Metabolomics of welding fume exposure

Final Report for OHS Futures Research Grant, 2017-2018

Bernadette Quémerais Division of Preventive Medicine, University of Alberta
6/20/2018

Metabolomics Group

Previous principal applicant: Paige Lacy[1]

Research assistants and students: Samineh Kamravaei[1]
Monisha Alam[1]
Meghan Dueck[1]
Marc Cassiède[1]
Lei Pei[1]

Collaborators: Pascal Mercier[2]
David Broadhurst[1]
Beatriz Bicalho[3]

[1]559 HMRC, Department of Medicine, University of Alberta
[2]NANUC, Department of Biochemistry, University of Alberta
[3]SWAMP Lab, Department of Renewable Resources, University of Alberta
Edmonton, AB

Acknowledgements

We would like to thank Beatriz Bicalho at the Soil, Air, Manure, and Plant Laboratory, Faculty of Agriculture, Life, and Environmental Sciences, University of Alberta for performing all the metal analysis of air and urine samples. We also would like to specially thank Pascal Mercier at the National High Field Nuclear Magnetic Resonance Centre, University of Alberta for carrying out the ^{1}H-NMR analysis and for helping us with data analysis. We also want to thank all the health and safety representatives, occupational hygienists, and managers from participating companies for allowing us to collect samples in their workplaces. Finally, we thank all the welders that accepted to participate in the study.

This research project was supported by the Government of Alberta OHS Futures – Research Funding Program (http://www.work.alberta.ca/ohsfutures).

Summary for general public audience

Welding is the process of joining two metal pieces together. It has been known for a long time that exposure to welding fumes is hazardous to the health of workers. In addition, exposure increase when welders need to work in poorly ventilated areas or in confined spaces. The Occupational Health and Safety Code from the Government of Alberta sets Occupational Exposure Limits for workers. In the case of welders, the limits that occupational hygienists use relate to the levels of particulates and metals in air samples. Normal monitoring is performed by collecting particles on a filter from the breathing zone of the welder. However, this type of monitoring does not necessarily correlate to the dose received by the welder, particularly when the worker uses a respirator. Biomonitoring is an alternative to air sampling that may provide better information about the dose received by the workers.

In our study we determine if exposure to welding fumes will result in changes in metals and metabolites found in urine samples from welders. The choice of urinary metals as a biomarker of exposure has been done because welders are exposed to large amount of metals, particularly iron and manganese. The metabolites have been chosen since a previous study showed differences in urinary metabolites between welders and controls. In addition, urinary metabolites can be used to determine if exposure to welding fumes promotes early metabolic changes and be used as biomarkers of early health effects.

Collection of urine samples was carried out from professional welders who had been exposed to welding fumes for more than three years in various workplaces in Edmonton, AB. The collection of samples from the companies was done with the assistance of health and safety representatives, occupational hygienists, unions (boilermakers and pipefitters) and managers in the industry. In addition, air samples were collected the previous day from the breathing zones of welders to determine the total particles and metal content.

The goal for professional welders was to collect three samples per year from 20 welders. However, mainly for economic reasons, it was very difficult to recruit subjects that would have work long enough to give 3 samples. The project was modified to recruit 40 welders (20 smoking and 20 non-smoking) and 40 controls, and sample them only once during the year. Unfortunately, only 19 air and urine samples were collected from welders by the end of the project (16 non-smokers and 3 smokers). Filter samples were weighed to determine the level of exposure to particulates.

The report also collates results from last year project on smoking welder apprentices as results were not available at the time the report was written. Results show that, as for non-smokers, exposure to welding fumes increases as apprentice skills are improving during their training.

Results also show difference in some metal contents, such as manganese, chromium, zinc, and antimony, between welders and controls with higher concentrations found in welders. However, no difference was found for metabolites between welders and controls.

Table of Contents

- Metabolomics of welding fume exposure i

Acknowledgements ii

Summary for general public audience iii

Table of Contents iv

List of figures v

List of tables vi

List of acronyms, abbreviations vii

Introduction 1

Methodology 1

  Recruitment of subjects 1

  Sampling and analysis 2

Results and Discussion – Smoking apprentices 2

  NMR quantification of metabolite concentrations in urine samples using MC computational methods 2

  Air samples 2

  Urinary metal concentrations for non-smokers 3

  Metabolomics analysis of urine samples from non-smoking welder apprentices 3

Results and Discussion – Professional welders 3

  Information on subjects 3

  Mass concentration of particles 3

Conclusions 3

References 4

List of figures

Figure 2. PCA of urinary metabolite concentrations (log[mmole/mole creatinine x 10^3]) shows no variation between controls and welders on day 50. 11

List of tables

Table 1. List of metabolites passed (achieved <20% RSD with QC sample) using Monte Carlo computational methods in smokers and non-smokers. Metabolites with “u” indicate unknown entities. 6
Table 2. Particle and metal concentrations in smoking controls and welders. 7
Table 3. Urinary metal concentrations for smoking welders and controls in µg/g creatinine. 8
Table 4. Information on professional welders... 9
Table 5. Mass concentration of particles for professional welders 10

List of acronyms, abbreviations

^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectroscopy
LEV	Local Exhaust Ventilation
NAIT	Northern Alberta Institute of Technology
OEL	Occupational Exposure Limit
PCA	Principal Component Analysis
RSD	Relative Standard Deviation
TWA	Time Weighted Average

Introduction

Welding is the process of joining pieces of metal together by causing coalescence. This process results in the release of metal fumes and toxic gases [1]. It is well established that occupational exposure to welding fumes has adverse pulmonary health effects. The most common acute health effect historically reported in welders is metal fume fever, which presents with flu-like symptoms [2].

Recent studies on the size distribution of particles in welding fumes have shown that particles produced during the welding processes are in the fine to ultrafine range (< 5 μm) and are therefore likely to deposit in the small airways of the lungs and alveolar spaces [3-6]. Toxicology studies have demonstrated that metal oxide particles persist for many years in the lungs of welders [7, 8].

It has been demonstrated that a decreased pulmonary lung function caused by occupational exposure to welding fumes is associated with an increased risk of chronic obstructive pulmonary disease (COPD) as well as occupational asthma [9, 10] and pneumonia [11]. Long-term exposure to high concentrations of welding fumes is associated with an increased incidence of lung cancer and interstitial pulmonary fibrosis, which are associated with a higher incidence of premature death [12]. Finally, continued exposure to welding fumes, particularly in poorly ventilated areas, contributes to poor quality of life and shortened life expectancy [13].

Our project was to collect air and urine samples in two groups, welders and controls, with controls being in another trade and not exposed to welding fumes. Particle and metal concentrations were measured in air samples while metals and metabolites were analyzed in fasting urine samples. The objectives of the project were to 1) evaluate exposure to welding fumes in welding facilities, and 2) evaluate metals and metabolites in urine as biomarker of exposure to welding fumes. In addition, we hypothesized that early metabolic changes may occur in welders and that urinary metabolites may be used as biomarkers of early health effects in healthy welders.

The first year of the project (2015-2016) was dedicated to non-smoking welder and instrumentation apprentices and results were presented in last year's report. The second year of the project (2016-2017) we sampled smoking welder and power line technician apprentices. Sampling was completed only in March 2017, samples were sent for analysis in Spring 2017 and results obtained only in Summer 2017, after sending the report. Results for smokers are therefore presented in this report. For the third year of the project we sampled professional welders in various workplaces. Unfortunately, sampling was not completed, and this report gives only information on subjects as well as gravimetry data for air samples.

Methodology

Recruitment of subjects

Due to economical problems in the province, many welding companies were struggling finding contracts and had to lay down their welders. This situation made the recruitment of professional welders very slow as many companies did not have long term contracts allowing us to collect three samples per welder. In addition, a piece of sampling equipment broke, and sampling had to be performed again as most air samples were considered not reliable. To allow for collection of samples, sampling design was modified to collect only one air and one urine sample per welder for 20 smoking and 20 non-smoking individuals. Unfortunately, the project was stopped before completion and only 19 air and corresponding urine samples were collected in three workplaces and two union training facilities.

Sampling and analysis

Sampling, sample preparation, sample analysis, and data analysis was performed as described in last year's report.

In the case of metals, detection limits for filter samples were calculated by using three times the standard deviation of all field blanks [14]. Detection limits for urine samples were calculated using the United States Environmental Protection Agency method [15]. Briefly, a low-level urine sample was analyzed in 22 replicates and the t (n-1, α = 0.99) and standard deviation (σ) were calculated. The limit of detection was calculated as $t\ (n\text{-}1,\ \alpha = 0.99)\ x\ \sigma$. Field blanks for both urine and filters were below the detection limits for all metals, therefore they were not subtracted from samples. Non-detected values were not replaced for filters while they were replaced by LOD/$\sqrt{2}$ for urine samples o allow for statistical analysis [16].

Silver, bismuth, gallium and lead was not detected in urine while selenium was not detected in filters. Aluminum showed a lot of variation on the blanks for both filters and urine, therefore is not included in the results. Cobalt also presented some elevated values in field blanks for urine and is not included in results.

Results and Discussion – Smoking apprentices

NMR quantification of metabolite concentrations in urine samples using MC computational methods

Fitting of ^{1}H-NMR spectra was performed using the Monte Carlo method as described in last year's report. In the case of smokers, 22 replicates of the quality control samples were analyzed and fitted, and the mean and standard deviation were calculated for each metabolite. As previously, only metabolites showing an RSD below 20% were considered as valid. Using this criterion, only 78 metabolites "passed" for the smoking group. When comparing with results obtained for non-smokers, only 51 metabolites passed for both smokers and non-smokers. The list of "passed" metabolites for both smokers and non-smokers is shown in Table 1.

Air samples

Particle and metal concentrations for smokers are presented Table 2. Results show that concentrations for welders were more elevated than concentrations for controls. Although we did not calculate a TWA, since most samples were collected for a total of 180 minutes, we can estimate that all samples were below the Alberta 8-hour Occupational Exposure for metals. However, some samples were above the 8-hour OEL for respirable particles (3 mg/m^3) since the maximum concentration was 10 mg/m^3 (3.75 mg/m^3 when corrected to 8-hour wok shift). As observed previously, the LEV system used at NAIT may not be properly used by students or not efficient enough to remove welding particles. It is suggested to train student in using the LEV and to verify it is functioning properly.

In addition, air concentrations increased from day 0 to day 50 (see Supplementary Data file), as for the non-smokers, suggesting that apprentice welders are more exposed as they become more skilled.

Urinary metal concentrations for non-smokers

Results for urinary metals normalized to creatinine are presented in Table 3. Chromium, manganese, and antimony were slightly more elevated in welders than in controls however it was significant only for antimony (see Supplementary Data file). Both Cr and Mn were slightly elevated for all sampling days (0, 1, 7 and 50). All welders had been previously exposed to welding fumes, which explains why Cr and Mn were already elevated on day 0. However, antimony levels were significantly different in welders at day 50 as compared to the other days and also significantly different between controls and welders at day 50 (see Supplementary Data file). Our results are consistent with previous studies and shows that these metals accumulate in welders even after a short period of exposure [17-19].

Metabolomics analysis of urine samples from non-smoking welder apprentices

PCA analysis did not show any differences between welders and controls at day 50 (Figure 1). If groups were significantly different, then the PCA plot would have shown controls and welders in separate clusters. It is quite possible we did not have enough samples to see any different or that only 50 days of training were insufficient to see any difference.

Results and Discussion – Professional welders

Information on subjects

A summary of information on subjects is presented in Table 4. Subjects were generally older than apprentices, but other parameters, such as BMI or health issues, were similar. The average number of years of experience was 15 years. All subjects were using a form of control, 53% used LEV, while 47% used respirators. All respirators were fit tested.

Mass concentration of particles

Results are presented in Table 5. Contrarily to apprentices, professional welders were sampled for a full day shift and sampling time was between 5 and 8 hours, except for one subject for which welding lasted just over 3 hours. The highest concentrations were found in facilities using either general or dilution ventilation. The highest concentration (18 mg/m^3) was found for a worker mainly grinding, which is consistent with a previous study in a steel construction facility [20].

Conclusions

Our results confirm that there is an increase in particles and metals exposure in apprentices as welders become more skilled during their training. In addition, chromium, manganese, and antimony show slightly elevated levels in the urine of apprentice welders as compared to controls. We did not observe any difference in metabolites between apprentice controls and welders as previously reported.

There were limitations to the study: 1) it is possible we did not have enough samples to see real patterns; 2) 50 days of exposure may not have been sufficient to see significant changes; and 3) more than 90% of apprentice welders had been exposed to welding fumes before; 4) urine is a waste product influence by diet and may not be the best body fluid to use for exposure assessment. We plan to test exhaled breath condensates in future projects.

The group of professional welders was slightly older than the apprentices, but other parameters were found to be similar. All professional welders sampled were using some control, either LEV or respirator. As for NAIT, even when using LEV, mass concentration of particles still elevated showing that LEV may not be the most efficient method to remove welding fume particles. It is also possible that LEV are not properly used by welders and that training might be a good option to reduce exposure. However, many welders are reluctant to locate the capturing hood close to the weld when they use gas shielding since they are concerned the ventilation system may also remove the protecting gases.

Our OHS Futures-supported work has resulted in the publication of one scientific paper [21]. In addition, Meghan Dueck submitted successfully her Master's thesis and it has been published [22]. A presentation was made on metals in urine samples of apprentice welders at the Inhaled Particle XII conference held in Glasgow in September 2017 [23]. All results for smoking and non-smoking apprentices were presented at the American Industrial Hygiene Association, local section, in March 2018.

References

1. Dunn, W.B. and D.I. Ellis, *Metabolomics: current analytical platforms and methodologies.* TrAC Trends in Analytical Chemistry, 2005. **24**(4): p. 285-294.
2. El-Zein, M., et al., *Is metal fume fever a determinant of welding related respiratory symptoms and/or increased bronchial responsiveness? A longitudinal study.* Occupational and environmental medicine, 2005. **62**(10): p. 688-694.
3. Brand, P., et al., *Number size distribution of fine and ultrafine fume particles from various welding processes.* Annals of occupational hygiene, 2012: p. mes070.
4. Debia, M., S. Weichenthal, and A. Dufresne, *Case study: ultrafine particles exposure in apprentice welders.* Journal of occupational and environmental hygiene, 2014. **11**(1): p. D1-D9.
5. Lehnert, M., et al., *Exposure to inhalable, respirable, and ultrafine particles in welding fume.* Annals of occupational hygiene, 2012. **56**(5): p. 557-567.
6. Pfefferkorn, F.E., et al., *Characterization of exposures to airborne nanoscale particles during friction stir welding of aluminum.* Annals of Occupational Hygiene, 2010. **54**(5): p. 486-503.
7. Antonini, J.M., et al., *Persistence of deposited metals in the lungs after stainless steel and mild steel welding fume inhalation in rats.* Archives of toxicology, 2011. **85**(5): p. 487-498.
8. Beach, J.R., et al., *An epidemiologic investigation of asthma in welders.* American journal of respiratory and critical care medicine, 1996. **154**(5): p. 1394-1400.
9. Temel, O., et al., *Occupational asthma in welders and painters.* Tuberk Toraks, 2010. **58**(1): p. 64-70.
10. Vandenplas, O., et al., *Occupational asthma caused by aluminium welding.* European Respiratory Journal, 1998. **11**(5): p. 1182-1184.

11. Buerke, U., et al., *Interstitial pulmonary fibrosis after severe exposure to welding fumes.* American journal of industrial medicine, 2002. **41**(4): p. 259-268.
12. Guha, N., et al., *Carcinogenicity of welding, molybdenum trioxide, and indium tin oxide.* The Lancet Oncology, 2017. **18**(5): p. 581-582.
13. Antonini, J.M., *Health Effects of Welding.* Critical Reviews in Toxicology, 2003. **33**(1): p. 61.
14. Howard, A.G. and P.J. Statham, *Inorganic Trace Analysis: Philosophy and Practice*. 1997, Chichester Sussex England: John Wiley & Sons. 182.
15. US EPA, *Appendix B to Part 136 - Definition and Procedure for the Determination of the Method Detection Limit - Revision 2* in *40 CFR part 136*, U.E.P. Agency, Editor. 2017, US Government: United States.
16. Croghan, C. and P. Egeghy, *Methods of dealing with values below the limit of detection using SAS.* Southern SAS User Group, 2003: p. 22-24.
17. Arshad, R., et al., *Determination of manganese and chromium in welders and non-welders population in Lahore, Pakistan.* Bulletin of Environmental Studies, 2016. **1**(3): p. 75-81.
18. Bertram, J., et al., *Human Biomonitoring of Chromium and Nickel from an Experimental Exposure to Manual Metal Arc Welding Fumes of Low and High Alloyed Steel.* Annals of Occupational Hygiene, 2014.
19. Ellingsen, D.G., et al., *Biological monitoring of welders' exposure to chromium, molybdenum, tungsten and vanadium.* Journal of Trace Elements in Medicine and Biology, 2017. **41**: p. 99-106.
20. Mino, J. and B. Quemerais, *Using a Particle Counter to Inform the Creation of Similar Exposure Groups and Sampling Protocols in a Structural Steel Fabrication Facility.* Toxics, 2017. **5**(34): p. 1-17.
21. Cassiede, M., et al., *Comparison of computational approaches for identification and quantification of urinary metabolites in 1H NMR spectra.* Analytical Methods, 2018. **10**(18): p. 2129-2137.
22. Dueck, M., *Metabolomics analysis of early exposure to welding fumes in apprentice welders*, in *Medicine*. 2017, University of Alberta, Department of Medicine: Edmonton, Alberta. p. 138.
23. Quemerais, B., et al. *Metal concentrations in urine samples of welder apprentices*. in *Inhaled Particles XII*. 2017. Glasgow, Scotland.

Table 1. List of metabolites passed (achieved <20% RSD with QC sample) using Monte Carlo computational methods in smokers and non-smokers. Metabolites with "u" indicate unknown entities.

	"Passed" both smokers and non-smokers		**"Passed" both smokers and non-smokers**
1	1-Methylnicotinamide	27	Lactate
2	2-Aminoadipate	28	Malonate
3	2-Hydroxyisobutyrate	29	Methylguanidine
4	3-Aminoisobutyrate	30	N-Acetylglutamine
5	3-Hydroxy-3-methylglutarate	31	N-AcetylglutamineDerivative
6	3-Hydroxybutyrate	32	N-Acetylornithine
7	3-Hydroxyisobutyrate	33	N-Methylhydantoin
8	3-Hydroxyisovalerate	34	N,N-Dimethylglycine
9	3-Indoxylsulfate	35	Proline
10	4-Hydroxybenzoate	36	Pyroglutamate
11	5-Aminolevulinate	37	Sarcosine
12	Adipate	38	Threonine
13	Alanine	39	Trigonelline
14	Asparagine	40	Trimethylamine
15	Azelate	41	Trimethylamine N-oxide
16	Butanone	42	u075
17	Chlorogenate	43	u11
18	cis-Aconitate	44	u122
19	Citrate	45	u122triplet
20	Dimethylamine	46	u144
21	Ethanolamine	47	u217
22	Formate	48	u233
23	Fumarate	49	uarm2
24	Glutamine	50	Valine
25	Glycine	51	β-Alanine
26	Hippurate		

Table 2. Particle and metal concentrations in smoking controls and welders.

	Controls		Welders	
	Range	**GM (95% CI)**	**Range**	**GM (95% CI)**
Part (mg/m^3)	ND-0.09	0.046 (0.041-0.052)	ND-10	0.74 (0.51-1.08)
Ag (µg/m^3)	ND-0.013	0.008 (0.007-0.010)	ND-0.083	0.021 (0.014-0.030)
As (µg/m^3)	ND	ND	ND-1.9	0.28 (0.21-0.36)
Bi (µg/m^3)	ND	ND	ND-0.17	0.0038 (0.0026-0.0057)
Cd (µg/m^3)	ND-0.004	0.0034 (0.0032-0.0037)	ND-0.26	0.018 (0.005-0.060)
Co (µg/m^3)	ND	ND	ND-0.39	0.087 (0.069-0.11)
Cr (µg/m^3)	ND-2.3	1.3 (1.25-1.37)	ND-5.6	2.5 (2.2-2.8)
Cu (µg/m^3)	ND-0.063	0.063†	ND-37	4.2 (2.6-6.6)
Fe (mg/m^3)	ND-0.004	0.004†	ND-2.8	0.16 (0.085-0.29)
Ga (µg/m^3)	ND-0.003	0.0023 (0.0022-0.0024)	ND-0.74	0.028 (0.017-0.045)
Mn (µg/m^3)	ND	ND	ND-365	30 (19-47)
Mo (µg/m^3)	ND-0.002	0.002†	ND-1.2	0.083 (0.051-0.14)
Ni (µg/m^3)	ND-0.16	0.16†	ND-6.7	0.68 (0.45-1.0)
Pb (µg/m^3)	ND-0.005	0.005†	ND-3.0	0.18 (0.12-0.29)
Sb (µg/m^3)	ND-0.032	0.016 (0.011-0.023)	ND-11	0.15 (0.080-0.29)
Tl (µg/m^3)	ND	ND	ND	ND
V (µg/m^3)	ND-0.002	0.002†	ND-0.55	0.023 (0.015-0.035)
Zn (µg/m^3)	ND	ND	ND-28	3.2 (2.3-4.3)

†Only one value was detected

Table 3. Urinary metal concentrations for smoking welders and controls in µg/g creatinine.

	Controls		Welders	
	Range	**GM (95% CI)**	**Range**	**GM (95% CI)**
As	0.89-50	4.6 (3.8-5.5)	1.3-31	4.3 (3.7-5.1)
Cd	0.060-0.55	0.16 (0.14-0.18)	0.040-0.75	0.14 (0.12-0.16)
Cr	0.040-1.8	0.16 (0.13-0.19)	0.050-1.4	0.22 (0.19-0.26)
Cu	2.6-27	6.5 (5.9-7.0)	3.9-447	7.4 (6.3-8.7)
Fe	2.8-25	7.9-8.8	2.3-165	8.5 (7.4-9.8)
Mn	0.010-0.78	0.058 (0.048-0.070)	0.020-8.1	0.10 (0.079-0.13)
Mo	1.7-108	27 (23-31)	4.0-218	23 (20-27)
Ni	0.17-8.1	1.0 (0.84-1.2)	0.35-110	1.4 (1.1-1.6)
Sb	0.014-0.19	0.036 (0.032-0.041)	0.011-0.30	0.054 (0.047-0.062)
Se	17-71	33 (31-36)	18-67	34 (32-37)
Tl	0.050-0.73	0.17 (0.15-0.19)	0.060-0.60	0.16 (0.14-0.18)
V	0.021-0.62	0.090 (0.078-0.10)	0.024-0.39	0.089 (0.077-0.10)
Zn	81-634	275 (246-308)	26-1112	354 (319-392)

Table 4. Information on professional welders

	Professional welders
Number of subjects	19
Age	39.2 ± 13.6
Ethnicity - European descent	89%
BMI	27.4 ± 3.1
Smokers	3
Cigarettes/day†	6.3 ± 1.2
Alcohol consumption	84%
Drug use	32%
High blood pressure	11%
Heart problems	
Kidney problems	
Liver problems	
Asthma	5%
Prescription drugs	32%
Over the counter medicine	5%
Vitamins and supplements	32%
Experience as a welder (years)	15 ± 13
Local Exhaust Ventilation	53%
Wearing a respirator	47%
Respirator fit tested	47%

†For smokers only

Table 5. Mass concentration of particles for professional welders

	Range	GM (95% CI)
Particles (mg/m^3)	0.47-18	2.8 (1.6-5.0)

Figure 1. PCA of urinary metabolite concentrations (log[mmole/mole creatinine x 10^3]) shows no variation between controls and welders on day 50.