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A METHOD FOR PRESERVATION AND DETERMINATION OF WELDING FUME NANOPARTICLES IN EXHALED BREATH CONDENSATE

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ABSTRACT

Analysis of exhaled breath condensate (EBC) represents a non-invasive method for detecting inhaled nanoparticles (NP) associated with various occupational and environmental exposures. However, the few studies that have investigated inhaled NPs in EBC often assess only bulk, ionic intensities to provide information on overall elemental content, rather than on particulate content. In an attempt to assess inhaled metallic particles in their original particulate form, we developed a methodology for the preservation and determination of inhaled welding fume NPs in EBC. Two EBC preservation strategies were tested: either flash freezing EBC immediately after collection, or keeping EBC at room temperature until analysis. Particle content of the differently preserved samples was assessed by Microdroplet Generation Inductively Coupled Plasma Mass Spectrometry (MDG-ICP-MS), and with Electron Microscopy.

- Welding fume NPs in EBC may quickly and uncontrollably degrade, thereby losing their original form and hampering effective characterization analysis.
- We demonstrate the importance of flash freezing EBC samples immediately after collection and defrosting them shortly before analysis at a temperature that does not affect proteins and peptides (<38°C) in order to effectively preserve NPs in particulate form.
- This methodology can be applied easily, effectively, and inexpensively to preserve EBC samples for future NP content determination and characterization.

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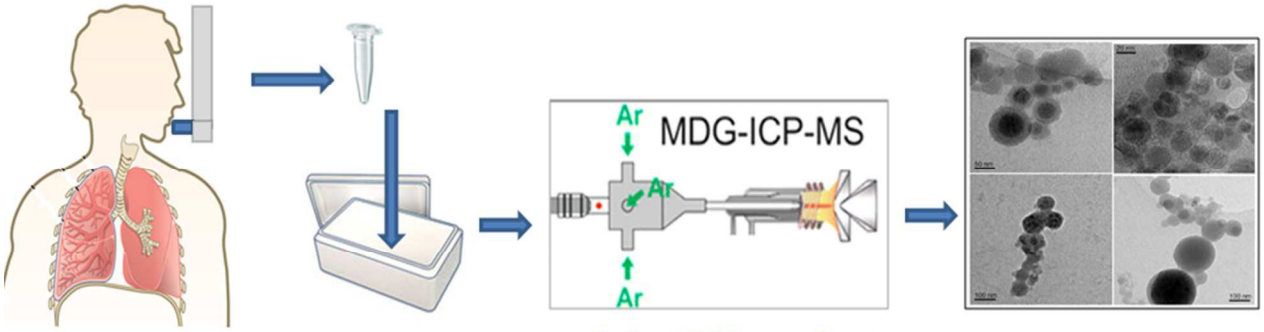
GRAPHICAL ABSTRACT



NANO-IMPACT

Humans are exposed to a wide variety of inhaled nanoparticles (NP) from environmental and occupational sources. Analysis of exhaled breath condensate (EBC) represents a non-invasive method for detecting inhaled NPs; however, biological interactions may promote rapid degradation of NPs, hampering characterization efforts. We developed a methodology for the preservation and characterization of inhaled NPs by first, flash freezing EBC immediately after collection, and then assessing the defrosted liquid via Microdroplet Generation Inductively Coupled Plasma Mass Spectrometry (MDG-ICP-MS). This investigation highlights the importance of preserving and characterizing NPs in their original particulate form for understanding their unique behavior and potential fate once inhaled. This methodology can be applied in a variety of exposure scenarios to effectively preserve NPs in EBC for future characterization efforts.

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INTRODUCTION

Analysis of exhaled breath condensate (EBC) which contains many different constituents such as ions, molecules and proteins represents a non-invasive method for studying the composition of epithelial lining fluid.[1] Collection of EBC has been proposed as a method for assessing biomarkers of various lung diseases, such as inflammation and can provide information on NP exposure directly from the target side.[1,2] EBC has been previously used a matrix for the assessment of biological effects[3] and also for the detection of toxic metals and trace elements associated with various occupational and environmental exposures such as welding fumes,[4,5] cigarette smoke,[6,7] and other pollutant exposures.[8] Some studies have specifically investigated inhaled metals and their content from occupational exposures in EBC; however, it is important to note that these studies have only assessed bulk intensities to provide information on overall metal content in EBC.[5,9]

Welding fumes contain high concentrations of metallic particles that can occur at the nanoscale, depending on the specific type of welding process.[10] Particularly, Tungsten Inert Gas (TIG) welding processes has been shown to generate primary particles with the majority below 100 nm.[11,12] Assessing and characterizing the inhaled metals in their inherent NP form is of particular interest, especially when assessing health relevant and toxicological questions such as inhaled particle translocation to systemic circulation. It has already been evidenced that EBC collected after welding exposure may contain Mn, Fe, Cr, Ni depending on the welding technique used.[13] However, current research in this field has yet to investigate and characterize the NP form of these inhaled metal fume exposures.

Recently, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was proposed as a fast and sensitive method for the analysis of liquid NP suspensions. Mainly, two different approaches are

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used: single particle (sp)-ICP-MS which is based on conventional nebulization of a highly diluted NP suspension and introducing the produced polydisperse aerosol through a spray chamber into the ICP;[14-17] and microdroplet generation (MDG)-ICP-MS which is based on a piezoelectrically driven dispenser head generating monodisperse droplets with a distinct frequency, transporting the droplets in a gas stream through an adapter into the ICP and which allows for close to 100% transport efficiency (TE).[18-21] Both methods enable the quantification of the ionic and the particulate fraction, however, sp-ICP-MS is dependent on NP reference material which is currently not available for most NPs and matrices[17] whereas MDG-ICP-MS provide the possibility of directly quantify ionic and/or particulate fraction by internal or external calibration.[20] Furthermore, MDG-ICP-MS require only little sample amount and due to the small single droplets introduced into the ICP, matrices will not alter the ICP conditions tremendously.

In this proof-of-concept communication we will show the first analysis of EBC obtained from a welder performed by MDG-ICP-MS determining the signals of various metals. We will compare the obtained results for flash frozen EBC and EBC stored at room temperature, and complement the results with electron microscopy analysis. Problems, limitations and prospective directions in the field of analytical chemistry and occupational exposure assessment related to this preservation methodology will be discussed.

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MATERIAL AND METHODS

INSTRUMENTATION

As part of a larger occupational exposure study[12] EBC was collected from a healthy, adult male volunteer who conducted four hours of TIG welding as part of his occupational tasks. The study was approved by the Ethics Committee of the Canton de Vaud (protocol 389/13), and was conducted in accordance with the Declaration of Helsinki. All participants provided informed written consent prior to participating in the study. An RTube (Respiratory Research, Atlanta, USA) was used to collect EBC for 10 minutes following the tested procedure early described.[22] The volunteer was asked to rinse out his mouth with water prior to collection to avoid contamination with welding particles deposited in the mouth. The EBC was extracted from the RTube and split into two 0.5 mL samples sealed in Eppendorf tubes. One Eppendorf tube was placed immediately in dry ice to induce flash-freezing and the second was kept at room temperature for approximately 12 hours. For comparison, an EBC sample was collected from a healthy, adult male volunteer who was not exposed to welding fumes.

Shortly before measurement, the frozen sample was defrosted and both samples and blank were transferred into a 4 mL HDPE vial, connected consecutively to a microdroplet dispenser head (Microdrop Technologies GmBh, Norderstedt, Germany) and the isotopes of interest where measured for 3 min each on a Sector Field (SF)-ICP-MS (Element 2, ThermoFisher, Bremen, Germany) according to the experimental procedure described earlier.[18,20] The sequence was repeated three times.

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In Table 1 and Table 2 the ICP-MS and dispenser settings used for the experiment are summarized. Sodium, which is highly present in EBC, was monitored for dispenser stability and transport efficiency (TE).

MICROSCOPY

Approximately 1 µL of each sample was pipetted and let to dry on a Si wafer for scanning electron microscopy (SEM-EDX, FEI Quanta 200 FEG) or on a Copper grid for transmission electron microscopy (TEM, FEI Tecnai F30 FEG) measurements.

EVALUATION

For determination of the limits of detection (LOD) for each measured isotope, mean and standard deviation was calculated. The mean of the blank signals was used for background correction of the intensities obtained from the samples.

For the samples containing a particulate and/or an ionic fraction of the analyzed elements, it is important to apply a so called "split correction" which is necessary using integration times smaller than 10 ms. This correction was applied using a custom written macro in MATLAB (R2012a, MathWorks, Natick, USA), afterwards the mean of the background was subtracted from each signal. The obtained intensities were plotted as intensity distribution in Origin 8.6 (Origin 8.6, OriginLab Corporation, Northampton, USA).[20]

Intensity distributions of the ionic fraction were fitted with a Gauss function and the particulate fraction with a LogNormal function.[14] Mean and standard deviation were calculated from the fitted functions. The blanks were used for calculating the limits of detection (LOD). The LOD for each isotope was calculated using the following formula:

$$LOD = \overline{x_{blank}} + 3 \times \sigma_{blank} \quad (1)$$

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where $\overline{x_{blank}}$ is the mean of the background signal and σ_{blank} its standard deviation.

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RESULTS AND DISCUSSION

MDG-ICP-MS

In single-droplet ICP-MS, a first differentiation between dissolved ions and particles can be done by analyzing the distribution of the signal intensity. An ionic solution will make a narrow peak with the average intensity corresponding to the average concentration, whereas dispersed particles will only create a detectable event when there is a particle in the droplet, which will result in a larger relative standard deviation (RSD). A so-called "split correction" needs to be applied to eliminate signals corresponding to background and summing up signals belonging to one single event.[18]

For all expected elements, signals could be detected in both differently stored EBC samples of the exposed welder, whereas the sample from the non-exposed volunteer shows only background signals. Results for the measured isotopes are summarized in Table 3.

For the differently stored samples, clear differences were observed for Al and Ni. Droplets produced from the unfrozen sample contained the two elements homogeneously distributed, therefore most likely revealing ionic form. The signal intensity is proportional to the amount of introduced material and to the third power of the radius. This means that the larger the width of the signal (standard deviation), the higher the mass difference of introduced material. The introduced droplets have a size variation of approximately 1%, therefore the width of the signal corresponding to homogeneously distributed ionic material would be small. For the unfrozen sample, the RSD of the intensity distribution was relatively small (16% for Al and 22% for Ni) which is similar to an ionic solution aspirated and introduced via the MDG into the ICP; whereas the RSD of the intensity distribution corresponding to particles is normally much larger

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(>30%).[14,18,20,21] These preliminary results suggest that the elements in the unfrozen sample were most likely present as an ionic fraction. Additionally, establishing a stable dispensing and 100% transport efficiency, each droplet should contain a similar amount of ions, while particles should be less frequently detected when assuming $<10^6$ particles mL^{-1}. As such, their intensities produced had a more irregular pattern due to the size dependence. The flash-frozen sample had similar Al and Ni background intensities as the unfrozen sample; however, there was no background corrected distribution detectable. There are only few signals at higher intensities, which are attributed to large Al or Ni particles (confirmed in the SEM microscopy results below, showing a large Ni particle, Figure 7).

Overall, the high ionic content detected in the unfrozen sample allows the assumption that the longer the particles are stored in a liquid medium such as EBC, the more likely they tend to degrade. The time after which this degradation starts to play a significant role for welding particles is likely in the range of hours: We only saw minimal amounts of ions from particles (as indicated by the absence of background) when samples were rapidly frozen. These particles had been dispersed in liquids first in the lungs during the exposure, and then inside the RTube during the EBC-collection. This suggests that the short time (up to 20 minutes) from start of exposure until the samples were flash frozen did not lead to relevant dissolution, whereas the twelve hours until analysis used for the non-frozen samples were too long to avoid dissolution.

The other elements (Fe, Mn and Sn) were present as particles in samples stored by both of the different strategies. Their intensities were above the LODs and the intensity distributions appeared lognormal which is typical for polydispersed NPs (see Figure 1 and Figure 2) Nevertheless, the distributions are very broad (relative standard deviation >50%). An explanation might be that the particles are very polydisperse and their sizes spread over a wide range, or the

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concentration is too high to have only one particle per droplet and the intensity distributions for one particle per droplet is interfered with the distributions for two or more particles per droplet. Considering the results obtained by TEM (presented below), which show very similar particle shape and size especially for the flash frozen sample (Figure 6), it might be more probable that the particles were analyzed as agglomerates consisting of more than one particle.

Comparing signal intensities (Table 4), the flash frozen sample demonstrated higher intensities for Fe and Sn, whereas the sample kept at room temperature showed higher intensity for Mn. Due to the fact that the intensity is proportional to the mass of the analyte introduced into the mass analyzer during a certain time, it is most likely that also Fe and Sn particles degrade relatively slowly within EBC stored at room temperature. It is probable that Mn particles agglomerate over time and therefore the mean intensity is shifted to higher intensity.

MICROSCOPY

The "blank" EBC sample, collected from a volunteer not exposed to welding fumes and kept frozen did not appear to have any particles, and only contained non-particulate organic material (Figure 3). Interestingly, the EBC sample obtained from an exposed welder and stored at room temperature produced similar microscopy results to the blank sample, in that no particles were visible in TEM images and mostly non-particulate organic material was witnessed (Figure 4). In this unfrozen, room temperature sample, very few particles were found with SEM. One relatively small particle (<1 μm) was found, and corresponded to Fe and Mn according to EDX analysis (Figure 5).

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Conversely, the TEM images of the flash frozen sample obtained from the exposed welder demonstrated a very high concentration of NPs (Figure 6). Most of the particles have a spherical shape and seem to agglomerate. Some appear to be core shell particles while others seem to be composed of different components. SEM analysis also revealed the presence of particles, which were relatively larger than the particle found in the unfrozen sample. Figure 7 presents a large particle (>3 μm) that was composed of Ni according to EDX analysis.

In all micrograph images, a high amount of organic matter is visible which also contains high amounts of salts. It is possible that the salts present may form crystals or particles influencing the detection of the particles produced by the welding process.

Overall, our microscopy results show that the unfrozen sample stored at room temperature had very few particles, with only one relatively small particle found in the SEM image. On the other hand, the flash frozen sample revealed a large number of particles in TEM analysis, and relatively large Ni particle in SEM analysis. These findings work to support the conclusions of the MDG-ICP-MS measurements, in that welding NP stored in EBC at room temperature may be more likely to degrade than those stored in flash frozen EBC.

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CONCLUSIONS

This study provides the first results of EBC analysis performed by MDG-ICP-MS. The use of EBC as a biological matrix for analysis of inhaled particle exposure has been increasingly applied in the medical and occupational safety and health fields. With the recent progress in NP characterization studies, it has been recognized that understanding and predicting NP behavior strongly depends on the methods of preparation and preservation until analysis. MDG-ICP-MS is capable of determining the signals of various metals and does additionally allow for distinguishing between ionic and particulate matter.

Preliminary findings by MDG-ICP-MS support the assumption that some of the particles generated by TIG welding, at least those which are composed of Ni and Al, undergo degradation in EBC within a few hours when kept at room temperature. Signals were detected for all expected elements Al, Ni, Mn, Fe and Sn. The sample kept at room temperature contained dissolved Al and Ni, whereas the other elements appear to be in particulate form.

Further to these findings, MDG-ICP-MS measurements are supported by evidence from the TEM and SEM images. For the unfrozen sample, SEM and TEM images do not show a significant amount of particles, and consist mainly of organic matter. On the other hand, the flash frozen sample contains a significant number of spherical particles, in which some appear as core shell particles that seem to agglomerate. SEM analysis also revealed the presence of a relatively larger particle when compared to the unfrozen sample. Most droplets generated by the MDG produced a signal above LOD. Therefore, it will be necessary for further investigations to dilute the original EBC shortly before measurements to reduce the particle number concentration and minimize the probability of coincidence of two or more NPs per droplet. Phosphate buffered saline (PBS) diluted at a similar ratio as lung lining fluid in EBC (about 1:100) may serve as an

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effective liquid, providing a pH similar to body fluids and therefore minimizing the possibility that peptides or proteins degenerate. Additionally, it must be guaranteed that particle appearance (shape, agglomeration, dissolution) is not influenced.

Quantification of NPs contained in EBC might be possible by internal calibration or matrix matched external calibration.[20] However, it will be necessary to find a suitable reference material. Standard solutions which are used in liquid ICP-MS are mainly stabilized in acid which might alter the particles, e.g. by dissolution or agglomeration as well as proteins, e.g. by denaturation. Further studies might also be conducted on a Time-of-Flight (TOF)-ICP-MS which allows much higher time resolution as well as multi-elemental capabilities.[21]

Preliminary results of this investigation revealed the necessity of careful investigation of ongoing processes in a biological matrix such as EBC when dealing with particles and other substances, which may be unstable or sensitive to parameters present, and may therefore change their appearance rapidly. For particle detection in EBC, our results demonstrate the importance of flash freezing EBC samples immediately after collection and defrosting them shortly before analysis at a temperature that does not affect proteins and peptides (<38°C) in order to minimize degradation or alteration of present particles. This storage technique can be applied easily, effectively, and inexpensively to preserve EBC samples, particularly those collected in a field setting for future NP content determination and characterization. While the results presented in this case study offer a preliminary proof of concept, further experiments and more detailed evaluation of the fate of NPs in EBC should be carried out in future studies.

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Environmental Science: Nano Accepted Manuscript

Manuscript ID: ***EN-COM-11-2015-000240***

REFERENCES

1. Hunt, J. Exhaled breath condensate: an evolving tool for non-invasive evaluation of lung disease. J Allergy Clin Immunol 110 (2002) 28–34.

2. Montuschi P, Barnes PJ, Analysis of exhaled breath condensate for monitoring airway inflammation, Trends Pharmacol. Sci. 23 (2002) 232-237.

3. Hartmann L, Bauer M, Bertram J, Gube M, Lenz K, Reisgen U, Schettgen T, Kraus T, Brand P, Assessment of the biological effects of welding fumes emitted from metal inert gas welding processes of aluminium and zinc-plated materials in humans, Int J Hyg Environ Health. 217 (2014) 160–168.

4. Gube M, Ebel J, Brand P, Göen T, Holzinger K, Reisgen U, Kraus T, Biological effect markers in exhaled breath condensate and biomonitoring in welders: impact of smoking and protection equipment, Int. Arch. Occup. Environ. Health 83 (2010) 803-811.

5. Bredberg A, Ljungkvist G, Taube F, Ljungstrom E, Larsson P, Mirgorodskaya E, Isaxon C, Gudmundsson A, Forsgard N, Olin AC, Analysis of manganese and iron in exhaled endogenous particles, J. Anal. At. Spectrom. 29 (2014) 730-735.

6. Bredberg A, Josefson M, Almstrand AC, Lausmaa J, Sjövall P, Levinsson A, Larsson P, Olin AC, Comparison of Exhaled Endogenous Particles from Smokers and Non-Smokers Using Multivariate Analysis, Respiration 86 (2013) 135-142.

7. Sauvain JJ, Sánchez S, Hohl M, Wild P, Pralong JA, Riediker M, Exhaled Breath Condensate as a Matrix for Combustion-Based Nanoparticle Exposure and Health Effect Evaluation J. Aerosol Med. Pulm. Drug Delivery (2014).

8. Almeida SM, Felix PM, Franco C, Freitas MDC, Alves L, Pinheiro T, Barreiros M, Garcia S,Using the exhaled breath condensate as a tool for non-invasive evaluation of pollutant exposure, Int. J. Environ. Health 4 (2010) 293-304.

9. Fox JR, Spannhake EW, Macri KK, Torrey CM, Mihalic JN, Eftim SE, Lees PSJ, Geyh AS, Characterization of a portable method for the collection of exhaled breath condensate and subsequent analysis of metal content, Environ. Sci.: Processes Impacts 15 (2013) 721-729.

10. Jenkins NT, Eagar TW, Chemical analysis of welding fume particles, Weld. J. 84 (2005) 87 93.

11. Lehnert M, Pesch B, Lotz A, Pelzer J, Kendzia B, Gawrych K, Heinze E, Van Gelder R, Punkenburg E, Weiss T, Mattenklott M, Hahn JU, Möhlmann C, Berges M, Hartwig A, Brüning T, Group TWS, Exposure to Inhalable, Respirable, and Ultrafine Particles in Welding Fume, Ann. Occup. Hyg. 56 (2012) 557-567.

12. Graczyk H, Lewinski N, Zhao J, Concha-Lozano N, Riediker M. Characterization of

Environmental Science: Nano Accepted Manuscript

Manuscript ID: ***EN-COM-11-2015-000240***

Tungsten Inert Gas (TIG) Welding Fume Generated by Apprentice Welders. Annals of Occupational Hygiene; 2015 – in press.

13. Flynn MR, Susi P, Manganese, Iron, and Total Particulate Exposures to Welders, J. Occup. Environ. Hyg. 7 (2009) 115-126.

14. Laborda F, Jimenez-Lamana J, Bolea E, Castillo JR, Selective identification, characterization and determination of dissolved silver(i) and silver nanoparticles based on single particle detection by inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom. 26 (2011) 1362-1371.

15. Pace HE, Rogers NJ, Jarolimek C, Coleman VA, Higgins CP, Ranville JF, Determining Transport Efficiency for the Purpose of Counting and Sizing Nanoparticles via Single Particle Inductively Coupled Plasma Mass Spectrometry, Anal. Chem. 83 (2011) 9361-9369.

16. Tuoriniemi J, Cornelis G, Hassellöv M, Size Discrimination and Detection Capabilities of Single-Particle ICPMS for Environmental Analysis of Silver Nanoparticles, Anal. Chem. 84 (2012) 3965-3972.

17. Mitrano DM, Lesher EK, Bednar A, Monserud J, Higgins CP, Ranville JF, Detecting nanoparticulate silver using single-particle inductively coupled plasma–mass spectrometry, Environ. Toxicol. Chem. 31 (2012) 115-121.

18. Gschwind S, Flamigni L, Koch J, Borovinskaya O, Groh S, Niemax K, Gunther D, Capabilities of inductively coupled plasma mass spectrometry for the detection of nanoparticles carried by monodisperse microdroplets, J. Anal. At. Spectrom. 26 (2011) 1166-1174.

19. Olesik JW, Gray PJ, Considerations for measurement of individual nanoparticles or microparticles by ICP-MS: determination of the number of particles and the analyte mass in each particle, J. Anal. At. Spectrom. 27 (2012) 1143-1155.

20. Gschwind S, Hagendorfer H, Frick DA, Günther D, Mass Quantification of Nanoparticles by Single Droplet Calibration Using Inductively Coupled Plasma Mass Spectrometry, Anal. Chem. 85 (2013) 5875-5883.

21. Borovinskaya O, Hattendorf B, Tanner M, Gschwind S, Gunther D, A prototype of a new inductively coupled plasma time-of-flight mass spectrometer providing temporally resolved, multi-element detection of short signals generated by single particles and droplets, J. Anal. At. Spectrom. 28 (2013) 226-233.

22. Riediker M, Danuser B, Exhaled Breath Condensate pH Is Increased after Moderate Exercise, J Aerosol Med. 20 (2007) 13-18.

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TABLES

Table 1: ICP-MS settings.

RF Power	1280	W
He at Dispenser	0.4	L/min
Ar at Adapter	1.004	L/min
Auxiliary Gas Flow	0.81	L/min
Plasma Gas Flow	16	L/min
Measured Isotopes	(^{23}Na), ^{27}Al, ^{55}Mn, ^{57}Fe, ^{58}Ni, ^{120}Sn	
Dwell Time	1	ms
Samples/Peak	1000	
Integration Window	10%	
Resolution	Low	

Table 2: Dispenser settings.

Dispenser Frequency	100	Hz
Droplet Diameter	~40	µm
Droplet Volume	~34	pL

Table 3: Limits of detection for the determined isotopes in the sample kept at room temperature or frozen.

	^{27}Al	**^{55}Mn**	**^{57}Fe**	**^{58}Ni**	**^{120}Sn**
LOD RT [counts]	660	13	13	33	12
LOD frozen [counts]	710	13	13	47	12

Table 4: Summarized intensities for the determined isotopes in the sample kept at room temperature and the flash frozen sample.

	^{27}Al	**^{55}Mn**	**^{57}Fe**	**^{58}Ni**	**^{120}Sn**
Signal RT [counts]	420±70	670±380	45±36	62±14	46±62
Signal frozen [counts]	-	320±350	75±41	-	100±320

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FIGURE DESCRIPTIONS

Figure 1: Signal distributions for the isotopes present in the EBC sample kept at room temperature; the blue line represents a LogNormal fit, the purple line a Gauss fit.

Figure 2: Signal distributions for the isotopes present in the EBC sample flash frozen immediately after collection; the blue line represents a LogNormal fit (intensity distributions after applying the split correction to eliminate signals corresponding to background and summing up signals belonging to one single event).

Figure 3: TEM images of a blank EBC sample obtained from a control volunteer not exposed to welding fumes.

Figure 4: Microscopy images of an exposed EBC sample obtained from a welder and kept at room temperature. Mainly non-particulate organic material is visible.

Figure 5 : SEM image of an exposed EBC sample obtained from a welder and kept at room temperature. EDX analysis revealed an elemental composition of Fe and Mn.

Figure 6: TEM images of an exposed EBC sample obtained from a welder, immediately flash frozen after collection. Numerous particles are visible: most appear spherical and some seem to be core shell particles (dark metal core with light grey organic shell).

Figure 7: SEM image of the flash frozen EBC sample obtained from a welder shows a large Ni particle, as determined by EDX.

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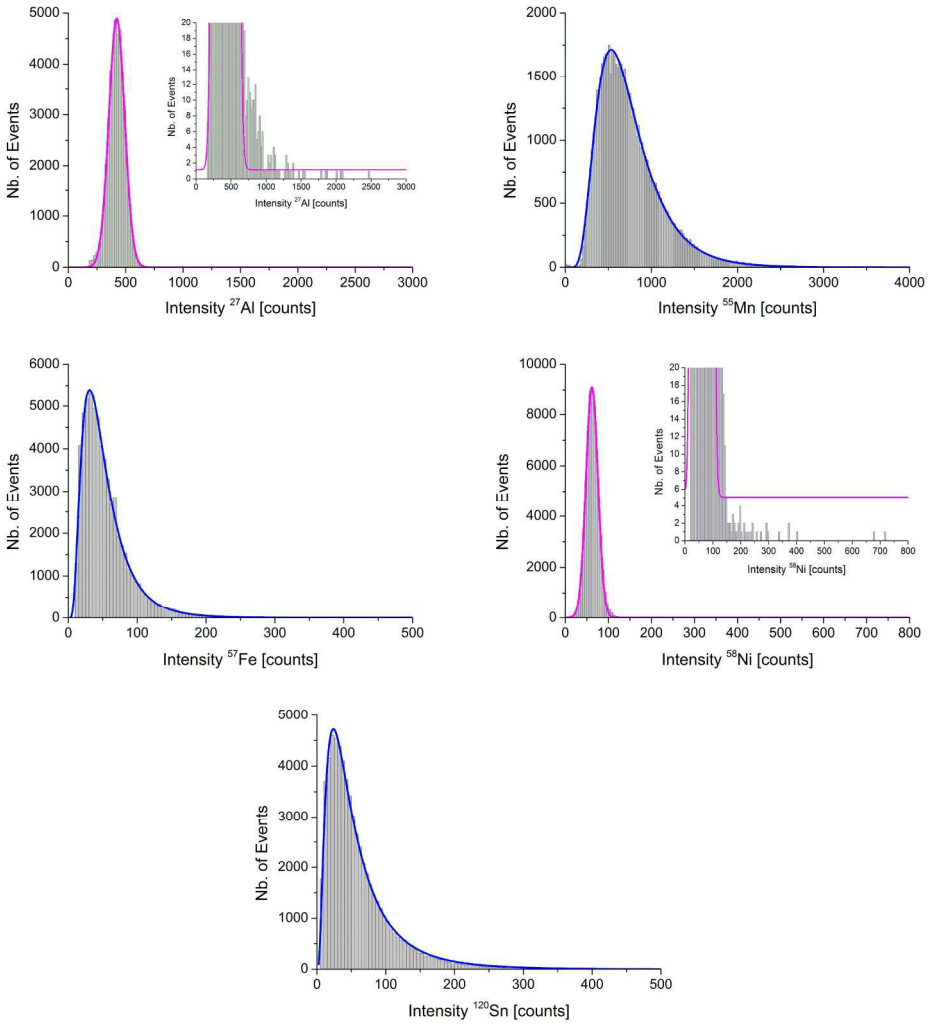

Figure 1: Signal distributions for the isotopes present in the EBC sample kept at room temperature; the blue line represents a LogNormal fit, the purple line a Gauss fit.
623x658mm (96 x 96 DPI)

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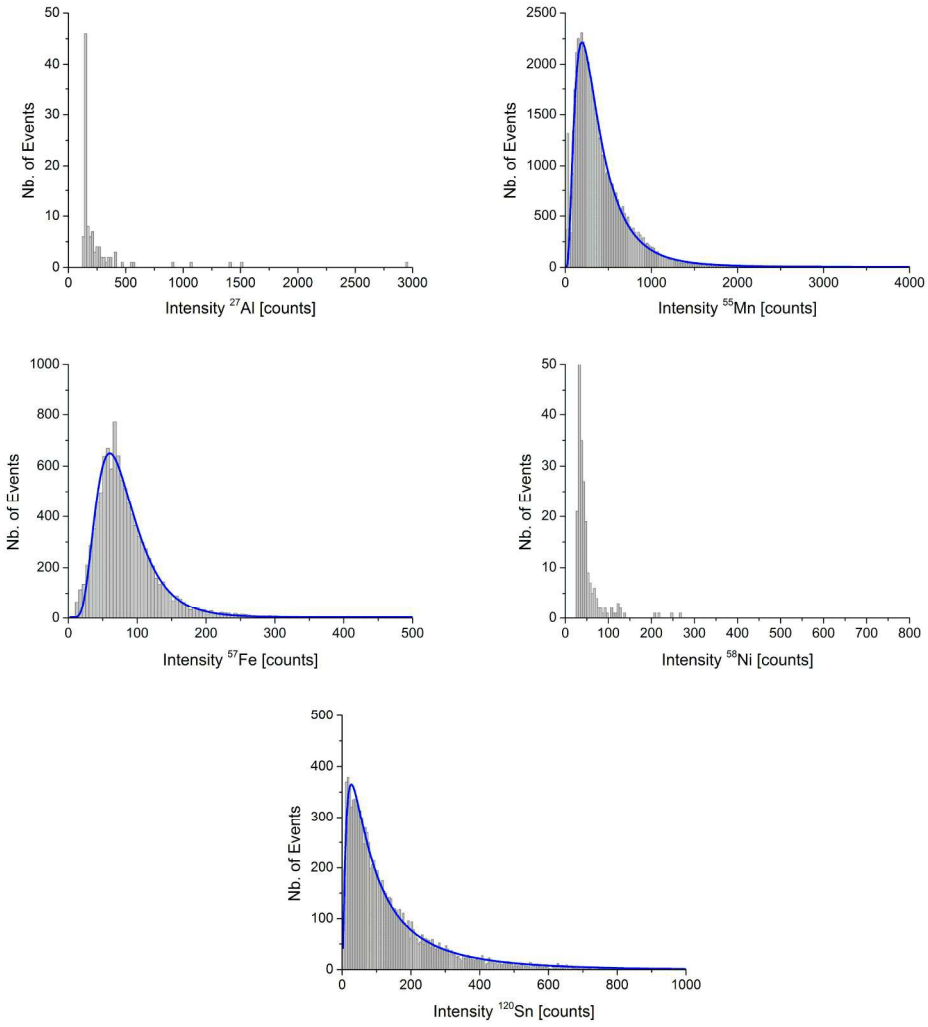

Figure 2: Signal distributions for the isotopes present in the EBC sample flash frozen immediately after collection; the blue line represents a LogNormal fit (intensity distributions after applying the split correction to eliminate signals corresponding to background and summing up signals belonging to one single event).
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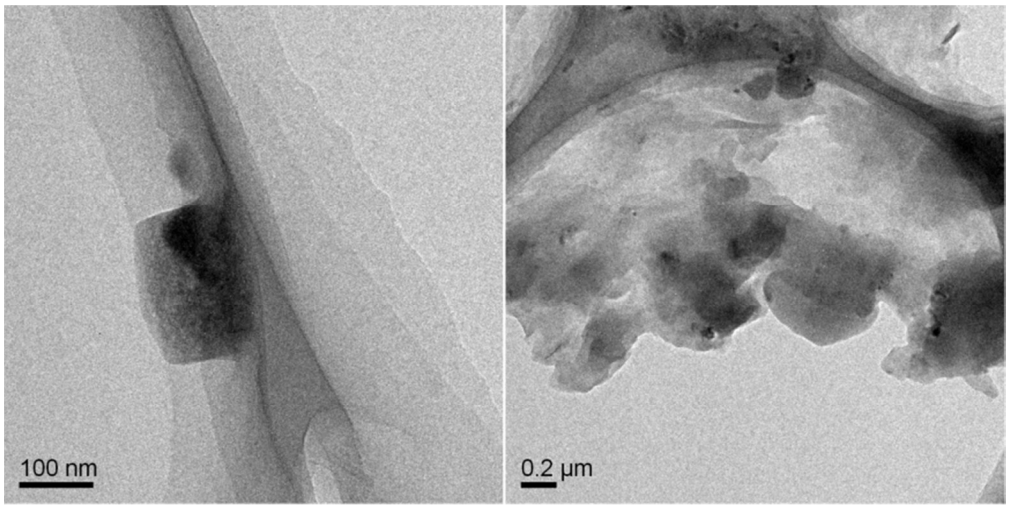

Figure 3: TEM images of a blank EBC sample obtained from a control volunteer not exposed to welding fumes.
229x114mm (96 x 96 DPI)

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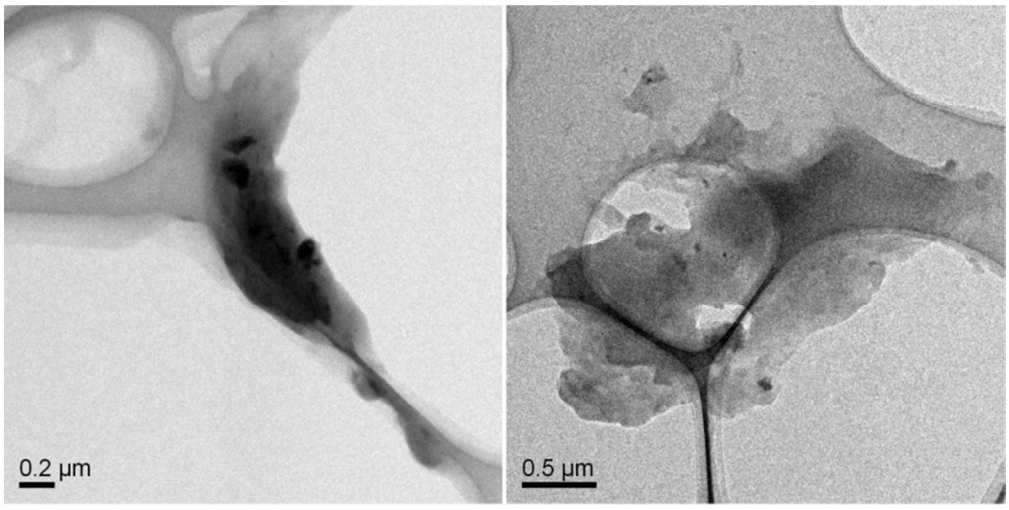

Figure 4: Microscopy images of an exposed EBC sample obtained from a welder and kept at room temperature. Mainly non-particulate organic material is visible.
229x114mm (96 x 96 DPI)

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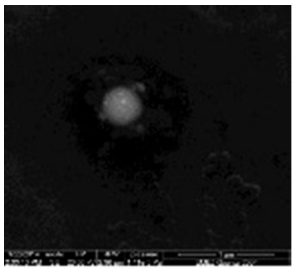

Figure 5 : SEM image of an exposed EBC sample obtained from a welder and kept at room temperature. EDX analysis revealed an elemental composition of Fe and Mn.

50x46mm (72 x 72 DPI)

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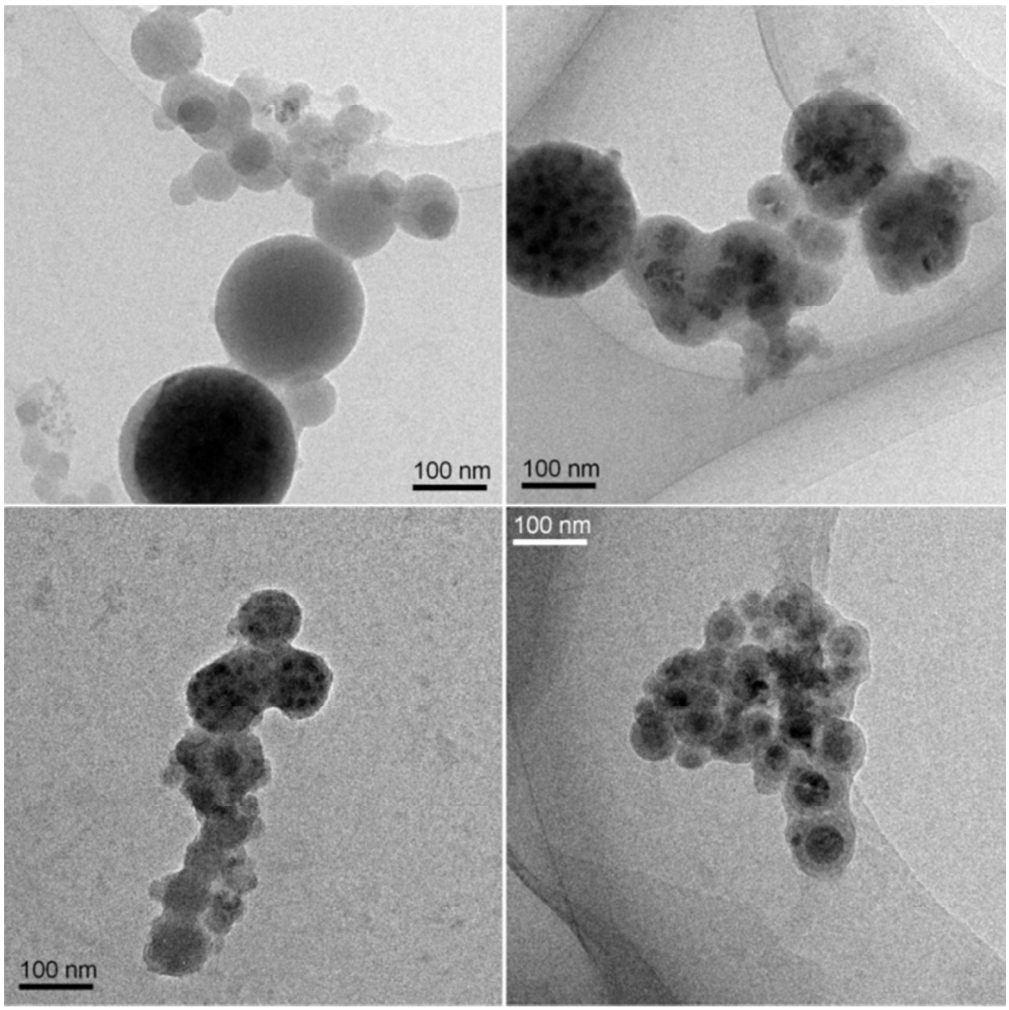

Figure 6: TEM images of an exposed EBC sample obtained from a welder, immediately flash frozen after collection. Numerous particles are visible: most appear spherical and some seem to be core shell particles (dark metal core with light grey organic shell).
186x186mm (96 x 96 DPI)

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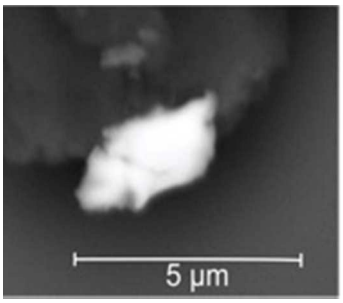

\r\nFigure 7: SEM image of the flash frozen EBC sample obtained from a welder shows a large Ni particle, as determined by EDX.\r\n
59x51mm (72 x 72 DPI)

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