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Analysis of manganese and iron in exhaled endogenous particles

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Background: many full-time welders experience some sort of respiratory disorder *e.g.*, asthma, bronchitis and metal fume fever. Thus, welding aerosols are thought to cause airway inflammation. There is a need for markers of welding aerosols in exposure assessments, and as most welding aerosols contain manganese and iron, these metals may possibly be used as an indicator. We have previously developed a novel non-invasive technique to collect endogenous particles in exhaled air (PEx). This study is designed to (i) develop a method for analysis of manganese and iron in PEx and (ii) investigate whether the manganese and/or iron content of PEx changes after exposure to welding aerosols. *Methods*: nine individuals were experimentally exposed to welding fumes. PEx was collected at three time points for each individual; before, after and 24 hours after exposure. Analyses of PEx samples were performed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). *Results*: four out of nine individuals showed an increase in manganese and iron levels after exposure to welding aerosols. The mean manganese and iron concentration increased from, <LOD to 82–84 pg L^{-1} (range from 0 to LOD for values <LOD) and 20–86 to 2600 pg L^{-1} of exhaled air respectively. *Conclusions*: an ICP-MS method for analysis of manganese and iron in PEx has been developed. The method could easily be expanded to include other trace metals of interest, such as cadmium, nickel or chromium. This first attempt to evaluate PEx as a tool for exposure assessments of airborne metals indicates that the method has potential.

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Introduction

More than two million workers around the world have welding as part of their work duties.[1] Welding processes generates an aerosol consisting of potentially harmful particles and gases.[1] The primary particles formed are in the range 0.01–0.1 μm, but quickly agglomerate into larger particles in the 0.1–0.6 μm range, which have a high degree of peripheral deposition.[1,2] Particles of a larger size may deposit in the nasal airway region and reach the brain *via* the olfactory transport from the nose to the brain.[1]

Inhaled welding aerosols have been suspected to be a pulmonary irritant which can induce airway inflammation. Most full-time welders experience some type of respiratory disorder *e.g.*, asthma, bronchitis, decrease in the lung function, metal fume fever, and increased susceptibility to infections.[1,3–5] Most welding aerosols contain manganese (Mn) since the welding rod and steels contain manganese to improve the metallurgical properties of the weld.[1,4] Manganese may therefore be used as an indicator for exposure to welding aerosols.

A fraction of the welding aerosol of the inhalable size is deposited in the respiratory tract lining fluid (RTLF), thus, sampling of the RTLF is of interest in exposure assessments. Therefore, a non-invasive method, suitable for repeated sampling of the RTLF, would be useful for measuring the dose of airborne pneumotoxic metals. The non-invasive method Exhaled Breath Condensate (EBC) has shown potential in assessment of the lung dose. However, the EBC method is foremost a method optimized for collection of volatile compounds. We hypothesized that the deposited aerosol is absorbed by the RTLF and that the material may be incorporated into endogenous particles formed during exhalation.

Our novel equipment designed for collection of particles in exhaled air, PEx, has been described in detail previously.[6] PEx are produced in the peripheral airways during breathing and originates from the RTLF, which covers the airways as a protective interface.[7] We have previously shown that PEx contain phospholipids and proteins and that the composition is altered in individuals who smoke or have asthma.[6,8–11] Furthermore, a recent unpublished pilot study has indicated

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that PEx from smokers contain more cadmium than PEx from non-smokers.

The present study is the first step to investigate whether PEx can be used for exposure assessment of metals in occupational settings.

The specific aim of this study was to test the following hypotheses: (i) quantification of manganese and iron in PEx can be performed using inductively coupled plasma mass spectrometry (ICP-MS), (ii) the manganese and iron content of PEx is increased after experimental exposure to welding fumes.

Method

To test the two hypotheses the study comprises two subprojects. First method development and evaluation to confirm that quantification of manganese and iron in PEx can be performed using ICP-MS. Secondly an experimental exposure was conducted to test whether the manganese and iron content of PEx is increased after experimental exposure to welding fumes.

Method development and evaluation

Collection of PEx. PEx were counted and collected using a system developed in-house.[6] In all experiments the individuals performed a standardised four-step breathing manoeuvre: (i) full exhalation to the residual volume, (ii) breath holding for five seconds, (iii) a quick full inspiration to total lung capacity, (iv) relaxed exhalation back to the residual volume. Collection of PEx solely in the fourth step was achieved by using an airflow diverting valve. The breathing manoeuvre was repeated until 100–120 L had been collected, which was achieved in about 20 minutes. Particle free air was breathed for two minutes prior to the first exhalation and for a few breaths between each breathing manoeuvre. A nose clip was worn throughout the procedure.

Exhaled particles with an aerodynamic diameter of 0.4–4.6 μm impacted on a hydrophilic membrane filter (hydrophilic 0.45 μm FHLC, http://www.millipore.com) that was subsequently analysed. Particles were counted and sized using an online optical particle counter (Grimm Model 1.108, Grimm Aerosol Technik, Ainring, Germany).

ICP-MS analysis of manganese and iron in PEx. All extractions of samples were performed in laminar flow hoods to minimize contamination. All containers used for samples and solutions were acid leached with 5% nitric acid (suprapur, http://www.merck.com) for at least one week and rinsed three times with ultrapure water from a Milli-Q advantage ultrapure water system (http://www.millipore.com) combined with a Q-POD Element unit (http://www.millipore.com). The handling of filters prior to, and after sampling, could for practical reasons not be performed in laminar flow hoods, but was achieved rapidly. PEx is sampled in an in-house system mainly constructed of stainless steel and aluminium. It was therefore necessary to test possible contamination from the system by multiple blank samples. The collection procedure involves cutting the membrane filter with a scalpel, and the blank samples were treated in the same way. To determine the manganese and iron (Fe) content of filters additional analyses of filters taken directly from the supply package were performed.

Standard and sample preparation. Standards were prepared from a multi-element calibration standard (http://www.agilent.com) containing 10 mg L^{-1} Mn and Fe. Standards were prepared on a daily basis and diluted with 2% nitric acid. Germanium was used as the internal standard and was added from an internal standard mix (http://www.agilent.com) containing 10 mg L^{-1} to give a final concentration of 5 μg L^{-1}. Calibration was achieved by external calibration with standard solutions from 0.1 to 500 μg L^{-1}.

PEx samples were extracted by ultrasonication for 10 minutes, in 300 μL of 2% nitric acid with addition of the internal standard, and then centrifuged for 1 minute before discarding the filter. Mean extraction recovery was determined through analyses of three consecutive extractions of five different PEx samples (each sample concentration after first extraction divided by its total concentration from all three extractions). All PEx samples were analysed within 8 weeks from collection.

Instrumentation. An Agilent 7700x ICP-MS (http://www.agilent.com) with an octopole reaction system was used for all analyses. The reaction system was operated in the helium collision cell mode to eliminate interference from isobaric polyatomic species *via* kinetic energy discrimination. Typical operating conditions are given in Table 1. Samples were introduced with a peristaltic pump and nebulization was carried out with a MicroMist concentric nebulizer (http://www.agilent.com). The spray chamber used was a Scott-type double-pass operated at 2 °C and a standard quartz torch with 2.5 mm internal diameter injector was used. The instrument was equipped with an Agilent I-AS integrated autosampler. The 1.5 mL Eppendorf microtubes, used for extraction, were fitted inside standard autosampler tubes to minimize the volume of the autosampler vials. Before each batch, an autotune was performed with a 1 μg L^{-1} tune solution containing Ce, Co, Li, Mg, Tl and Y and using the 7700 MassHunter software to optimize sensitivity and oxide levels. The sample uptake time was adjusted before each run to be as short as possible to maximize

Table 1 Standard operating conditions in the ICP-MS method

Instrument parameters	
RF power	1550 W
Carrier gas	1.08 L min^{-1}
Sample depth	8 mm
He flow (ORS)	5.0 mL min^{-1}
Energy discrimination	3.0 V
Acquisition parameters	
Points/mass	3 points
Replicates	3
Integration time/point	
Mn (*m*/*z* 55)	1.00 s
Fe (*m*/*z* 56)	0.30 s
Ge (*m*/*z* 72)	0.20 s
Sweeps/replicate	100

measurement time but still give a constant signal before measurement. Total analysis time per sample including sample uptake and wash-out was 2 min.

The developed method for quantification of Mn and Fe in PEx was first applied in a pilot study of ten healthy non-smokers (5F/5M), and then in the experimental exposure study where nine participants were included.

Experimental exposure study

The main study comprised PEx collection at three time points; before, within five minutes after, and 24 hours after two hours exposure to welding fumes. The Mn and Fe content of the PEx samples was established using the developed method. Nine healthy non-smokers (4F/5M) between 29 and 63 years of age, volunteered for the exposure study. An informed consent was obtained from all individuals and the study was approved by the Ethics Committee of the University of Gothenburg.

The exposure chamber has been described in detail by Isaxon *et al.* 2012. In short, the chamber is a 21.6 m^3 room made out of stainless steel where the welding aerosol is premixed with the supply air (air exchange rate 5.8 h^{-1}). The system is under slight positive pressure to avoid contamination or dilution by surrounding air. In this study the chamber was equipped with four resting chairs and the temperature is kept at 23 °C, to provide a comfortable environment for the participants.

Welding fumes were generated by gas metal arc welding (GMAW), with a commonly used 1 mm electrode (ESAB, Aritorod 12.50, Gothenburg, Sweden) and shielding gas, Ar/CO$_2$ mixture (Air liquid, Arcal MAG, Paris, France), to resemble real-life working conditions. Welding was performed in short intervals at 125 A, 5.5 V to create an average fume particle concentration below the Swedish threshold limit value, 0.1 mg m^{-3} (8 hour mean level) for manganese in respirable dust and 3.5 mg m^{-3} for iron (from iron oxides in respirable dust). A Scanning Mobility Particle Sizer (SMPS) system was used to measure the particle number concentration and mobility size distribution of the welding fumes. A tapered element oscillating microbalance (TEOM) was used to measure the particle mass concentration. The chemical composition of the fumes was determined to be 14% manganese and 49% iron using particle-induced X-ray emission (PIXE) and X-ray energy dispersive spectroscopy (XEDS). A more detailed description can be found elsewhere.[12]

Results

Method development and evaluation

Calibrations were linear over the calibration range (0–500 μg L^{-1}) with determination coefficients (R^2) > 0.9995 for both elements. Mean recovery was 88% for manganese and 80% for iron. The limit of detection (LOD = mean$_{\text{blank}}$ + 3 × SD$_{\text{blank}}$) for the ICP-MS analysis (LOD$_{\text{ICP-MS}}$) was determined to be 1 ng L^{-1} and 20 ng L^{-1} for Mn and Fe respectively. Assuming 300 μL extraction volume, this corresponds to an LOD$_{\text{ICP-MS}}$ of 0.3 pg (Mn) and 6 pg (Fe) per sample extracted.

However, the LOD of the method in field (LOD$_{\text{METHOD}}$) was considerably higher due to the metal content of the filter and to the handling procedure. Analyses of filters (without any contact to sampling equipment) and blank samples (filters placed in sampling equipment for 30 min and then excised using a scalpel) were performed, using 300 μL extraction volumes. Data showed that essentially all manganese in the blank samples originated from the filters, 270 pg in filters as well as in blank samples (with a range for filters 230–370 pg and for blanks 170–350 pg). Most of the iron in the blank samples originated from the handling procedure, 1900 pg (450–6500 pg) while 420 pg (140–760 pg) for filters alone. The LOD$_{\text{METHOD}}$ was calculated to be Mn: 450 pg and Fe: 7800 pg per sample extracted, and samples below these values are reported as <LOD. For samples above LOD$_{\text{METHOD}}$, averaged field blank values were subtracted and results were reported as mass per litre exhaled air.

None of the ten samples from unexposed individuals, in the method development, showed manganese levels above LOD and only one sample showed detectable levels of iron.

Experimental exposure study

The particle concentrations in exhaled air, collected in the exposure study, are presented in Table 2. The intra-individual difference of number concentrations was relatively low. There was however, a large inter-individual difference with a range from 4000 to 45 000 particles per litre of exhaled air.

The first 7 individuals were exposed to 2.7 mg m^{-3} total dust, while the last two individuals were exposed to 4.5 mg m^{-3} total dust. Given that the welding fumes have a Mn and Fe content of 14% and 49%, respectively, that all individuals were exposed for two hours, and that the average individual inhale 7 L min^{-1}, the inhaled dose of Mn and Fe would be approximately 0.3 mg and 1.1 mg in the first exposure. For individual number 8 and 9, in the second exposure, the inhaled dose was estimated to be 0.5 mg and 1.9 mg.

The metal concentration is presented in Table 3 and expressed as pg L^{-1} exhaled air. In total 4 and 6 of the 27 samples had concentrations above the limit of detection for Mn and Fe respectively. Four individuals showed higher levels of Mn as well as Fe after exposure. The 24 hour samples show no measurable lasting effects on the manganese and iron

Table 2 Number concentrations of exhaled particles (0.4–4.6 μm) per liter of exhaled air collected; before, immediately after and 24 hours after exposure. Samples showing a Mn and/or Fe content in the subsequent analysis (Table 3) are highlighted

	Number of particles (0.41–4.55 μm) L^{-1} exhaled air		
Subject	Before exposure	After exposure	24 h after exposure
1	4000	4900	4900
2	6300	6000	5900
3	13 000	13 000	18 000
4	25 000	33 000	32 000
5	6500	8100	13 000
6	29 000	27 000	29 000
7	45 000	44 000	39 000
8	28 000	22 000	28 000
9	13 000	15 000	8800

Table 3 Concentrations of manganese and iron in exhaled air before and after exposure to welding aerosols

	Before exposure		After exposure		24 hours after exposure	
Subject	Mn (pg L^{-1})	Fe (pg L^{-1})	Mn (pg L^{-1})	Fe (pg L^{-1})	Mn (pg L^{-1})	Fe (pg L^{-1})
1	<LOD	<LOD	120	2600	<LOD	<LOD
2	<LOD	72	28	750	<LOD	<LOD
3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
6	<LOD	<LOD	560	19 000	<LOD	<LOD
7	<LOD	250	30	1100	<LOD	<LOD
8	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
9	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

concentration after the exposure. All samples containing manganese also contained iron, revealing a ratio of 3–5% manganese.

Discussion

An ICP-MS method for the analysis of manganese and iron in PEx has for the first time been developed. The developed method was applied in an experimental exposure study where 4 out of 9 test subjects showed a substantial increase in manganese and iron levels after exposure.

We achieved a limit of detection for the analysis (LOD_{ICP-MS}) of manganese: 1 ng L^{-1} and iron: 20 ng L^{-1}, this corresponds to 0.3 pg and 6 pg per sample extracted. However, the detection limit of the full method, including filters and sampling procedure, was considerably higher. The filters used in this study were the major source of Mn in the LOD_{METHOD}. The relatively high and variable background of manganese in the filters suggests that ultra-pure filters should be used in further studies. The sampling equipment contributed to the Fe LOD_{METHOD} to a large extent and part of the tubing could possibly be exchanged into Teflon. Besides this, great care has to be taken to avoid contamination from ambient air and laminar flow hoods should be used in all sample handling. Still, manganese and iron in four of the samples from exposed individuals reached levels of at least five times the LOD_{METHOD}. The non-invasive method EBC has also described methodological difficulties, such as contamination and low concentrations, and general methodological recommendations suggest that all materials in contact with the EBC sample should be tested for possible contamination.[13,14] The EBC method has nevertheless shown potential with significant differences, on a group level, in EBC metal content from controls and individuals exposed to *e.g.* tobacco smoke or welding fumes.[13,15,16]

Unexposed individuals had manganese levels below the LOD_{METHOD} (in total 19 samples as samples from the pilot study were included). Iron was detected in three of the 19 samples from unexposed individuals giving a mean of 20–86 pg L^{-1} (range from 0 to LOD_{METHOD} for values <LOD_{METHOD}). The 9 samples from exposed individuals had increased mean levels of manganese, 82–84 pg L^{-1}, and iron, 2600 pg L^{-1} exhaled air. The literature on manganese and/or iron content of EBC is scarce, but relatively consistent, with manganese levels in ng L^{-1} condensate and iron levels in μg L^{-1} condensate.[13,17–21] As the PEx sample is dry and can be dissolved in any volume, we decided to report the mass per litre of exhaled air. EBC studies report levels per litre of condensed water, from the exhaled air, and in order to get comparable results we need to make estimations of the metal content per litre of exhaled air in the EBC experiments. Measurement of the exhaled volume is not a standard procedure in the EBC method, but rather a set time for collection, normally 10–15 minutes, to produce a sufficient amount of condensed water. Moreover, different condensers may differ in efficiency and hence produce different amounts of condensate.[22] A fair estimation would be that 10–15 minutes of breathing yield approximately 100 L of air and 1 mL of condensate. The concentration of Mn and Fe in EBC would then be comparable to PEx, *i.e.* in the pg L^{-1} exhaled air region.

Five out of nine individuals showed no measurable increase in Mn and Fe levels after exposure. The reason for this is unclear, but may be due to differences in airway geometry, which may cause a different deposition of the welding aerosols in these individuals.[23] If the deposition is located to an area without any airway closure, *i.e.* an area unable to generate PEx, no welding aerosol particles would be present in the exhaled aerosol. This is a considerable problem for estimation of acute exposure, but may be of less importance assessing long-term exposure. As ciliated transport is constantly moving the RTLF upward toward the glottis, to clear the lungs from inhaled substances, a deposited aerosol may move up through the respiratory tree to a PEx generating area.[24] The non-invasiveness of PEx offers a possibility to monitor this by repeated measurements after exposure.

It was noted that the Mn/Fe ratio was lower in the exhaled particles, 0.03–0.05, compared to the inhaled welding aerosol which had a Mn/Fe ratio of 0.29. It appears unlikely that the manganese and iron oxides, comprising a large part of the particles, will dissolve at biological pH, thus the particles most likely are cleared through ciliated transport.[4] Another possibility may be that manganese and iron oxides accumulate in particles of different sizes and therefore deposit at locations with different tendencies for airway closure and thus different capabilities to generate PEx. A mechanism to explain such a fractionation could be based on the boiling points of the melted metals since iron has a boiling point of 2750 °C while manganese boils already at 1962 °C.[25]

Our results are in line with previous studies regarding the large inter-individual difference in the number concentration of PEx. This phenomenon is suggested to be a consequence of different compositions of the RTLF and/or more extensive airway closure in individuals with higher particle amount.[26,27] Furthermore, we found a positive association between the number concentration of PEx collected and age, which is in agreement with previous studies showing more frequent airway closure with increasing age.[28,29] The low intra individual difference in the number concentration of PEx collected before and after exposure indicates that this type of acute short term exposure does not alter the actual production of PEx, *i.e.* the extent of airway closure.

Conclusion

An ICP-MS method for analysis of manganese and iron in PEx has been developed. The method may be of particular interest in preventive occupational medicine and could easily be expanded to include other trace metals of interest, such as cadmium, nickel or chromium. Four out of nine individuals showed an increase in manganese and iron levels after exposure to welding aerosols. This first attempt to evaluate PEx as a tool for exposure assessments of airborne metals indicates that the method has potential. In future exposure studies, frequent repeated measurements of PEx are desirable to monitor the transport of the deposited material in the respiratory tree.

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