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Exhaled Breath Condensate: A Novel Matrix for Biological Monitoring to Assess Occupational Exposure to Respirable Crystalline Silica

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Abstract

Biological monitoring (BM) is a useful way of determining overall exposures to chemical substances; however, in the case of respirable crystalline silica (RCS), this has not been analytically feasible in conventional biological matrices. The aim of this study was to investigate the utility of exhaled breath condensate (EBC) as a potential biological matrix in which to determine exposure to RCS. A small pilot study was undertaken collecting EBC from six quarry workers and six occupationally unexposed persons; the samples were analysed using both single particle inductively coupled plasma mass spectrometry (spICP-MS) and transmission electron microscopy (TEM). The results showed that EBC obtained from the occupationally unexposed persons exhibited low background levels of dissolved silica whilst silica particles of various sizes were present in samples from quarry workers. This is the first study to report EBC as a potential biological matrix that allows differentiation of RCS concentrations between samples from workers and occupationally unexposed controls. The results shown here confirm the presence of RCS in EBC by both spICP-MS and TEM. However, there are difficult analytical challenges still to be overcome before this can be used as a BM method to determine workplace exposure, these are currently being investigated.

Keywords: exhaled breath condensate; respirable crystalline silica; single particle ICP-MS; TEM and quarry workers

Introduction

The hazards and health outcomes of exposure to respirable crystalline silica (RCS) have been well reported. RCS has been classified as a carcinogen when inhaled in its quartz or cristobalite forms by NIOSH (The National Institute for Occupational Safety and Health, 2016), the US National Toxicology Program (National Toxicology Program 2014), and IARC (Group1; IARC 2016). Occupational exposure to RCS induces distinctive lung toxicity in the form of silicosis (a fibrotic pneumoconiosis), which is characterized by pulmonary oedema, interstitial inflammation, fibrotic granuloma, and silicotic nodules (Kawasaki 2015).

Biological effect markers of exposure, such as elevated levels of inflammatory cytokines, have been

reported as result of exposure to RCS (Kawasaki 2015). The use of biological monitoring (BM) allows the assessment of all routes of exposure and can subsequently help control and reduce exposures to a wide range of chemicals. This is not the case with silica, until now the possibility of a direct BM marker of exposure has eluded researchers. This is as a consequence of the lack of solubility and analytical sensitivity of silica and silicon using spectroscopic methods (resulting from interferences and inherent elevated background levels). Therefore, it has not been possible to use common BM matrices because of the relatively high concentrations of silicon in samples such as urine (Roberts and Williams, 1990; Kobayahsi *et al.*, 1995; Widner *et al.*, 1998) making it difficult to differentiate between workers with potential exposures and unexposed persons. Furthermore, measuring total silicon would not be a direct measurement of RCS, and if an inhalation exposure had occurred it is not known whether or not a urine sample would appropriately reflect this exposure.

Recent developments in instrumental sensitivity and software now offer the potential to measure individual particles of known elemental status. Techniques such as single particle inductively coupled plasma mass spectrometry (spICP-MS) can measure to low concentrations of individual silica particles (as silicon at m/z 28) without the need for aggressive sample digestion (Laborda *et al.*, 2014). In combination with direct particle-sizing instrumentation such as electron microscopy, this offers a comprehensive analytical approach to help identify and quantify the presence of RCS in a suitable biological matrix. Whilst the measurement of silica nanoparticles has been achieved previously using an array of analytical techniques such as asymmetric field flow fractionation, multi-angle light scattering as well as transmission electron microscopy (TEM), spICP-MS, and ICP-MS/MS (Aureli *et al.*, 2015; Barahona *et al.*, 2015; Bartczak *et al.*, 2015); there are no reports of RCS being analysed in biological samples in this way.

Exhaled breath condensate (EBC) may be an appropriate 'biological matrix' for biomonitoring purposes for RCS in workplace exposures where silica particles are being inhaled. An EBC sample is composed of mostly water vapour and droplets of epithelial lung lining fluid from the bronchial and alveolar regions of the lungs (Hoffmeyer *et al.*, 2011).

The novel work presented here investigates the measurement of RCS in EBC samples from a small cohort of sandstone quarry workers and occupationally unexposed controls (laboratory workers) with analysis by spICP-MS and TEM with energy dispersive X-ray spectroscopy (EDX).

Method

Sample collection

The study group consisted of six workers from a sandstone quarry in North Yorkshire, England, and six control volunteers from the Health and Safety Executive's Health and Safety Laboratory who were not occupationally exposed. All participating volunteers gave informed consent in accordance with HSG 167 (HSE, 1997).

EBC samples were collected using a TURBO-DECCS (Transportable Unit for Research on Biomarkers Obtained from Disposable Exhaled Condensate Collection Systems) by ItalChill (Parma, Italy). The TURBO-DECCS technique is as previously described (Leese *et al.*, 2016). The collection of EBC is by regular, calm tidal breathing through the mouth via a disposable mouthpiece for 15 minutes. The exhaled air produced passes into a temperature-controlled chamber where the EBC is cooled and collected as a liquid condensate on the surface of a collection tube. EBC samples from both the quarry workers and the control group were frozen at −20°C immediately after collection and stored at −80°C at the laboratory until analysed. The collection of EBC results in very small sample volumes, usually <1 ml. Therefore, not all EBC samples could be analysed by both spICP-MS and TEM analysis.

A commercially available Health and Safety Laboratory RCS certified reference material (CRM) (89% certified crystallinity) HSL A9950 (Respirable α-Quartz powder) was analysed by all methods. This was prepared with ultra-pure deionized water and diluted to various concentrations depending on the analytical technique.

Analysis

spICP-MS

Analysis was performed on an ICAP Q ICP-MS (Thermo Scientific, Hemel Hempstead, UK) using kinetic energy discrimination (KED) mode with helium as the collision cell gas. The analytical method and sample preparation was performed as outlined in RIKILT's spICP-MS procedure (RIKILT Wageningen UR, https://www.wur.nl/en/show/Single-Particle-Calculation-tool.htm, accessed 17 November 2016). Dwell times were 3 ms for both ^{197}Au and ^{28}Si; sample acquisition was for 60 s. The nebulisation efficiency was calculated at 6.5% by determining the sample flow rate (0.24 ml/min) using a 30 nm gold nanoparticle reference material (NIST 8012). All EBC samples were diluted 10-fold with ultra-pure deionized water. The time-resolved data for both the gold and silicon analyses were exported into the RIKILT single particle calculation tool (RIKILT Wageningen UR, https://www.wur.nl/en/show/Single-Particle-Calculation-tool.htm, accessed 17 November 2016).

Transmission electron microscopy

Analysis was performed using a Tecnai G2 Spirit 120 kV TEM (FEI, Cambridge, UK), with attached X-MAX energy dispersive spectroscopy (EDX) system (Oxford Instruments, Abingdon, UK). For each sample, 20 µl was dropped onto an ultrathin carbon-coated 200-mesh nickel grid (Agar Scientific, Stansted, UK) and air dried. Electron micrographs were captured at ×30 000 plate magnification (approximately ×300 000 including magnification by the camera). Particles were analysed by EDX for elemental composition and an electron diffraction (ED) pattern obtained to confirm crystallinity

Results and Discussion

As shown in Fig. 1, there was an obvious difference between the spICP-MS time scans for the control EBC sample and the quarry worker EBC sample, whilst the time scan of the RCS CRM appeared visibly similar to the quarry worker EBC sample.

Exporting the ICP-MS data into the RIKILT spreadsheet showed that it was possible to determine the number of silica particles in control EBC, worker EBC, and RCS CRM samples. Using this approach over a concentration range of 1–100 µg/l of the RCS CRM, the number of particles showed an incremental increase, from 2 to 245 particles. Results showed a significant difference between the number of particles detected in control EBC samples compared with worker samples. The number of particles detected in workers' EBC ranged from 11 to 354 whilst the control samples showed between 0 and 8 particles. The EBC sample from the quarry worker cited in Fig. 1 contained 354 particles with particles ranging in size from ~300 to 1200 nm.

There are limitations of the spICP-MS approach for RCS determinations and this impact upon the accurate quantitative reporting of particle size. Firstly, there are no commercially available certified standard or reference materials of size-characterized crystalline silica particles to fully validate the spICP-MS method. Sec-

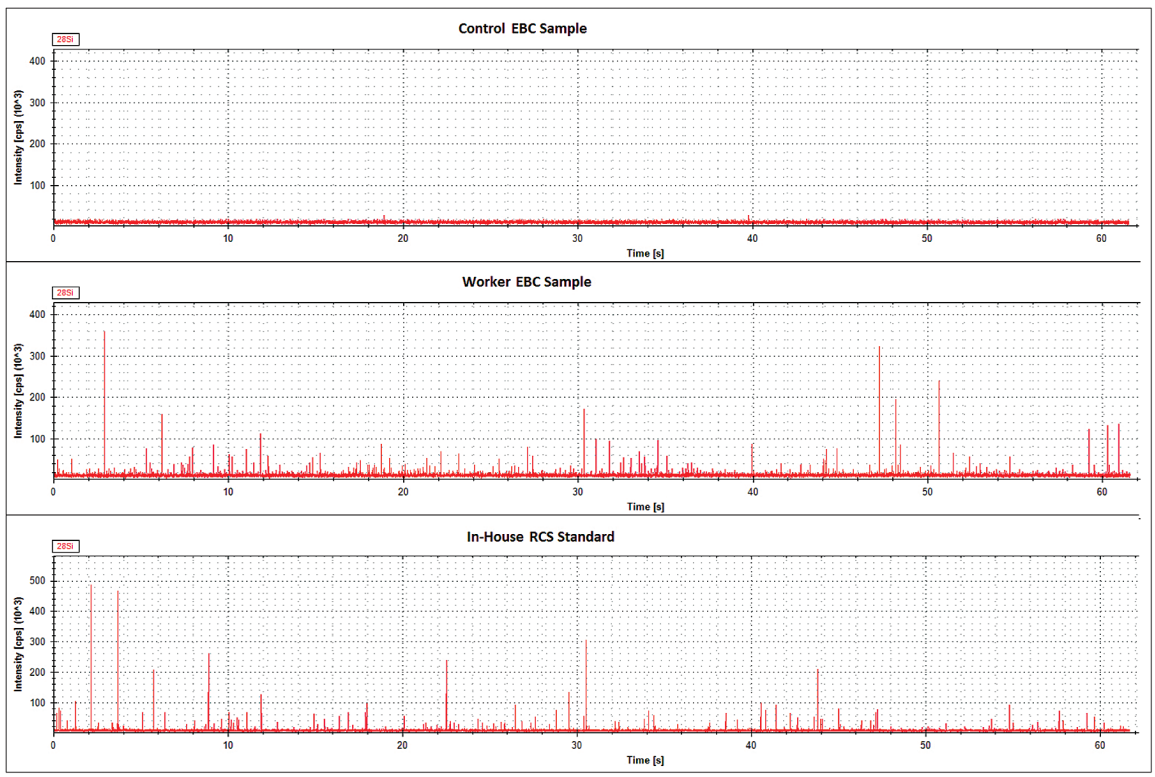

Figure 1. Time scan of silica particles as ^{28}Si in EBC samples from (A) occupationally unexposed control (B) quarry worker (C) RCS CRM ~50 µg/l (HSL A9950 (Respirable α-Quartz powder), when measured by spICP-MS in KED mode. Note that the single particle peaks are detected randomly throughout the 1-minute sample acquisition and so the scans will not be replicated.

ondly, the RIKILT calculations (RIKILT Wageningen UR, https://www.wur.nl/en/show/Single-Particle-Calculation-tool.htm, accessed 17 November 2016) assume spherical particles, but RCS is neither spherical nor a consistent shape and so all references to particle size using this approach at this point only give a qualitative value. Finally, the current limit of size detection using single quadruple ICP-MS is estimated to be around 300 nm.

TEM was able to confirm the presence of RCS in EBC from the quarry workers in all four post-shift samples analysed. No RCS was observed by TEM in EBC samples from the control samples.

Using TEM, analysis of an EBC sample from the quarry worker with the most particles detected by spICP-MS exhibited 10 crystalline silica particles ranging in diameter from 100 nm to 9000 nm (one is shown in Fig. 2). One particle was observed approximately every 5–10 grid openings inspected.

It is important to note that not all the silicon containing particles observed with TEM were crystalline silica (SiO_2). Using EDX, it was possible to identify some par-

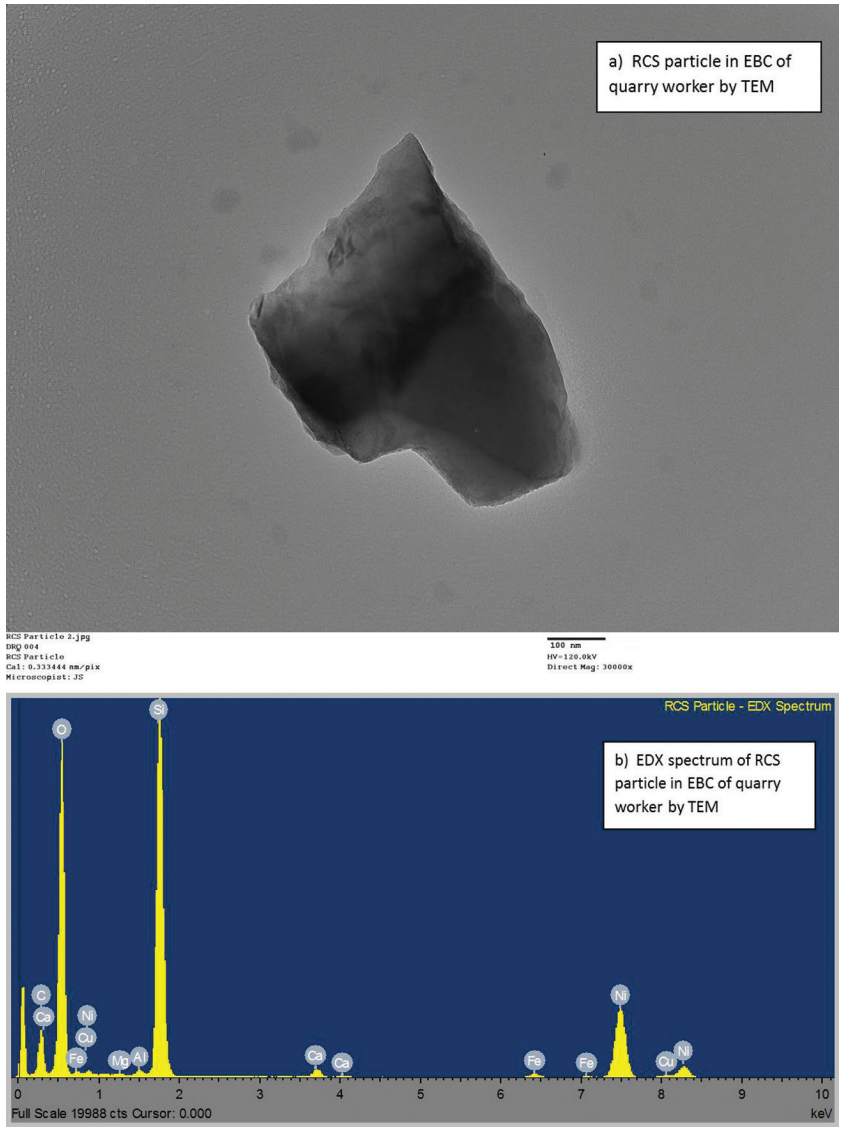

Figure 2. (a)TEM micrograph of a RCS particle observed in an EBC sample from a quarry worker (same sample as seen in Fig. 1), confirmed as silica by EDX (b).

ticles as aluminium silicates. This information is helpful in identifying the composition of the 'baseline' observed with spICP-MS, i.e. the contribution of other silicates as well as amorphous silica. Another contributing factor is likely to be from the quartz background of the ICP-MS sample introduction system, residual contamination/impurities of all solutions including standards and of the argon gas used in the plasma.

Conclusion

This work demonstrates that it is possible to detect crystalline silica particles in EBC of quarry workers. However, as the collection of EBC only produces a small volume of sample we need to be able to acquire a lot of information efficiently. At this stage, the analysis and characterisation of the silica particles still require further method development. There are several analytical challenges to overcome; specifically that when using spICP-MS, the particles are easily detected but not sized; and with the current TEM methodology, a large sample area must be searched to provide high analytical sensitivity. Future developments of these methods will include sample pre-concentration methods for the TEM and the use of a shorter dwell time in spICP-MS. The removal of interferences using ICP-MS/MS may also offer further analytical improvements. It may also be worth considering calculations using particle mass instead of size, or determining the hydrodynamic radius of RCS particles in solution.

The potential of EBC as a matrix for determining exposure to RCS has been realized. It is now likely that future EBC measurements will be able to show actual worker exposures and assist with the improvement of workplace controls.

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Declaration

Its contents, including any opinions and/or conclusions expressed, are those of the authors alone and do not necessarily reflect HSE policy.

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