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# Manganese in exhaled breath condensate: A new marker of exposure to welding fumes

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## HIGHLIGHTS

- Our study evaluates Mn and Ni in EBC as an indicator of exposure to welding fumes.
- Concentrations of Mn and Ni in EBC of welders are higher than unexposed subjects.
- Levels of Mn and Ni in EBC are correlated with the cumulative indices of exposure.
- Levels of Mn and Ni in EBC are not correlated with their respective levels in urine.
- Mn and Ni levels in EBC are both complimentary indices of exposure to those in urine.

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## ABSTRACT

*Objective:* To evaluate manganese in exhaled breath condensate (Mn–EBC) as an indicator of exposure to fumes from metal inert gas welding process.
*Methods:* We collected EBC and urine from 17 welders and 16 unexposed control subjects after 5 days exposure. Concentrations of manganese (Mn), nickel (Ni), iron (Fe) and chromium (Cr) were measured in EBC and urine samples and correlated with cumulative exposure indices for the working week (CIW) and for the total welding years (WY), based on duration of welding activity and atmospheric metal measurements.
*Results:* Concentrations of Mn and Ni in EBC were significantly higher among welders than controls whereas this difference was not significant for Mn in urine. Levels of Mn and Ni in EBC were not correlated with their respective levels in urine. The linear regressions found significant positive coefficients between Mn–EBC, Ni–EBC, Ni–U and Cr–U concentrations and the cumulative exposure indices. Taking into account tobacco use, statistical analysis showed the same trends except for the relationship between Mn–U and CIW.
*Conclusion:* This pilot study showed that Mn–EBC, as well as Ni–EBC, can serve as reliable indices of occupational exposure to welding fumes and provide complimentary toxicokinetic information to that provided by urine analyses.


*Abbreviations:* ACGIH, American Conference of Governmental Industrial Hygienists; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AWS, American Welding Society; CHRU, Centre Hospitalier Régional et Universitaire; CIW, cumulative index of exposure for a week; Cr, chromium; EBC, exhaled breath condensate; Fe, iron; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in a second; FVC, forced vital capacity; GMAW, Gas Metal Arc Welding; ICP-AES, inductively coupled plasma atomic emission spectrometer; ICP-MS, inductively coupled plasma mass spectrometer; IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; MIG, metal inert gas; MMEF, maximal mid-expiratory flow; Mn, manganese; MS, mild steel; Ni, nickel; SD, standard deviation; SE, standard error; TLV, threshold limit value; TWA, time weighted average; WY, welding years.

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# 1. Background

Several hundreds of thousands of people work as welders in the world. There are different welding processes according to the metal parts to be joined and the American Welding Society (AWS) has listed over 80 different processes. The most common type of welding process used in industry is Gas Metal Arc Welding (GMAW). In this process, a shielding gas prevents weakening of the weld produced by heating a continuous filler metal electrode (Antonini, 2003). The metal inert gas (MIG) process, in which the shielding gas is not involved in the formation of the weld, is used to join two mild steel (MIG/MS) or stainless steel pieces. The main constituents of welding fumes from MIG/MS are iron (Fe) and manganese (Mn) (Antonini et al., 2004).

The main uses of Mn are in iron and steel production by supplementing steel with ferromanganese on the one hand to reduce the dioxygen and sulfur content, and on the other hand to increase the rigidity, hardness and strength of the steel. Manganese is also used as an oxidizing agent for electrode coating in welding rods. The concentration of Mn in welding fumes from the welding of mild steel by the MIG technique is between 3.9% and 7.3% (IARC, 1990).

Occupational chronic exposure to manganese by inhalation is associated with increased respiratory symptoms like bronchitis but also to a change in spirometric parameters (Bowler et al., 2007; Roels et al., 1987, 1985). Recent studies have focused on early neurological effects that may occur at low occupational exposure (Laohaudomchok et al., 2011); this is one reason why the American Conference of Governmental Industrial Hygienists (ACGIH) has proposed, in a Notice of Intended Change for Manganese, to reduce the threshold limit value (TLV) —time weighted average (8-h TWA) for respirable particulates from 0.2 to 0.02 mg $m^{-3}$.

Manganese is known to accumulate in the lungs (Leem et al., 2000). Manganese in blood and urine are controversial biomarkers of exposure due to the variability of the metabolism and excretion of this metal (Hoet et al., 2012; Järvisalo et al., 1992; Roels et al., 1992; Zheng et al., 2011); it is thus important to develop a more reliable biological index of exposure that could explain the burden of Mn in lungs and better link it to occupational exposure via inhalation.

Exhaled breath condensate (EBC) is a matrix that can be sampled non-invasively and has the potential to reflect occupational exposure to metallic particles (Chérot-Kornobis et al., 2012; Mutti and Corradi, 2006; Smolders et al., 2009). Hoffmeyer et al. have demonstrated the usefulness of EBC for the medical surveillance of welders, assaying metals such as nickel (Ni), iron, and chromium (Cr), as well as biomarkers of effect in the EBC (Hoffmeyer et al., 2012). Mutti et al. have previously determined manganese in the EBC of unwell subjects >but, to our knowledge, no study has ever studied Mn in EBC as a biomarker of occupational exposure (Mutti et al., 2006). The aim of this study was therefore to evaluate, in an exposed-control study, the utility of assaying Mn in EBC at the same time as other metals already studied (Ni, Fe and Cr), and to relate their concentrations in EBC to the atmospheric concentrations of metals in MIG welding fumes.

## 2. Materials and methods

### 2.1. Study population

We conducted an exposed-control study at the end of a working week on a population of 17 welders and 16 unexposed control subjects. We selected a plant in which welders were exposed to manganese in welding fumes during the assembly of metal structures for the manufacture of railway vehicles. The MIG welding process is used at the plant to assemble the mild steel structures of trains and the welding workstation conditions have remained unchanged for several years. During our study, welders were working on two production lines, which we named "A" and "B"; the welding process and the composition of both filler metal and metal electrode were similar for the two production lines. According to the company's own data, the welding process used metal electrodes containing between 1.4% and 1.62% manganese, 0.005% and 1.55% nickel, and 0.03% and 0.35% chromium. All welders worked in a naturally ventilated hall and wore personal breathing protection during the welding process in the form of supplied-air respirators (3M Speedglas™ 9100 Series Welding Shields, 3 M Company, St. Paul, US). Recruitment took place from June 2011 to March 2012 and the inclusion criteria of welders were:

- no history of respiratory diseases and no current respiratory symptoms;
- only mild steel should be welded and only MIG welding used at the time of the study;
- a history of at least two years of continuous welding work at the plant.

Controls were identified in the same or a different plant than the welders and had no current or previous welding exposure. We excluded all controls working in the vicinity of the welding area or at other points that would potentially expose them to air pollutants from the welding process. The control group was recruited after the welders so as to obtain similar characteristics according to their sex, age, and tobacco consumption. All subjects in this study were male. This study was performed during the regulatory follow-up of the subjects imposed by the French Labour Code after approval of the Committee of Health and Safety Working Conditions. All subjects have been fully informed about the aims of the study, and have given their prior, free and informed consent.

### 2.2. Study design

All subjects answered a questionnaire in two parts:

- The first part of the questionnaire concerned subjects' background, in particular respiratory conditions, respiratory symptoms, smoking habits, and tobacco consumption. The questionnaire on respiratory symptoms was a version of the Medical Research Council questionnaire, modified by the British Occupational Hygiene Society, translated into French, and previously validated by Marez and coworkers (British Occupational Hygiene Society Committee on Hygiene Standards, 1980; Marez et al., 1993).
- The second part of the questionnaire was about occupational history, particularly the duration of welding carried out and the working conditions during the five preceding days, as well as the number of welding years during working lifetime.

We made the following measurements and collected the following samples at the end of the working week, after 5 days exposure to welding fumes for the welders, at occupational health units located in buildings away from the workplace:

- spirometric measurements;
- exhaled nitric oxide measurement;
- exhaled breath condensate (EBC) sample;
- urine sample.

All EBC and urine samples were stored at −80 and −20 °C, respectively, and analysed for metals at the end of the recruitment.

### 2.3. Spirometric measurements

Pulmonary function tests were performed according to the 2005 guidelines of the European Respiratory Society (Miller et al., 2005). Lung volume (forced vital capacity (FVC), maximum forced expiratory volume in a second (FEV1), FEV1/FVC, maximal mid-expiratory flow (MMEF)) was measured using the KoKo spirometer system (Pulmonary Data Services, Inc, Louisville, Colo). Data were expressed as percentages of ratios of observed/predicted values (% pred).

### 2.4. Exhaled nitric oxide (FeNO) measurements

Fractional exhaled nitric oxide (FeNO) levels were measured with a chemiluminescence NO analyzer (NOx 8000, SERES Aix en Provence, France). The examinations were conducted according to the ATS guidelines (American Thoracic Society and European Respiratory Society, 2005). Subjects were seated and noseclips were not used. After deep inspiration of NO-free air, NO concentrations were measured during controlled expiration (50 mL/s), at the first stable FeNO plateau of at least three seconds (FeNO variations < 10% or 1 ppb). The mean FeNO concentrations from two reproducible measurements were then recorded.

### 2.5. Assessment of exposure to welding fumes

#### 2.5.1. Welders

The exposure to inhalable metallic dust was assessed 3 times for the production line "A" and twice for production line "B" (A1, A2, A3, B1, B2) using personal air sampling equipment that was worn by a welder for one full work shift (7 h). We used GilAir ™ pumps (flow rate 2 L/min; Gilian Instrument Co., USA) with 37-mm filter cassette (MERCK KGaA, Darmstadt, Germany) and quartz microfibers filters for sampling of inhalable dust (37 mm diameter and 2.5 μm pore size, Whatman™