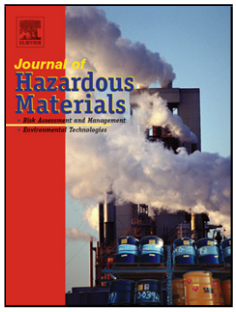

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Authors: Timothy Jones, Kelly BéruBé

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The bioreactivity of the sub-10 micron component of volcanic ash: Soufrière Hills volcano, Montserrat.

Timothy Jones[a1]*, **Kelly BéruBé**[b1]

[a]School of Earth and Ocean Sciences, Cardiff University, Wales, UK, CF10 3YE

[b]School of Biosciences, Cardiff University, Wales, UK, CF10 3US

* Corresponding author tel +44(0)2920 874924 fax +44(0)2920 874326 jonestp@cf.ac.uk

[1] These authors contributed equally to this work.

ABSTRACT

With the recent eruption of the Icelandic volcano Eyafallajökull and resulting ash cloud over much of Europe there was considerable concern about possible respiratory hazards. Volcanic ash can contain minerals that are known human respiratory health hazards such as cristobalite. Short-term ash exposures can cause skin sores, respiratory and ocular irritations and exacerbation of pre-existing lung conditions such as asthma. Long-term occupational level exposures to crystalline silicon dioxide can cause lung inflammation, oedema, fibrosis and cancer. The potential health effects would be dependent on factors including mineralogy, surface chemistry, size, and levels and duration of exposure. Bulk ash from the Soufrière Hills volcano was sourced and inhalable (<2.5μm) ash samples prepared and physicochemically characterised. The fine ash samples were tested for bioreactivity by SDS-PAGE which determined the strength of binding between mineral grains and lung proteins. Selected proteins bound tightly to cristobalite, and bound loosely to other ash components. A positive correlation was seen between the amount of SiO_2 in

the sample and the strength of the binding. The strength of binding is a function of the mineral's bioreactivity, and therefore, a potential geo-biomarker of respiratory risk.

Keywords: Soufrière Hills, volcano, ash, cristobalite, bioreactivity, lung proteins

1. Introduction

When we are threatened with potential respiratory hazards, such as airborne fine volcanic ash, a health risk assessment needs to be undertaken immediately, so that action can be taken and the risk minimised. The fast options available are a geological (usually mineralogical) analysis of the dust and making comparisons with similar dusts, or chemo-biological screening assays (Figure 1). These rapid assays are based on chemical, biological components or cell reactions. Each of these screening assays have their advantages and disadvantages, however, different assays can give quite different results for the same dusts; which is problematic for any meaningful risk assessment. Different assays target different potential causes of adverse health effects, such as generation of Reactive Oxygen Species (ROS), bioreactive particle surfaces, and leachable (e.g. toxic metals) components. In addition to the leachable transition metals, concerns have also been raised about interactions between water-soluble components on particle surfaces and bronchoalveolar fluids [1], with elements such as yttrium and the lanthanides crystallising as phosphates in the interstitial lung spaces. In extreme occupational exposures this can result in dendriform pulmonary ossification forming in the lung [2]. Trace element values for the Montserrat volcanic ash is given in BéruBé et al. [3]. The main concerns with volcanic ash are minerals with bioreactive surfaces, although there are minor concerns about iron levels in some ashes.

The Soufrière Hills volcano is an active stratovolcano found on the Lesser Antilles island of Montserrat. The volcano has been erupting since July 1995 and continues to be

active today [4, 5, 6]. Due to its continued eruptive state it is important to determine the long-term health risks of the volcanic ash [7]. The volcanic activity includes ash and steam venting, occasional pyroclastic flows and explosive phreatic eruptions [8, 9, 10]. The Soufrière Hills volcanic dome lava is predominantly a porphyritic andesite [4] and consists of approximately 45 - 55 wt% phenocrysts (>300μm), 15 - 20 wt% microphenocrysts (300 – 100μm) and 20 – 30 wt% microlites (<100μm) and a residual high silica rhyolite glass (76 – 79% SiO_2) [11]. The phenocrysts predominantly comprise of plagioclase, hornblende, orthopyroxene, titanomagnetite and minor quartz, whereas the microphenocrysts comprise clinopyroxenes, apatite and ilmenite [12]. Recent research suggests that gas-charged magma injection could act as a trigger for dome collapse [13]. The crystalline SiO_2 in the ash includes cristobalite, the high-temperature low-pressure polymorph, and is thought to be the most bioreactive SiO_2 strain as it is most likely to produce surface radicals [14]. When particulate material (PM) 10μm or less in diameter (i.e. PM10), is erupted or remobilised into the atmosphere by natural or anthropogenic disturbance (such as clean-up operations after eruptions), it can be inhaled and lodge into the upper (nose and mouth), lower (thoracic cavity) or distal (alveolar region) respiratory tracts (RT; Figure 2). Coarse particles (2.5 to 10μm) are only able to penetrate into the extra-thoracic region of the upper RT. Finer particles (2.5 to 1μm), readily translocate into the lower RT, whereas ultrafine particles (<1μm) can penetrate even deeper into the lung, reaching the alveoli, where oxygen and carbon dioxide exchange [15].

In the human lung, the first line of defence against inhaled PM such as volcanic ash is the specialised epithelial surface of the conducting airways [16, 17]. This is coated in epithelial lining fluid (ELF) containing defence proteins (e.g. surfactants, mucous, anti-oxidants and anti-bacterial molecules). It is these molecules that either react against, or bind to, the surface of any foreign bodies [18]. The strength of the response of these chemicals can be seen as a measure of the bioreactivity of the respired particle [19].

In the lower RT, bronchial epithelial cells (e.g. ciliated cells) also have hair-like cilia, which beats a layer of mucus (adhesive glycoprotein secreted by goblet cells) that encapsulates the dust particles and carries them up the pharynx by the muco-ciliary escalator to be swallowed (Figure 2). In the distal RT (i.e. alveolar region) mobile cells called alveolar macrophages are the main defence against airborne particles (Figure 2) [17, 20]. These cells are responsible for the phagocytosis (ingestion) of micro-organisms and PM that has been inhaled.

For PM that is highly-respirable and able to deposit into the distal lung region, the first visible damage response takes place at the alveolar surface with abnormal leakage of fluid into the alveolar gas spaces. Swelling (oedema), the first stage of inflammation, is caused by secretions from damaged cells and release of inflammatory mediators (e.g. cytokines and chemokines) into the alveolar air spaces. Inflammation may become chronic and this can lead to increased proliferation of epithelial cells (i.e. hyperplasia) and in the longer-term, pulmonary fibrosis or emphysema (i.e. COPD [Chronic Obstructive Pulmonary Disease] through metaplasia (i.e. abnormal transformation of columnar cells into squamous cells; squamous metaplasia) of the airway epithelia [21, 22, 23].

It has been established that crystalline silica is more harmful in the lung when the particles have 'fresh' surfaces created by fracturing, resulting in a charged surface [24]. Thus, the highly attritional and corrosive environment of an erupting eruption should in theory generate particles with reactive surfaces that would interact with the lung cells and fluids. The alveolar macrophages, which are involved in cleaning-up of the lung, perform phagocytosis and 'ingest' the ash particles. However, cristobalite is highly cytotoxic for the macrophages [21] and leads to the release of ROS causing hydroxyl (·OH) and oxygen ($\cdot O_2^-$) radicals to form. These radicals can damage DNA in the cells of the lung and cause mutations [22] The

potential for damage being caused to the lung is increased with prolonged exposures to the bioreactive components.

Despite the recognised disease pathways, to date, few significant adverse health effects from volcanic ash have been seen in the inhabitants of Montserrat, and despite many investigations few studies have shown significant bioreactivity. Sang Hee and Richards [25] noted that the volcanic ash produces inflammatory reactions in lymph nodes at 13 weeks after instillation in rats, and lung inflammation is delayed until 49 weeks post-exposure. However, despite the cristobalite content there is no evidence of lung fibrogenic responses. This is generally mirrored in health studies of other volcanic ashes Worldwide, with the significant exception of Newnham et al. [26] whose epidemiological research on the Mt Ruapehu (NZ) eruption of 1996 suggested that the diffuse fine ash component could present a hazard even at large distances from the volcano. There is also the caveat that the eruption at Montserrat has continued for an unusually long period [6]; subjecting the locals to a long-term exposure to respirable ash, and this could have unknown adverse health effects. The conclusion from many researchers is the fortuitous situation that respired volcanic ash does not appear to be anywhere near as bioreactive as it should be based on the mineral components [3]; but we do not know why. The integration of results from geological analysis and bioreactivity assays is central if we are ever to properly understand the potential bioreactivity of volcanic ash; and achieving a genuine understanding of ash/lung interactions and human safety assessments (Figure 1).

2. Methods

2.1 Geology methods

Since it was not logistically practical to collect sufficient quantities of respirable airborne volcanic ash, methods were adopted that best reproduced the physicochemical characteristics of freshly erupted or re-suspended ash. Crushing or corrosive chemicals were not used during the preparation of the samples, as these could have altered the bioreactivity. Bulk volcanic ash samples were collected from Montserrat in 1998 at seven locations; Trants, Farm River Gorge, Dyers Bridge, Tar River Valley, Lovers Lane, the American Medical School and Olveston. The ash was collected by government scientists in response to the British Government concerns over adverse respiratory health effects, and was distributed to laboratories UK-wide; including Cardiff University. The locations chosen will have been selected on access and safety grounds. A comparison of the geochemistry of the different samples shows some moderate variation, and as such they are believed to be a representative selection of ash compositions. The bulk ash samples were collected off the ground, and therefore consisted of both non-respirable larger fragments and fine respirable dusts. The analytical work was done between 1999 and 2005, with the ash being stored in dry air-tight containers. The respirable constituents were separated from the coarse components by a dry re-suspension filtration system. This comprised a rotating horizontal drum with a baffle, able to process approximately 400g of sample [27]. The re-suspended dust was extracted from the drum by an air flow of five litres/minute; a rate calibrated to collected particles of 2.5 microns or less. The respirable dust was finally collected inside a Negretti PM10 Head [28] on a polycarbonate filter. This method avoids the need to use wet cyclones or sedimentation in water. Figure 3 shows two scanning electron micrographs of the separated respirable component. Virtually all the grains are less than 4 microns, with the vast majority less than 2.5 microns (Figure 3a). An analysis of grain shapes is given in BeruBe et al. [3], with the grains sub-angular to angular, with an aspect ratio of 1:1.7. There is no indication of fibrous habits (aspect ratios exceeding 1:3) in any of the grains. Figure 3b shows some larger grains

of ash with much smaller volcanic nanoparticles [26] adhering to their surface. Nanoparticles are defined as being less than 100 nanometres, and are currently a major respiratory health concern [29]. The percentage of cristobalite in the samples was determined following the methods of Baxter et al. [4] using wet chemistry, electron probe microanalysis (EPMA) and X-ray diffraction. The values obtained for our samples and those of Baxter et al. [4] from the same location, American School, showed close agreement. A pure cristobalite sample was obtained from a glass manufacturer; the cristobalite forming in the splash zone around the glass furnace [30].

The bulk and respirable components of the samples were analysed for major and minor trace elements by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). ICP-OES preparation involved a 900^{o}C loss of ignition (LOI) procedure to release volatiles such as H_2O and CO_2. Solutions were prepared using a FLUXY fusion system and analysed for Si, Ti, Al, Fe, Mg, Mn, Ca, K, Na and P major elements as oxides and Ni, Cu, Co, Cr, Ba, Sr, Zr, Y, Sc, and V as minor elements using a JY Horiba ULTIMA2 ICP-OES. The system was calibrated against known reference materials JB1a, BHVO1-a and NIM-G and corrections for drift made using an internal spike of Rh. In this manuscript the sample collected at Olveston is used as the example for the bioreactivity assay. This sample was chosen following the geochemical analyses as it was determined that the mineral values were 'mid-range' and as such it could be considered to represent a 'typical' ash from the volcano.

2.2 Bioreactivity methods

The SDS-PAGE (sodium dodecyl sulfate – polyacrylamide gel electrophoresis) bioreactivity assay is based on the adsorptive capacity of specific mineral dusts to mixtures of organic macromolecules such as lung proteins. Initial research [19] concentrated on asbestos

dusts, and the 'strong' adsorption of serum protein to specific minerals was considered to be the most important physiologically.

Rat lung lavage fluid (BALF: Bronchio Alveolar Lung Fluid) was prepared by washing out the lungs of healthy rats with sterile saline solution, according to the methods previously described in BéruBé et al., [3]. Ten milligrams of each volcanic ash sample and the positive control cristobalite were incubated for 24hrs in 0.5ml of lavage fluid; the negative control was pure and particle-free BALF. The samples were then agitated for 30 minutes at room temperature, followed by centrifuging for 20 minutes at 1300g (4000rpm). The supernatant was discarded so no non-specific binding protein remained. The remaining pellet was re-suspended in 0.5ml of running buffer before the same agitation (30 minutes) and centrifuging at 1300g for 20 minutes. The supernatant from this stage was collected and labelled as a loosely-bound protein sample (i.e. L1, L2, L3). A further round of agitation (30 minutes) and centrifuging (20 minutes at 1300g) in 0.5ml of running buffer was carried out and the supernatant from this final stage was labelled as tightly-bound proteins (i.e. T1, T2, T3).

SDS-PAGE [19] was undertaken on all the loosely and tightly bound protein with ash and cristobalite samples and the lavage and marker proteins. Twenty microlitres of each sample was run alongside 20µl of a molecular weight marker that corresponded to the range of known proteins in rat BALF, and 20µl of the initial whole lavage to observe differences in protein content and molecular weight of bands resolved following gel electrophoresis. A Mighty Small, Hoefer Scientific Gel former with a Mighty Small SE245 Dual caster was used to form the gels and a Mighty Small SE250 Dual Gel Tank for electrophoresis. After electrophoresis the gels were stained with silver nitrate (Invitrogen, UK) to show the protein bands within the gel. This involved four stages of fixative enhancer, washing, staining and

stopping. The gels were imaged using an Amersham Image Scanner and the images saved as TIFF files for further analysis.

3. Results

3.1 Geological results

The ICP-OES results for the respirable component (Table 1) demonstrated that all samples had characteristic rhyolitic compositions with moderately high SiO_2 and Al_2O_3, with the exception of the Lovers Lane sample, which exhibited the lowest SiO_2 and K_2O that was more indicative of an evolved andesitic source. The SiO_2 levels were comparable to the values of Baxter *et al*. [4] for PM <10μm explosion ash falls and pyroclastic flow samples. The data also determined that all the ash samples were depleted in Al_2O_3 and Fe_2O_3 relative to the bulk ash results (Table 1). Cristobalite values ranged from 16.3% (Olveston; mixed ash) to 31.1% (Farm River Gorge; dome collapse ash). Generally, the dome collapse/pyroclastic flow material contained more cristobalite and higher levels of plagioclase than the mixed ash and pumice. Other minerals of note were the iron silicates, which have been thought to act as a catalyst and reactivity enhancer achieved through the Hyber-Weiss cycle [31].

3.2 Bioreactivity results

The results of the SDS-PAGE analysis are shown in Table 1; with two columns showing the numbers of loosely-bound and tightly-bound bands. The cristobalite positive control was run with every ash sample. An example of one of the gels, for the ash collected at Olveston, is shown in Figure 4a, with a graphic interpretation in Figure 4b. The first column (M) shows the controls' protein marker weights in kDa. The BALF sample (B), without volcanic ash, and therefore the bioreactivity negative control, produced 18 protein bands. The cristobalite sample, the bioreactivity positive control, generated 4 loosely-bound (L_{cr}) bands

and 1 tightly-bound (T_{cr}) band. The Olveston volcanic ash had an average of 11.7 (13, 13 and 9 as a triplicate) loosely-bound (L2), and an average 1 (1, 1 and 1 as a triplicate) tightly-bound (T2). The tightly-bound band for the cristobalite corresponded with the tightly-bound band for the volcanic ash.

All of the volcanic ash samples created decreased banding when compared to BALF (Table 1). The cristobalite standard yielded a loosely-bound maximum of 5 and a minimum of 2, tightly-bound maximum of 2 and minimum 0 bands. All of the volcanic ash samples showed a significant decrease between the loosely-bound (maximum 13, minimum 5) and tightly-bound (maximum 3, minimum 0) bands. Using the protein weight markers on the gels, bands can be tentatively linked to certain proteins [32]; although more advanced 2D proteomic gels are required for more conclusive protein identification. There is strong banding at 75kDa for the loose bound samples, and to a lesser degree for the tightly bound samples. Banding at this molecular weight can be attributed to proteins such as Albumin, α-1 β Glycoprotein, Complement C3, Hemopexin and $_{L}$-Plastin [32].

4. Discussion and conclusions

The geochemistry indicated that the respirable fraction in Montserrat volcanic ash contained high levels of silica dioxide when compared to whole rock averages (Table 1); agreeing with the results of previous investigators [4]. Conversely some of the other major element oxides are depleted; most notably iron oxide. This enrichment of silica dioxide in the respirable fraction is probably the result of physical fractionation and crushing, resulting in a process of particle size separation within the settling ash clouds [12, 33, 34]. This process possibly being enhanced by the relative 'crushability' of cristobalite, compared with other minerals in the ash. The lava domes, reaching temperatures up to 800^{o}C, build over time until gas-charged magma injection, over-loading or seismicity results in collapses generating

pyroclastic flows and associated fine ash clouds. The time-span of the domes before collapse is important, as during this time devitrification can occur, converting amorphous glassy lava into crystalline matter by slow cooling and vapour-phase crystallisation [4]; this results in the formation of cristobalite. The mineralogical composition of the ash is therefore a function of the original composition of the magma, the amount of time and conditions available for devitrification, and the physical parameters of the flow or explosion. For example, the Farm River Gorge ash sample was produced in a large dome collapse and resulting pyroclastic flow, and contained 31.1% cristobalite and 66.92 wt % SiO_2.

Taking the Olveston site ash as a typical example, an average of 11.7 proteins, out of a possible 18 proteins (found in BALF), loosely binds onto the surface of the ash particles (Figures 4a and 4b). Of those 11.7, one protein, band 5, also tightly binds. Protein 5 also loosely and tightly binds with the positive control cristobalite. If we take the strength of the binding as an indication of the degree of bioreactivity, then loosely-bound proteins can be considered to be weakly bioreactive and tightly-bound proteins strongly bioreactive (Figure 5). The tightly-bound proteins are therefore also indicators of possible respiratory toxicity as cristobalite is an established respiratory hazard (Figure 6). However, given that a maximum of two proteins tightly-bind to the ash, they are not that useful for predicting the level of toxicity. On the other hand, with a range of 12 to 5 (Figure 4) proteins loosely binding to the ash, they show a variance that might be more useful in establishing lower-level toxicity. Overall, the comparison of the number of protein bands against the ash SiO_2 content (wt %) shows a correlation for both the tightly and loosely-bound proteins; however the correlation for the loosely-bound proteins is not statistically significant. The correlation with the tightly-bound proteins to SiO_2 has a higher R^2 value, due to the much lower number of bands.

These results have possible implications for the role that iron might play in bioreactivity, with Horwell et al. [8] suggesting that iron radicals soak up the loose bonds produced during crystal fracturing. As the amount of SiO_2 is enriched in the fine ash component (Table 1, +2.73% to +9.04%), the Fe_2O_3 component is correspondingly decreased (Table 1, -1.56% to -2.61%). This means for the SDS-PAGE assay, there is a correlation between a decrease in iron and increase in bioreactivity. This supports the suggestion of Horwell et al [8] about the possible role of iron, but also it could simply mean that the bioreactivity is related to the SiO_2 and for that component to increase the other ash components must decrease.

The interaction between the mineral grains and the lung proteins is clearly part of the lung's defence mechanisms, but raises questions about the outcome of that role. The binding between the charged surface of crystalline SiO_2 particles and polar lung proteins is clearly 'bioreactivity'. As outlined earlier, the reactive surfaces of the mineral grains interact with the lung's cells, fluids and cells (e.g. macrophages [mild inflammation] and neutrophils [severe inflammation]) leading to damage of the epithelium and the release of radicals, initiating disease pathways (Figure 7). The respiratory epithelium that lines the airways of the lung is the primary site of initial exposure to inhaled particulate matter. The primary non-enzymatic, water-soluble antioxidants uric acid (UA), ascorbic acid (AA) and reduced glutathione (GSH) are the ROS-scavenging molecules vital in protecting the underlying epithelial tissue from cellular damage [35]. They are the first line of defence against inhaled xenobiotics which may be oxidative, and the respiratory tract mucosal surfaces are lined with a rich milieu of antioxidants. If the antioxidant defences are overwhelmed or depleted, for example, during high-dose chronic exposures (Figure 7), the excess ROS can oxidise carbohydrates, (CHO) lipids, and protein, resulting in a wounded epithelium (i.e. dedifferentiation of epithelial cells to produce a squamous cell covering to maintain barrier

integrity), with concomitant leakage of cellular products onto the surface. The activation of redox-sensitive transcription factors (e.g. NFκB and AP-1) propagates a severe cellular inflammatory response leading to cytokine production and recruitment of inflammatory cells, in the form of neutrophils [15]. Repeated insults or a delay in the repair of the epithelium results in 'squamous metaplasia' that becomes irreversible, leading to pulmonary fibrosis. In contrast, low-dose acute exposures that do not diminish antioxidant defences would be able to ameliorate excess ROS, along with the recruitment of macrophages (i.e. mild inflammation) for assistance to limit injury. Consequently, the respiratory epithelial barrier integrity would be maintained.

It has been suggested [19] that the bound proteins on the surface of the mineral grains act like a protective organic sheath, isolating the grains from vulnerable lung cell surfaces and minimising the release of potentially harmful radicals. If this hypothesis is correct, then it appears to support some evidence that ash exposure is potentially harmful to people with pre-existing lung conditions (Chronic Obstructive Pulmonary Disease [COPD], lung infections or asthma), whose antioxidant pools are diminished, and therefore have compromised defence mechanisms [36]. There is also the consideration that even people with healthy lungs who are exposed to levels of mineral grains that exceed the available defence proteins (i.e. lung overload), or longer-term (i.e. chronic) exposures that have depleted those proteins, are more likely to be vulnerable to adverse health effects [37] (Figure 8).

Overall the question still remains, why is the respirable ash generated by the Montserrat eruption not as toxic as indicated by the mineralogical content, specifically the cristobalite [3]? Although this is good news for people exposed to the ash, it is important to try and understand the lack of bioreactivity. In the short-term, as a rapid response to a crisis, the application of geological proxies such as crystalline SiO_2 content to determine the human safety risk assessments might be acceptable (Figure 1). In the longer-term, only a proper

understanding of the potential disease pathways and geo-biological causes will enable a more reliable risk assessment.

In conclusion, these results show the clear advantages of rapid biological screening assays for the risk assessment of potentially harmful mineral dusts. Specifically, the SDS-PAGE offers an insight into the bioreactivity between specific minerals and lung proteins, which is critical for predictions of adverse health effects. It is possible to make the SDS-PAGE more accessible to potential users (geologists and biologists alike), by using artificially-created BALFs and ready-made consumables (e.g. pre-cast gels and pre-mixed reagents). The role in the lung of known bioreactive minerals, such as cristobalite, appears crucial, however, aspects of the disease mechanistic pathways, mineral-lung interactions, and individuals' susceptibility (e.g. pre-disposing genetic factors) to mineral dusts, remains unclear.

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Highlights

- Human lung proteins adhere to inhaled mineral grains.
- Specific proteins have been recognised that tightly adhere to the recognised hazardous mineral cristobalite; a potential geo-biomarkers of respiratory harm.
- A range of lung proteins loosely adhere to 'non-toxic' volcanic ash minerals; however the effects of long-term ash exposures are unknown.
- There is a positive correlation between Si content and level of protein adherence.
- SDS-PAGE is a rapid bioreactivity assay, generating results to be imported into human health risk assessments.

Sample	Size	SiO_2	Al_2O_3	Fe_2O_3	Na_2O	K_2O	TiO_2	L-bound	T-bound
Olveston Mixed Ash (16.3% Cristobalite)	$PM_{2.5}$	65.12	15.41	4.97	3.29	0.89	0.35	13 13 9	1 1 1
Trants Pumice (22.3% Cristobalite)	$PM_{2.5}$	68.42	14.58	4.07	3.33	1.29	0.34	6 5 5	0 0 0
Farm River Gorge Dome Collapse Ash (31.1% Cristobalite)	$PM_{2.5}$	66.92	16.47	5.70	4.13	1.12	0.45	6 6 7	1 1 1
Dyers Bridge Dome Collapse Ash	$PM_{2.5}$	65.12	15.59	4.87	3.33	0.92	0.35	5 5 6	3 1 1
Tar River Valley Pyroclastic Flow (28.1% Cristobalite)	$PM_{2.5}$	65.84	14.83	5.13	3.26	0.99	0.37	8 8 8	2 2 2
Lovers Lane Dome Collapse Ash (21.6% Cristobalite)	$PM_{2.5}$	63.97	15.54	5.90	3.15	0.80	0.41	6 6 8	3 2 2
AMS Court Yard Mixed Ash (13.5% Cristobalite)	$PM_{2.5}$	65.34	14.90	5.05	3.22	0.88	0.35	12 10 9	2 2 2
Average $PM_{2.5}$ Ash (n-8) (22.2% cristobalite)	$PM_{2.5}$	65.82	15.33	5.10	3.39	0.98	0.37	n/a	n/a
Average Ash (n=8)	Bulk	61.29	17.72	6.66	2.51	0.86	0.58	n/a	n/a
$PM_{2.5}$:Bulk fractionation		**+4.53**	-1.89	**-1.56**	+0.88	+0.12	-0.21	n/a	n/a
Pyroclastic Flow Ash*	PM_{10}	67.39	16.69	3.29	4.44	1.48	0.24	n/a	n/a
Pyroclastic Flow Ash*	Bulk	64.66	16.23	5.47	3.63	1.04	0.51	n/a	n/a
PM_{10}:Bulk fractionation*		**+2.73**	+0.46	**-2.18**	+0.81	+0.44	-0.27	n/a	n/a
Explosive Ash Fall*	PM_{10}	69.84	14.60	3.47	4.13	1.69	0.33	n/a	n/a
Explosive Ash Fall*	Bulk	60.80	18.04	6.08	3.55	0.86	0.58	n/a	n/a
PM_{10}:Bulk fractionation*		**+9.04**	-3.44	**-2.61**	+0.58	+0.83	-0.25	n/a	n/a

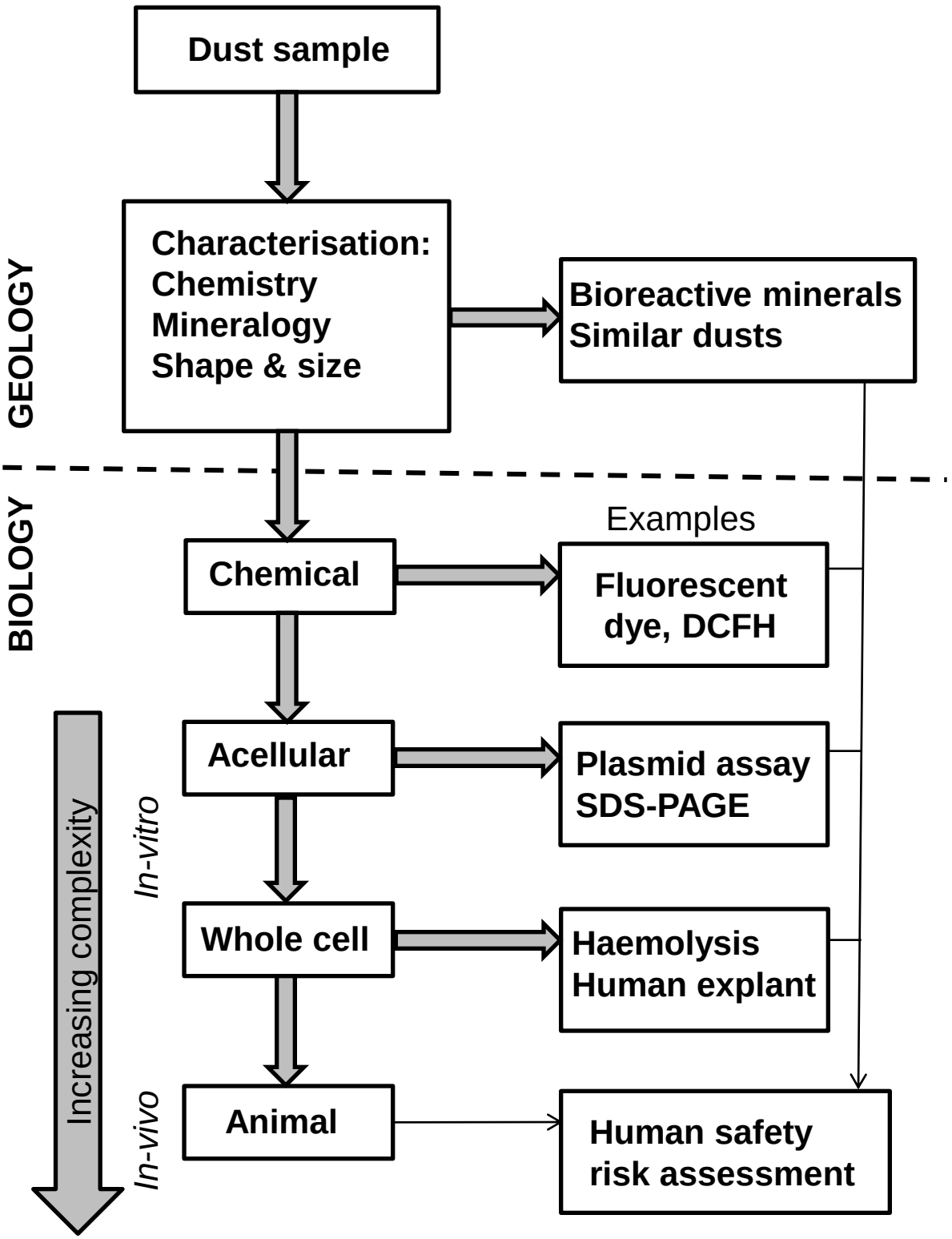
Table 1.

ICP-OES results for the Montserrat volcanic ash samples of 2.5μm size fraction compared to XRF data (taken from Baxter et al., 1999)* of andesitic ash from the Soufriere Hills Volcano. The results are given as oxides in weight %. All data shows a enrichment of SiO_2 and a depletion of Fe_2O_3 in the PM fraction (highlighted).

L-bound = number of loosely-bound proteins. T-bound = number of tightly-bound proteins. Column 3 highlighted = SiO_2 enrichment in PM. Column 5 highlighted = Fe_2O_3 depletion in PM.

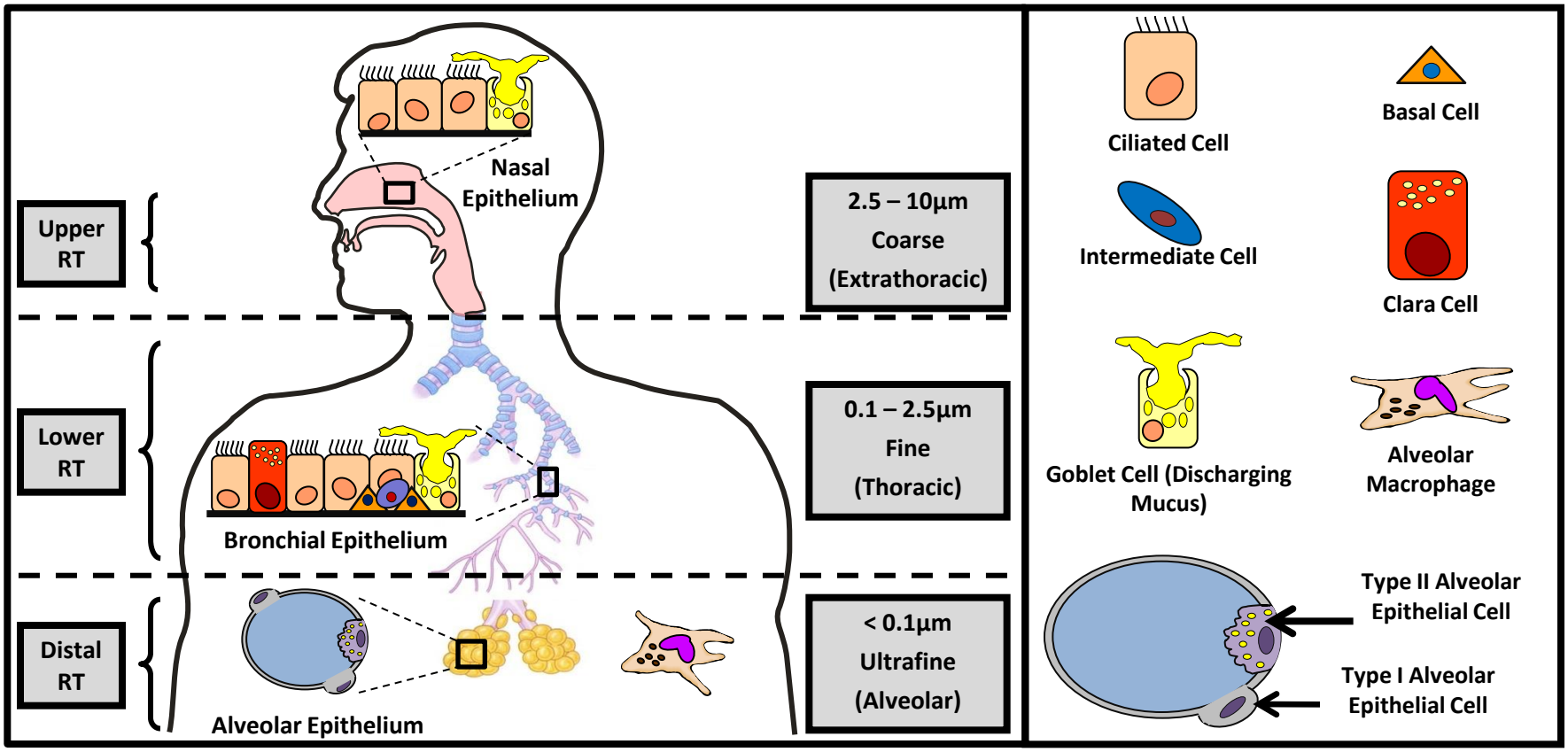
Figure(s)

Figure 1: A flow diagram of the pathways to assess mineral dust bioreactivity and human safety risk assessment



Figure(s)

Figure 2: Human respiratory tract



Figure(s)

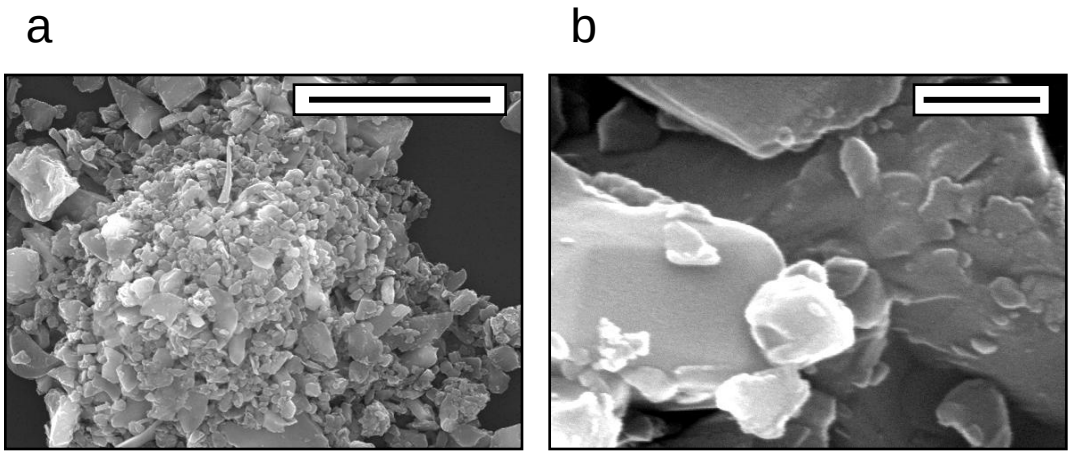
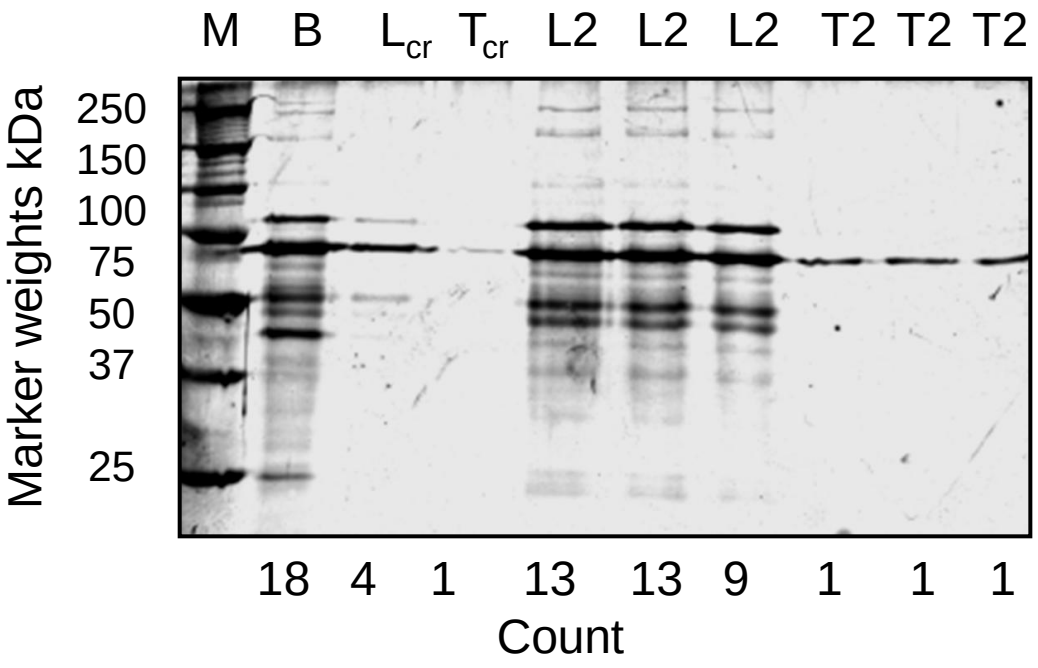


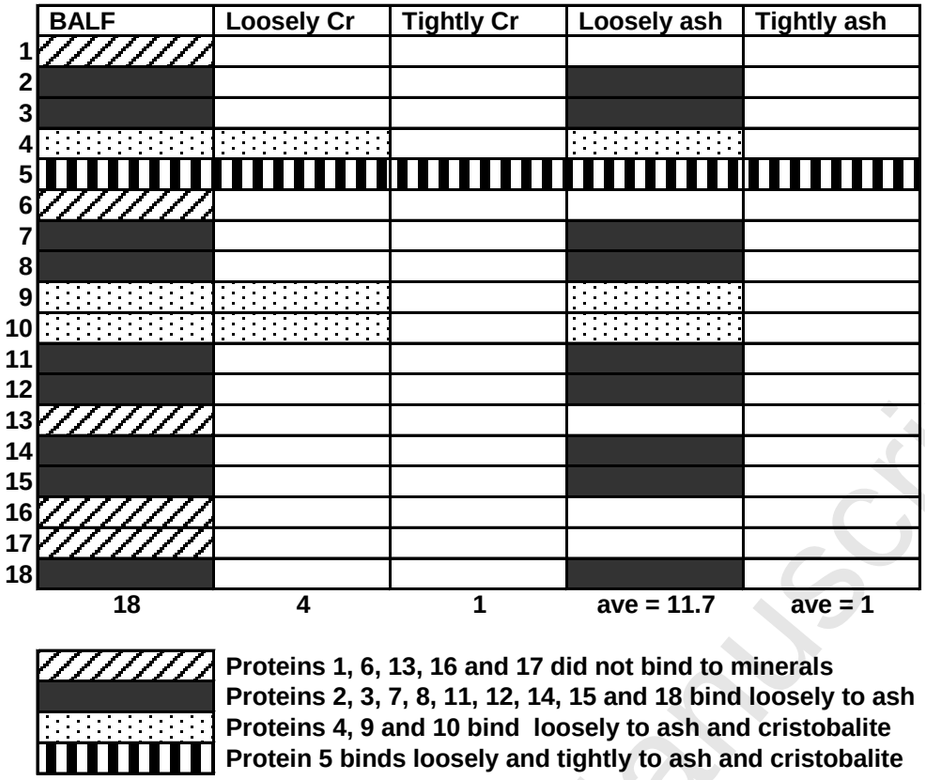
Figure 3: Scanning electron micrographs of the size fractionated $PM_{2.5}$ volcanic ash. (a) All the particles are less than 4 microns. Scale bar 10 microns. (b) The ash contains some larger particles with sub-micron particles adhering to the surface, including some less than 100 nanometres that can be classified as volcanic nanoparticles. Scale bar 500 nm.

Figure(s)

Figure 4a



4a. Olveston site volcanic ash sample showing gel banding detail. M = marker weights in kDa: B = bronchio-alveolar lung fluid; L_{cr} = loosely-bound cristobalite; T_{cr} = tightly-bound cristobalite; L2 = loosely-bound Olveston sample run in triplicate; T2 = tightly-bound Olveston sample run in triplicate.



4b. Graphic interpretation of the gel lines (Figure 3a). Ave values ash, n=3.

Figure(s)

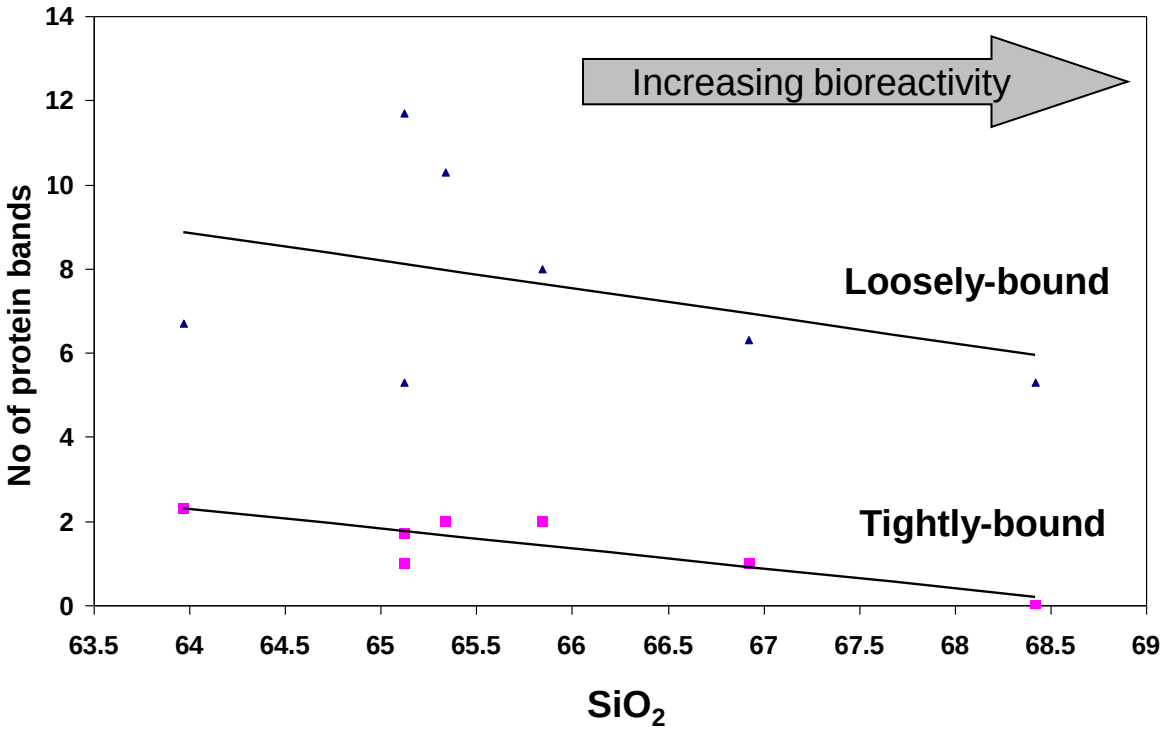


Figure 5: Graph showing correlation of silica dioxide content (wt%) with number of loose and tight binding proteins. Tightly-bound proteins $R^2 = 0.7215$, loosely-bound proteins $R^2 = 0.1462$. As the percentage of SiO_2 increases, then less bands are seen in the supernatant, indicating that more proteins have bound to the surface of the grains.

Figure(s)

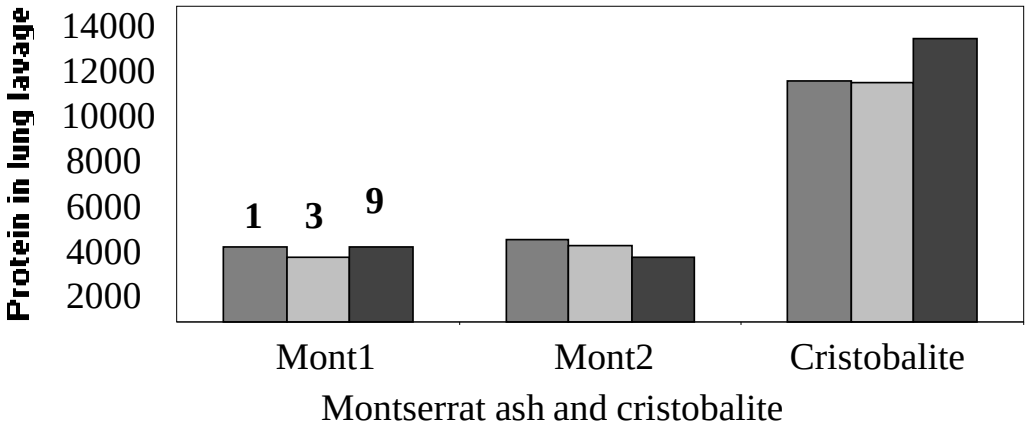


Figure 6: Data from BéruBé *et al*., [3]. Volcanic ash and a cristobalite standard instilled into rat lung; 1, 3 and 9 weeks. The lungs instilled with cristobalite have approximately three times the levels of protein in the lavage fluid. This protein could either be generated as part of the lung's defence mechanism (secretion of surfactant) or be proteins released from damaged epithelial cells.

Figure 7: Simplified mechanism of oxidative stress and biochemical response in the airway following exposure to particulate matter (e.g. volcanic ash).