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# Physicochemical and toxicological profiling of ash from the 2010 and 2011 eruptions of Eyjafjallajökull and Grímsvötn volcanoes, Iceland using a rapid respiratory hazard assessment protocol ☆

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ABSTRACT

The six week eruption of Eyjafjallajökull volcano in 2010 produced heavy ash fall in a sparsely populated area of southern and south eastern Iceland and disrupted European commercial flights for at least 6 days. We adopted a protocol for the rapid analysis of volcanic ash particles, for the purpose of informing respiratory health risk assessments. Ash collected from deposits underwent a multi-laboratory physicochemical and toxicological investigation of their mineralogical parameters associated with bio-reactivity, and selected *in vitro* toxicology assays related to pulmonary inflammatory responses. Ash from the eruption of Grímsvötn, Iceland, in 2011 was also studied. The results were benchmarked against ash from Soufrière Hills volcano, Montserrat, which has been extensively studied since the onset of eruptive activity in 1995.

For Eyjafjallajökull, the grain size distributions were variable: 2–13 vol% of the bulk samples were $<4\,\mu m$, with the most explosive phases of the eruption generating abundant respirable particulate matter. In contrast, the Grímsvötn ash was almost uniformly coarse ($<3.5$ vol% $<4\,\mu m$ material). Surface area ranged from 0.3 to 7.7 $m^2\,g^{-1}$ for Eyjafjallajökull but was very low for Grímsvötn ($<0.6\,m^2\,g^{-1}$). There were few fibre-like particles (which were unrelated to asbestos) and the crystalline silica content was negligible in both eruptions, whereas Soufrière Hills ash was cristobalite-rich with a known potential to cause silicosis. All samples displayed a low ability to deplete lung antioxidant defences, showed little haemolysis and low acute cytotoxicity in human alveolar type-1 like epithelial cells (TT1). However, cell-free tests showed substantial hydroxyl radical generation in the presence of hydrogen peroxide for Grímsvötn samples, as expected for basaltic, Fe-rich ash. Cellular mediators MCP-1, IL-6, and IL-8 showed chronic pro-inflammatory responses in Eyjafjallajökull, Grímsvötn and Soufrière Hills samples, despite substantial differences in the sample mineralogy and eruptive styles.

The value of the pro-inflammatory profiles in differentiating the potential respiratory health hazard of volcanic ashes remains uncertain in a protocol designed to inform public health risk assessment, and further research on their role in volcanic crises is warranted.

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## 1. Introduction

In April 2010, the UK and Europe suffered unprecedented disruption to commercial aviation due to the sudden eruption of Eyjafjallajökull volcano in Iceland. A year later, a second Icelandic volcano, Grímsvötn, erupted and, again, volcanic ash affected northern Europe. Volcanologists believe that the eruptions heralded a predicted period of increased volcanic activity in Iceland, which occurs within a 130–140 year cycle (Larsen et al., 1998). In addition to air traffic disruption and its economic consequences, Iceland and Europe became concerned about the potential impacts of any ashfall on animal health, including chemical contamination of pasture and crops, and were unprepared for assessing and informing the public of the potential human health hazard. Whilst the Grímsvötn eruption only lasted a week, the Eyjafjallajökull eruption was sustained for five and a half weeks and was followed by significant re-suspension of the ash by wind and human activity in the local farmed area, leading to particular concern regarding the respiratory hazard from ash inhalation.

Despite drawing on experience at an increasing number of global eruptions, the small number of medical and epidemiological investigations of human and animal health that have been undertaken means the evidence base on respiratory health effects of ash inhalation remains relatively sparse. Acute clinical manifestations in communities affected by ashfalls have included asthmatic and bronchitic symptoms, with those people suffering from pre-existing chronic lung conditions being the most vulnerable and likely to suffer exacerbations of their conditions (Baxter et al., 1981, 1983; Forbes et al., 2003). Some volcanic eruptions generate ash with abundant crystalline silica in the form of cristobalite, quartz and tridymite, which may present a chronic hazard of fibrotic lung disease (e.g., silicosis or mixed dust fibrosis) in continuously and heavily exposed groups (Baxter et al., 1999). This is a well-recognised disease process in workers exposed to siliceous dusts in certain industries, but has not yet been documented in areas of active volcanism (Baxter et al., in press; Buist et al., 1986; Yano et al., 1986).

Information pamphlets on the health effects of volcanic ash and advice on preventive measures are available through the International Volcanic Health Hazard Network (www.ivhhn.org), but are not specific to individual volcanoes, eruption styles or magma types. At the onset of the Eyjafjallajökull eruption, Icelandic agencies rapidly translated the IVHHN pamphlets for public distribution, to provide basic information on the respiratory hazard of volcanic ash.

### 1.1. IVHHN rapid analysis protocol

To offset the limited number of health studies on volcanic ash, and the time taken to carry out new studies, we developed a laboratory protocol (Fig. 1) for rapid ash analysis to determine whether it is possible to identify physicochemical features and inflammatory biomarkers which might provide an early indication of the potential acute and chronic respiratory hazard to populations needing urgent advice following an eruption. The protocol involves physicochemical and toxicological analyses which assess health-pertinent characteristics of the ash and likely biological interactions. It also allows rapid screening of numerous ash samples which may have varying pathogenic properties, depending on eruption style. *In vitro* tests are only general indicators of reactivity *in vivo* but could be useful for rapidly comparing the pro-inflammatory potential of different types of freshly-erupted volcanic ash.

The protocol was first applied during the 2007-8 eruption of Rabaul volcano, Papua New Guinea and has been used since during eruption crises at Kilauea (Hawai'i, 2008), Chaitén (Chile, 2008), Merapi (Indonesia, 2010) and Puyehue Cordón Caulle (Chile, 2011-12) (Damby et al., 2013; Horwell et al., 2010a; Le Blond et al., 2010) as well as Iceland in 2010 and 2011 (present study). In addition, the methods have been further tested and refined on ash from a number of other volcanoes (e.g. Vesuvius and Etna, Italy, Soufrière Hills, Montserrat, and Sakurajima, Japan) (Hillman et al., 2012; Horwell et al., 2010b). The protocol embodies recent developments in methodologies for ash analysis (e.g., Horwell, 2007; Horwell et al., 2007; Le Blond et al., 2009) and undergoes revision as new techniques become available or are deemed more informative. The protocol is divided into phases, allowing immediate dissemination of data for decisions on the need for respiratory protection, with later dissemination of a report on the full analyses usually within a month of sample receipt. Data on individual analyses such as crystalline silica content may be disseminated prior to the final report if warranted.

For the protocol, we adopt a suite of selected physicochemical analyses and *in vitro* assays. The physicochemical characteristics of the ash may vary widely among volcanoes with different magma composition and eruptive styles; in particular, in the proportion of respiratory-sized particles and their crystalline silica content (Horwell, 2007; Horwell and Baxter, 2006). In addition to particle size, large specific surface area is known to lead to increased biological reactivity (Duffin et al., 2002), as is surface free-radical (hydroxyl) generation and oxidative potential in lung lining fluid, all of which can be tested rapidly, in cell-free *in vitro* analyses (Ayres et al., 2008; Fubini et al., 1995). Iron-catalysed hydroxyl radical generation is a potential lung inflammation factor and a carcinogenic factor (Hardy and Aust, 1995; Kane, 1996). The depletion of antioxidants in the presence of particles may have special significance in triggering airway inflammation in asthma sufferers (Kelly et al., 1999).

The human erythrocyte haemolysis test is a well-tried means of measuring the interaction of the particle surface with a red blood cell membrane, and it may have value as an *in vitro* predictor of *in vivo* pathogenicity of crystalline silica (Clouter et al., 2001).

To establish the cellular reactivity of volcanic ash, the cytotoxic and acute and chronic inflammatory potential responses of human lung epithelial type 1-like (TT1) cells were also assessed as part of the protocol. Type-1 alveolar epithelial cells cover $>95$ % of the alveolar surface area and are a major target for respirable material in the lung. The well-characterised (Swain et al., 2010; van den Bogaard et al., 2009) lung epithelial type 1-like cells used in this study are human-derived and recently immortalised (Kemp et al., 2008) to provide a unique model of this important particle deposition site. This model has been used by us previously, for example, to reproducibly confirm the highly-toxic potential of positively-charged polystyrene latex nanoparticles and the comparatively low toxic potential of their neutral and negatively charged counterparts (Ruenraroengsak et al., 2012).

### 1.2. Soufrière Hills benchmark

To help contextualise the results of our laboratory work we benchmarked our findings by including ash samples from the eruption of Soufrière Hills volcano, Montserrat, which are rich in crystalline silica and therefore may pose a silicosis risk, and have been extensively characterised (e.g. Horwell et al., 2003a, 2003b). Soufrière Hills volcano began its current eruption in July 1995 and has had several phases of lava dome growth (and collapse), interspersed with periods of explosions and pauses in activity. The ash has been repeatedly studied over time (Baxter et al., in press) to monitor its cristobalite concentration during the different phases of the eruption, which are marked by episodes of growth of a lava dome in its crater. Cristobalite forms within the Soufrière Hills volcano lava domes and, when these collapse to form

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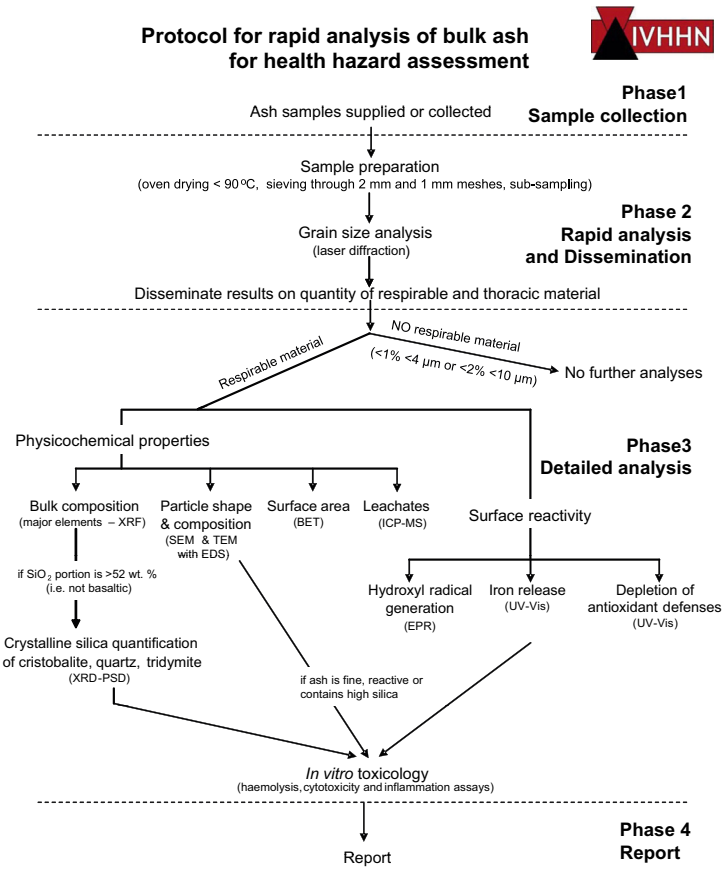


**Fig. 1.** Protocol used for rapid assessment of health hazard of volcanic ash, adapted from Le Blond et al. (2010) and Damby et al. (2013). The report on the initial analyses (end of Phase 2) was disseminated to local agencies at the end of May 2010 for Eyjafjallajökull ash, with the full report available from the International Volcanic Health Hazard Network website in September 2010.

pyroclastic density currents, the rock fragments, generating clouds of respirable particles that fall out to form ash deposits (Baxter et al., 1999). The longevity of the eruption and frequency of population exposure to elevated levels of respirable crystalline silica makes the Soufrière Hills volcano eruption unique in recent times (Searl et al., 2002).

In 2002, a group of experts reviewed all the toxicological data on the Soufrière Hills ash which had become available up to that time (Baxter et al., in press). Their judgement was used to inform a probabilistic assessment on the silicosis risk amongst the islanders from continuing exposure to the ash and its high concentration of cristobalite. The main concern was that the volcano showed no sign of decreasing activity, and the risk from the exposure continuing for the foreseeable future needed to be quantified. The principal toxicological studies were:

- Cullen and Searl (1998): *in vitro* testing on human epithelial cells and rat alveolar macrophages.
- Wilson et al. (2000): competence of human epithelial cells and the ability of volcanic ash to induce sheep blood haemolysis.
- Cullen et al. (2002): inhalation and instillation studies in two groups of rats and further *in vitro* studies.
- Housley et al. (2002): instillation study to assess the effects of ash on the lungs of rats.
- Lee and Richards (2004): instillation in rats sacrificed at intervals up to 49 weeks.

The expert group interpreted the findings of reduced bio-reactivity in these studies to infer that the toxicity of the cristobalite in the Soufrière Hills ash was significantly less than the bio-reactivity expected based on the abundance of cristobalite in the ash alone, perhaps because the toxicity of pure cristobalite was being masked by the heterogeneous mineral matrix in which it was embedded. A similar conclusion had been reached in the 1980s after comparable laboratory experiments were performed on ash from Mount St. Helens, USA (Baxter et al., in press).

The most comparable example of industrial exposure to cristobalite-laden dust is that of diatomaceous earth workers in California (Hughes et al., 1998). The dose-response model used in the Hughes et al. (1998) study formed the basis for the risk assessment methodology that incorporated modelling of volcanic ash exposure levels and duration, including variable cristobalite concentrations, based on the 1995–1997 volcanic activity and assuming that the eruption could continue for years into the future. The probabilistic model assessed the possible development of silicosis in the island population after a total of 20 years of exposure to ash and found, for example, a 2–4 wt% risk of developing early detectable radiological evidence of silicosis in gardeners (the highest exposed occupational group) (Hincks et al., 2006). The results formed the basis of applying more stringent measures to reduce future exposure to ash in the population.

Benchmarking toxicological testing between ash from different volcanoes has not been formally undertaken before and we decided to investigate its value in communicating our assay results to health experts with responsibility for advising patients and the general population in Iceland on the health risks from the eruptions.

### 1.3. Eyjafjallajökull and Grímsvötn volcanoes

Eyjafjallajökull volcano is located in southern Iceland (Fig. 2) and last erupted intermittently between 1821-23. After a period of enhanced seismic activity and ground deformation, particularly

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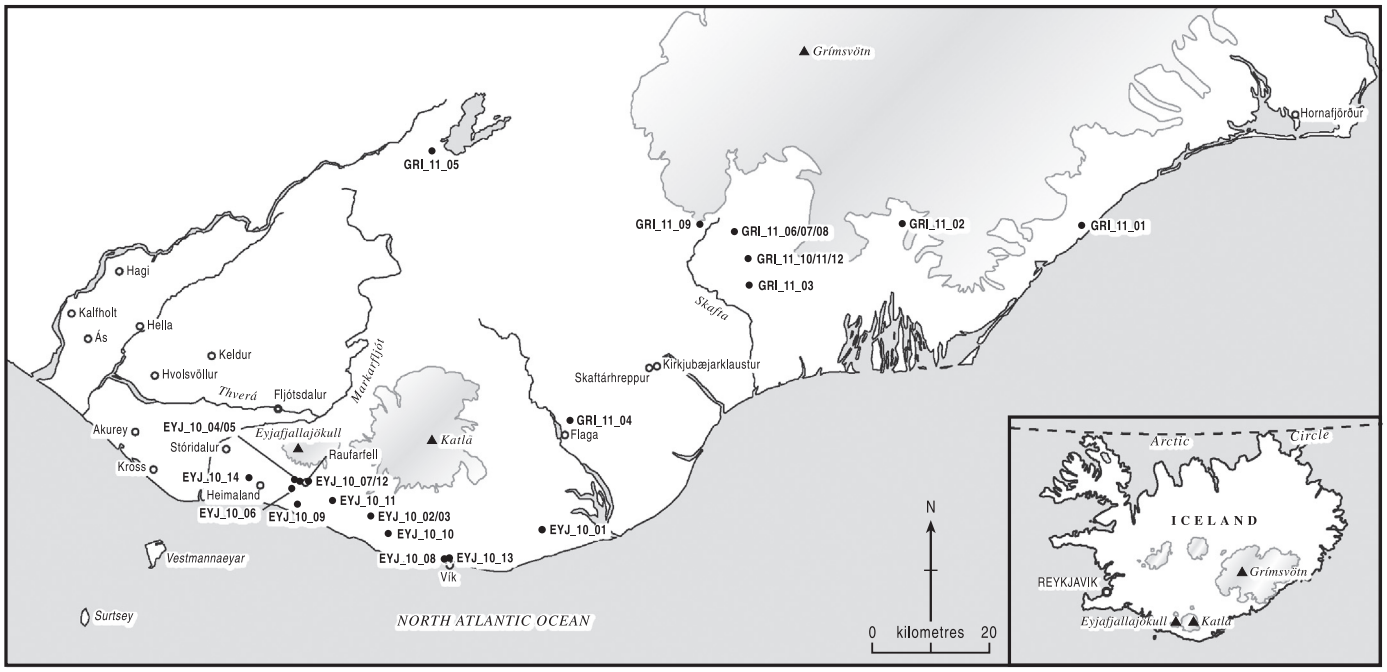


**Fig. 2.** Map of sample collection locations in relation to Eyjafjallajökull and Grímsvötn volcanoes and surrounding landmarks. Towns and villages are marked by open circles and include those with air quality monitoring stations plotted in Fig. 5. Ice caps are shaded grey.

during 1994, 1999, and from December 2009, Eyjafjallajökull erupted on 20 March 2010 (Sigmundsson et al., 2010). The initial Fimmvörðuháls eruption was effusive (non-explosive, thereby generating little to no ash) and basaltic (defined as $<52$ wt% bulk $SiO_2$), and the eruption fissure was located on the north eastern flank of the volcano, just outside of the ice cap. Activity at Fimmvörðuháls stopped on 12 April and two days later a new fissure opened within the summit crater of Eyjafjallajökull volcano, heralding the new phase of explosive activity. The explosive eruption at the summit vent lasted from 14 April to 22 May and can be divided into four phases (Gudmundsson et al., 2012b): phase I (14–18 April) was characterised by explosive eruption of benmorite magma (60–61 wt% $SiO_2$) that was somewhat enhanced by water–magma interaction, especially during the first day when the vent was partly filled with glacial melt-water. Phase II (18 April–4 May) was characterised by lower discharge and lava effusion, with some weak but sustained explosive activity, of slightly less silicic magma (58–59 wt% $SiO_2$; still classed as benmorite). Phase III (5–17 May) involved a rejuvenation of more silicic explosive activity, and Phase IV (18–22 May) signalled the decline of the eruption, with reduced explosivity and magma discharge. For detailed information on location of proximal ash fallout, see Supplementary material.

Ash was reported over Europe between 15–21 April and 6–17 May, but the amount of ash that fell over Europe was negligible (Stevenson et al., 2012), and syndromic surveillance over the UK did not find any unusual increases in presented respiratory ailments (Elliot et al., 2010). In contrast, ashfall was substantial on the small farming community in southern Iceland near the volcano (up to 4 cm was deposited in the lowland south of the volcano on 17 April, G. Larson, personal communication). During the eruption, there was little precipitation in the area and ash deposits continued to be re-suspended by wind and human activity for months after the eruption ceased. Ambient air monitoring stations were installed by Icelandic scientists in the impacted areas (Thorsteinsson et al., 2012).

Grímsvötn, a basaltic volcano in south-eastern Iceland, is Iceland's most frequently active volcano in historical times and is mostly covered by the Vatnajökull icecap. The recent eruption began on 21 May 2011 and rapidly broke the ice cover, producing an eruption plume up to 20 km high which was sustained as a 50–100 km wide umbrella cloud until 22 May (Gudmundsson et al., 2012a). Heavy ash fall occurred on populations 70–100 km south of the volcano. This explosive eruption was far greater in intensity than the 2010 summit eruption at Eyjafjallajökull, releasing more tephra in the first day than during the earlier five and a half-week eruption (Gudmundsson et al., 2012a, 2012b). The eruption continued at a lower intensity until 28 May, with tephra mainly falling on Vatnajökull glacier, which surrounds the volcano (Fig. 2). There was sporadic air traffic disruption to northern Europe during this time.

The Eyjafjallajökull and Grímsvötn eruptions were examples of the ability of Iceland's volcanoes to produce sustained, as well as sizeable, explosive eruptions, which are likely to produce ash of inhalable size. In particular, Grímsvötn produces explosive basaltic eruptions, in contrast to the more 'stereotypic' effusive basaltic eruptions typical of Hawaii and Etna (Thordarson and Hoskuldsson, 2008). Basaltic eruptions in 'dry' environments are mostly effusive, featuring lava fountains that produce tephra dominated by lapilli sized fragments (2–64 mm) and, for that reason, are considered not to pose a respiratory health hazard. Within-glacier eruptions of basaltic magma increases the explosivity significantly. Such events produce fine, iron-rich ash which may present a respiratory hazard through iron-catalysed free radical generation (Horwell et al., 2007).

The different phases of an eruption may produce ash with differing physicochemical profiles of pertinence to health hazard assessment, particularly in terms of grain-size distribution, depending on the explosivity. Therefore, samples were sought which covered the range of eruptive styles during the two eruptions. Samples of ash were collected on Iceland, downwind of the eruption plume, and sent to Durham University, UK where they were distributed to laboratories in the UK and Europe for co-ordinated, rapid analysis.

## 2. Material and methods

The protocol methods are described in full elsewhere (Damby et al., 2013; Horwell, 2007; Horwell et al., 2007; Le Blond et al., 2009, 2010) so are described briefly here with some further detail in the Supplementary material, together with information on sample preparation.

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**Table 1**
Sample summary information for Eyjafjallajökull (sample numbers starting EYJ), Grimsvötn (sample numbers starting GRI), and SHV samples (sample numbers starting MBA=Montserrat Bulk Ash). Further sample information can be found in Table S1 of Supplementary material. Question marks in the 'state of sample' column refer to slight uncertainty based on lack of information from sample collection (i.e. whether there had been rainfall between deposition and collection.

| Sample I.D. | Date erupted | Date collected | Location | Distance from source (km) | Collected by | Collection information | State of sample |
|---|---|---|---|---|---|---|---|
| **EYJ_10_01** | 15.4.10 | 15.4.10 | Myrdalssandur | 58 | Gislason[a] | Phase I[h] | Not known |
| **EYJ_10_02** | 16–17.4.10 | 17.4.10 | Solheimakot | 20 | Larsen and Höskuldsson[a] | Phase I[h] | Dry |
| **EYJ_10_03** | 17.4.10 | 18.4.10 | Solheimakot | 20 | Larsen and Höskuldsson[a] | Phase I[h] | Damp |
| **EYJ_10_04** | 17.4.10 | 18.4.10 | Thorvaldseyri power station | 8 | Larsen and Höskuldsson[a] | Phase I[h] | Wet |
| **EYJ_10_05** | 17.4.10 | 18.4.10 | Thorvaldseyri power station | 8 | Larsen and Höskuldsson[a] | Phase I[h] | Wet |
| **EYJ_10_06** | 19.4.10 | 20.4.10 | Thorvaldseyri | 9–10 | Larsen and Sverrisdóttir[a] | Phase I[h] | Dry |
| **EYJ_10_07** | 14–18.4.10 | 22.4.10 | Seljavellir | ~6.5 | Delmelle[b] | Phase I | Fresh? |
| **EYJ_10_08** | 14–18.4.10 | 24.4.10 | Vik | ~37.5 | Delmelle[b] | Phase I | Snowed on |
| **EYJ_10_09** | 14-18.4.10 | 23.4.10 | Onundarhorn farm | ~13 | Delmelle[b] | Phase I | Fresh? |
| **EYJ_10_10** | 14-18.4.10 | 22.4.10 | Klifandi | 27 | Delmelle[b] | Phase I | Snowed on |
| **EYJ_10_11** | 14-18.4.10 | 22.4.10 | Hotel Skogar | ~14 | Delmelle[b] | Phase I | Rained on |
| **EYJ_10_12** | 9.5.10 | 9.5.10 | Seljavellir | 7.9 | Baxter and Jenkins[c] | Phase III | Damp |
| **EYJ_10_13** | 6-7.5.10 | 7.5.10 | Vik | 38.3 | Baxter and Jenkins[c] | Phase III | Fresh/dry |
| **EYJ_10_14** | 17.4.10 | 17.4.10 | Bridge over Holtsa river on highway 1 | ~5 | Ilyinskaya[c,d] | Phase I | Fresh |
| **GRI_11_01** | 21-22.5.11 | 22.5.11 | Jokulsarlon | ~70 | Hoskuldsson, Larsen, Oladottir, Sigmarsson[a] | Collected from a rooftop | Dry |
| **GRI_11_02** | 21-22.5.11 | 22.5.11 | Skaftafell National Park | ~45 | Hoskuldsson, Larsen, Oladottir, Sigmarsson[a] | Collected from a wooden table/bench | Dry |
| **GRI_11_03** | 21-23.5.11 | 23.5.11 | Djupa river | ~50 | Hoskuldsson, Larsen, Oladottir, Sigmarsson[a] | | Dry, collected as falling |
| **GRI_11_04** | 21-22.5.11 | 22.5.11 | Eldvatn resting area | ~90 | Hoskuldsson, Larsen, Oladottir, Sigmarsson[a] | Collected from a wooden table/bench. | Dry after slight drizzle |
| **GRI_11_05** | 21-23.5.11 | 1.6.11 | Vatnsfell power station | ~80 | Hoskuldsson, Larsen, Oladottir, Sigmarsson[a] | Collected dry from a concrete floor in a sheltered outdoor area, disturbed by wind | Rained on? Contaminated by sand-silt size material from floor |
| **GRI_11_06** | 21-23.5.11 | 3.6.11 | Fossarbrekkur, Djupardalur | 34 | Thordarson and Hoskuldsson[a,e] | Collected from ground-moraine. Total deposit thickness of 16 cm | Moist |
| **GRI_11_07** | 21-23.5.11 | 3.6.11 | Fossarbrekkur, Djupardalur | 34 | Thordarson and Hoskuldsson[a,e] | " | Moist |
| **GRI_11_08** | 21-23.5.11 | 3.6.11 | Fossarbrekkur, Djupardalur | 34 | Thordarson and Hoskuldsson[a,e] | " | Moist |
| **GRI_11_09** | 21-23.5.11 | 2.6.11 | Nupahraun Raudholl 2 | 50 | Thordarson and Hoskuldsson[a,e] | On surface of lava, near lava flow front. Top and bottom wet–took dry middle 20 cm | Dry |
| **GRI_11_10** | 21-23.5.11 | 3.6.11 | Djupardalur | 45 | Thordarson and Hoskuldsson[a,e] | Collected on Nupahraun lava. Total deposit thickness of 24 cm. | Moist |
| **GRI_11_11** | 21-23.5.11 | 3.6.11 | Djupardalur | 45 | Thordarson and Hoskuldsson[a,e] | " | Moist |
| **GRI_11_12** | 21-23.5.11 | 3.6.11 | Djupardalur | 45 | Thordarson and Hoskuldsson[a,e] | " | Moist |
| **MBA5/6/99** | 5.6.99 | 5.6.99 | Salem, Montserrat | 4 | Horwell[f] | Collected as fell | Dry |
| **MBA12/7/03** | 12.7.03 | 12.7.03 | Olveston, Montserrat | 4 | MVO[g] | Collected as fell | Dry |
| **MBA20/12/09** | 20.12.09 | 20.12.09 | Olveston, Montserrat | 4 | MVO[g] | Collected as fell | Dry |

Affiliations of sample collectors:
Further details of EYJ_10_14 can be found in Ilyinskaya et al. (2011).

[a] University of Iceland.
[b] University of York, UK.
[c] University of Cambridge, UK.
[d] Icelandic Meteorological Office.
[e] University of Edinburgh, UK.
[f] Durham University, UK.
[g] Montserrat Volcano Observatory.
[h] For samples EYJ_10_01–06, all samples were dried at 40 °C in Iceland. These samples were in the field for several hours – up to two days before collection.

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### 2.1. Sample collection

For Eyjafjallajökull, fourteen samples of ground-deposited ash were collected from the ash fallout on Iceland (Fig. 2), with twelve of the samples from 14 to 18 April (i.e. Phase I) and the two days following the end of this phase. Two further samples were from ash erupted from 6 to 9 May during Phase III. Details of sample information and collection are given in Table 1.

For Grímsvötn, twelve samples of ground-deposited ash were collected (Fig. 2), all from ash deposited between 21 and 23 May 2011. Samples GRI_11_01-04 were collected during the eruption but samples GRI_11_05-12 were all collected 1–2 weeks after deposition.

Three samples of Soufrière Hills dome collapse ash were chosen (from archived samples at Durham University, from collapses in 1999, 2003 and 2009) based on existing physicochemical data for these samples (Horwell, 2007; Horwell et al., 2007, 2010a).

Due to the prevailing weather on Iceland, collection of pristine samples was challenging for this study. Where possible, samples were collected from clean, dry surfaces but systematic ash collection (e.g. from a well-maintained ash tray network) was not possible. Most samples were collected from single ashfall events but some were the result of several days' accumulation, as detailed in Table 1. Samples which were not collected immediately upon deposition may have been exposed to moisture, thereby potentially affecting the surface composition and reactivity of the particles. Table 1 therefore gives information on the condition of the sample upon collection as well as the date of collection (in comparison to the date of deposition).

Key mineralogical analyses were carried out on all samples (bulk composition, grain size and crystalline silica content), and all other tests were carried out on selected samples (outlined in Tables S1 and S2; Supplementary material) according to the grain-size results (finest samples chosen) and parameters such as eruption phase and condition of sample (i.e. collected dry; information in Table 1).

One of us (PJB) also undertook a field study of the eruption impact in collaboration with Icelandic scientists from 4 to 11 May 2010, which included meeting local health professionals and veterinarians, and also establishing a network of diffusion tubes for sulphur dioxide monitoring in the inhabited area most impacted by ash fall from the eruption plume.

### 2.2. Mineralogical and geochemical analyses

The cumulative grain-size distribution (0.02–2000 μm) including the 'respirable' ($<4$ μm) and 'thoracic' ($<10$ μm) material in the samples was measured by laser diffraction. The crystalline silica (cristobalite, quartz and tridymite) content was determined using X-ray diffraction with a position sensitive detector following a method most suitable for quantifying individual phases in volcanic ash (Le Blond et al., 2009). Scanning electron microscopy was used to image the morphology of the ash particles, and any fibre-like particles were then identified by transmission electron microscopy diffraction pattern indexing. The specific surface area of the ash samples was derived from nitrogen gas adsorption data by applying the Brunauer Emmett Teller (BET) method. The major elemental composition of the bulk ash was determined by X-ray fluorescence, to confirm the magmatic composition of the ash, variations in which may help to explain differences in toxicity.

The concentrations of water-soluble metal elements in the ash that may relate to its bio-reactivity (Fe, Mn, As, Cd, Cr, Cu, Ni, Pb and Zn) were measured by leaching ash samples in water followed by inductively coupled plasma mass spectrometry. The pH of the water leachates was also measured. Anion analysis (as F, Cl, and $SO_4$) of ash leachates was carried out as part of a separate project to determine environmental effects and bioaccessibility of leachable elements (J. Calkins, Ph.D. thesis, University of York, 2012).

### 2.3. In vitro bio-reactivity, cytotoxicity and pro-inflammatory marker assays

Volcanic ash may generate significant hydroxyl radicals ($HO^{\bullet}$), particularly in Fe-rich ash types (Horwell et al., 2007, 2003a). Electron paramagnetic resonance spectroscopy, in association with the 'spin trap' technique, was used to measure hydroxyl radical generation in solution from the samples through cell-free replication of the Fe-catalysed Fenton reaction, where ferrous iron reacts with endogenous hydrogen peroxide following a standard methodology for volcanic ash (Horwell et al., 2007). The amount of iron available to participate in the reaction was also measured following chelation using spectrophotometry. The data were compared to standard samples from Soufrière Hills, Cerro Negro and Etna volcanoes (Horwell et al., 2007).

The oxidative capacity of the ash was tested in two assays: an ascorbate screening assay and a composite respiratory tract lining fluid assay (Ayres et al., 2008). The human erythrocyte lysis assay was used to examine the potential for ash particles to cause rupture of red blood cell membranes (Clouter et al., 2001).

Specific samples were chosen for further toxicological assessment. One Eyjafjallajökull sample and one Soufrière Hills sample were tested for cytotoxicity (MTT colorimetric assay) against mono-layers of human alveolar epithelial type 1-like cells (TT1). The potential of samples from Eyjafjallajökull, Grímsvötn and Soufrière Hills to incite acute and chronic inflammation was measured by the induction of inflammatory markers: release of interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) (see Table S2 in Supplementary material) (Ruenraroengsak et al., 2012). Two different ash sample preparations were used: 'as supplied' and 'ground'. Grinding can re-activate weathered ash surfaces (Horwell et al., 2003a) and may represent abrasion and crushing of particles by humans and vehicles after an ashfall event, thereby potentially altering the grain-size distribution of re-suspended particulate. Significant differences between samples (at each dose) and an unexposed control were assessed using a one way ANOVA where $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

For further detail on all methods, please see the Supplementary material.

## 3. Results

### 3.1. Mineralogical and particle characteristics

Bulk composition analyses classified the Eyjafjallajökull samples as ranging from basaltic trachy-andesitic to trachy-andesite (53–59 wt% $SiO_2$, see Table S3 in Supplementary material) and there was no obvious trend in composition over the course of the eruption. The Grímsvötn samples were all basaltic, ranging from 49 to 51 wt% $SiO_2$, and the Soufrière Hills volcano samples were andesitic/dacitic (62–63 wt% $SiO_2$) (Fig. S1 in Supplementary material).

The grain-size distribution of fine ash from Eyjafjallajökull was variable: ~2–13 vol% $<4$ μm and ~4–26 vol% $<10$ μm (see Table S4 in Supplementary material). In samples from the early stage of the eruption (Phase I), higher respirable and thoracic concentrations are generally observed (up to 13.3 vol% $<4$ μm) than in Phase III (up to 5.1 vol% $<4$ μm). The Grímsvötn samples were coarser than at Eyjafjallajökull despite the initial eruption having greater intensity: 0.0–3.5 vol% $<4$ μm and 0.0–8.4 vol% $<10$ μm.

Crystalline silica abundance (quartz or cristobalite; no tridymite) was low for all samples (1.4–3.2 wt% for Eyjafjallajökull and un-detectable for Grímsvötn) as expected from the magma composition and lack of dome growth. By contrast, the Soufrière Hills samples contained 5.2–15.2 wt% cristobalite and 1.2–1.6 wt% quartz (Horwell et al., in press, 2010a) (Table S5 in Supplementary material), generated through vapour-phase deposition and devitrification within the cooling andesitic lava dome (Baxter et al., 1999; Horwell et al., 2013).

Particle morphology for all samples was typical of volcanic ash from explosive eruptions (blocky, angular and glassy, with conchoidal fractures), though the very occasional respirable, fibre-like particle was identified in sample EYJ_10_13 (Fig. S3 in Supplementary material). These fibre-like particles were composed of glass, feldspar, gypsum or an Fe/Mg silicate which could not be classified but was not compositionally related to asbestos fibres (Fig. S4 in Supplementary material).

The specific surface area ranged, for Eyjafjallajökull, from 0.3–7.7 $m^2\ g^{-1}$ (Table S6 in Supplementary material). The data were loosely correlated to the cumulative grain size data ($R^2=0.56$ for the sub-4 μm material). The specific surface area of ash from Grímsvötn was consistently low ($<0.6\ m^2\ g^{-1}$) as were the data for Soufrière Hills volcano ($<1.4\ m^2\ g^{-1}$).

### 3.2. Water-leachate analysis

Two ash samples (EYJ_10_12 and 13) corresponding to Phase III of the Eyjafjallajökull eruption produced significantly lower pH (4.4) than the Phase I samples (5.0-9.1), suggesting the presence of acids adsorbed onto the ash surface (Table S7 in Supplementary material). Sample EYJ_10_13 was also notably enriched in all the elements analysed compared to the other Eyjafjallajökull ash materials. In general, Fe dominated the transition and trace metal compositions measured in the leachates. Manganese was the second highest soluble metal extracted, although dissolved Zn exceeded Mn in a few samples. Copper and Ni amounted to a few up to several hundred $\mu g\ kg^{-1}$. Most of the Grímsvötn ash samples produced ash leachate compositions

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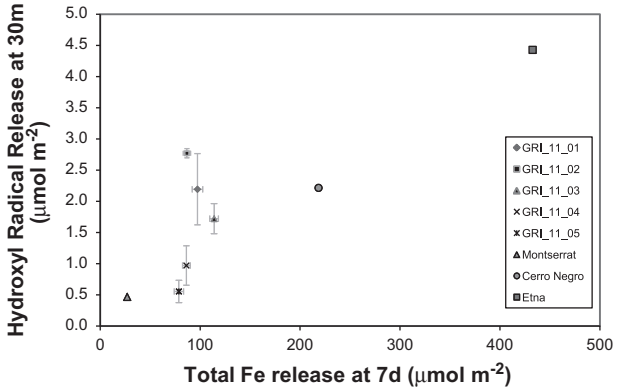


**Fig. 3.** Total iron release vs. hydroxyl radical generation for Grímsvötn samples showing a range of abilities to generate radicals independent of iron release. Error bars represent one standard deviation. Montserrat = Soufrière Hills MBA5/6/99; Cerro Negro (1995 basaltic eruption, Nicaragua); Etna (2002 trachy-basaltic eruption, Italy).

with significantly higher dissolved contents of Zn, Cu and Cr than those found for Eyjafjallajökull.

### 3.3. Bio-reactivity assays

The abundance of hydroxyl radicals generated by all Eyjafjallajökull samples was very low ($<0.5$ μmol m$^{-2}$ after 30 min) in comparison to a suite of standard ash samples analysed concurrently (from Soufrière Hills, Etna and Cerro Negro volcanoes), with the exception of EYJ_10_13 which generated radicals and released iron on a scale akin to iron-rich basaltic samples (1.7 μmol m$^{-2}$ after 30 min, Fig. S5 in Supplementary material). There was a strong correlation between hydroxyl radical generation and iron release for the Eyjafjallajökull samples (Fig. S6, Supplementary material).

For the basaltic Grímsvötn eruption, the ash generated between 0.6 and 2.8 μmol m$^{-2}$ hydroxyl radicals after 30 min (Fig. 3). The upper values are expected for iron rich samples, despite low total Fe release ($\sim$ 100 μmol m$^{-2}$ at 7 days compared to $\sim$200 and 400 μmol m$^{-2}$ for Cerro Negro and Etna standard basaltic analyses and 30 μmol m$^{-2}$ for Montserrat (sample MBA5/6/99)) (Horwell et al., 2007). The range of hydroxyl radical values, however, was independent of iron release, which was relatively constant (Fig. 3). Soufrière Hills sample MBA5/6/99 generated few hydroxyl radicals (average = 0.6 μmol m$^{-2}$ for 2010 and 2011 analyses), typical of iron-poor ash types.

No evidence of oxidative activity was observed for any samples in either the composite respiratory tract lining fluid or ascorbate depletion kinetics assays (Table S8 in Supplementary material), and the propensity for haemolysis was low for all samples ($<5$ % at $\leq 1$ mg ml$^{-1}$ dose).

### 3.4. Cytotoxicity and pro-inflammatory marker assays

Sample EYJ_10_13 generated more hydroxyl radicals, was more acidic and contained higher abundances of leachable elements than any other sample, as well as containing fibre-like particles. However, it had the lowest surface area of all samples. Collected by PJB, we are certain that it was pristine, whilst all other Eyjafjallajökull samples were probably exposed to some moisture before collection. Given these results, we chose this sample for cytotoxicity along with Soufrière Hills sample MBA20/12/09. Two further samples from Eyjafjallajökull, 2 samples from Grímsvötn and 3 Soufrière Hills volcano samples were chosen for their fineness (EYJ_10_08 and 14, GRI_11_02 and 04 and Soufrière Hills samples MBA5/6/99, 12/7/03 and 20/12/09) for the pro-inflammatory assays (see Table S2 in Supplementary material).

EYJ_10_13 and MBA20/12/09 showed significant acute TT1 cell toxicity (30–40 % cell death) at physiologically-unrealistic doses (500 and 1000 μg ml$^{-1}$; $P<0.001$) but at doses $<50$ μg ml$^{-1}$ MBA20/12/09 caused up to 20 % cell death and EYJ_10_13 caused $\sim$10 % (see Fig. S7 in Supplementary material).

In the acute pro-inflammatory mediator assay (24 hr), the only significant observation for Eyjafjallajökull ash exposure was a slight increase in MCP-1 released for EYJ_10_14 at 10 μg ml$^{-1}$ (1.3-fold over the unexposed control; $P<0.05$). Only one Grímsvötn sample, GRI_11_04, produced a significant increase in IL-8 (1.3-fold) and MCP-1 (1.3-fold), both at 50 μg ml$^{-1}$ ($P<0.05$), while IL-6 remained unchanged (Tables S9 and S10, Supplementary material). In contrast, significant changes in IL-6, IL-8 and MCP-1 release were observed to varying degrees in all three 'as supplied' Soufrière Hills samples (Tables S9 and S10 in Supplementary material).

Each of the Eyjafjallajökull, Grímsvötn and Soufrière Hills volcano samples showed the ability to elicit a pro-inflammatory response in TT1 cells over an extended period (up to 5 days) at doses ($\leq 50$ μg ml$^{-1}$) considered patho-physiologically relevant for 'hot-spot' deposition (Phalen et al., 2006). Grinding of samples increased this reactivity further (see Fig. 4 for EYJ_10_13 observations, Tables S9 and S10 in Supplementary material and accompanying text for detailed discussion of data). Critically, we found that removal of ash from exposed TT1 cells did not resolve the pro-inflammatory response and a lasting, significant effect on cytokine production was apparent.

## 4. Discussion

### 4.1. The IVHHN protocol

Our protocol was successfully implemented following the eruptions of both Eyjafjallajökull and Grímsvötn. Airport closures meant that there were delays with sample receipt, but the data from the Eyjafjallajökull study report were used to inform Carlsen et al.'s (2012a) clinical study of early health effects in local residents.

For this study, samples were analysed by a suite of laboratories most of whom are members of IVHHN and/or the UK Natural Dust & Health Network; analysis of samples in parallel, with co-ordination from one institution, allows for efficient analyses and results dissemination. However, the number of analyses is substantial; while we build an evidence base of the health-pertinent characteristics of different volcanic ash types, it is necessary to carry out the full suite of analyses in the protocol. Clearly, a more concise version would be useful for those wishing to carry out the assessment independently and this will be developed as our results database grows and the necessity for each technique becomes apparent. Certain techniques, such as grain size analysis, are critical but there are issues with the availability of state-of-the-art instruments such as laser diffractometers. To counter this, Horwell (2007) has published an equation for estimating the amount of respirable material in a bulk ash sample following sieving to 63 μm (4∅, the lowest practicable mesh size). As we grow our database following each new eruption, it is becoming possible to predict data ranges for some analyses so, eventually, estimates of likely characteristics might be possible without the need for full enactment of the protocol.

### 4.2. Population exposure

The potential for ash re-suspension and further abrasion after deposition, by wind and human activity, is likely for long periods after an eruption unless it is physically removed, incorporated into the soil or subjected to weathering processes (Searl et al., 2002). About 60 farms (over 200 people) occupied the sparsely popu-

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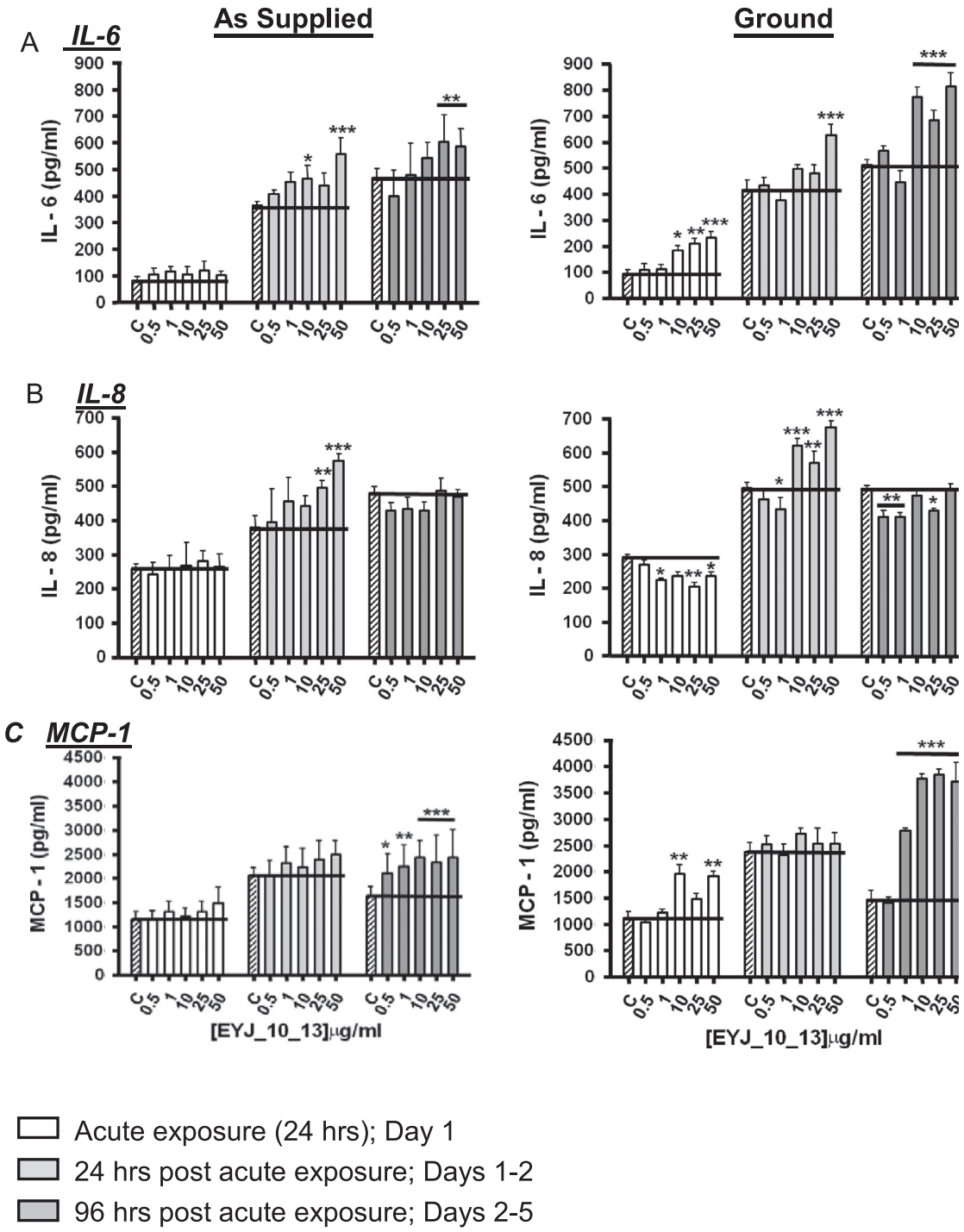


**Fig. 4.** Pro-inflammatory mediator release from Eyjafjallajökull-exposed (sample EYJ_10_13) TT1 cells. Data comprises the release of (A) IL-6, (B) IL-8 and (C) MCP-1 after acute exposure (24 h) and at 24 and 96 h post-acute dose removal. TT1 cells were exposed to either an 'as supplied' sample preparation or a 'ground' sample preparation. Data are expressed as mean ± SEM ($n=3$). Hatched bar indicates respective control for each time interval.

lated region most impacted by the ash around Eyjafjallajökull and around 3000 people were exposed to the Grímsvötn ashfall, particularly in the communities of Hornafjörður (2119 people) and Skaftárhreppur (446 people) (Statistics Iceland, 2012).

Whereas the Grímsvötn ash was coarse ($<3.5$ vol% $<4$ μm material), some of the Phase I Eyjafjallajökull ash samples comprised a substantial respirable component (up to 13 vol% $<4$ μm) comparable to that seen from large, explosive eruptions elsewhere, e.g. Vesuvius, Italy, AD79 (Horwell et al., 2010b). The two samples collected during Phase III were more comparable to mildly-explosive Vulcanian eruptions at other volcanoes (e.g. 5.9 vol% at Soufrière Hills volcano (Horwell, 2007), in contrast to the Soufrière Hills volcano silica-rich dome-collapse ash tested here, ~11 vol%, Table S4 in Supplementary material), but as we only obtained 2 samples from this phase they may not be fully representative.

For Eyjafjallajökull, exposure was prolonged due to the eruption duration (5.5 weeks) and the abundant, re-suspended fine ash causing the $PM_{10}$ levels to frequently peak well above the WHO 24 h average guideline value for environmental particulate matter (50 μg $m^{-3}$) (World Health Organization, 2000) (Fig. 5 and data in Thorsteinsson et al., 2012). This resulted in the potential for high

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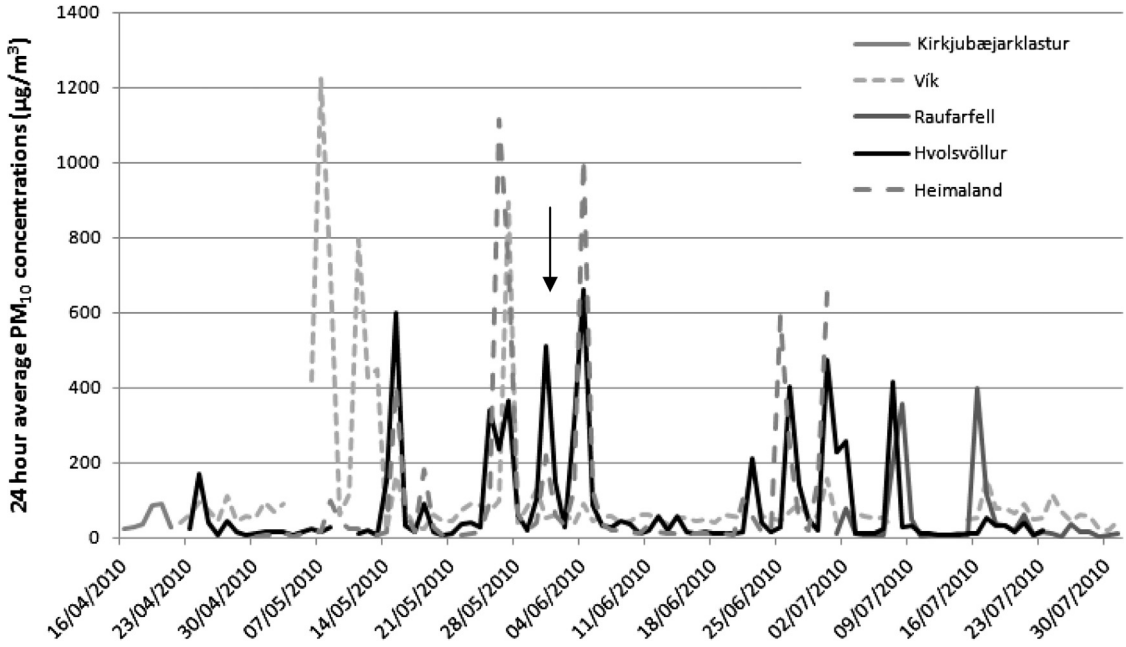


**Fig. 5.** 24 h average air quality data ($PM_{10}$) from stations around Eyjafjallajökull (see Fig. 2) showing decline in resuspended ash episodes after emissions ceased on May 22. Peaks after 1 June (arrow) are due to re-suspension of ash deposits by wind, not direct ash fall. Kirkjubæjarklaustur has a population of ~112; Vík has a population of ~291; Hvolsvöllur has a population of ~878; the Heimaland station is a rural community centre and Raufarfell is a farm (Statistics Iceland, 2012; data for 1 Jan 2011–1 Jan 2012). Data for Vík, Raufarfell and Heimaland coincide with sample locations used in this study. Fig. supplied by Þorsteinn Jóhannsson, Environment Agency of Iceland. See Thorsteinsson et al. (2012) for further information.

exposure for outdoor workers and also the general population in dry weather periods (e.g. on 4 June 2010 in Heimaland, ~10 km from the volcano, Fig. 5) (Statistics Iceland, 2012), indicating the need for continued monitoring of ambient air quality by local scientists.

Early reported symptoms of upper airway and eye irritation and exacerbation of pre-existing asthma were limited amongst residents of the most ash-exposed rural area close to Eyjafjallajökull (Carlsen et al., 2012a, 2012b). One possible explanation was the high standard of weather-proofing of houses and buildings in Iceland which would have prevented significant infiltration of ash into the indoor air and limited the incidence and severity of acute symptoms. In addition, high efficiency dust masks were rapidly distributed by the authorities. Analyses of the network of sulphur dioxide diffusion tubes in the populated area confirmed that the gas plume had not grounded, at least in the last three weeks of the Eyjafjallajökull eruption, and so the gases themselves should not have contributed to acute respiratory symptoms.

### 4.3. Data interpretation

#### 4.3.1. Physicochemical characterisation

Despite preventative measures taken by the authorities and local population, concerns arose over the risk of chronic respiratory effects if the ash deposits persisted in the environment for several months, or more permanently. The ash from both Icelandic eruptions contained little to no crystalline silica, which was reassuring as well as expected from the magma composition, and only included substantial respirable material under certain eruptive conditions. Recently, several authors have found fibre-like particles in volcanic ash, raising concerns because of potential similarities to asbestos fibrils and the presence of nano-sized cristobalite in ash from Chaitén volcano (Horwell et al., 2010a; Le Blond et al., 2010; Reich et al., 2009). However, none of the observed fibre-like particles in the present study had asbestiform morphologies or compositions, nor contained cristobalite, and constituted a volumetrically negligible component of the ash.

The range of specific surface areas for the Eyjafjallajökull ash was unexpected as volcanic ash generally has a surface area of $<2\ m^2\ g^{-1}$ (e.g. Le Blond et al., 2010) and there is no obvious reason for the high surface areas ($>2\ m^2\ g^{-1}$) for six out of eight samples tested (see Table S6, Supplementary material). One might expect the surface areas to influence the toxicology results yet sample EYJ_10_13, which had the lowest surface area of the Eyjafjallajökull samples, demonstrated similar chronic pro-inflammatory responses to EYJ_10_08 and _14, except in the IL-8 assay where no significant change occurred (compared to the control) for EYJ_10_13. In these assays, the samples appear to have a very low toxicity surface.

Trace elements adsorbed by the ash from the plume may be relevant to respiratory health (Witham et al., 2005), but their concentrations in the leachate analyses were low in most samples and, given the weak intensity of ash emission during Phase III of the Eyjafjallajökull eruption, when the highest concentrations were observed, these findings are likely to have little implication in terms of respiratory health hazard. Overall, the dissolved metal concentrations measured in the Icelandic ash fell within the range reported for other volcanoes (e.g., Armienta et al., 2002; Christenson, 2000; Cronin et al., 1998; Hinkley and Smith, 1982), but were lower than those determined using the same leachate method for the 2010 Merapi ash (Damby et al., 2013). However, extraction in water is not necessarily representative of lung conditions, so a simulated lung fluid leach is recommended for future analyses (Caboche et al., 2011). Further studies are also required in order to assess if the systematic presence of significant (but variable) concentrations of soluble Mn, Zn, Cu and Ni in ash leachates play a role in determining the potential toxicity of ash deposited into the lungs (e.g. Lemire et al., 2008; Rice et al., 2001).

#### 4.3.2. Bio-reactivity, cytotoxicity and pro-inflammatory assays

Although there are some important differences in the physicochemical characteristics of the ash samples from the volcanoes studied here, in particular, the contrast in crystalline silica content between the Icelandic and Soufrière Hills samples, the assay

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results and their pro-inflammatory profiles were surprisingly similar and showed few signs of pro-inflammatory activity. The potential for the basaltic Grímsvötn ash to generate hydroxyl radicals is somewhat offset by the fact that there was so little respirable material in the samples. A range in the abundance of hydroxyl radicals generated amongst samples was seen for the Grímsvötn ash, which was independent of the amount of available iron (Fig. 3). This pattern is usually observed for more silicic samples (Damby et al., 2013; Horwell et al., 2007; Le Blond et al., 2010). However, for the Eyjafjallajökull samples, a strong correlation between hydroxyl radical abundance and iron release was observed ($R^2=0.9957$, Fig. S6, Supplementary material). It has been hypothesised that reactivity may be dependent on isolated iron on the surface of the particles, and that an excess of iron may actually reduce reactivity (Fubini et al., 1995).

In this study, the indicator of particle reactivity to emerge is the chronic pro-inflammatory response in ash from all three volcanoes studied, perhaps indicating an as-yet undefined inflammatory pathway. The persistent inflammatory effect of the ash after the extracellular particles had been removed suggests the cells may have either entered a pro-inflammatory, positive feedback signalling cycle or may have passively taken up ash particles which continued to react with the cell. Importantly, the activity of these inflammatory mediators is affected by very low, physiologically relevant doses. Damby et al. (2013) used the same assay on ash from Merapi volcano, Indonesia, as part of a rapid study, but that ash was mostly non-reactive against the TT1 cell model, with no chronic pro-inflammatory effect (or acute response). This cell model is new to volcanic ash studies, so further work is required to unravel the causes of the varying outcomes of the assays to date.

### 4.4. Crystalline silica in Icelandic ash

A key question is whether a future Icelandic dome-forming eruption, for example at Katla volcano, could generate substantial crystalline silica and present similar health concerns to the Soufrière Hills ash. Although this is still a concern, there is growing evidence that the inflammatory potential of volcanic crystalline silica is masked; Horwell et al. (2012) recently showed that the potentially reactive surface of Soufrière Hills cristobalite particles is masked by other adhered phases (such as volcanic glass and feldspar) and that the cristobalite itself contains substituted elements such as aluminium. Duffin et al. (2001) demonstrated that occlusion of the DQ12 quartz surface with Al-lactate prevents it from producing the classical inflammatory silica response. The potential for the release of activated oxygen species capable of causing mutations in the epithelial cells of the lung, which could lead to the development of epithelial cancer (Clouter et al., 2001), would also be limited by modification of the silica surface. Those findings are also supported by the lack of haemolytic activity we have found in ash samples from Soufrière Hills volcano as part of this study. Some earlier investigators found a positive response in haemolysis assays with Soufrière Hills and Mt St Helens ash (Vallyathan et al., 1983; Wilson et al., 2000), a discrepancy which we now attribute to their use of sheep erythrocytes which are more fragile than the human erythrocytes used here (Matsuzawa and Ikarashi, 1979).

### 4.5. Summary and conclusions

The goal of this research was to define the physicochemical characteristics and *in vitro* toxicological behaviour of volcanic ash, from Eyjafjallajökull and Grímsvötn volcanoes, through implementation of a protocol for rapid ash testing which can be widely adopted to inform health-risk assessment in volcanic eruptions and allows inferences about the acute and chronic respiratory hazard of volcanic ash to be made.

The toxicology assays in use in current lung research that we chose did not discriminate between the pro-inflammatory potential of ash from the three different volcanoes, and our finding of a chronic pro-inflammatory effect in all three ash types warrants further study. Future work involving toxicological assays should benchmark the pro-inflammatory profiles of a wide range of polluting natural mineral particles, as well as volcanic ash. Ideally, toxicological benchmarking needs further development using a wider range of *in vitro* bio-reactivity assays applied to a larger archive of ash samples from future eruptions, in particular where health studies of human subjects are also undertaken. We are currently carrying out a study on a suite of ash and dome samples from previous eruptions of Icelandic volcanoes to assess whether the wide spectrum of potential magmatic types and eruption styles might produce ash with more potentially-toxic characteristics.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.08.011.

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