Metabolomics Analysis of Early Exposure to Welding Fumes in Apprentice Welders

by

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Abstract

Welding is defined as the joining of metals with extreme heat, producing fumes, which consist of harmful metals and ultrafine particulates that may lead to detrimental health effects. Currently, air sampling is the primary method to determine welding fume exposure, but is not always feasible. Biomarkers of welding fume exposure are sought for reliable measurement of exposure. Here I propose that urinary metabolomics may be applicable in screening for potential biomarkers for early exposure to welding fumes, and correlated with metal analysis to determine levels of urinary metals. Non-smoking, male apprentice welders (*n* = 23) and an age/sex-matched control group (*n* = 20) were recruited from the Northern Alberta Institute of Technology (NAIT) for this study. Air exposure samples were collected on days 0, 1, 7, and 50 of the welding program at NAIT, and 12 h fasting urine samples were collected on each occasion. Urinary metabolites and metal concentrations were analyzed using single proton nuclear magnetic resonance (^{1}H-NMR) and inductively coupled plasma mass spectrometry (ICP-MS). A pooled urine sample was used as a quality control to determine reliable metabolites. Air samples demonstrated that welding participants were exposed to higher particle and metal concentrations compared to controls. Urinary metal analysis presented conflicting results, with measurements at or near the limit of detection. A total of 151 metabolites were fit to ^{1}H-NMR spectra, with 61 validated as reliable (< 20% relative standard deviation) based on the pooled quality control sample (*n* = 33). Urinary metabolite, 2-hydroxyisobutyrate and three unknown metabolites, indicate relative promising differences on day 50, that were not observed in earlier sampling days between controls and welders. Metabolomics analysis shows promise in the detection of biomarkers of welding fume exposure, however further research is required.

Preface

The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, “Metabolomics of welding fume exposure: a novel biomarker approach for monitoring health in welder apprentices,” No. Pro00054536, January 20, 2016.

In addition, this thesis received research ethics approval from the Northern Alberta Institute of Technology Research Ethics Board, “Metabolomics of welding fume exposure: a novel biomarker approach for monitoring health in welder apprentices,” No. 2015-05, March 2015.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

BDA	Bayesian Decision Analysis
BMI	Body mass index
CRM	Certified Reference Material
DSS	4,4-dimethyl-4-silapentane-1-sulfonic acid
EDTA	Ethylenediaminetetraacetic acid
FDR	False discovery rate
FID	Free induction decay
GC-MS	Gas chromatography mass spectrometry
GMAW	Gas metal arc welding
HMDB	Human metabolome database
ICP-MS	Inductively coupled plasma mass spectrometry
IHDA	Interactive Health Data Application
LC-MS	Liquid chromatography mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
N/A	Not applicable
NAIT	Northern Alberta Institute of Technology
NANUC	National High Field Nuclear Magnetic Resonance Center at University of Alberta
ND	Not detectable

NMR	Proton nuclear magnetic resonance, ^{1}H-NMR, where ^{1}H represents single proton
OEL	Occupational exposure limits
PCA	Principal component analysis
PLS-DA	Partial least squares-discriminant analysis
PSRR	Progressive spectral region reconstruction
PTFE	Polytetrafluoroethylene
PVC	Polyvinyl chloride
QC	Quality control
RM-ANOVA	Repeated measures-analysis of variance
ROC	Receiver operating characteristic
RSD	Relative standard deviation
SD	Standard deviation
SWAMP	Soils, Water, Air, Manures and Plants, the Department of Renewable Resources, Faculty of Agricultural, Life, & Environmental Sciences at University of Alberta
TWA	Time weighted average
VIP	Variable importance in projection
WEKA	Waikato Environment for Knowledge Analysis

Chapter 1

Introduction

1.0 Introduction

The following chapter will address what welding is and its importance on our society. Fumes generated from the process of welding may have negative health consequences. How these consequences are currently dealt with in the workplace may not be enough to prevent over exposure, therefore this thesis proposes using metabolomics to potentially detect welding fume exposure.

1.1 The importance of welding

Welding is the efficient process of joining two or more pieces of metal together under extreme heat [1, 2]. A highly variable process, welding is crucial for almost all industries and metal products and can be conducted in a wide variety of environments, from inside to outside to underwater and even in outer space [2, 3]. Welders are responsible for fabricating materials made of metal, construction processes, such as building bridges or oil rigs, and many other products [2]. It is estimated that > 50% of the United States' gross domestic product is related to welding [2].

As of 2004, there were an estimated 800,000 full time welders worldwide, and approximately 1-2 million individuals perform some type of welding in their jobs [3, 4]. More than 20,100 Albertans are employed as welders or related machine operators, this number is expected to continue to grow annually by 1.3% from 2016 to 2020 [5]. The Northern Alberta Institute of Technology (NAIT) in Edmonton, AB is responsible for the training of welder apprentices. Over 2,000 apprentice welders a year attend NAIT for technical training, in addition to their work experience hours to receive their journeyman qualification [6, 7].

1.2 Welding background

There are three main components to welding. These include: a heat source, most often an electric arc, a shielding material, most often a gas, and a filler material, which joins two pieces together [1]. Prolonged exposure to welding fumes can be potentially toxic. These fumes consist of a complex mix of particles and gases, such as nitrogen oxides, from the electrode and material being welded and shielding gases that protect the weld [8, 9]. Therefore, welding

fumes consist of a mixture of vaporized metals and gases that react with the air to create particulates [3].

There are over 70 different types of welding processes [1, 3]. The most common of these processes includes differing varieties of arc welding, such as shielded arc welding, gas tungsten arc welding, gas metal arc welding (GMAW) and flux core arc welding. GMAW is a form of gas-shielding welding process, where the arc and welding zone are protected with a shielding gas to prevent oxygen from contaminating the weld and is one of the main types of welding taught to first year apprentices attending NAIT [6, 10]. Different fume compositions occur depending on the type of welding process and materials used. The type of welding material and process influences the particle size distribution and number of ultrafine particles [11]. Ultrafine particles include nanoparticles, which may have an additional impact on welders' health if over exposure continually occurs. Ultimately, a wide diversity of factors influences the fume composition, toxicity, and exposure.

1.3 Welding fumes

A profuse amount of fumes is released from the welding process, which are potentially hazardous to those exposed [8]. The harm and damage caused by welding fume exposure are affected by many different factors, such as the chemical nature, particle size, solubility, quantity absorbed, the duration and frequency of exposure, occupational environment, and susceptibility of the individual [10, 12]. Most welding fumes originate from the consumable and consist of metal particulates and oxides, along with shielding gases and any fumes produced from coatings, if present [3, 13, 14]. It has been found that a higher current intensity delivered to the welding process will release a larger amount of welding fumes, and decreasing the current intensity can decrease the welding fume exposure [15]. Beyond fumes created from the welding process directly, if the material is coated, for example with paints or solvents, fumes may be even more toxic, and extra care and ventilation will be required [8, 10]. Common metals used in welding include mild and stainless steel. Stainless steel welding may have a larger impact on health as it contains more toxic metals in its fumes compared to mild steel [16]. Mild

steel releases an abundance of Fe and Mn, whereas Cr and Ni are released in higher concentrations in stainless steel welding [17].

Chronic exposure to welding fumes is a potential hazard. Due to variability, welding fume exposure is unique and complex, which can make it difficult to consistently measure or compare. For this reason, the United States' National Institute for Occupational Safety and Health has estimated that it is not feasible to establish an exposure limit for welding fumes, but instead to limit exposure to different components in welding fumes [3].

1.3.1 Ultrafine particles in welding fumes

Welding fume particulates are aggregates of fine to ultrafine particles [15, 18-21]. Analysis of welding fumes found almost all particulate matter fell in the respirable fraction and has the potential to reach the lungs [18, 22-24]. Particle size determines how long particles remain suspended in the air, and theoretically how far down the respiratory path particles can reach [10, 12]. Therefore, smaller particles often remain suspended longer and may end up further down the respiratory path [10, 12]. For example, particles with a cut-off point of 4 μm will enter alveolar regions in the lungs and may take a substantial amount of time to be cleared [10]. Welding fume particulates are reported to have a mass median aerodynamic diameter between 190 nm to 260 nm, which is well below the respirable fraction limit, influencing their deposition in the lungs and with the largest portion falling in the alveolar range [24]. In addition, ultrafine particles may directly enter respiratory epithelial cells, which facilitates entry into blood and lymph circulation, potentially transferring ultrafine particulates to other sensitive organs in the body [11]. Ultrafine particles have a much greater surface area compared to larger particles, which generate free radicals and increase oxidative stress because of chemical interactions with body fluids [25].

1.4 Health impacts of welding fume exposure

Occupational exposure to welding fumes has been shown to result in adverse pulmonary health effects. In regards to general lung function, it was reported that non-smoking welders have a significant decline in forced expiratory volume, which was correlated to the duration of exposure to welding fumes [26]. Specific health effects of welding fume exposure

include metal fume fever and welder's siderosis [27, 28]. Metal fume fever causes flu-like symptoms but is resolved following the removal of exposure [3]. Metal fume fever is caused by exposure to zinc fumes, which is commonly generated from welding galvanized steel [29]. Siderosis, or welders' lungs, is a pathological condition caused by prolonged exposure to Fe oxides that causes an accumulation of Fe particles in the lower lung, and although there are no symptoms, siderosis can often lead to other respiratory diseases [28]. Further, welders have been found to be at an increased risk for chronic obstructive pulmonary disease, occupational asthma, and pneumonia [13, 20, 30]. Welding fume exposure throughout a lifetime can contribute to a poor quality of life and premature death [31]. Occupational welders have been found to be at an increased risk to develop lung cancer, laryngeal cancer, esophageal cancer, and leukemia [32]. This year, the International Agency for Research on Cancer has now recognized welding fumes as carcinogenic to humans [33]. Various studies have attempted to establish the effects of fume exposure and individual components. Overall, welding populations and environments greatly vary, making it difficult to accurately determine and interpret human exposure and toxicological effects [34]. The toxicity of welding fumes may be due to interactions of the differing fume components, adding even more confounders to determining harmful exposure levels [34]. To address this, a variety of animal studies have been conducted to test for direct effects of exposure, described below.

1.4.1 Animal studies

When exposed to metal fumes, rats demonstrate an accumulation of metal oxide particles in their small airways [16]. Welding fumes from stainless steel have been observed to induce pneumotoxicity, and particulates were cleared at a slower rate from the lungs compared to the particulates generated from mild steel [35]. An increase in lung injuries and inflammation in rats is observed when exposed to welding fumes generated from flux-covered electrodes [35]. A study by Antonini *et al.* (1999) found a difference in lung cell responses depending on the type of welding fume exposure, and concluded that stainless steel increased pulmonary toxicity [35]. When rats were continuously exposed to stainless steel welding fumes an accumulation of agglomerates, mass of particulates, formed from the fume particulates were found in the lung [36].

1.4.2 Early health impacts

Welding fumes have been an occupational health concern for many years. Case studies of respiratory illnesses and the accumulation of Fe particulates in lungs have been recorded since the early 1900's. Symptoms such as continuous coughing, chest pain and metal fume fever were prevalent among welders, but symptoms would cease following cessation of fume exposure [37]. While early experimental work demonstrated that Fe would accumulate in the lung, it was not shown to be responsible for causing fibrosis [38]. However, as materials changed and welding fume compositions became more complex, the hazardous properties of welding fumes has changed [38].

1.4.3 Respiratory impacts

Individuals who have been welding for many years have been found to have agglomerates in the lung tissue, typically in alveolar macrophages [16]. When comparing welders to a control group, increased levels of chromosome and DNA damage was reported in buccal and nasal cells; furthermore, DNA synthesis in lymphocytes was halted [4, 39]. This damage is believed to occur through the elevation of pro-inflammatory cytokines and oxidative stress in the airways of the lungs [4, 40]. Increased levels of cytokines in blood samples and nasal lavage fluid of welders have been reported, suggesting inflammation [13, 27, 34]. The inhalation of metal particulates is thought to be responsible for the disruption of cellular homeostasis and cellular damage, which can lead to a wide variety of pulmonary disorders [17].

1.4.4 Individual metal component impacts

Specific components of welding fumes may have different consequences on the health of the welder. The potential toxicity of individual metals will often depend on the oxidation state that the metal exists at in the welding fumes [3]. Some oxidative states of metals, such as Mn and Ni, have the capacity to promote redox reactions, which releases cytotoxic free radicals potentially impacting the health of the individual [3]. Cr (VI) and Ni or Co oxides, welding fume components are considered class 1 carcinogens [15, 17]. Prolonged exposure to Cr may cause lung fibrosis, skin irritation, and increases lung cancer risk [8, 10]. Fe exposure may lead to siderosis and lung scarring, whereas Ni exposure may cause skin, eye, nose and throat sensitization, and is a suspected carcinogen [8, 10].

Finally, Mn has been a metal of specific interest due to the potential detrimental effect it has on the nervous system. Mn has been noted to be a common contaminant in air exposures due to high density traffic and subway systems [41, 42]. However, inhalation of Mn in the workplace has been associated with inflammatory lung responses, bronchitis, pneumonia, decreased pulmonary function, impotence in men and, of particular concern, neurotoxicity [43]. Specifically, Mn exposure has been linked with manganism, a disorder characterized by Parkinson-like symptoms [44-46]. Initial symptoms of manganism are often overlooked and with progression, symptoms become more severe and ultimately irreversible [46, 47]. Mn concentrations were found to be higher in blood and urine of occupationally exposed subjects [47]. In addition, Cowan *et al.* (2009) found that Mn/Fe ratios for erythrocytes and plasma exhibited a significant increase in occupationally exposed subjects compared to controls [47]. Beyond biological markers, those exposed to high levels of Mn at work have been recorded to perform poorer on motor function tasks, report higher occurrences of fatigue, tension and anger, and have decreased cognitive flexibility [46].

1.4.5 Overall health impacts

In addition to health deficits in the respiratory system, recent research has also shown that exposure to welding fumes and particulate matter may have a detrimental effect on cardiovascular health. Those with occupational exposure to welding fumes may have an increased mortality from ischaemic heart diseases, possibly due to systemic inflammation from exposure [48]. Proinflammatory cytokine expression in cardiac macrophages exacerbates the autonomic function of the heart as a result of the of inhaled particulates that cause lung inflammation [9]. A study conducted by Kim *et al.* (2005) found acute exposure to welding fumes was associated with an increase in levels of systemic inflammatory markers, that non-smokers had an increase in white blood cells, specifically neutrophils, and in fibrinogen levels [48]. Both smokers and non-smokers had an increase in C-reactive protein, which increases in the presence of inflammation, 16 h following welding fume exposure [48]. A meta-analysis conducted in 2014 suggested borderline significance for an increased risk of ischemic heart diseases among workers exposed to welding fumes [49]. Occupational exposure to particulate matter was associated with a decrease in heart rate variability in welders overall,

and a significant decrease in welders who did not use respiratory protective equipment [9]. There have also been mixed results regarding negative impact on reproductive health, although a decrease in median sperm density was recorded in welders who have been exposed to stainless steel fumes [50].

1.5 "Healthy worker" effect

Due to conflicting evidence of welding fumes having negative consequences on health, the "healthy worker" effect is suggested to play a role. This is the concept that workers who develop respiratory problems or occupation-related diseases leave their jobs without citing health concerns, leaving a selection of individuals who are healthy and tolerant of welding fume exposure, although this is difficult to assess [22]. A study conducted by Thaon *et al.* (2012) found that smoking had a larger impact on lung function in non-occupationally exposed subjects than those who were exposed to welding fumes, suggesting those who are resistant to respiratory health deficits caused by occupational exposure are also likely to have increased resistance to any effects caused by smoking, supporting the "healthy worker" effect [26]. Another study found that employed welders maintained consistent healthy lung function while employed, but once left their employment they experienced increased respiratory symptoms [13].

1.6 Prevention and protection

Generally, the body is exceptionally efficient at metabolizing and eliminating most contaminants and particulates. However, excessive exposure may occur, and currently the only way of determining this is when health effects become apparent [12]. To ensure that welders work within healthy limits, the evaluation of workplace exposures are important. Therefore, occupational exposure limits (OELs) are set depending on the environmental contaminant and the length of exposure based on research [12]. There are many precautions that can be taken by the employer and welder to prevent exposure to welding hazards, specifically welding fumes, and any possible detrimental health effects. Appropriate ventilation and respiratory protective equipment should be used depending on the working environment. Figure 1.1 represents an example of the local exhaust ventilation systems used at the NAIT South campus.

To ensure safe working conditions, the best practice is to frequently assess hazardous exposures.

1.6.1 Air sampling

Air sampling helps determine exposure to airborne substances in the workplace and ensure a safe working environment. It is commonly used to measure worker exposure and characterize the source of hazards [12]. There are two main types of air sampling, (i) background and (ii) personal measurements from the breathing zone of welders. Background measurements, or ambient air samples, quantify the amount of fumes present in the general air, whereas personal samples involve sampling in the breathing zone of the individual, as close to their nose and mouth as possible to collect a true exposure sample [10, 12]. There are a wide variety of air collection procedures, such as absorption, gas/adsorbents and diffusive samplers [12]. When measuring particulates, air sample collection on a filter is a common method [12].

Following air sample collection, there are a wide variety of analysis techniques for filters that can be used depending on the type of particulates collected, such as gravimetry or instrumental analysis [12]. A large portion of occupational assessments to check compliance rely on gravimetric analysis of filters and airborne particulates collected [51]. However, there can often be variations within and between laboratories, and it is important to ensure reproducibility [51].

1.6.2 Measuring welding fume exposure

One way to measure welding fume exposure is to measure particulates in fumes by gravimetric analysis. Theoretically, it is possible to separately collect the respirable and inhalable fractions based on the type of sampler [10]. Further, chemical analysis of filters allows individual metals to be quantified [10]. There are a wide variety of techniques that can be used to determine individual metals. Some examples include graphite furnace atomic absorption spectrometry or inductively coupled plasma mass spectrometry (ICP-MS).

Figure 1.1 The local exhaust ventilation system at NAIT Souch Campus. A standard in all welding labs at NAIT, Souch Campus. [52].

1.6.2.1 ICP-MS

ICP-MS is a commonly used method for the determination of metallic elements in air samples. This technique allows the analysis of a range of metal concentrations at once. Filters previously analyzed for overall particulate matter can be digested in an acid mixture, and analyzed using ICP-MS to determine metal components of the fume. ICP-MS is known for detecting trace elements within a sample, and is considered the gold standard for characterizing trace elements in biological samples, such as urine [53, 54]. The following study has employed ICP-MS to analyze exposure levels, which is important in the determination of biomarkers to environmental exposures. Figure 1.2 outlines the principles of ICP-MS for metal detection.

1.7 Current biomarkers for welding fume exposure

The current approach for monitoring welding fume exposure is medical surveillance programs. These include yearly check-ups and x-rays to ensure there is no accumulation of iron oxide particles in the lungs and that the worker is healthy. Checking lung function may also be a key component to ensure there is no respiratory damage. As beneficial as these surveillance programs are, significant time passes in the exposed welder before any respiratory health deficits become evident [17]. Therefore, it may be beneficial to determine appropriate and specific biomarkers for early exposure to welding fumes.

As welding fume exposure may result in damage at the cellular level and overall health damage, it is important to detect early exposure impacts. Urine and blood samples are common biological fluids used for determination of biomarkers. There are mixed results with urinary metal analysis. One report (*n* = 137) demonstrated that metal concentrations in urine samples of welders were significantly higher compared to non-occupationally exposed subjects using ICP-MS [15]. Specifically, Cr and Mn urinary concentrations were elevated in elderly welders and welders who worked in confined spaces or long hours, and Mn increased in welding participants who were involved in grinding, a metal cutting process [15]. Another report showed increases of urinary metal concentrations, Cr, Ni and Al, in welders (*n* =45) compared to control (n = 24) populations using graphite furnace atomic absorption spectrometry [40].

However, little to no differences between occupationally exposed welders (*n* = 115) and controls (*n* = 145) have also been reported using flameless atomic absorption spectrometry [46]. When looking at trace metals using ICP-MS Morton *et al.* found no difference in occupationally exposed (*n* =167) and controls (n = 62) in urine [55]. As urinary metals have resulted in inconsistent results as a biomarker for welding fume exposure, other approaches may resolve the disparity in observations.

Additional studies have considered other mediums to measure welding fume exposure, such as scalp hair, or oxidative stress biomarkers [4, 56, 57]. Metal concentrations in exhaled breath condensate showed increase Cr concentrations with exposure to respirable welding fumes [17]. A significant correlation between Fe exposure and Fe concentration in exhaled breath condensate was found in welders who did not wear proper respiratory protection [17]. Exposed welding participants, who did not wear respiratory protective equipment, were found to have increased nitrite/tyrosine and nitrate/tyrosine ratios [40]. Although these methods suggest promising results they are not always practical and additional research is necessary. Metabolomics, a potentially reliable and robust method, is used in the following study as a method to determine biomarkers for early exposure to welding fumes.

1.8 Metabolomics

The metabolome, which is the sum of all the metabolites in an organism, is expansively large, is in the early stages of being understood, and has the potential to be beneficial in determining phenotypes [53]. The analysis of the metabolome of an organism is conducted through metabolomics, a relatively new branch of “omics” that measures an organism’s interaction with its environment and allows insight into the effects of lifestyle factors, gender, environmental stressors, and diseases in real time [53, 58]. In comparison to other “omic” methods, metabolomics is still in its early stages. Metabolomics focuses on comprehensive characterization of small molecules, such as those found in cells or organisms, in response to environmental exposure [59]. Recently, metabolomics has demonstrated to be particularly useful in the identification of biomarkers, drug discovery and in studying environment-gene interactions [53].

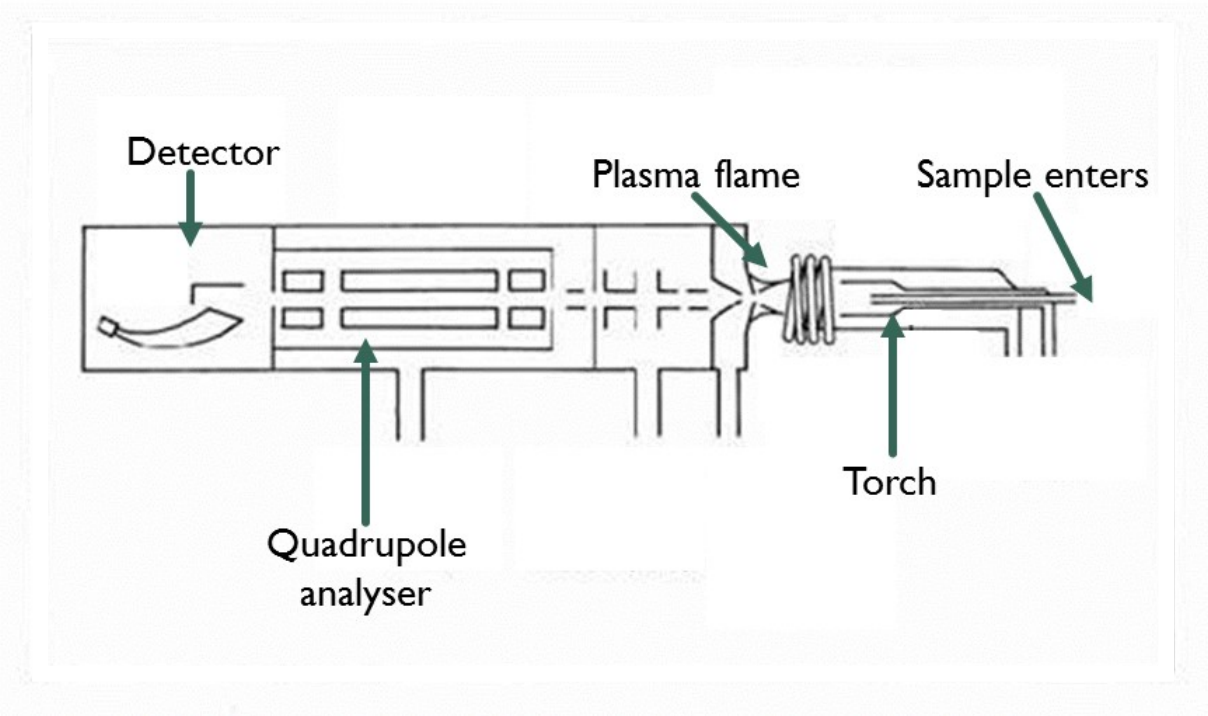


Figure 1.2 ICP-MS analysis. The sample enters on the left, where it passes through the plasma flame that ionizes the atoms, then enters the quadrupole analyzer that separates the ions based on their mass-to-charge ratio before being detected. Modified from meetcolab.com [60].

Initial research has discovered variations in metabolomic profiles based on gender, diet, age, diurnal changes, and ethnicity [61]. This suggests that metabolomics is a sensitive technique to environmental changes, and allows the detection of changes due to diseases and toxin exposure [61]. Metabolomics is a potentially powerful tool that allows for the identification of perturbed biochemical pathways, allowing disease fingerprinting and biomarker discovery [62, 63]. There are a wide variety of techniques that can be used to analyze metabolites, such as gas and liquid chromatography mass spectrometry (GC-MS and LC-MS), and nuclear magnetic resonance (NMR), which is quite commonly used and was utilized in the following project.

1.9 NMR

Single proton (^{1}H) NMR utilizes a powerful magnet that aligns the protons present in a sample and subjects them to radio frequency pulses [64]. A high-power radio frequency pulse is applied to the sample causing protons to absorb and then release electromagnetic radiation. The release of energy will vary for different compounds based on protons in the sample, and leads to the generation of a free induction decay (FID) curve [64]. Data from a FID undergo a Fourier transformation, which allows the frequency components of a wave to be extracted, creating a spectrum that can be used to quantify metabolite concentrations [65]. The use of chemical shifts and spin-spin couplings can provide information on metabolite structure, where information on metabolite concentrations and interactions are obtained with chemical shifts, line shapes and relaxation properties [66]. Each metabolite exhibits a unique chemical signature or “fingerprint” that is composed of either a single or multiple clusters of peaks across the spectrum, based on its composition of protons [64]. These peaks allow the identification of metabolites in biological samples, provided that specific physical conditions are met [64]. There are a variety of magnet types, ranging from 400 to 900 MHz in strength, with larger magnets having increased sensitivity [64].

A typical NMR spectrum of urine contains hundreds of possibly overlapping peaks, representing metabolites of low molecular weight (Figure 1.3) [61, 67]. Although biomarkers – a measurable substance or substances that indicate a disease or exposure - can be established

with a range of techniques, NMR analysis of urine allows quantification of metabolites based on the chemical shifts, spectral peaks and addition of an internal standard [67]. NMR visualizes hundreds of distinct peaks in human urine, allowing the detection and quantification of approximately > 100 compounds, providing a metabolic profile [63]. The patterns generated by the large quantities of metabolites present in urine are useful in providing insight into underlying disease processes and physiological changes induced by interactions with environmental stimuli [34].

1.9.1 Advantages of NMR

Qualitative and quantitative measurements can be obtained while measuring multiple compounds in a sample using NMR [61, 62]. NMR is a non-invasive and non-destructive technique, leaving the sample intact and it has been found to have high reproducibility, making it a robust and reliable technique for biomarker discovery [53, 61, 62, 67]. When measured on different magnets, normalized metabolite concentrations are consistent [68]. NMR allows a wide range of metabolites to be simultaneously detected in a short acquisition time [61]. Minimum sample preparation is required, in comparison to other techniques where samples undergo frequently extensive derivatization processes [63, 65, 68]. In comparison to other methods, NMR appears to be the most comprehensive and quantitative approach when analyzing biofluids [53]. Therefore NMR is considered a potentially powerful approach for the quantification and identification of metabolites [63].

1.9.2. Limitations of NMR

As with all techniques, NMR has limitations and challenges. Even small pH variations between samples can cause major chemical shifts along the baseline of the spectrum, which generally should not be a problem unless alkalinity or acidity is induced [61]. Samples with high ionic strengths or salt concentrations can influence spectrum acquisition, and even moderately diluted samples can be difficult to analyze [61]. In comparison to other methods, NMR has a relatively low sensitivity, restricting the detection limit and requiring relatively large sample volumes (200-500 µl) [59, 63]. For this reason, NMR and mass spectrometry methods are often used together as complementary approaches [59].

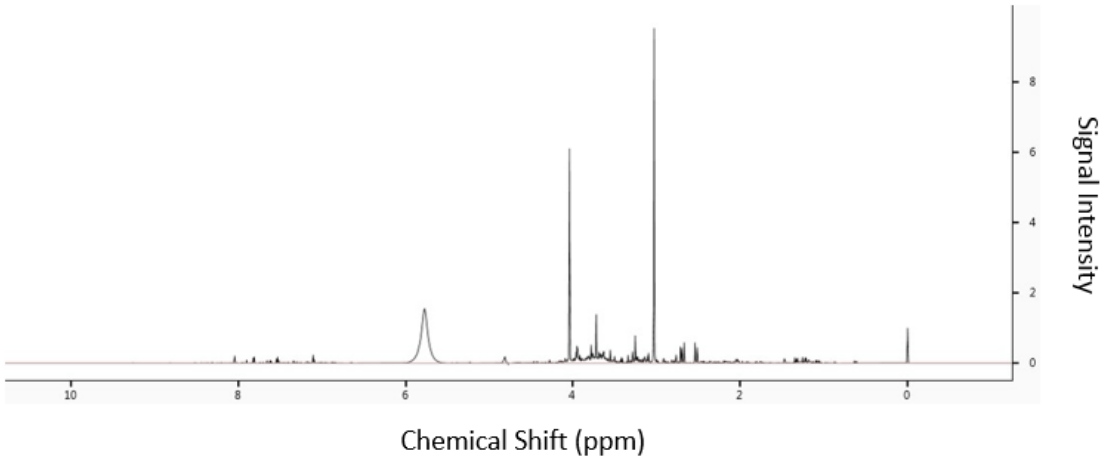


Figure 1.3 Example NMR spectrum. Spectrum collected on a Varian VNMRS 600 MHz spectrometer at the National High Field Nuclear Magnetic Resonance Center (NANUC), University of Alberta.

Another limitation of NMR can be intersample chemical-shift variations, which are attributable to different pHs, ionic strength variations, interactions between metabolites and between metabolites and proteins, and by any cations present in samples [66]. Compounds that overlap or have low intensity peaks can be more difficult to fit, and contribute to high variation [68]. These may be overcome by using peak-fitting algorithms, or controlled by using a consistent pH throughout the samples, or the addition of a chelating agent, such as ethylenediaminetetraacetic acid (EDTA) [66].

1.10 Biofluids for analysis

Metabolomics can be applied to the analysis of a variety of body fluids, such as cerebrospinal fluid, saliva, blood and urine. There are advantages and disadvantages to each. As a diagnostic biofluid, urine has been important throughout history, with different colours and tastes being used in early medicine to establish diagnoses [53]. Presently, urine continues to have considerable importance in determining health [53]. Urine is non-invasive, easy to obtain, and is relatively stable, which allows longitudinal analyses and collection of large sample quantities from healthy or diseased subjects [53, 62, 63]. Urine contains negligible protein and cellular content while remaining abundant in chemical composition [62, 63]. The metabolic composition of urine can vary. Factors such as diet, gender, ethnicity, gut microflora, or health status, may have an impact on the metabolites [63]. Diurnal patterns have also been recorded to have an impact on an individual's metabolic fingerprint; therefore, collection times should be standardized and other confounders controlled to avoid extreme variability [63].

1.11 NMR use in biomarker discovery

Many studies have previously used metabolomics to successfully identify metabolic fingerprints for diseases and exposures [34, 65, 69, 70]. Exposure to environmental toxins and human diseases lead to physiological changes that result in metabolite concentration variations [63, 65]. Greater changes in metabolite concentrations in urine have been recorded in comparison to changes in protein levels in response to human diseases, giving a diagnostic edge to metabolomic identification and profiling [63]. Individuals with inborn metabolic errors have

been recorded as having distinct metabolic profiles in comparison to healthy control groups using NMR and multivariate analysis, a key component in biomarker discovery [71].

However, caution is required when establishing biomarkers; Bouatra *et al.* (2013) found that even when urinary metabolites were normalized to creatinine concentrations, values in urine can vary by ± 50% depending on the metabolite [53]. In order for metabolomics to be beneficial as biomarkers and in disease diagnostics, reliable methods, and databases using multivariate analyses need to be generated [65].

1.11.1 Statistical analysis in metabolomics

As metabolomics generates large volumes of data, special multivariate techniques and analyses need to be in place to reduce the dimensionality of data. There are several different techniques available to quantify NMR spectra, including spectral binning or targeted profiling, which is to detect known metabolites [59]. Quantitative metabolomics is labour-intensive, although there have been recent advances in the development of computer-based algorithms and software that can automate this process, accelerating the process of metabolite quantification and generating robust data for biomarker determination [59, 72].

Data from metabolomics is generally analyzed using chemometrics, pattern recognition techniques and bioinformatics [61, 67]. Multivariate analysis and modelling are used to facilitate NMR pattern recognitions, which helps in identification of trends and hidden phenomena in the data [65, 68, 73]. Chemometric techniques may be used in biomarker discovery in comparison to targeted profiling [68]. Principal component analysis (PCA) is one form of unsupervised multivariate statistical analysis commonly used in metabolomics. It works by creating principal components, which consist of combinations of the original variables describing the maximum variation in the data, these principal components are then used to visualize differences [73]. This provides an unbiased understanding of the group structure and variation [74]. Whereas partial least squares projection to latent structures-discriminant analysis (PLS-DA) is a supervised multivariate method, using class membership to determine variation in the data [74]. This often leads to a better fit for the data, but presents a much

greater risk of overfitting [75]. Figure 1.4 represent an outline of the procedure for NMR from sample collection to multivariate analysis.

Machine learning methods is an alternative approach that can be applied to metabolomic data to generate robust models that can generalize to other data. Machine learned models use baseline metabolomic data to predict the development of diseases can be generated and tested [76, 77]. Classification algorithms, such as J48 decision trees and Naïve Bayes, have parameters that can be set to decrease overfitting and create models that generalize to other data sets [76]. Classification, supervised machine learning models, relies on a set of training data to build a predictive model [78].

1.11.2 Novel tool to determine welding fume exposure

Metabolomics represents a unique opportunity to assess occupational exposures because of its ability to determine individual phenotypes in response to environmental stimuli [34]. Previously, there has only been one other study to use NMR in urine samples from welders to determine metabolic differences in comparison to a control group [34]. This study conducted on workers exposed to welding fumes in Taiwan found increases of acetone, betaine, creatinine, gluconate, glycine, hippurate, serine, S-sulfocysteine, and taurine, and a decreased level of creatine in urine compared to controls [34]. Changes in these metabolites were thought to be important in modulating inflammation and oxidative stress [34]. Metabolomics has potential use in the determination of biomarkers to welding fume exposure, and ultimately may assist in screening for early health effects in welders.

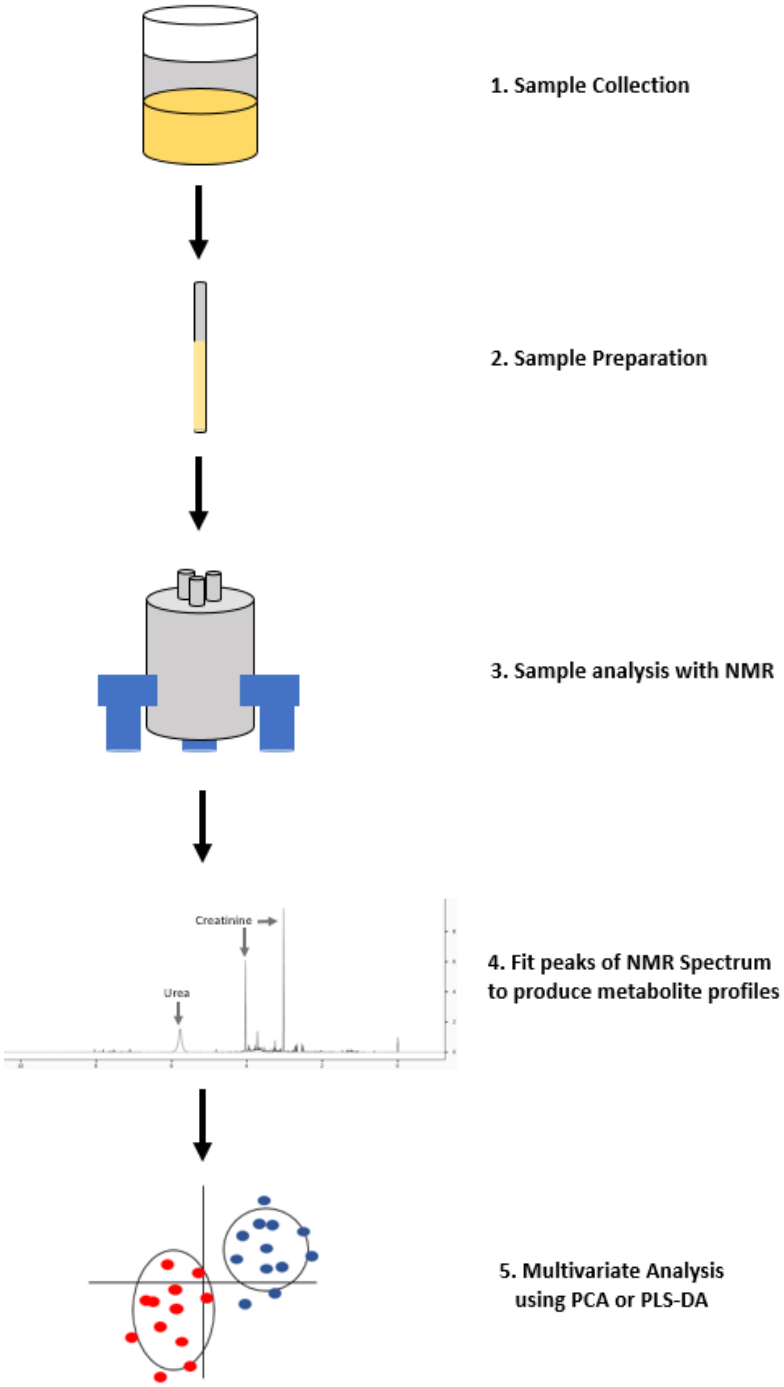


Figure 1.4 Metabolite analysis procedure with NMR. The five steps described above is the general procedure for determining metabolic profiles using NMR. Following sample collection (1), the sample is prepared and placed in an NMR tube (2) for analysis (3). FIDs undergo Fourier transform to create a NMR spectrum, which is fit for metabolite quantification (4), followed by multivariate analysis (5).

1.12 Rationale and hypothesis

1.12.1 Rationale

Welding is a major occupation in present society and those employed in the industry are exposed to welding fumes, often without appropriate protection. Although air sampling is helpful in determining overexposure, it is not always appropriate or feasible. Urinary metal analysis has resulted in conflicting outcomes and no biomarker assays are available to monitor welding fume exposure. Therefore, better monitoring techniques need to be developed. Here we proposed using metabolomics as a method to detect and monitor welding fume exposure (Figure 1.5). By starting with apprentice welders, early exposure effects can be observed when initial changes may be occurring. This will allow personalized profile trajectories to be built and detect any trends in metabolic changes.

1.12.2 Hypothesis

The exposure of welders to concentrations of fine and ultrafine particles in welding fumes was hypothesized to **result in changes in the levels of small molecules and metabolites in urine samples detected by metabolomics.** This is predicted to be evident when inadequate ventilation or respiratory protection is used, which was recorded and analyzed through air sampling over the period of the welding program. The chain of events hypothesized is welding fume exposure leads to an accumulation of particles in the airways of welders, which induces a cascade of inflammatory reactions leading to a spillover into systemic circulation, resulting in changes in small molecules and metabolites in urine samples.

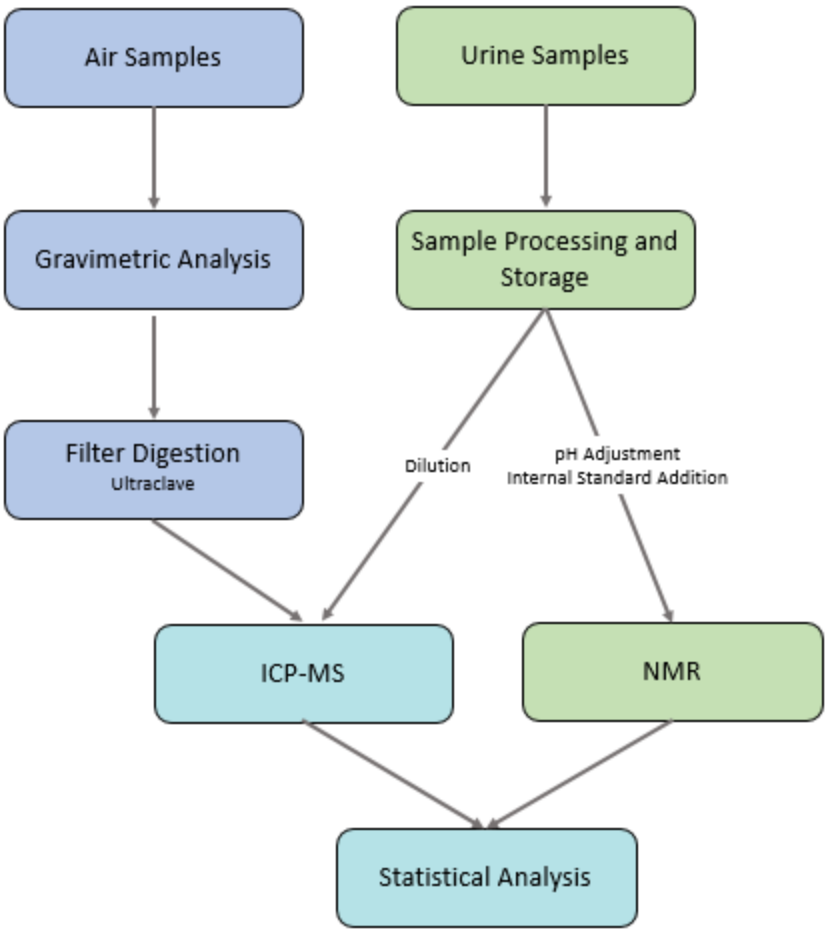


Figure 1.5 Flow chart of sample collection and analysis plan. Air sample collection and analysis is in blue, while urine collection and analysis is in green. The cyan color represents steps for air and urine sample analysis.

Chapter 2

Materials and Methods

2.0 Materials and Methods

A detailed overview of the study design and methods will be covered in the following chapter. The recruitment of participants, materials used, collection and processing of air and urine samples, along with the statistical analysis used on all samples follows.

2.1 Recruitment and participants

2.1.1 Ethics

Ethics approval was received from both the University of Alberta Research Ethics Board (Pro00054536, January 20, 2016) and the NAIT Research Ethics Board (No. 2015-05, March 2015) [79, 80]. The following methods were carried out in accordance with approved institutional guidelines. All subjects received both written and oral information prior to inclusion in the study, and provided informed consent. Participants were voluntary and allowed to drop out of the study at any time without additional explanation.

2.1.2 Participant recruitment

Students enrolled at NAIT, Edmonton AB, were recruited for the following study. Male, non-smoking first year welding apprentices (*n* = 23) were recruited from the Souch Campus of NAIT. Control subjects, who were age and sex-matched to welders (*n* = 20), consisted of students enrolled in the Instrumentation program at the North Campus of NAIT.

Recruitment occurred during program-specific orientation at NAIT. Participants were recruited and contributed between September 2015 and February 2016. There were three rounds of recruitment in August 2015, October 2015 and January 2016, until the minimum sample size of 20 controls and welders was reached. This sample size was selected based on a previous study of metabolite profiling between 16 welders and 35 controls (office workers) [34]. In addition, within the context of practical study design constraints - specifically related to the ability to voluntarily enroll and retain eligible subjects - 20% of an eligible sample population is considered a reasonable minimum for statistical analysis [81]. As our eligible sample population of welding students was estimated to be 100 throughout the study period, a minimum sample size of 20 was set. Subjects were informed of the objective of this study and

the benefits and possible risks to their health, which were minimal. Each participant was requested to fill out a questionnaire and consent form (Appendices A and B). Each subject also received an information sheet outlining the project and their rights (Appendix C).

2.2 Preparation of sampling and laboratory equipment

2.2.1 Equipment cleaning for metal analysis

2.2.1.1 Materials

Sub-boiled HNO_3 (Aristar Ultra BDH, Radnor, PA) was used for cleaning equipment, sub-boiled is a purification method for inorganic acids. In addition, Decon™ Contrex™ CA acid detergent (Decon Laboratories, PA) was used for cleaning equipment.

2.2.1.2 Procedure

Urine collection cups, 15 ml polyethylene metal tubes and 37 mm support pads were soaked in 2% Contrex acid detergent for 2-3 h, and thoroughly rinsed with Type II deionized water (ATEK at 18 MΩ, Edmonton, AB). Plasticware were then transferred and immersed in 5% HNO_3 for at least one week. At the end of the week, materials were rinsed 3x with deionized water, filled under the laminar flow hood with 2% sub-boiled HNO_3, and stored in a clean environment until needed.

Three-piece styrene cassettes and petri dishes (60 mm x 15 mm), where filters were stored, were washed as previously described, omitting the 2% HNO_3 solution step. These were dried under a laminar flow hood, and stored in plastic bags to prevent contamination. Finally, polytetrafluoroethylene (PTFE) tubing was washed in Contrex acid detergent, rinsed in deionized water, dried under laminar flow hood and stored until use.

2.2.2 Cassette preparation

2.2.2.1 Materials

Three-piece 37 mm clear styrene cassettes and 37 mm polyvinyl chloride (PVC) filters with 5 µm pores were obtained from Zefon International (Ocala, FL). These filters capture total dust particulates. Polypropylene 37 mm support pads were acquired from SKC (Eighty Four, PA).

2.2.2.2 Procedure

Three-piece clear styrene 37 mm cassettes were used to collect air samples. Cassettes were fit with 37 mm support pads and 37 mm, 5 μm PVC filter. Cassettes and support pads were pre-washed (2.2.1.2 Procedure) and filters were pre-weighed (2.4.1.1 Gravimetry). All assembly occurred under the laminar flow hood (Figure 2.1) Both ends of cassettes were plugged and cassettes were placed in appropriately labeled bags for transport to the sampling site.

2.2.3 Pump calibration

Air sampling was conducted with Gilian GilAir Plus Personal Air Sampling Pumps (Sensidyne Gilian, St. Petersburg, FL). The pumps were calibrated to a flowrate of 2 L/min prior to and immediately following air sampling using a primary calibrator, specifically, a Defender 530 Bios Calibrator (Mesa Labs, Lakewood, CO). The average flowrate was used to calculate sampling volume (Equation 1).

Equation 1: Sampling Volume calculation.

$$Sampling\ Volume\ (L) = Average\ Sampling\ Flowrate\ (L/min) \times Sampling\ Time\ (min)$$

2.3 Sampling

2.3.1 Sampling schedule and locations

The Welding and Instrumentation programs used to recruit welders and controls at NAIT are each approximately eight weeks long. Subjects participated on four sampling days: days 0, 1, 7, and 50. Day 0 provided a baseline measurement prior to starting the welding program, while day 50 was obtained at the end of the program. As important changes could occur at earlier time points of exposure, samples were collected following the first day of exposure, day 1, and one week into the welding program, day 7. This schedule allowed us to construct personalized trajectory profiles for participants. Fasting urine samples were collected the morning immediately after air sampling (Figure 2.2).

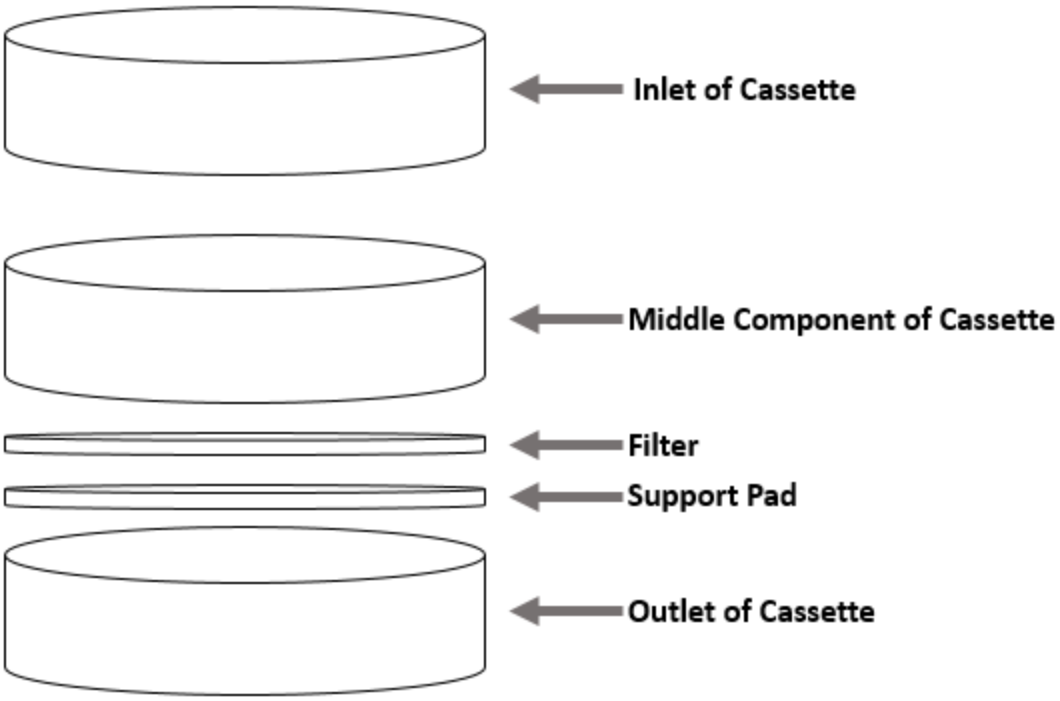


Figure 2.1 Assembly of the three-piece styrene cassette with 37 mm support pad and filter. All components were placed together, plugs were then placed in the inlet and outlet prior to transportation to and from the sampling site.

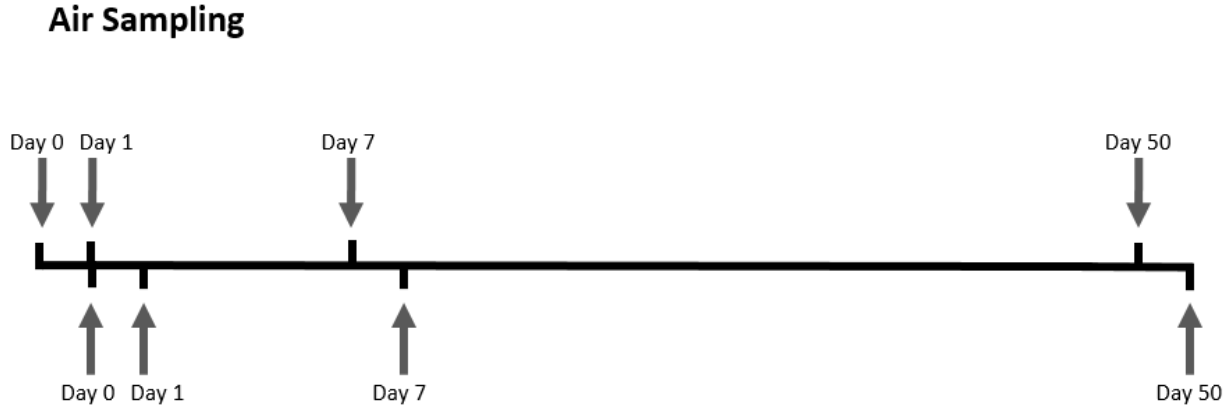


Figure 2.2 Eight-week sampling timeline. Ambient air samples were collected for instrumentation students on all four sampling days and for welding apprentices on day 0 and personal samples were collected for welding apprentices on days 1, 7, and 50.

2.3.2 Air sampling

Personal air sampling was performed for welders for days 1, 7 and 50, while area sampling was performed for controls and welders on day 0 (Figure 2.3). For personal air samples, pumps were attached to the belt of the subjects and PTFE tubing went underneath the welding jacket to prevent burning or melting. The cassette was clipped on the collar of the welding jacket, ensuring that the outlet was underneath their helmet and in the personal breathing zone. Area sampling was performed by collecting six samples simultaneously in the classroom or laboratories for controls and in the cafeteria of NAIT Souch campus for welders. Cassettes were taped to the top of the pumps for area sampling to keep the cassettes horizontal. Once back at the laboratory, cassettes were opened under the laminar flow hood and filters placed in pre-cleaned petri dishes in a desiccator until analysis.

Two field blanks were collected for each sampling day. Field blanks consisted of a cassette prepared with a support pad and filter that were brought to NAIT but not used. This allowed verification of no contamination during the handling and transportation of cassettes.

2.3.3 Urine sampling

2.3.3.1 Materials

Polystyrene urine collection cups were purchased from Fisher Scientific (Ottawa, ON), and white nitrile gloves from VWR (Mississauga, ON). Urine collection cups were cleaned as described (2.2.1.2 Procedure).

2.3.3.2 Procedure

Fasting urine samples were collected the morning after air sampling before subjects started their classes. Fasting urine is essential for reducing confounding effects of diet, which is known to perturb urinary metabolites [34, 63]. Subjects were requested to fast for a minimum of 12 h. All participants received a pre-washed collection cup, with a pair of white nitrile gloves. Participants were instructed to catch a mid-stream sample, as recommended, to decrease the number of cells and bacteria present [82]. Subjects were asked to avoid alcohol and drug consumption for 48 h and 10 days, respectively, and avoid exercising prior to sample collection. On the day of sample collection, participants were asked if they had followed the previous

requirements, and any deviation from these parameters was recorded. Once collected, the urine collection cup was put in a cooler with icepacks to keep samples close to 0°C for transportation back to the University of Alberta for processing.

For each urine collection, a field blank was prepared with deionized water in a collection cup. Field blanks followed the same processing procedure as urine (2.4.2 Urine samples). This ensured that no contamination occurred during transportation or processing of samples.

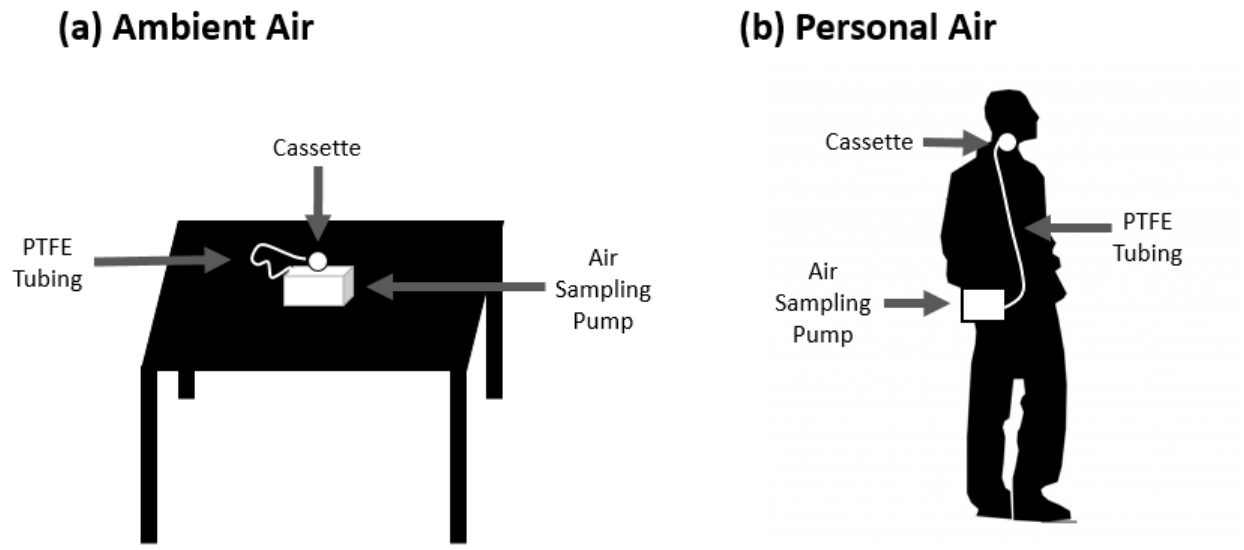
2.4 Sample preparation and analysis

2.4.1 Air samples

2.4.1.1 Gravimetry

Prior to weighing filters from air sampling, three quality control (QC) filters were weighed. These consisted of three blank filters labelled QC1, QC2 and QC3. This was important to ensure that there was no variation in the results over time due to static [14]. Each filter was weighed two times to ensure consistency. In addition, from September 2015 to November 2015 weighing was performed at the Natural Resources Engineering Facility, University of Alberta. From November 2015 weighing was performed at the Heritage Medical Research Center, University of Alberta. Two microbalances were used because our laboratory did not have a microbalance at the beginning of the project but was equipped with one half way through.

All filters were kept in a desiccator for a minimum of 24 h prior to weighing to minimize the effects of humidity. Weighing was performed on two microbalances (Sartorius, Elk Grove, IL and, Mettler Toledo, Columbus, OH). Since static was creating variations in filter weight, an antistatic device was used prior to weighing. Filters were weighed prior to and after sampling. The difference between pre- and post- sampling weight was used to determine the mass of particles on filters.



(c) Gillian Pumps used for Air Collection

Figure 2.3 Ambient and personal air sampling set-up. Representations of the placement and arrangement of the air sampling pumps for ambient (a) and personal (b) sampling. As seen in the diagram, the cassette was attached to the collar of the welders, in the personal breathing zone, the tubing would have looped under the welding jacket. The picture presented in (c) represents the pumps used and how they would have been set-up for ambient air collection.

Following calculation of the sample weight (mg) from the gravimetric analysis, the concentration (μg/m^3) of particles, the 8 h time weighted average (TWA) (mg/m^3) and dose (mg/kg/day) were calculated. The TWA is the standard measurement in occupational air exposure [83]. The following equations represent the calculations used for the concentration, 8 h TWA, and dose.

Equation 2: Calculation for concentration.

$$Concentration\ (\mu g/m^3) = \frac{Mass\ (\mu g)}{Sampling\ Volume\ (L)}$$

Equation 3: Calculation for 8 h TWA.

$$TWA = \frac{Concentration\ (\mu g/m^3) \times SamplingTime\ (min)}{480\ min}$$

Equation 4: Calculation for dose. The intake rate is for males between the age of 19-65 years. The exposure factor was calculated by dividing the number of mins sampled by the number of mins in a day (1440 min), as seen in the second equation below.

$$Dose = \frac{Concentration\ (mg/m^3) \times Intake\ Rate\ (15.2 m^3/day) \times Exposure\ Factor}{Body\ Weight\ (kg)}$$

$$Exposure\ Factor = \frac{Number\ of\ minutes\ sampled}{1440\ min}$$

2.4.1.2 Metals

2.4.1.2.1 Certified reference materials

Certified welding fume reference material was purchased from the Health & Safety Laboratory, both mild (MSWF-1) and stainless (SSWF-1) steel (Buxton, UK).

2.4.1.2.2 Procedures

Metal analysis was performed at the Soil, Water, Air, Manure, and Plant (SWAMP) laboratory (Department of Renewable Resources, Faculty of Agriculture, Life and Environmental Sciences, University of Alberta). Filters were placed in Teflon tubes with 3 ml sub-boiled HNO_3 acid and 1 ml sub-boiled H_3OBF_4. The digestions were performed in a MLS UltraClave

(Milestone, Sorisole, Italy) filled with a solution of 500 ml H_2O, 10 ml 30% H_2O_2, and 5 ml H_2SO_4 to create an oxidizing environment [84, 85]. Digestions lasted 2 h at 240°C and 160 Barr [84]. The high temperature and pressure allowed complete digestion of filters and particles.

All metals were determined on an iCAP-Q ICP-MS (Fisher Scientific). The conditions for the ICP-MS included using a PFA-400 Nebulizer (Elemental Scientific, Omaha, NE) to nebulize samples, with an introduction speed of 400 µl/min. To prevent larger particles from entering the torch, the spray chamber was cooled to 2.7°C. The data acquisition parameters include 60 sweeps per reading with a dwell time of 0.03 s for all metals. The metals measured were: Ag, Al, As, Bi, Cd, Co, Cr, Cu, Fe, Ga, Mn, Mo, Ni, Pb, Sb, Tl, V, and Zn.

Field blanks were analyzed with certified reference materials (CRM) (MSWF-1 and SSWF-1) every eight samples. In addition, three reagent blanks were analyzed per day. To calculate metal concentrations, field blanks were subtracted from samples.

2.4.2 Urine samples

2.4.2.1 Randomization

Samples sent for ICP-MS and NMR analysis were randomized and blinded. Randomization and blinding are important to prevent potential bias in subsequent analysis is prevented [86]. A random list of welding subjects and controls was created (https://www.random.org/lists/). The lists were paired to create block pairs of welders and controls, with all four days of sampling. The different sampling days were then randomized in each block pair. QCs were analyzed between each block pair. Table 2.1 demonstrates an example of randomized samples with the QCs. After samples were randomized, they were labeled in numerical order to blind them and were unblinded after receiving results.

Table 2.1 Example of randomization of samples and quality controls. Welding (1XX) and control (2XX) samples were randomly assigned to block pairs, as represented by 103 and 208 in the table below. The sampling days are randomized in these block pairs represented in the time column. QCs for the analysis were run between each block pair (every 8 samples).

INDEX	PARTICIPANT	TIME (0,1,7, 50)	CASE/CONTROL	QC
1	QC	QC	QC	1
2	103	7	Case	0
3	103	50	Case	0
4	103	1	Case	0
5	103	0	Case	0
6	208	50	Control	0
7	208	1	Control	0
8	208	0	Control	0
9	208	7	Control	0
10	QC	QC	QC	1
11	112	1	Case	0
12	112	50	Case	0

2.4.2.2 Metals

2.4.2.2.1 Materials

Pure grade EDTA powder was purchased from Sigma Aldrich (Oakville, ON). All other chemicals and solutions were from the SWAMP laboratory. The 15 ml polyethylene tubes were purchased from VWR.

2.4.2.2.2 Certified reference materials

Low and high-level CRMs ClinChek I and II were acquired from Recipe Chemicals (Munich, Germany).

2.4.2.2.3 Quality controls

QC samples were prepared and employed for urinary metal analysis. To be used as an internal QC, one urine sample was centrifuged (Eppendorf) at 4°C, 600 *g* for 10 min. The supernatant, 10 ml, was transferred to a 15 ml tube and frozen at -80°C. ClinChek level I and II samples were prepared, described in Cassiède *et al.* (2017), to ensure the ICP-MS accurately calculated metal concentrations [87]. Finally, field and acid blanks, consisting of 2% HNO_3, were tested to ensure there was no contamination from handling samples and determine the limit of detection (LOD) and limit of quantification (LOQ). Field blank concentrations were subtracted from samples. Metal concentrations were normalized to creatinine to account for the hydration status of the subject.

2.4.2.2.4 Procedure

All urine processing for metal analysis was carried out under a sterile laminar flow hood to prevent contamination of samples. Three aliquots of 0.5 ml of each urine sample were transferred to pre-cleaned 15 ml polyethylene tubes as soon as samples were back from NAIT. Urine samples were then frozen at -80°C until analysis. All samples analyzed at the SWAMP laboratory were diluted with 9.4 ml ultrapure H_2O and 0.1 ml sub-boiled HNO_3. To remove the effect of major ions 0.04% EDTA was added to the solutions. Metals were then determined on the iCAP-Q ICP-MS (Thermo Scientific) as previously described (2.4.1.2 Metals). The following metals were measured in urine: Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Ga, Fe, Mn, Mo, Ni, Pb, Sb,

Se, Tl, V, and Zn. All dilutions were performed at the SWAMP laboratory to avoid contamination of samples.

2.4.2.3 NMR

2.4.2.3.1 Materials

Chemicals and solutions used included: NaOH pellets (VWR), and concentrated HCl (12.1 M) (Fisher Scientific). The internal standard for NMR analysis, IS-1 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) with added imidazole, was purchased from Chenomx Inc. (Edmonton, AB). The 15 ml conical centrifuge tubes from Corning, Inc. (Corning, NY) and 2 ml tubes for freezer storage were purchased from Eppendorf (Hamburg, Germany). Glass 5 mm thin-walled NMR sample tubes were acquired from Wilmad Labglass (Vineland, NJ).

2.4.2.3.2 Quality controls

To serve as QC, a pooled urine sample was created by mixing together five urine samples from welding participants. The mixture was centrifuged and aliquoted into sterile 15 ml conical tubes and frozen at -80°C. This QC allowed us to check for variability in replicate measurements as well as batch effects between different days of spectrum acquisition. As there is no CRM for NMR, our QC sample verified metabolites that were fit with high precision consistently over time.

To verify the precision of the NMR analysis, the relative standard deviation (RSD) was calculated for each metabolite in the pooled QC urine samples (n = 33). Metabolites with < 20% RSD were considered reliable and reproducible, and used for further analysis. Equation 5 demonstrates how RSD was calculated in our QC sample. We fit peak profiles for 151 metabolites. From 151 metabolites that were fit to replicate QC spectra, a total of 61 had < 20% RSD. Metabolite concentrations were also normalized to creatinine to account for the hydration status of the subject.

Equation 5: Relative Standard Deviation Calculation.

$$\frac{Standard\ Deviation}{Mean} \times 100\%$$

2.4.2.3.3 Procedure

An aliquot of 10 ml of urine was transferred to a separate sterile 15 ml conical centrifuge tube and centrifuged at 4°C, 600 *g* for 10 min to remove precipitates and particulate matter including cells and bacteria. Supernatants were transferred to a new 15 ml conical tube, without disturbing the pellet, and three 1.8 ml aliquots were transferred into 2 ml tubes. Tubes were appropriately labeled for NMR analysis and placed in a -80°C freezer until analysis.

The day before NMR analysis, samples were removed from -80°C and thawed on ice. Once thawed, samples were vortexed to ensure uniformity. Each sample was placed in a 10 ml tube, where 200 µl of Chenomx IS-1 (Chenomx Inc.) containing the internal standard, DSS, was added, and the sample was gently vortexed. DSS was added to adjust for chemical shift when fitting peaks. The pH of each sample was measured and recorded using a pH meter equipped with a Orion™ 9157BNMD Triode™ 3-in-1 pH/ATC probe (Thermo Scientific, Ottawa, ON) with the pH meter calibrated using the commercial standard buffers (pH 4.01, 7.00, and 10.01) (Thermo Scientific). Each sample was adjusted to 7.0 ± 0.1 pH using varying amounts of 1 M NaOH and 1 M HCl. Finally, 750 µl of each sample was aliquoted into an appropriately labeled 5 mm NMR tube and placed in the 4°C fridge until analysis within 24 h.

NMR was performed at the National High Field Nuclear Magnetic Resonance Center (NANUC), University of Alberta. NMR spectra were acquired on an Oxford 14.09 Tesla (600 MHz) VNMRS spectrometer (Oxford, Abingdon, UK) equipped with a 5 mm HX probe with Z-axis gradient coil and Varian 768AS robotic system (Agilant Inc., Palo Alto, CA) by Dr. Pascal Mercier (NANUC, University of Alberta). The specifications used by the spectrometer match the Chenomx library for 600 MHz magnet profiles and those in our previously published work [87]. In a small set of samples, a 700 MHz Varian magnet (Agilant Inc.) was used (Department of Chemistry, University of Alberta).

Following spectral collection, peaks for 151 metabolites were fit to the spectra using specialized computer algorithms and Chenomx NMR Suite software, which corrects the spectrum to a baseline with a pH of 7.0 ± 0.1. Peak-fitting is a highly-specialized technique, and was carried out by Dr. Mercier. A computer-based algorithm using progressive spectral region

reconstruction (PSRR) developed by Dr. Mercier (NANUC, University of Alberta) was used to identify the metabolites that were present and their concentrations. Once all peaks and concentrations were identified, samples were unblinded and sorted for further analysis.

2.5 Calculations and statistical analysis

2.5.1 Materials

Data analysis was completed with the use of the following software. All NMR spectra were analyzed using the Chenomx 600 MHz library available on the Chenomx NMR Suite 8.0 Software. Initial and exploratory data analysis was conducted on Microsoft Excel. Metabolite concentrations were organized to an Excel friendly format using MatLab commercial software package (MATLAB 6.1, Natick, MA). All further analysis was done using STATA (StataCorp, College Station, TX), Prism 6 (GraphPad Software, Inc., San Diego, CA), Interactive Health Data Application (IHDA) (EASi, Inc, US.), Waikato Environment for Knowledge Analysis (WEKA) (Weka 3.8, University of Waikato, Hamilton, New Zealand), MetaboAnalyst 3.0 (McGill University, Montreal, QC) and R statistical package available online (R x64 3.3.1, Vienna, Austria). Two packages were installed and utilized for R, these include ggplot2 and ggfortify to graph the data.

2.5.2 Procedure

Data were log-transformed to generate a normal distribution for parametric analysis. Urinary metals and metabolites were normalized to creatinine. All error bars in graphs represent mean and standard deviation (SD) unless otherwise stated. To test for differences over time and between groups repeated measures analysis of variance (RM-ANOVA) was used with GraphPad Prism 6. To test for differences on day 50 between controls and welders in urinary metal and metabolite concentrations, independent *t*-tests with false discovery rate (FDR) completed. In addition, to longitudinally compare welding subjects from days 0 to 50, paired *t*-tests were carried out using Prism. Classification differences were tested with WEKA software for air exposure, urinary metal and metabolite concentrations. To test compliance to AB's OELs, IHDA software was implemented. Finally, to further test differences between the two groups and over time, PCA was calculated with R statistical package, and supervised classification, PLS-DA was done using MetaboAnalyst 3.0 [88].

Chapter 3

Results

3.0 Results

The following chapter will provide an overview on air exposure results, along with urinary metal and metabolite concentration results. Finally, at the end of this chapter, all results are combined to test for differences between welding and control groups and over 8 weeks of sampling.

3.1 Confirmation of creatinine measurements by NMR

A major concern for this study is that creatinine measurements have not previously been validated using NMR and compared with the gold standard Jaffe method used in hospital clinical laboratories for urinary creatinine assessment [89, 90]. NMR-measured creatinine has also never been used for normalization against urinary metals. Therefore, we sought to determine if NMR-measured creatinine was valid for use in normalization of metals and metabolites in urine samples. Ten 12 h fasting urine samples were collected from healthy, unexposed lab personnel (25-53 years old, 2 males and 8 females). These samples were processed for ICP-MS and NMR analysis (2.4 Sample preparation and analysis). Urine samples were analyzed on two separate magnets: a 600 MHz magnet at NANUC and a 700 MHz magnet at the Department of Chemistry, University of Alberta. In addition, urine samples were sent to the Department of Laboratory Medicine and Pathology, University of Alberta, to assess creatinine measurements using the Jaffe reaction. Creatinine concentrations obtained from the Jaffe analysis were compared to those obtained from the 600 and 700 MHz magnet. It was established that NMR analysis could reliably measure creatinine in comparison to the Jaffe method using a correlation as seen in Figure 3.1 (r^2 values of 0.988 and 0.984, respectively). Urinary metal concentrations for these 10 samples were then determined using ICP-MS, and normalized using the creatinine concentrations obtained from the 600 MHz magnet. Metal concentrations were comparable to those from the UK and Health Canada reports [54, 91]. These findings were published recently in *Clinica Chimica Acta* [87].

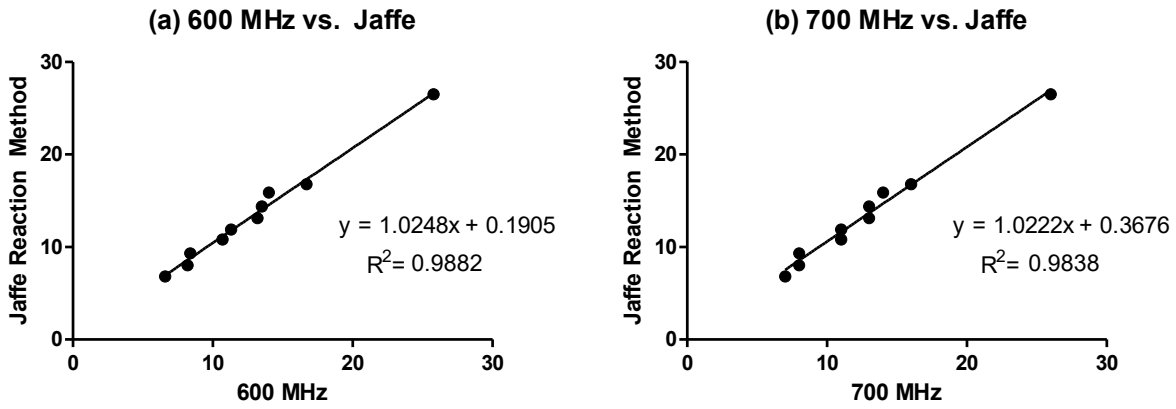


Figure 3.1 Creatinine concentration (mmol/l) comparison between Jaffe reaction method and NMR analysis (600 and 700 MHz). Urine samples were collected from 10 healthy control subjects and measured by Jaffe reaction method, 600 and 700 MHz. Creatinine values were compared using a Pearson's correlation between (a) Jaffe and 600 MHz and (b) Jaffe and 700 MHz.

3.2 Participant summary

A total of 23 welding apprentices from Souch Campus and 20 instrumentation students from North Campus, NAIT, were recruited (Table 3.1). All controls were non-smokers that were age- and sex-matched to the welding subjects between the ages of 18-40. To test similarities and differences between control and welding participants, independent *t*-tests were used to determine differences in the age, body mass index (BMI), and urinary creatinine concentrations of the two groups. The average age for the welding participants was 25 ± 5 (mean ± SD), whereas for the control participants it was 28 ± 6. Welding participants had an average (± SD) BMI of 26 ± 3, compared to control subjects, 25 ± 2. The average (± SD) creatinine concentration (g/l) for welders was 1.9 ± 0.44 and for controls was 1.6 ± 0.76. Therefore, there were no significant differences between welders and controls regarding their ages, BMIs or average urinary creatinine concentrations.

A McNemar's test, with a chi-square to determine significance, was used to test for any differences between medications, medical histories, and previous welding fume exposures. No welding or instrumentation (controls) students reported any history of high or low blood pressure, diabetes, hepatitis, kidney, or liver problems [92]. One welding participant reported a heart arrhythmia, and select welders (n = 3) and controls (n = 1) reported asthma. There was no significant difference ($p < 0.05$) between the two groups. Further, there was no significant differences between the two groups regarding prescriptions, over-the-counter medications, or vitamin use. A significant difference in previous exposure to welding fumes was found, with 22 welding participants and one control participant having previous exposure in the three months prior to participation. It was concluded that the groups were well-matched. Table 3.1 provides a summary of the participant information.

*Table 3.1 Summary of participant information for control and welding participants. Age and BMI are represented by mean and SD, with differences tested using an independent t-test. The remaining categories are represented by the number (%) of controls (n = 20) and welders (n = 23) with significance using a chi-square distribution. Both groups are well-matched overall. BMI represents body mass index, and N/A represents not applicable. Significance is indicated with an * p <0.05.*

	Welders No. (%)	Controls No. (%)	Difference
Sample Size	23	20	N/A
Age	25 ± 5	28 ± 6	0.0813
BMI	26 ± 3	25 ± 3	0.4301
Urinary Creatinine Concentration (g/l)	1.9 ± 0.55	1.6 ± 0.44	0.0860
Alcohol	15 (65)	19 (95)	0.0166*
Prescription Drugs	3 (13)	2 (10)	0.7562
Over-the-Counter Drugs	1 (4)	6 (30)	0.7562
Vitamins and other Supplements	11 (48)	6 (30)	0.2331
High Blood Pressure	0 (0)	0 (0)	N/A
Low Blood Pressure	0 (0)	0 (0)	N/A
Diabetes	0 (0)	0 (0)	N/A
Heart Problems	1 (4)	0 (0)	0.3454
Hepatitis	0 (0)	0 (0)	N/A
Kidney Problems	0 (0)	0 (0)	N/A
Liver Problems	0 (0)	0 (0)	N/A
Breathing Problems or Asthma	3 (13)	1 (5)	0.3651
Previous Exposure to Welding Fumes 3 months prior to Participation	22 (96)	1 (5)	< 0.0001*

3.3 Air sampling results

3.3.1 Gravimetry quality controls

There was a total of two gravimetry QCs used for analysis: (i) blank QC filters (3) and (ii) field blanks. The three blank QC filters were constant and had no weight differences throughout analysis. The RSD was calculated and found to be < 1% for each filter over time. In addition, a sign test was completed to ensure that there were no significant changes, and none were found. Table 3.2 summarizes the information from the three QC filters.

To confirm there were no significant differences between pre- and post-weighing of the field blanks, a paired *t*-test was executed. A *p*-value of 0.2226 verified that there were no significant differences overall in field blanks. Field blanks were collected by preparing filters as previously described (2.2 Preparation of sampling and laboratory equipment), once prepared the field blanks were transported to and from the sampling site but were not used and were processed the same as the used filters back at the lab to account for any contamination from processing and transportation. Further, the 95% confidence interval (-0.0033, 0.014) does not provide sufficient evidence against the null hypothesis, supporting that there was no significant variation in weights of the field blanks. In addition, previous work performed at the laboratory on 10 replicates showed that variation due to sampling and gravimetry analysis using PVC filters and cassettes was low with < 10% RSD [93].

3.3.2 Gravimetry

The total mass concentration (mg/m^3) of particles in air samples was determined by gravimetric analysis. PVC filters had 5 µm pores, that collected all total dust particles (1 nm to > 100 µm). Gravimetric data was log transformed to allow parametric analysis. The 8 h TWA was calculated for controls and welding apprentices based on the 3 h collection period (2.4.1.1 Gravimetry). A RM-ANOVA was used to test differences over time and between control and welding groups. A significant difference in particles from air samples was observed between controls and welders on days 1, 7, and 50 (**** $p < 0.0001$) (Figure 3.2). There was no significant difference over time for control participants, whereas welding participants had significant differences ($p < 0.0001$) between day 0 and all other sampling days (1, 7, and 50).

Therefore, welding apprentices were exposed to higher amounts of welding fumes compared to control participants on sampling days 1, 7 and 50 and compared to sampling day 0 (baseline).

The overall exposure dose (mg/kg/day) was calculated for each participant, according to Equation 4 (2.4.1.1 Gravimetry). This was based on their overall exposure, exposure factor, body weight (kg), and an assumed inhalation intake rate of 15.2 m^3/day. The intake rate represents males between the ages of 18-65, which includes the range of our subjects [94]. The intake rate exposure factor is a calculated value representing the average dose over a period of exposure [83]. Figure 3.3 shows doses (mg/kg/day) in total mass concentration (mg/m^3) for control and welding students. The results are comparable to the TWA for 8 h, with significant differences (** $p < 0.01$, **** $p < 0.0001$) between controls and welders on days 1, 7, and 50.

The total particle exposure that welding apprentices were exposed to on day 50 were analyzed using Bayesian decision analysis (BDA) with IHDA software. Descriptive and nonparametric statistics, as well as compliance, may be calculated using this software for workplace exposures and compared to OELs [95]. If it is unclear if exposure is possibly close to the OEL using compliance calculations, the IHDA software completes BDA to determine the probability of being above the OEL. Particles TWA were compared to AB's OELs, 10 mg/m^3 and 3 mg/m^3, for inhalable and respirable particles, respectively. Inhalable particles consist of a range from fine to large particles that settle in the whole respiratory tract [12]. Respirable particles, cut-off point of 4 μm, are smaller than inhalable particles and are found in the alveolar region [12]. Since welding fumes are mostly respirable, results were compared to both inhalable and respirable 8 h TWAs [18, 20]. The BDA initial reading was selected using the "Generic Professional Judgement" setting, with an initial arbitrary rating of "3-Controlled" and certainty level of "2-Medium" using IHDA software. Welders had a geometric mean at 0.97 mg/m^3, with 90% upper confidence limit at 5.548 mg/m^3 for sampling days 1, 7, and 50, which is substantially lower than the inhalable OEL in AB (10 mg/m^3). The BDA further supports that welding student TWAs were compliant and a category 4 exposure (overexposure) can be rejected with 99% confidence (Figure 3.4). However, the upper confidence limit was over the 8 h OEL for respirable particles (3 mg/m^3). The BDA supports that over 5% of the students may be overexposed to respirable particles with 99% confidence (Figure 3.4).

Table 3.2 Summary of three QC filters weighed for gravimetric analysis. All three filters have < 1% RSD demonstrating reliable and reproducible results. No significant differences were found using a sign test with the median (mg) as the null hypothesis, both the median and p-values can be seen below.

QC	RSD (%)	Median (mg)	*p*-Value
1	0.179	14.639	0.508
2	0.258	13.529	1.00
3	0.0486	14.190	1.00

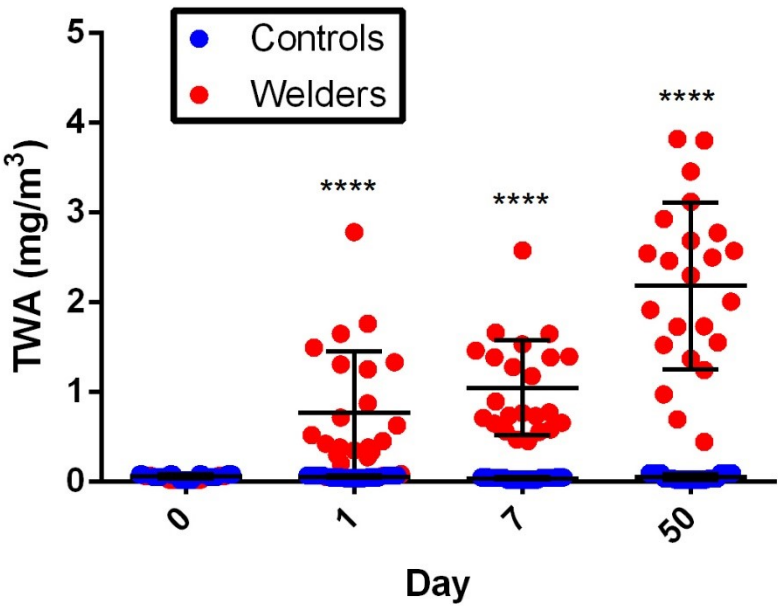


*Figure 3.2 TWA concentration (mg/m^3) of total particle exposure in welding apprentices on sampling days 0, 1, 7, and 50 compared to controls. Particle exposure is represented by 8 h TWA concentrations (mg/m^3). Data were collected on sampling days 0, 1, 7, and 50. Significance was calculated using a Tukey's test (**** $p < 0.0001$). TWA represents the time weighted average.*

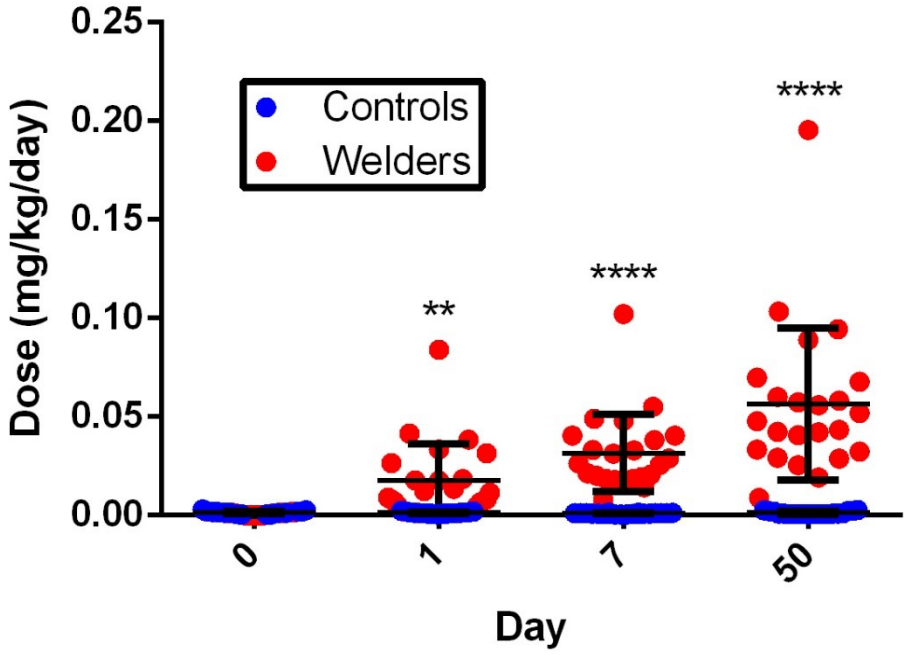


*Figure 3.3 Dose (mg/kg/day) of total particle exposure in welding apprentices on sampling days 0, 1, 7, and 50 compared to controls. The dose (mg/kg/day) was calculated individually for each participant based on their weight and exposure of the corresponding sampling day. Data was collected on sampling days 0, 1, 7, and 50. Significance was calculated using a Tukey's test (** p = 0.01, **** p < 0.0001).*

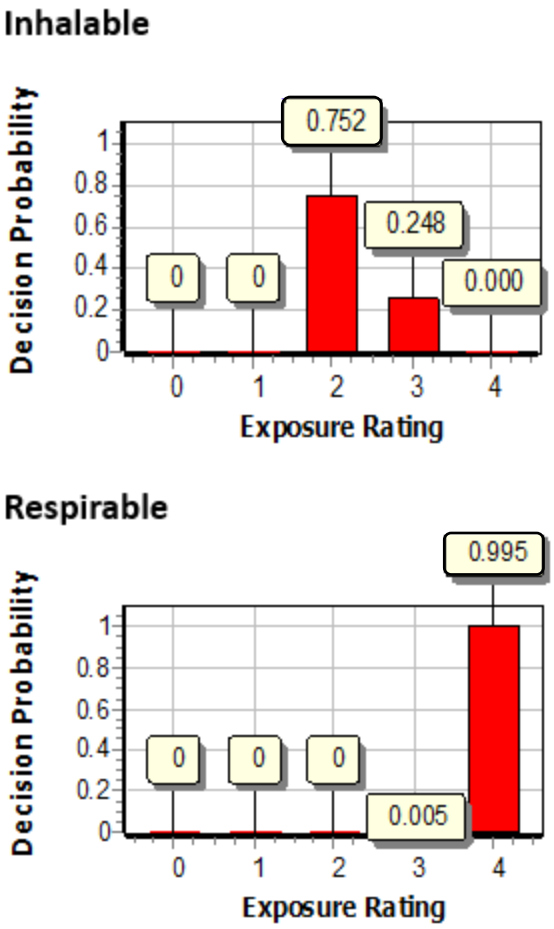


Figure 3.4 BDA of inhalable and respirable particles for welders on sampling days 1, 7, and 50 (n = 69). The following charts demonstrate that a category 4, > 5% exceedance of the OEL can be rejected with > 99% confidence for inhalable particles. When comparing respirable particles to AB's OEL, there is >99% chance that over 5% of the welding apprentices were overexposed.

3.3.3 Metals quality control

For each day of ICP-MS analysis, the LOD, and LOQ were reported because the LOD for each metal varies from day to day. For statistical purposes, non-detected metals were replaced by the corresponding LOD. The LOD was used, as replacing values with 0 may increase the overall error rate [96]. The mean, SD and recovery values were calculated for the CRMs.

Metal analysis of air exposure samples also contained two QCs, (i) field blanks and (ii) welding reference fume material. The following section summarizes findings from these QCs. The field blanks were analyzed using ICP-MS following gravimetric measurement. Table 3.3 summarizes the results of the field blank filters, along with the LOD.

The welding fume reference material, MSWF-1 and SSWF-1 were analyzed with each batch, and the average, SD, and RSD (%) were calculated. The RSD was ≤ 5% for all measured metals, demonstrating reliable determination of concentrations. The expected values, or the mean and SD of the certified reference values, were compared to the measured values, and the accuracy was calculated (%). Accuracy was > 90% for all metals (Table 3.4).

3.3.4 Metals

A total of 13 metals were quantified for air exposure samples using ICP-MS. These were: Al, As, Cd, Cr, Co, Cu, Fe, Mn, Mo, Ni, Pb, V, and Zn. The 8 h TWA (µg/m^3) for exposure to specific metal concentrations was calculated, like particle exposure. The dose (mg/kg/day) was also calculated for each metal (Equation 4). These values were compared using RM-ANOVA (Figure 3.5). Control and welder exposure was significantly different for all metals on day 50 ($p < 0.05$). When comparing day 0 to days 1, 7, and 50, there were significant differences for some metals in the welding participant's exposure. Metals with significant differences between day 0 and day 1 for welding participants were: As, Cu, Fe, Mn, Mo, Ni, Pb, V and Zn. Those with significant differences between day 0 and 7 and day 0 and 50 were all the same metals as day 0 to 1, along with Co and Cr. There was no difference for controls over time for any metal. Appendix D summarizes all TWA values and dose concentrations.

Table 3.3 Summary of filter field blank values and the LOD from ICP-MS analysis. The mean, SD (ng/filter), and LOD (ng) for each metal is presented (n = 54). The mean values from the field blank filters were later subtracted from the values of control and welder air exposure samples to control for any contamination from transportation and handling.

Metal	Mean (ng/filter)	SD (ng/filter)	LOD (ng)
Al	179.3	75.9669	8.16×10^{-3}
As	2.7	2.04137	1.17×10^{-2}
Cd	1.4	1.79454	8.0×10^{-4}
Co	2.7	2.17334	5.5×10^{-3}
Cr	350.9	149.998	4.98×10^{-2}
Cu	12.3	16.6162	1.32×10^{-1}
Fe	467.7	365.417	7.21×10^{-1}
Mn	58.7	33.8514	3.95×10^{-2}
Mo	0.6	0.9998	9.2×10^{-3}
Ni	21.5	32.57104	5.93×10^{-2}
Pb	0.5	0.47848	2.3×10^{-3}
V	0.7	1.21978	7.1×10^{-3}
Zn	39.0	51.2008	1.58

Table 3.4 Comparison of expected and measured values for welding fume reference material (MSWF-1 and SSWF-1) showing no significant difference between values and ICP-MS measurements. All concentrations (%) are represented as mean ± SD (n = 10). The accuracy was calculated as > 90% for metals. Valid values for recovery are in the 80-120% range, therefore the ICP-MS analysis reliably measured metal concentrations. The RSD was < 5% for all measured values, showing consistent measurements.

	Reference Values		Measured Values			
Metal	**MEAN**	**SD**	**MEAN**	**SD**	**RSD**	**% ACCURACY**
MSWF-1						
Fe	42.8	± 0.7	41.8	± 1.9	4	98
Mn	1.5	± 0.03	1.5	± 0.07	5	97
Zn	21.7	± 0.9	20.6	± 0.9	4	95
SSWF-1						
Cr	8.4	± 0.4	8.7	± 0.2	2	104
Fe	29.8	± 0.9	27.4	± 0.6	2	92
Mn	22.9	± 0.5	21.6	± 0.5	2	94
Ni	2.7	± 0.2	3.1	± 0.1	3	116

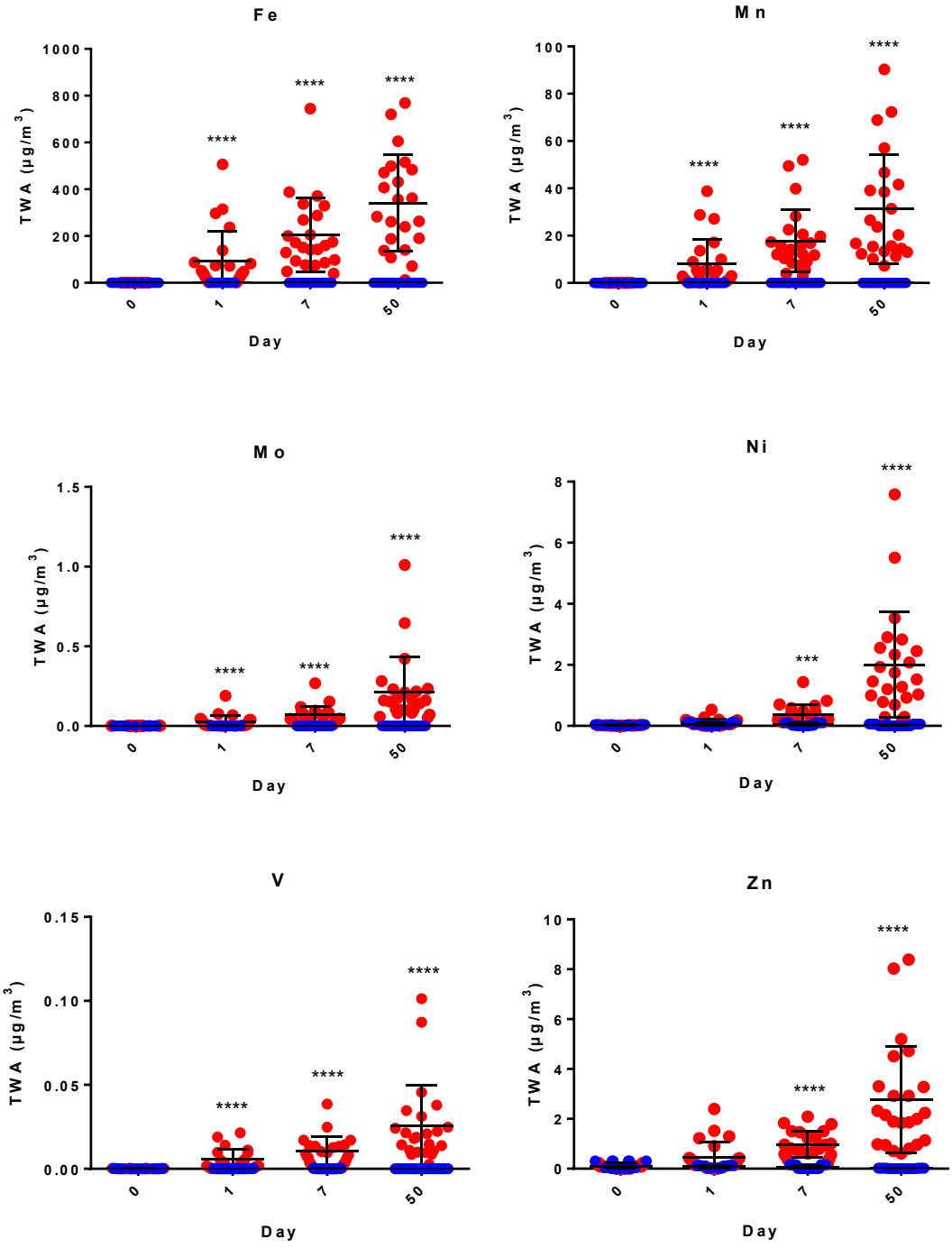


*Figure 3.5 TWA concentration (µg/m^3) of select metals in welding apprentices compared to controls. Metal exposure is represented by the 8 h TWA concentration (µg/m^3). Data was collected on sampling days 0, 1, 7, and 50. The represented metals are: Fe, Mn, Mo, Ni, V, and Zn. Significance was calculated using a Tukey's test (** p = 0.01, *** p < 0.001, **** p < 0.0001). TWA represents the time weighted average.*

A BDA was conducted using IHDA for exposure to individual metals for welding apprentices on sampling days 1, 7, and 50. The concentration ($\mu g/m^3$) of metal exposure was compared to AB's OELs (Table 3.5). As previously done with particles, the initial rating was at "3-Controlled" and the certainty level at "2-Medium" with the IHDA software. All metals were below their 8 h OEL for AB and a category 4 exposure could be rejected with 99% confidence (Table 3.5). Both Fe and Mn air exposure concentrations fell in exposure categories 2 and 3 and compliance is estimated but the values are close to the AB OEL (Figure 3.6). The remaining metals were not close to the AB OEL and overexposure was not an issue. A summary of the gravimetric results and metal analysis from air sampling for control and welders can be seen in Appendix D.

The particle concentrations (mg/m^3) and metal concentrations ($\mu g/m^3$) were combined and analysis was completed to classify subjects as welders or controls based on exposure levels on day 50 and to compare day 0 and 50 in welders. A PCA was conducted, comparing controls and welders on day 50. The first two principal components covered 85.16% of the variation, which is a strong model to determine between welders and controls (Figure 3.7). Baseline (day 0) values were compared to day 50 in welding participants, and explains 83.40% of the variation in the first two principal components.

A variety of machine learning models were applied to the data using WEKA to establish the strongest model to apply to the data. The intent of using machine learning models was to classify between welders and controls based on the collected data. All models were compared to the model generated by a zero rules algorithm, which selects one label and predicts every instance to belong to that group [97]. The different models tested include: zero rules, one rule, J48 decision tree, naïve Bayes, logistic regression, and IBk. The accuracy was determined by calculating a 10 x 10-fold cross-validation in WEKA. To summarize 10 different randomized sets of the data were generated in WEKA and underwent a 10-fold cross-validation. The cross-validation technique was selected instead of splitting data into a test and training set because the number of samples was quite low in comparison to the number of features, which can cause problems when splitting data. Specifically error estimates become inaccurate and the model quality decreases [75]. A J48 decision tree model was established to be reliable for two

models: (i) classifying welder and control subjects on day 50 and (ii) between day 0 and 50 in welders based on exposure data. The J48 decision tree method builds a model by identifying attributes that discriminate various instances most accurately in a training set, that can then classify a new item [98].

Figure 3.8 (a) the J48 decision tree model classifies controls and welders on day 50. A 10-fold cross-validation was used to generate the model. The cross-validation technique estimates how well the results of the analysis will generalize to an independent data set [99]. This is done in WEKA by splitting the data into 10 groups, the model is then trained on 9 of those groups and tested on the final 10^{th} group, which has not been used in training the model [97]. Out of 42 instances the final decision tree correctly classified all instances (100%), compared to the baseline model, zero rules (53.49%). The kappa statistic, a measure of inter-rate agreement between categorical variables, was 1.00 [100].

Figure 3.8 (b) represents J48 decision tree model for welders comparing day 0 and 50. A 10-fold cross-validation was implemented. All 45 instances were correctly identified (100%), compared to the zero rules (51.11%). A kappa statistic of 1.0 and the mean absolute error was 0. Therefore, the J48 decision tree model had high accuracy and predictability when classifying exposure data, and as V was indicated for both models demonstrates that it is a good predictor of welding fume exposure.

3.4 Urine sampling results

3.4.1 Quality controls for metals

Urinary metal analysis with ICP-MS included a vast number of QCs including, (i) field and acid blanks, (ii) single, repeated urine sample, and (iii) ClinChek levels I and II. Acid and field blanks were used as a background measurement and the means (Table 3.6) were subtracted from the subject's urine samples. The acid blank values were negligible and are not reported. The field blank values are summarized (mean, SD, RSD (%), LOD, and LOQ) in Table 3.6. Pooled urine was tested as a QC but precipitation caused interferences.

Table 3.5 Summary of AB 8 h OELs and compliance of exposure for welding apprentices on sampling days 1, 7, and 50 (n = 69). All metals may be rejected for overexposure with 99% confidence. The AB OEL is represented by the concentration (µg/m^3) and was obtained from the Occupational Health and Safety Code from 2009 [101]. The geometric mean, geometric standard deviation and 95% upper confidence limit are represented, calculated using IHDA-student software. OEL represents occupational exposure limit and GSD represents geometric standard deviation.

Metal	Alberta 8 hr OEL (µg/m^3)	Geometric Mean	GSD	Upper 90% Confidence Limit
Al	10 000	0.07	23.80	30.70
As	10	0.05	9.05	3.18
Cd	10	0.00	47.00	0.03
Co	20	0.01	11.60	1.08
Cr	50	0.25	7.87	12.90
Cu	200	0.91	11.30	93.50
Fe (respirable)	5000	123.00	3.94	$1.7x10^3$
Mn	200	10.70	4.35	178.00
Mo	10 000	0.04	8.00	2.05
Ni	20	0.06	6.30	2.15
Pb	50	0.06	6.30	2.15
V	50	0.01	5.10	0.15
Zn (respirable)	2000	0.61	6.53	22.00

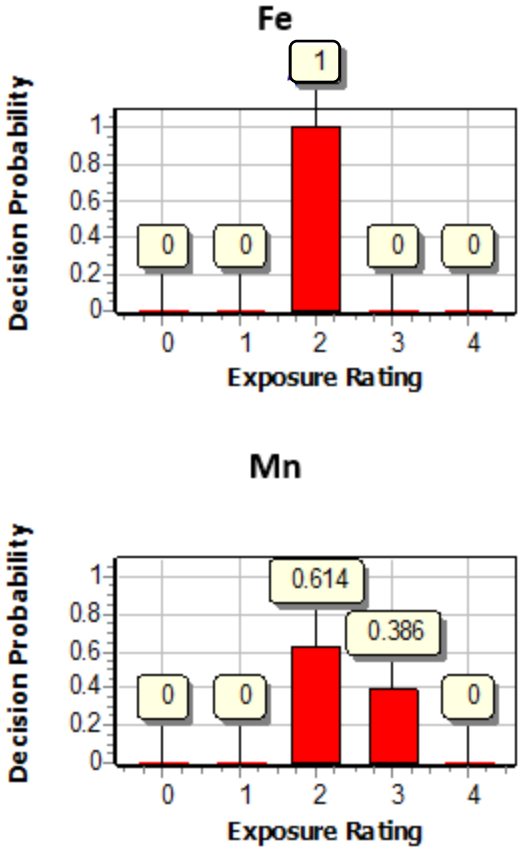


Figure 3.6 BDA of Fe and Mn for welders on sampling days 1, 7, and 50 (n = 69). The following charts demonstrate that there is > 60% chance that welding apprentices are exposed to category 2, (> 5% exceedance of 0.1 x OEL).

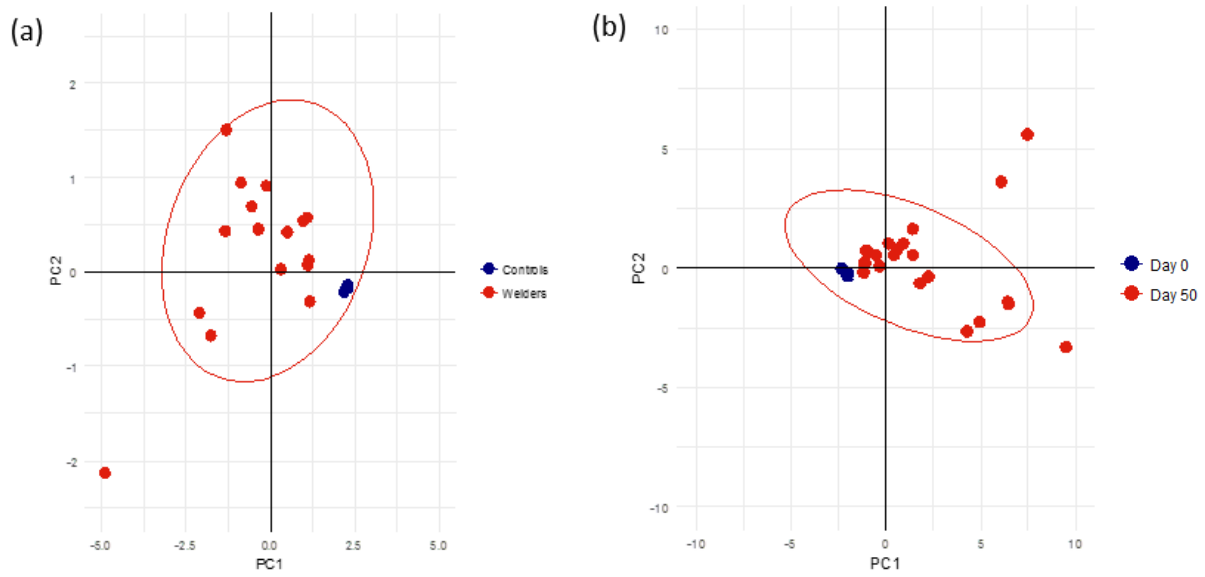
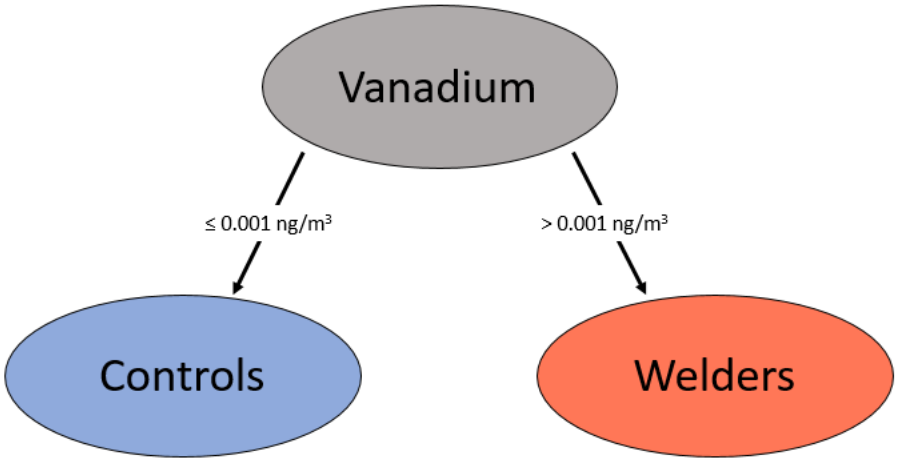


Figure 3.7 PCA of air exposure (µg/m^3). Comparison of controls and welders on day 50 (a) shows variation in the first two components, with 72.08% and 13.08%, representing principal component 1 and 2, respectively. When comparing welding subjects from day 0 with day 50 (b), principal component 1 represents 70.32% of the variance, and component 2 represents 13.08% of variance. Ellipses represent 95% confidence intervals.

(a) J48 decision tree for controls versus welders on day 50



(b) J48 decision tree for welders on day 0 versus day 50

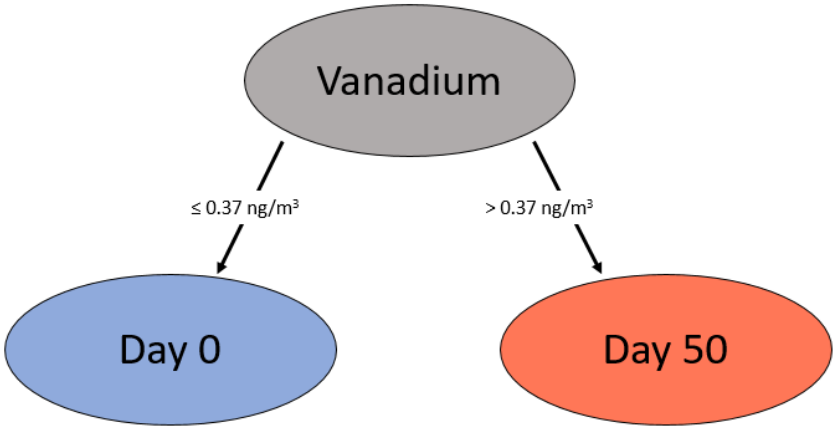


Figure 3.8 J48 decision tree model classifying exposure to welding fumes based on air exposure concentrations (ng/m^3). A 10-fold cross validation was employed in WEKA to evaluate the J48 machine learned model. Model (a) represents control and welder subjects on day 50, data is correctly classified for every instance. Model (b) represents welder subjects on day 0, baseline, and day 50, data is correctly classified for every instance. The grey circle represents the node, whereas the numbers indicate the concentration threshold.

Table 3.6 Summary of field blank values, and the LOD, and LOQ from ICP-MS analysis. The mean, SD, RSD (%), LOD, and LOQ (ng/l) is presented below (n = 26). The mean values from the field blanks were subtracted from the urinary metal concentrations to control for any contamination from transporting and processing of the samples. LOD represents the limit of detection, and LOQ represents the limit of quantification.

Metal	Mean (ng/l)	SD (ng/l)	RSD (%)	LOD (ng/l)	LOQ (ng/l)
Al	5898	6137	104	41.1	136.9
As	10	7	75	0.1	0.3
Be	5	3	58	3.1	10.4
Cd	3	6	181	0.1	0.2
Co	125	204	164	0.04	0.13
Cr	100	93	94	0.3	1.1
Cu	901	762	85	2.0	6.7
Fe	890	621	70	31.3	104.3
Mn	41	44	106	1.5	5.1
Mo	69	41	58	2.0	6.8
Ni	1604	2681	167	1.2	4.1
Pb	651	1509	232	0.1	0.2
V	13	14	105	0.4	1.4
Zn	7902	16278	206	59.4	198.1

A single QC urine sample (*n* = 23) was used as a control to verify reproducibility and determine batch effects. The RSD (%) was calculated for each metal, as represented in Table 3.7. The RSD was < 20% for four metals: As, Fe, Mo and Zn. However, the urine sample selected as a QC was quite dilute and had low metal concentrations present, occasionally lower than blank values, and was considered not a good sample for determining reliability of analysis. Further, this explains why some metals have a high RSD (> 20%) as they would have been close to the detection limit and therefore susceptible to interference or variations.

Finally, ClinChek levels I and II, CRM, were alternatively sampled between every eight samples (2.4.2.1 Randomization). The values obtained are summarized in Table 3.8, including, the mean, SD, RSD (%), certified value and the recovery (%) for each level. Level II had no values with > 20% RSD, whereas level I had three metals with > 20% RSD: Al, Ni, and Pb. The recovery percentage was greater than 85% for all metals at both levels, except for Pb, which had a recovery percentage of 68 and 70, for levels I and II, respectively. Level II ClinChek was the QC sample with higher metal concentrations. It is believed that poor RSD (> 20%) on the ClinChek level I was due to values being closer to blank levels. Therefore, they were subject to interference or variations, same as metal concentrations from the single QC urine sample. However, none of the metal measurements were discarded as higher levels would be accurate and appropriate for comparison.

When comparing the geometric mean and ranges of urinary metal concentrations in controls and welders, after subtracting field blank values, some metals (Be, Cr, Mn, and Pb) had maximum values that were lower than those present in ClinChek level I, indicating that they would not have been reliably detected at such low concentrations (Table 3.9). Further, some metals were present at high concentrations in field blanks, suggesting possible contamination from sample processing. This emphasizes the importance of using field blanks and subtracting these blank values in metal analysis. In general, urinary metal concentrations from controls and welders were close to the LOD and blank values to be accurately detected, removing select metals from further analysis (Al, Be, Co, Cr, Mn, Ni, and Pb). All subsequent analysis only looked at select metals (As, Cd, Cu, Fe, Mo, V, and Zn) that had higher urinary concentrations after subtracting field blank values.

3.4.2 Metal concentrations

The SWAMP laboratory provided a report for the metal urinary concentrations, as provided for air exposure samples. Data was unblinded and sorted according to groups. The mean from the field blanks was subtracted from each sample value to accommodate background measurements. Urinary metal concentrations were normalized to creatinine and log transformed to stabilize variance, as raw data showed nonparametric distribution. A variety of urinary metal concentrations were measured for controls and welders. These metals were: Al, As, Be, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, V, and Zn. However, only a handful (As, Cd, Cu, Fe, Mo, V, and Zn) were reliable based on field blank, QC and ClinChek values, and were considered for analysis (Table 3.9). One welding urine sample from day 0 was removed from analysis as an outlier because some urinary metal concentrations were exceptionally high. This was most likely not contamination as it was only one sample of $n = 250$, and metals that are commonly found as contaminants (Pb and Zn) were in normal ranges. Initially, RM-ANOVA was used to compare metals. Only V was significantly different (**** $p < 0.001$) between early sample collection (days 1 and 7) and day 50 in welding subjects, no other metals differed over time (Figure 3.9). When comparing between welders and controls on individual sampling days, As and Fe were significantly lower in welders on day 0, and Mo was significantly lower in welders on all sampling days (Figure 3.10). Controls exhibited higher urinary concentrations of Cd on all sampling days, whereas welders had higher concentrations of Cu on day 0, and V on day 0 and day 50.

*Table 3.7 Summary of single, repeated QC urine sample (n = 23) for ICP-MS urinary metal analysis shows four metals with < 20% RSD. The mean, SD (ng/ml) and RSD (%) are represented for each measured metal. Those metals with < 20% RSD (As, Fe, Mo, and Zn) show reliable and reproducible measurements in urine over time. Metals with < 20% RSD are indicated by * in the following table. ND represents not detectable values.*

Metal	Mean (ng/ml)	SD (ng/ml)	RSD (%)
Al	ND	ND	ND
As	1.06	0.08	8*
Be	0.00	0.00	119
Cd	0.03	0.01	24
Co	ND	ND	ND
Cr	ND	ND	ND
Cu	1.13	0.61	54
Fe	4.28	0.71	17*
Mn	0.03	0.05	155
Mo	10.28	0.54	5*
Ni	ND	ND	ND
Pb	ND	ND	ND
V	0.04	0.01	28
Zn	180.38	19.47	11*

Table 3.8 Summary of ClinChek values from ICP-MS analysis indicates almost all metals, except for Pb (**), have a recovery percentage > 85%. The mean, SD, RSD (%), certified value (CV), and recovery (Rec. (%)) are presented for measured metals both level I (n = 12) and II (n = 11). Metals with < 20% RSD are indicated (*) below showing reproducible measurements. CV represents the certified value.

	Level I					Level II				
Metal	**Mean (ng/ml)**	**SD (ng/ml)**	**RSD (%)**	**C.V.**	**Rec. (%)**	**Mean (ng/ml)**	**SD (ng/ml)**	**RSD (%)**	**C.V.**	**Rec. (%)**
Al	29.29	6.99	24	33	89	76.90	4.11	5*	86	90
As	49.72	3.46	7*	43	116	99.06	4.19	4*	83	119
Be	0.08	0.01	11*	0.07	104	0.26	0.02	8*	0.22	118
Cd	2.51	0.04	2*	2.5	102	15.01	0.59	4*	14	104
Co	1.91	0.19	10*	2.0	94	34.52	1.21	4*	35	99
Cr	3.89	0.38	10*	4.1	96	20.33	0.86	4*	20	102
Cu	38.20	3.17	8*	37	104	96.66	3.54	4*	92	105
Fe	38.96	6.02	15*	39	101	216.1	7.61	4*	224	96
Mn	3.74	0.41	11*	3.9	96	19.42	0.75	4*	19	100
Mo	22.98	0.85	4*	24	96	99.78	3.16	3*	101	99
Ni	6.75	2.92	43	5.9	114	42.61	1.62	4*	43	99
Pb	16.43	3.91	24	24	68**	45.37	4.34	10*	65	70**
V	20.55	2.24	11*	20	102	54.21	2.53	5*	49	110
Zn	222.5	24.47	11*	204	109	607.7	19.65	3*	535	114

*Table 3.9 Geometric mean and range (ng/ml) of combined welder and control (n = 171) urinary metals. Low concentrations are found throughout for all metals detected in urine. Metals with higher concentrations that were determined reliable for further analysis based off blank and ClinChek values are indicated by *.*

Metal	Geometric Mean (ng/ml)	Range (ng/ml)
Al	3.5	0-41
As*	8.6	0.5-144
Be	0.01	0-0.02
Cd*	0.2	0.01-1.8
Co	0.09	0-2.0
Cr	0.3	0-3.4
Cu*	8.7	0.5-27
Fe*	13	0.6-75
Mn	0.07	0-2.0
Mo*	47.7	4.3-250
Ni	0.8	0-19
Pb	1.0	0.03-4.1
V*	0.19	0.01-1.1
Zn*	545	32-2024

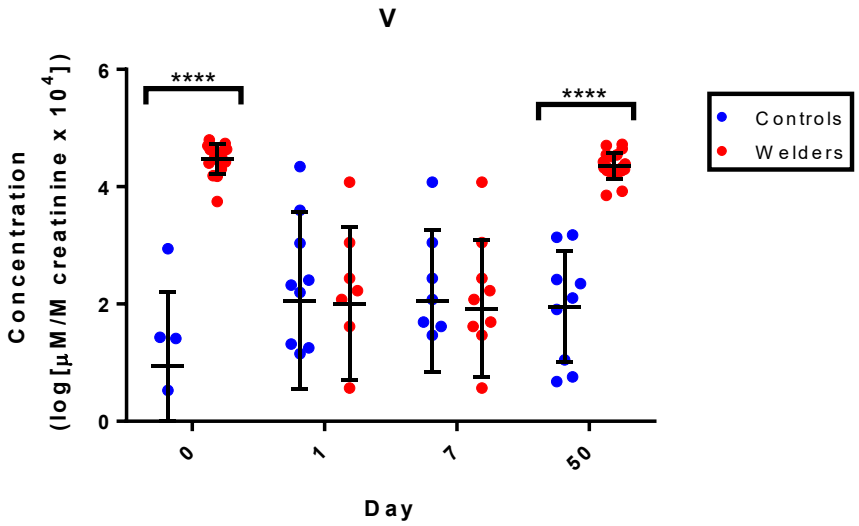


*Figure 3.9 Urinary concentration (log [µM/M creatinine x 10^4]) of V in controls and welders on sampling days 0, 1, 7, and 50. With significantly higher concentrations (**** $p < 0.0001$) on days 0 and 50 in welders. Significance was*

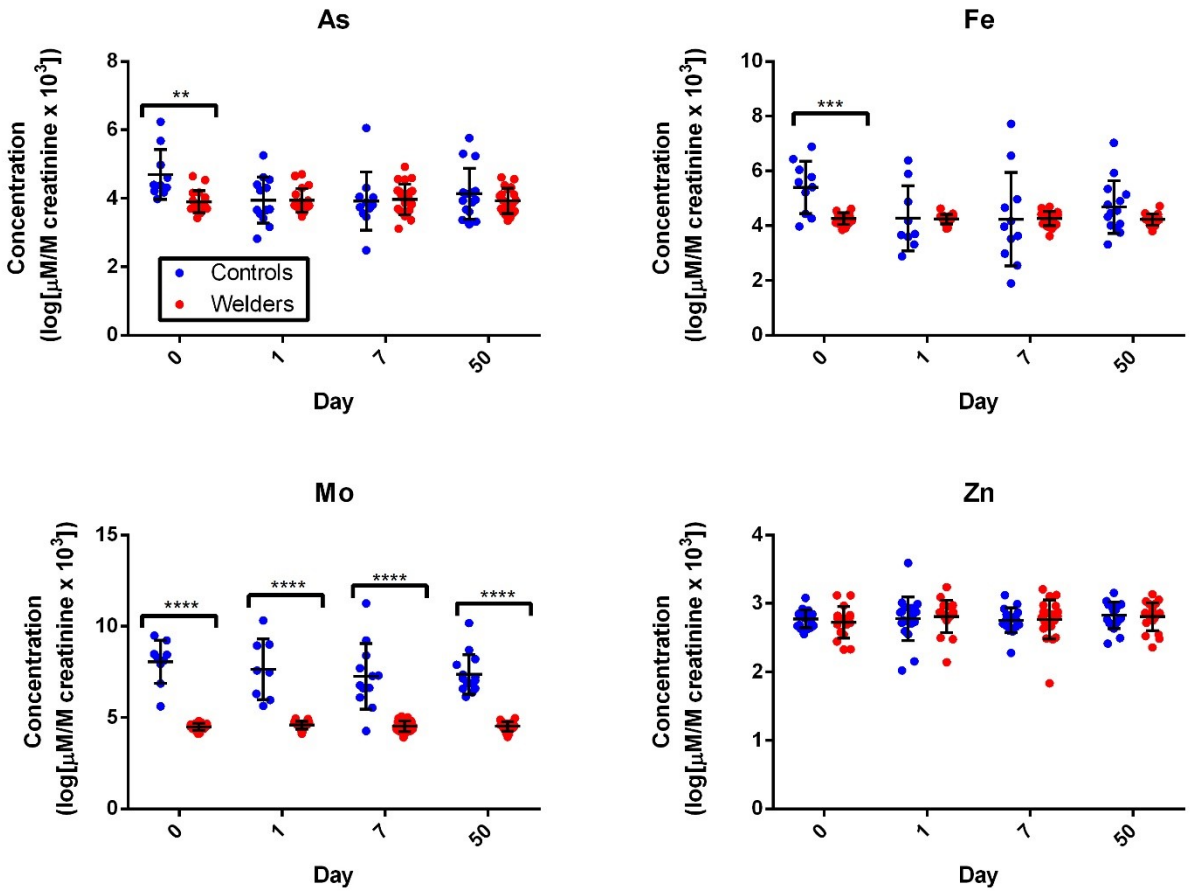


*Figure 3.10 Select urinary metal concentrations (log [µM/M creatinine x 10^3]) in controls and welders on sampling days 0, 1, 7, and 50. Represented metals are As, Fe, Mo and Zn. Significant differences found between controls and welders on sampling day 0 for As and Fe (** p = 0.01, *** p = 0.001). Control subjects were found to have significantly (**** p < 0.001) higher concentrations of Mo on all sampling days. All data was normalized to creatinine and log transformed.*

Independent *t*-tests were calculated with FDR (q = 20) between controls and welders on day 50, and Cd, Fe, Mo and V were found to be significantly different (* p = 0.05) (Figure 3.11). Levels were higher in controls compared to welders for Cd, Fe, and Mo, whereas welders had higher concentrations of V on days 0 and 50. As Cd, Fe, and Mo are commonly detected in the environment, it is possible that controls were exposed to these metals that were relatively diminished at Souch Campus. Urinary concentrations in metals are quite low in general, and substantial metal concentrations in the environment are required for urine to be reliable as an indicator of excessive exposure. However, V has potential as an indicator of welding fume exposure as it was found to be increased in welders on day 50, and is an impurity in metals used for welding [8, 102]. A linear regression analysis was conducted between the dose (mg/kg/day) of V welders were exposed to and their urinary concentration (µM/M creatinine) of V on day 50, and a significant correlation ($p < 0.05$) was found (Figure 3.12). Correlation was tested with a Pearson's correlation and was not seen for any other metal tested. It is important to note that Cd and V had > 20% RSD with the single QC urine sample. This could be because metal concentrations in the QC urine were very close to the detection limit, making it difficult to get an accurate measurement, although ClinChek level I and II V values were well within < 20% RSD. All urinary metal concentrations for controls and welders are shown in Appendix E.

Reliable urinary metal concentrations were combined and fit to a variety of classification models with a 10-fold cross-validation using WEKA. This was done to compare welders and controls on day 50, and welders on day 0 and 50. All models were compared to that generated by the zero rules model. When classifying welders versus controls on day 50, the zero rules model correctly identified 51.28% of instances. All other models tested correctly classified welders and controls > 97% over 10 random seeds. The one rule algorithm was the strongest model and correctly classified 100% of instances. This model is based on an algorithm that selects one attribute in the data set and just uses that attribute to classify instances [97]. The rule generated for this model was based on urinary V concentrations (log [µM/M creatinine x 10^4]). The J48 decision tree model had a classification accuracy of 97.44% and was also based off urinary V concentrations (Figure 3.13). Reliable classification models can be built to distinguish between controls and welders on day 50 using urinary metals, suggesting

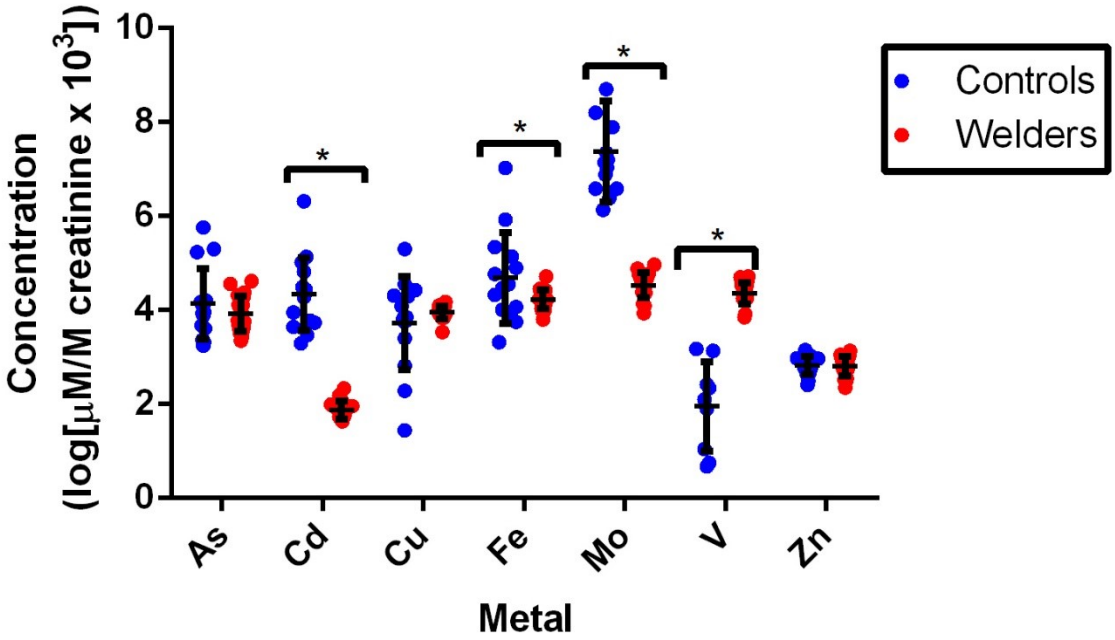


Figure 3.11 Reliable urinary metal concentrations (log µM/M creatinine x 10^3]) in controls and welders on day 50. All metals were tested using FDR (q = 20), and Cd, Fe, and Mo were significantly ($p < 0.05$) higher in controls compared to welding apprentices, whereas V was higher in welders. All data was normalized to creatinine and log transformed.*

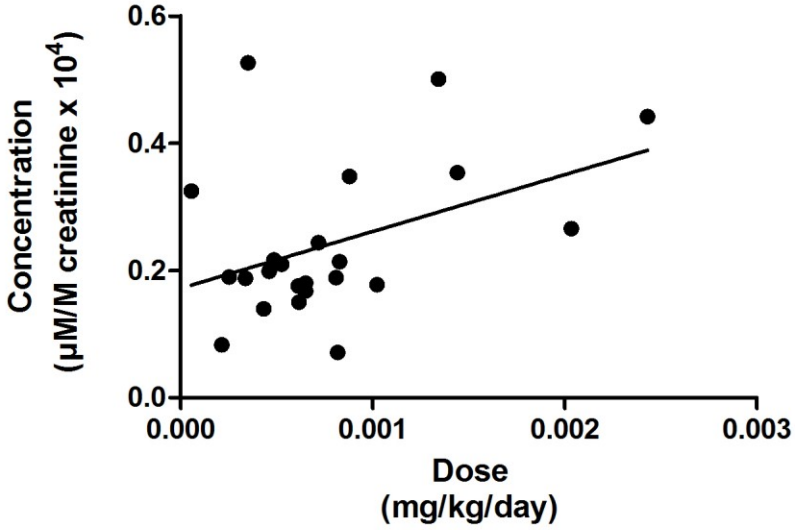


Figure 3.12 Significant correlation between the dose (mg/kg/day) of V and urinary concentration (µM/M creatinine x 10^4) on day 50 in welding participants. The correlation had an R^2 value of 0.42, with significance of p = 0.047.

differences between the two groups. In addition, as classification models revolved around urinary V concentrations, this indicates that V is possibly a good predictor for welding fume exposure in urine.

Classification models to determine the difference between day 0 and 50 in welding participants were also tested in WEKA. The baseline accuracy, determined using zero rules algorithm was 54.05%. Models were built using reliable urinary metal concentrations and all models tested had accuracies below the zero rules model. This suggests these features found no differences in welding subjects between day 0 and 50. This could result from welding apprentices having previous exposure before day 0 and starting at NAIT or 8 weeks may not be a long enough exposure period.

3.4.3 Metabolite quality control

The pooled QC urine sample from 5 welders was analyzed once every 8 samples throughout NMR analysis. Spectra from replicate QC measurements underwent PSRR to fit 151 predicted urinary metabolite peak profiles (n = 33). QC data allowed us to determine which metabolites were reliable by calculating the RSD (%). Metabolites with < 20% RSD were considered reproducible and reliable. A total of 61 out of 151 (40%) urinary metabolites were verified as reliable and used for further analysis. Identified metabolites are summarized, with mean, SD and RSD, shown in Appendix F.

3.4.4 Metabolite concentrations

The “passed” 61 metabolites were normalized to creatinine and log transformed prior to analysis as the raw data exhibited nonparametric distribution. This allowed parametric analyses, such as RM-ANOVA and independent *t*-tests to be used on the data. Radial plots comparing the means of transformed data for control and welding participants on days 0 and 50 were initially created to visualize differences in data (Figure 3.14). The World Health Organization recommends healthy creatinine values are between 0.3 – 3.0 g/l although, our participants had a wider range of 0.2 – 4.3 g/l [87]. Most participants were in the healthy range, as the mean ± SD was 1.78 ± 0.81, indicating creatinine values were in the healthy range for normalization overall.

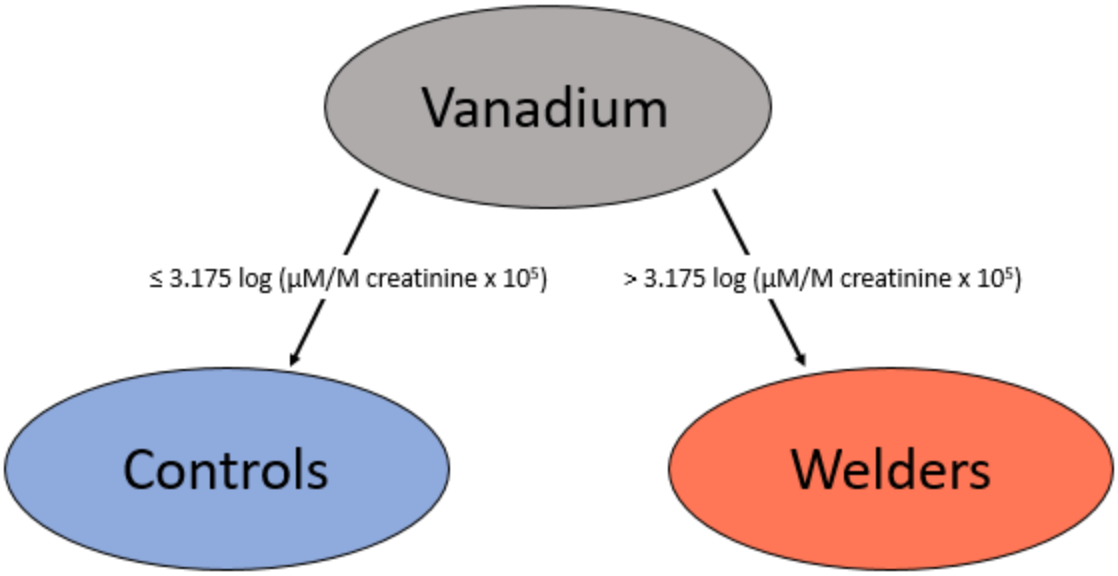


Figure 3.13 J48 decision tree model classifying exposure to welding fumes based on urinary metal concentrations (log [µM/M creatinine x 10^4]). A 10-fold cross-validation was employed in WEKA to create the J48 machine learned model and represents control and welder subjects on day 50, data was correctly classified for every instance. The grey circle represents the node, whereas the numbers indicate the concentration threshold.

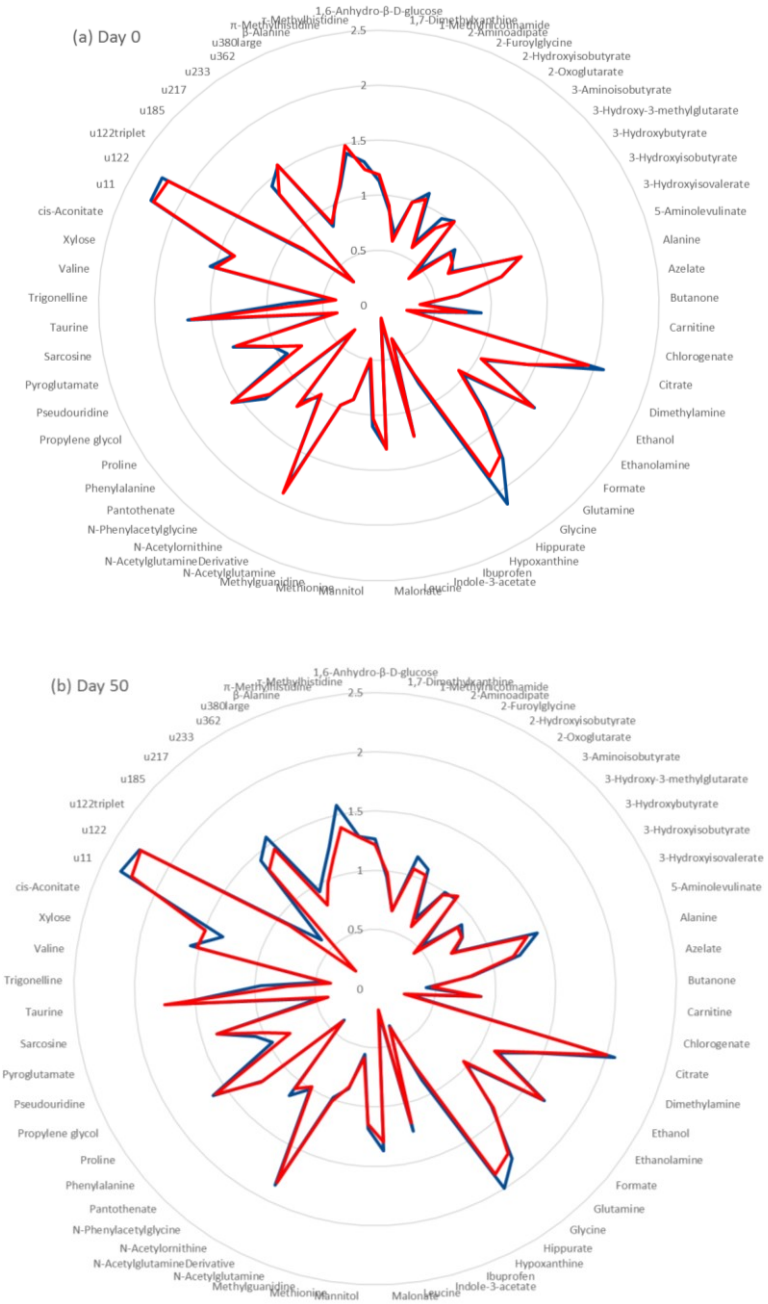


Figure 3.14 Radial plots of mean urinary metabolite concentrations (log [mM/M creatinine]) for 61 "passed" metabolites for controls and welders on days 0 and 50. Day 0 (a) demonstrates little difference between control and welder subjects suggesting well-matched groups, whereas day 50 (b) comparisons suggest some differences occur in the means of metabolites. Control subjects are represented in blue, welding apprentices in red. All data was normalized to creatinine and log transformed.

Individual metabolites were compared between controls and welders and over time using RM-ANOVA. A significant difference was found on day 50 between welders and controls for an unknown metabolite, u185 (Figure 3.15). Unknown metabolites are peaks that consistently appear on the NMR spectrum but the Chenomx library does not know which metabolite it corresponds to; the number corresponds to where it is found on the x-axis. No significant differences between all other 60 metabolites were observed. To determine individual differences in metabolites, an independent *t*-test combined with FDR (q = 20) was used to check for differences between controls and welders, as well as a paired *t*-test with FDR (q = 20) to check for differences over time. Three metabolites (2-hydroxyisobutyrate, u11, and u362) were significantly decreased in welders versus controls on day 50 using independent *t*-tests (Figure 3.16). This suggests that there may be differences between these two groups if tested with a larger sample size or welding apprentices were exposed over a longer period. Figure 3.17 shows selected urinary metabolites that were unchanged in controls and welders over the four sampling days. Appendix G summarizes the remaining "passed" metabolite concentrations for controls and welders.

All metabolite concentrations from day 50 were combined and evaluated by PCA. The first principal component described 18.68% of variance, with the second describing 11.81% (Figure 3.18). Therefore, the PCA model describes 30.48% of the variance - using the first 2 principal components - between controls and welders on day 50, which is insufficient to achieve significance (80% variance is required for the model to be considered robust). Further, a total of 40 principal components were required to fully explain the model. These findings demonstrate that there were no overall detectable differences between controls and welders in their urinary metabolites using PCA.

PLS-DA was also carried out, and demonstrated some separation between welders and controls on day 50 using metabolite concentrations. It is important to remember as a supervised method, PLS-DA will always maximize differences between the two groups, and is prone to overfitting. The first three components covered 28.8% of the variance, which is less than that covered by the PCA model (Figure 3.18). Further, the accuracy of the PLS-DA was 58%, with an R^2 value of 0.29 and a Q^2 value of -0.02. As in linear models, R^2 represents the overall fit

of the data to the model, whereas Q^2 is the measurement of the predictive power of the model, and as a negative value implies that this model is not robust or reliable. Variable importance in projection (VIP) plots were generated for the PLS-DA models; VIP plots indicate variables with high loadings or weights that influence components (Figure 3.19). The first two components of the PLS-DA model have the same top three urinary metabolites influencing the variation (taurine, π-methylhistidine, and u185). This indicates that these metabolites may have some variation between welders and controls.

A PCA model comparing urinary metabolite concentrations was also constructed for welder apprentices from day 0, baseline, to day 50, the final collection day. A total of 37 principal components were required to describe the variation in the model, with PC1 and PC2 describing 19.81% and 9.33% of the variance, just under 30% (Figure 3.20). In addition, PLS-DA was carried out, showing more separation than the unsupervised PCA, but still little variation between baseline (day 0) and day 50 with the first three components representing 28.9% (Figure 3.20). Further the accuracy was below chance, at 0.40 (R^2 = 0.27 and Q^2 = -0.38) for the first component. A VIP plot (Figure 3.21) was generated to indicate metabolites that influenced the observed variation and found that the top two metabolites with the highest weightings in the first two components were the same (N-phenylacetylglycine and π-methylhistidine). It is important to remember that PLS-DA overfits data and these results need to be considered with caution [75].

Passed metabolites were then used to test different classification models. When comparing welders and controls on day 50, the zero rules algorithm had an accuracy of 53.84%. The IBk algorithm did have a 64.35% accuracy, compared to all other models tested with accuracies lower than the zero rules model. An IBk model is based on a *k*-nearest neighbours algorithm, where classification is based on the neighboring points [97]. Classification models were built to compare day 0 and 50 in welders, where the zero rules accuracy was 51.11% and all models had a lower accuracy, except for the one rule algorithm, which correctly identified instances 67.33% of the time based on the urinary concentration of mannitol. The data for these models is not shown. Overall, predictive models based on urinary metabolites were ineffective in classifying the two groups based on the current data.

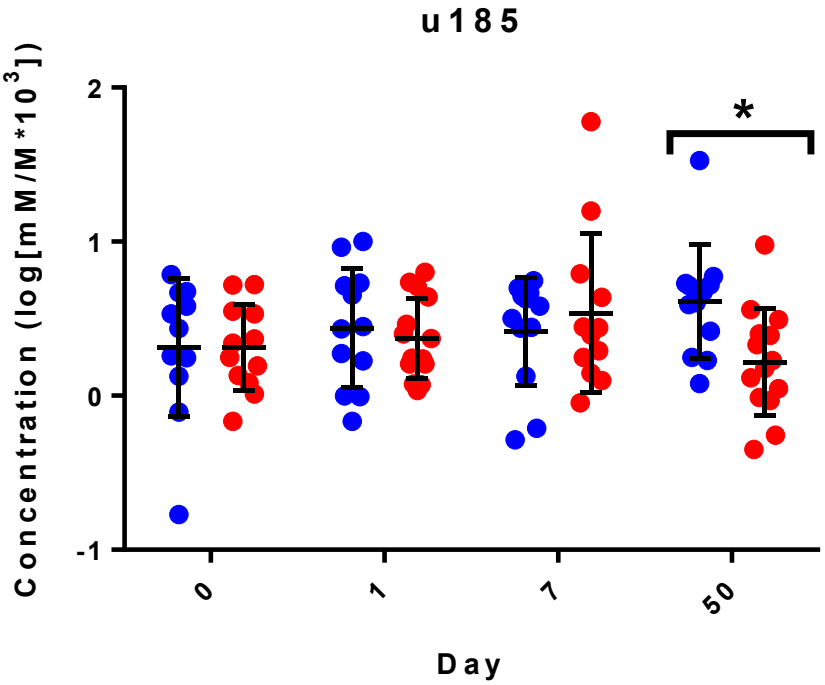


Figure 3.15 Urinary metabolite concentration (log [mM/M creatinine x 10^3]) of u185, an unknown metabolite in controls and welders on day 50. Controls had significantly higher concentrations compared to welding apprentices on day 50. Significance ($p < 0.05$) was tested using RM-ANOVA. All data was normalized to creatinine and log transformed.*

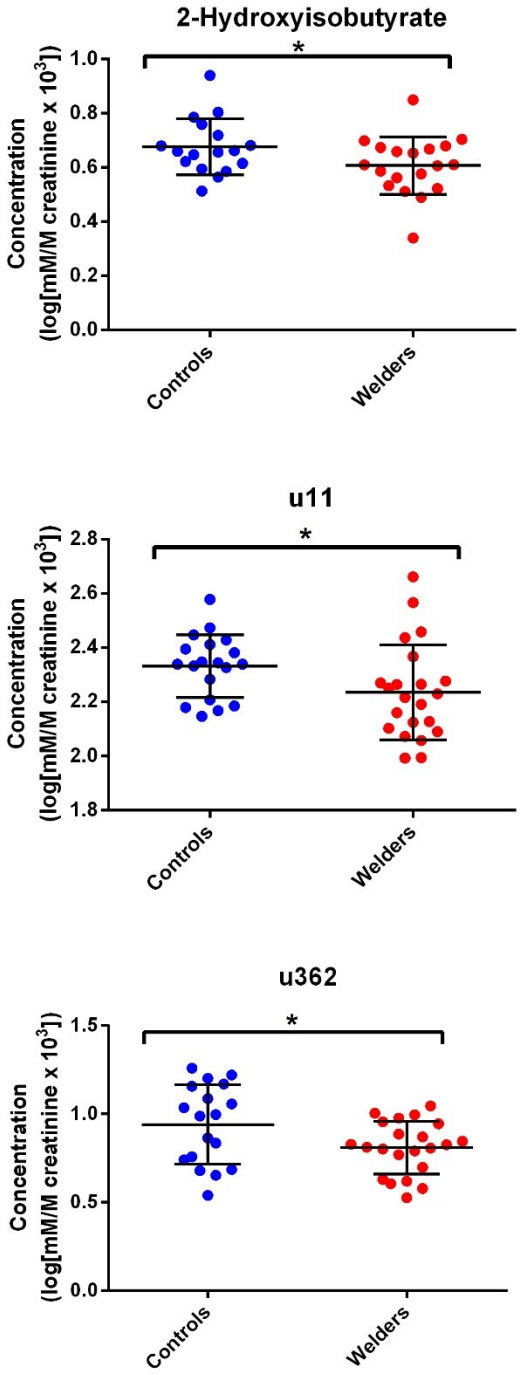


Figure 3.16 Urinary metabolite concentrations (log [mM/M creatinine x 10^3]) between controls and welders on day 50. Control subjects had significantly higher concentrations of 2-hydroxyisobutyrate, u11, and u362 compared to welding apprentices. Significance ($p < 0.05$) was tested using independent t-test. All data was normalized to creatinine and log transformed.*

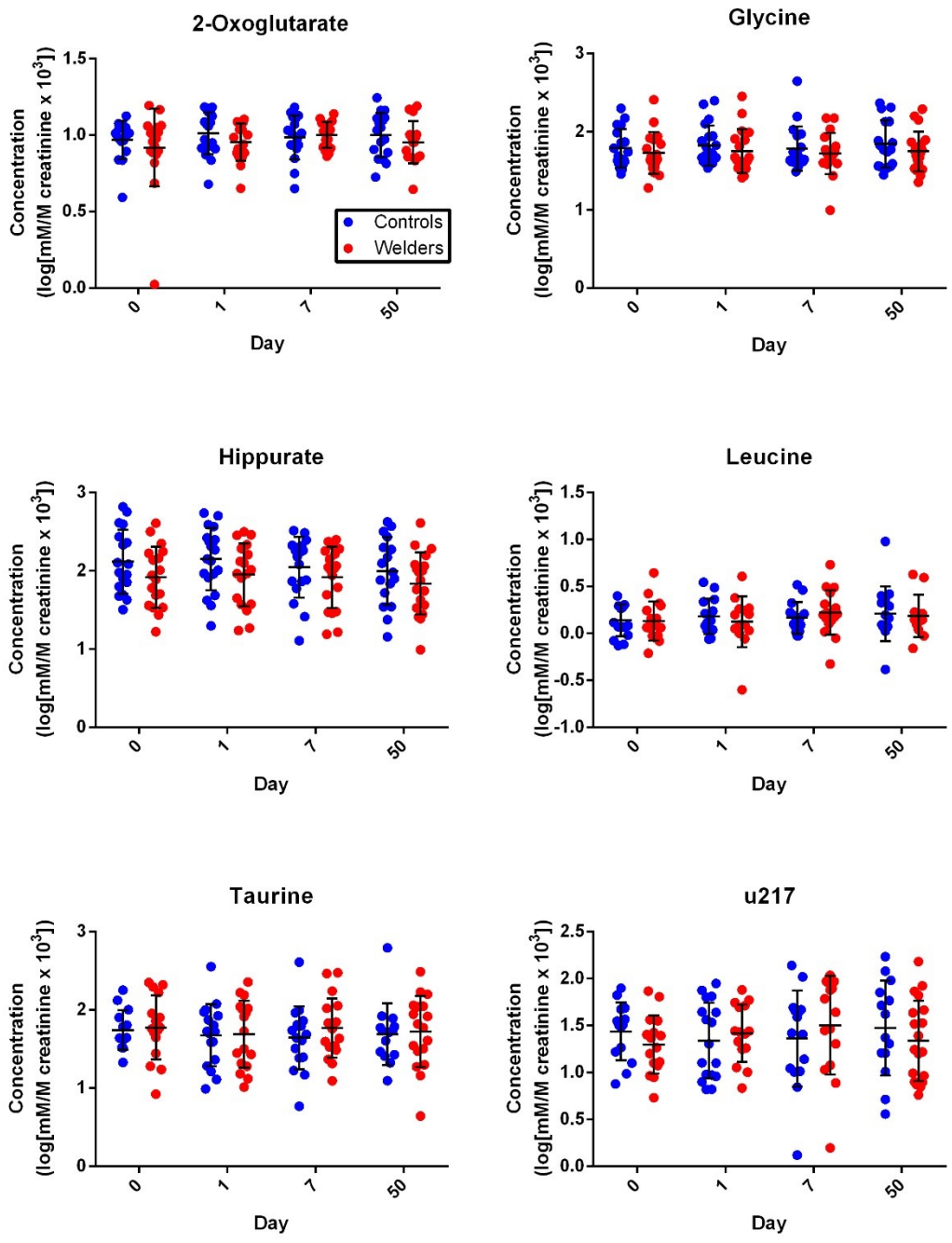


Figure 3.17 Select urinary metabolite concentration (log [mM/M creatinine]) between controls and welders on sampling days 0, 1, 7, and 50. Represented metabolites shown are 2-oxoglutarate, glycine, hippurate, leucine, taurine, and u217. No significant differences were found between controls and welders on any sampling days for the represented metals above using RM-ANOVA and independent t-test. All data has been normalized to creatinine and log transformed.

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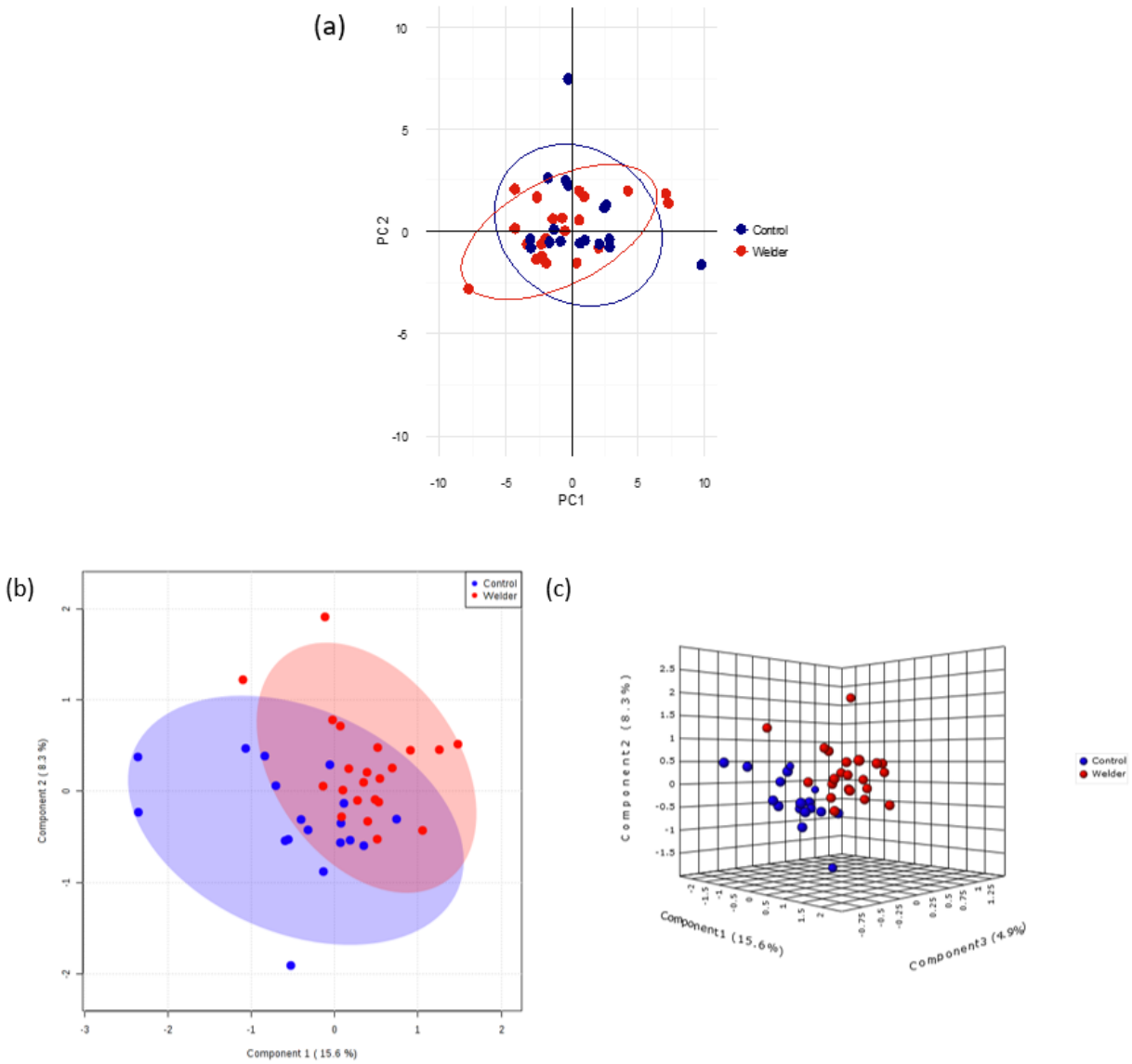


Figure 3.18 PCA and PLS-DA of urinary metabolite concentrations (log [mM/M creatinine x 10^3]) between controls and welders on day 50. (a) PCA consisted of 36 principal components, with the first representing 20.42%, and the second 12.51% of the variance. (b) 2D PLS-DA and (c) 3D PLS-DA plots use supervised methods, with the first component representing 15.6%, 8.3% the second component, and the third 4.9% of the variance. Ellipses represent 95% confidence intervals.

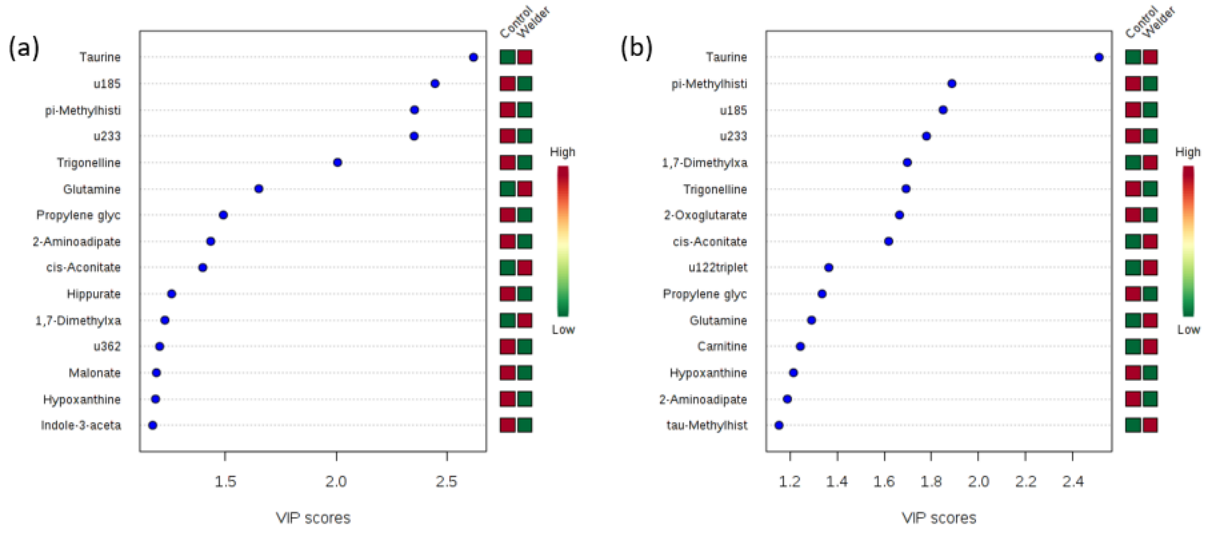


Figure 3.19 VIP plots for urinary metabolite concentrations (log [mM/M creatinine x 10^3]) for welder and control participants on day 50. The weightings for the top 15 metabolites in each component are expressed in (a) and (b) for components 1 and 2, respectively. Components correspond to PLS-DA in Figure 3.18.

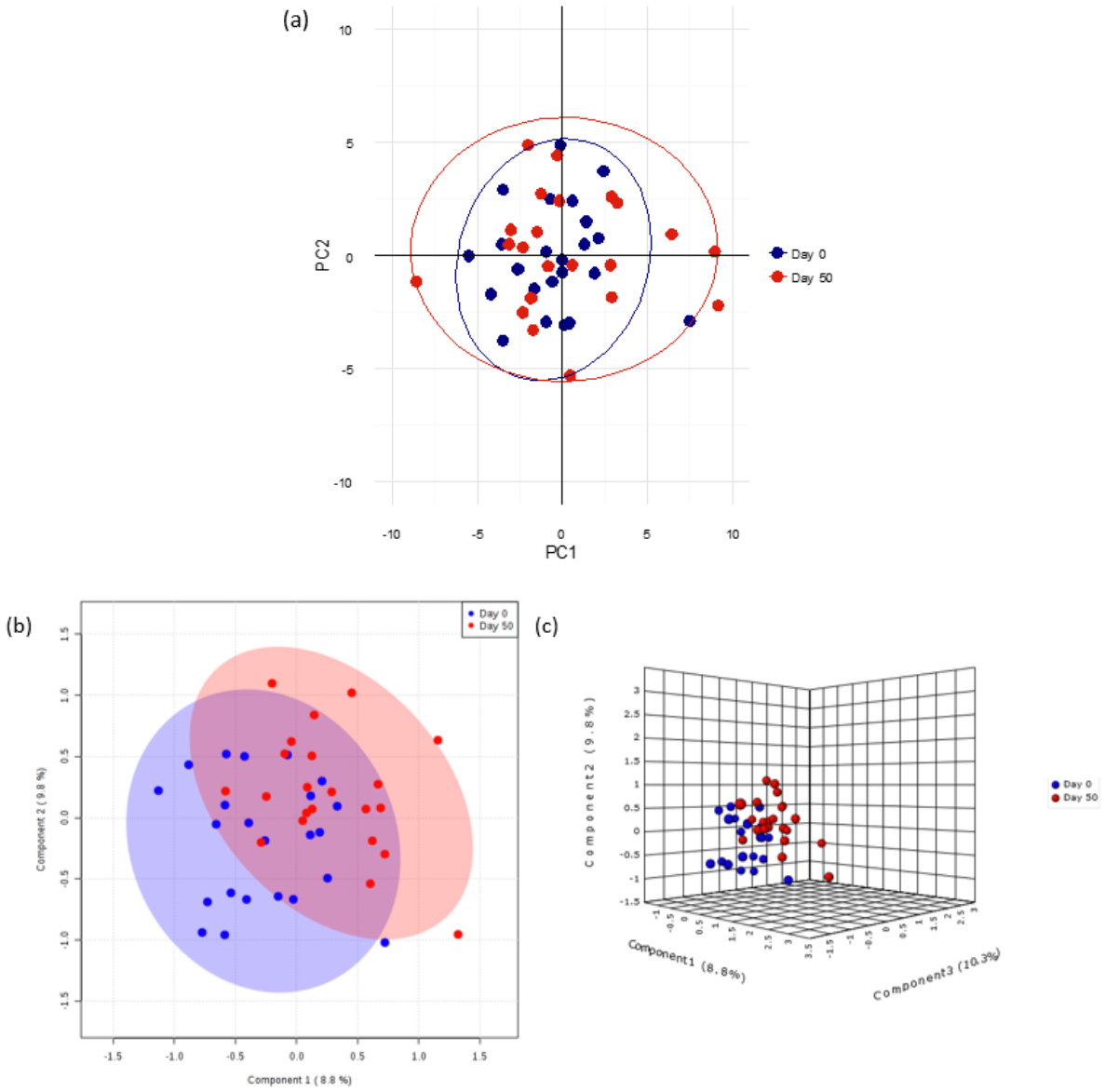


Figure 3.20 PCA and PLS-DA of urinary metabolite concentrations (log [mM/M creatinine x 10^3]) between day 0 and 50 in welders. (a) PCA consisted of 37 principal components, with the first representing 19.81% of the variance, and the second 9.33% of the variance. (b) 2D PLS-DA and (c) 3D PLS-DA plots use supervised methods, with the first component representing 8.8%, the second 9.8% and the third 10.3% of the variance. Ellipses represent 95% confidence intervals.

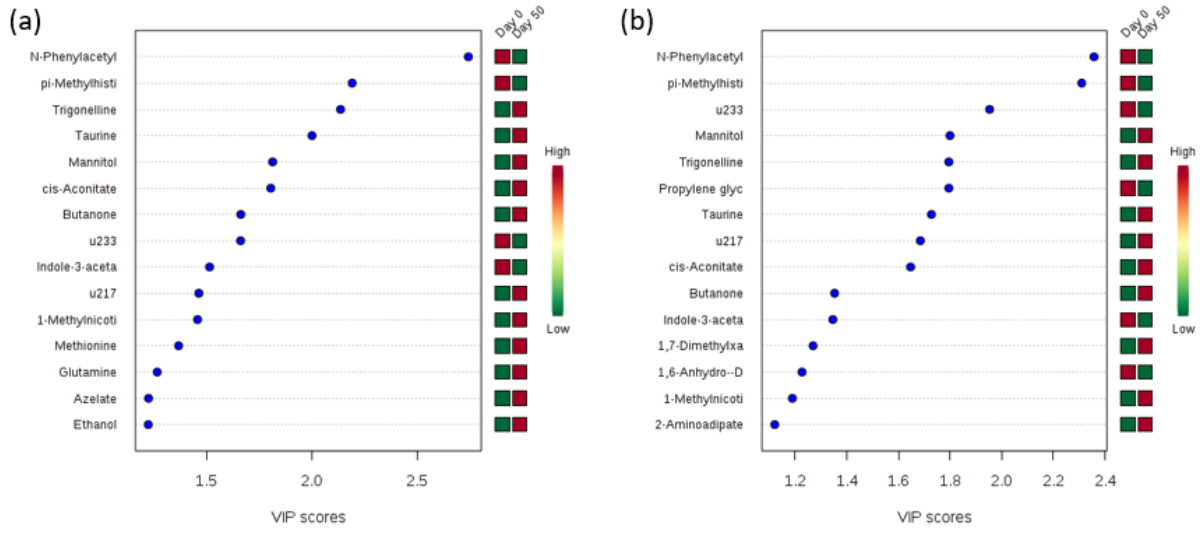


Figure 3.21 VIP plot for urinary metabolite concentrations (log [mM/M creatinine x 10^3]) for welders on day 0 and day 50. The weightings for the top 15 metabolites in each component are expressed in (a) and (b) for components 1 and 2, respectively. Components correspond to PLS-DA in Figure 3.20.

3.5 Models including combined data

All air exposure, urinary metal, and metabolite concentrations were combined and analyzed to determine if stronger classification models could be generated. PCA was carried out comparing controls and welders on day 50 (Figure 3.22). The first principal component described 18.2% of the variance, with the second principal component covering 10.73%. Overall the first two principal components explained 28.94% of the variance between control and welder groups. Controls were more closely clustered than welders although no separation was evident.

The PLS-DA plot was also generated for air exposure, urinary metal, and metabolite concentrations for welders and controls on day 50 (Figure 3.22). Control participants were grouped together quite closely, where a larger variation in welding participants is observed. The first component explains 96.3% of the model. The 2D PLS-DA plot has all control subjects appearing in one spot, likely formed by similar air concentrations as there was little to no exposure, whereas welding subjects had a variety of air concentration exposures. As the first two components explained 100% of the variation in the model, a 3D PLS-DA plot was not generated. Although the accuracy of the current model increased (73% with the first component), there was still a poor fit (R^2 = 0.20) and poor predictive power (Q^2 = -0.57). When looking at the features with the largest influence on the components (VIP plots in Figure 3.23), both components 1 and 2 rely heavily on Fe and Mn air exposure concentrations, which were increased.

PCA was also conducted for welding subjects comparing day 0 and day 50. The first two principal components described 28.3% of the variance, with 15.22% and 13.08% being explained by the first and second components, respectively (Figure 3.24).

Baseline (day 0) and day 50 air exposure, urinary metals, and urinary metabolite concentrations were combined to create a 2D PLS-DA model in welding apprentices (Figure 3.24). Overall, day 0 measurements were quite similar, while day 50 measurements varied substantially. The first component explained 96.3% of the model, which is a large portion, but air exposure concentrations were largely responsible, as seen in the VIP score plots (Figure

3.25). It is important to remember that overfitting is quite common in PLS-DA, especially when there is a large number of variables compared to participants [75]. The PLS-DA model had a high accuracy (74%) for the first component, but poorly fit the data (R^2 = 0.22). The predictive power of the model was quite poor as it was negative (Q^2 = -0.18), establishing that multivariate models with all data were poor at distinguishing between these groups with supervised and unsupervised methods.

Finally, classification models in WEKA were created. Initially, the air concentration data was included with the urinary metal and metabolite concentrations. The resulting models were identical to those generated using only air concentrations (3.3.4 Metals). Therefore, air concentrations were removed and models were built using just urinary metal and metabolite concentrations. When classifying controls and welders on day 50 the zero rules algorithm had an accuracy of 55%. All other models tested had higher accuracies over ten random seeds, with the one rules algorithm having an accuracy of 99.5%. The model was based around the urinary concentration of V, where ≥ 3.51 mM/M creatinine x 10^5 were classified as welders. Classification models of welders on day 0 and day 50 found the one rules algorithm to accurately distinguish between day 0 and day 50 in welding subjects 67.33% of the time based on mannitol concentrations, compared to the zero rules algorithm which had 51.11%. The remaining models tested had accuracies lower than that of the zero rules algorithm.

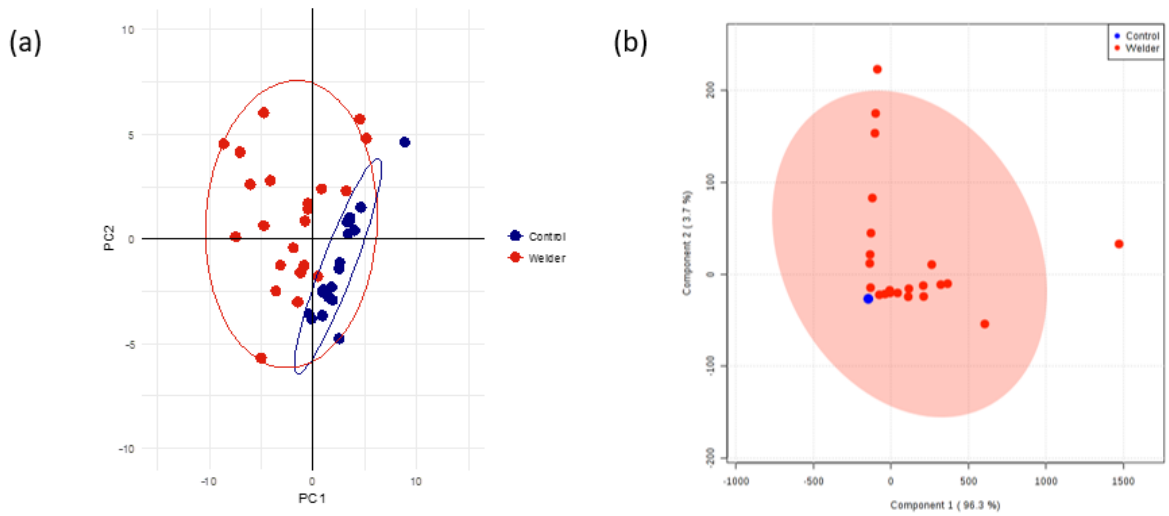


Figure 3.22 PCA and PLS-DA of air exposure (µg/m^3), urinary metal (log [µg/g creatinine x 10^x]), and metabolite (log [mM/M creatinine x 10^3]) concentrations between controls and welding apprentices on day 50. The first principal component in (a) PCA represents 18.2%, and the second 10.73% of the variance. (b) 2D PLS-DA plots represent 96.3%, and 3.7% in the first two components, respectively. Ellipses represent 95% confidence intervals.

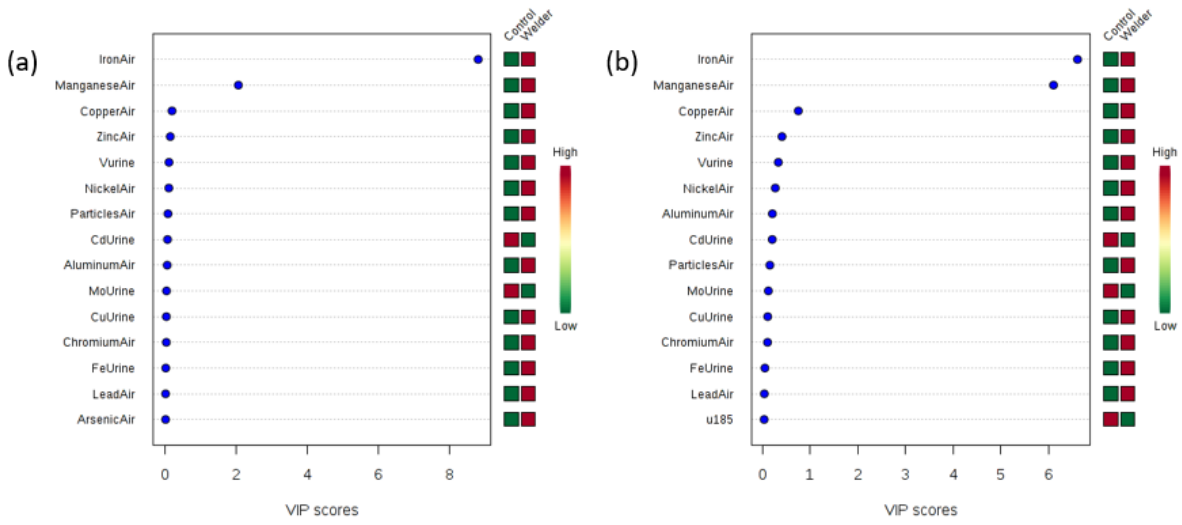


Figure 3.23 VIP plots for air exposure (µg/m^3), urinary metal (log [µg/g creatinine x 10^x]), and metabolite (log [mM/M creatinine x 10^3]) concentrations for welders and controls on day 50. The weightings for the top 15 metabolites in each component are expressed in (a) and (b) for components 1 and 2, respectively. Components correspond to PLS-DA in Figure 3.22.

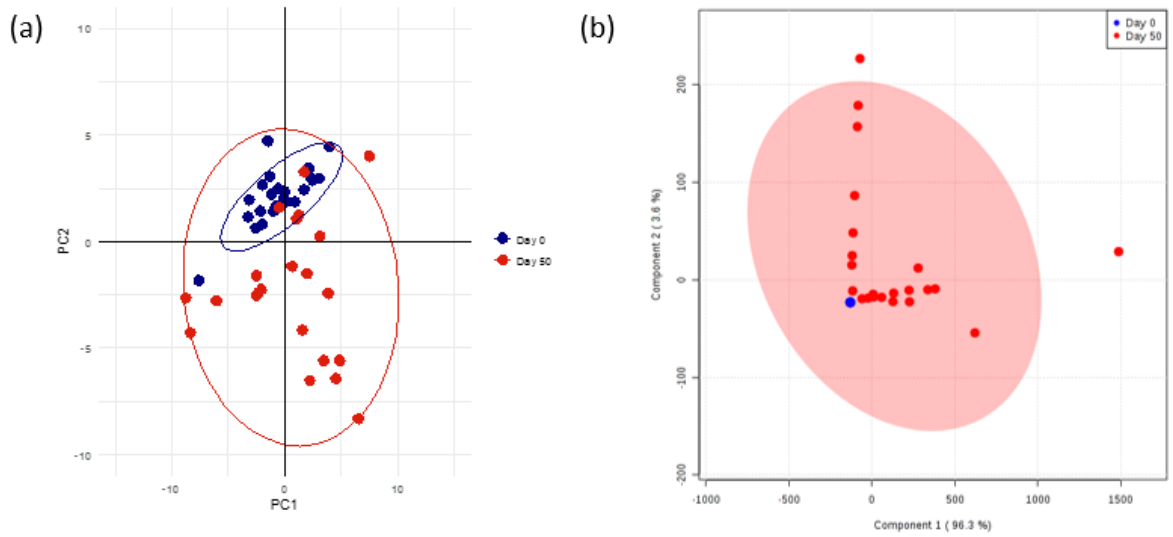


Figure 3.24 PCA and PLS-DA of air exposure (µg/m^3), urinary metals (log [µg/g creatinine x 10^x]), and metabolite (log [mM/M creatinine x 10^3]) concentrations between day 0 and 50 in welders. The first principal component for (a) PCA represents 15.22% and the second 13.08% of the variance. (b) 2D PLS-DA represent 96.3% and 3.6%, in the first two components, respectively. Ellipses represent the 95% confidence intervals.

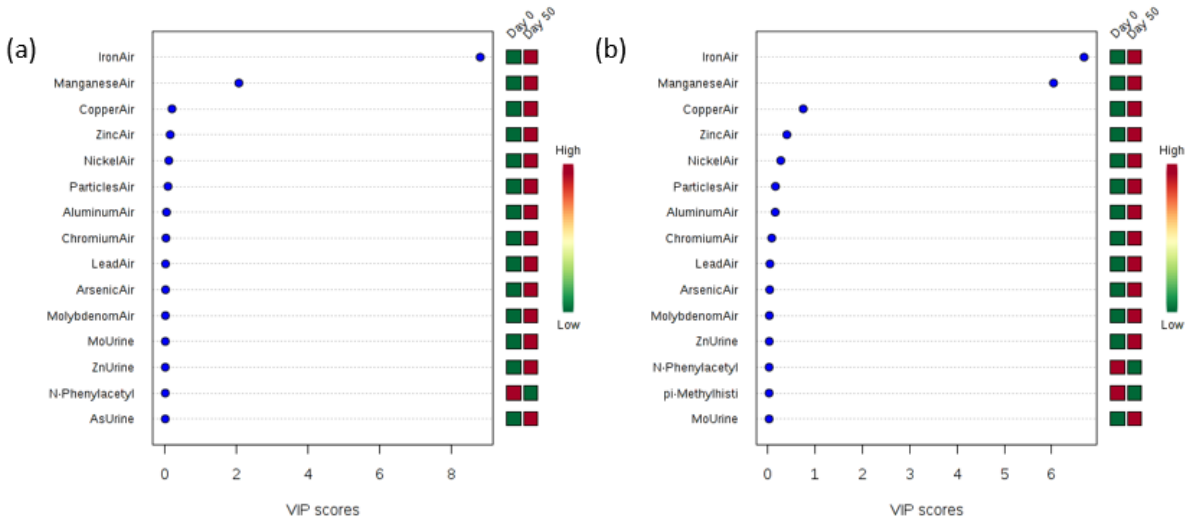


Figure 3.25 VIP plots for air exposure (µg/m^3), urinary metal (log [µg/g creatinine x 10^x]), and metabolite (log [mM/M creatinine x 10^3]) concentrations for welders and controls on day 50. The weightings for the top 15 metabolites in each component are expressed in (a) and (b) for components 1 and 2, respectively. Components correspond to PLS-DA in Figure 3.24.

Chapter 4

Discussion

4.0 Discussion

A summary of the results from the thesis are presented in this chapter. This includes the initial work done to establish that creatinine concentrations measured by NMR are reliable for normalization of urinary metals and metabolites, as well as air exposure and urinary metal and metabolite data from controls and welders. These findings are related to the current literature. Finally, some of the strengths and limitations of this study are addressed.

4.1 The importance of creatinine

Creatinine is an important metabolite in studies using urine as a marker of glomerular clearance and for normalization against other urinary components because it is excreted from the body at a constant rate with minimal reabsorption, and can be used to indicate the hydration status of the subject or renal disease. Molecules measured with NMR or metals analyzed with ICP-MS can be normalized against creatinine to adjust for dehydration [103]. Typically, urinary creatinine is measured in clinical labs using the Jaffe method, which is a colorimetric reaction [89, 90]. Prior to beginning this study, a comparison of creatinine values fit using NMR methods to values found with the Jaffe method was carried out [87]. The results showed NMR is reliable, accurate, and precise for urinary creatinine concentrations, thus allowing creatinine concentrations quantified by NMR to be used for normalization of urinary metals and metabolites.

4.2 Air exposure

The standard method for determining exposure to airborne contaminants such as welding fumes, is air sampling. In this study, particulate matter was collected that welding apprentices were exposed to, over their 3 h welding lab, and TWA values were calculated and compared to the total particles and individual metal 8 h OELs for AB. No participants surpassed the compliance limits on any of the sampling days, although a few participants were close to the OEL limit at 200 $\mu g/m^3$ over an 8 h period for Mn exposure on day 50 [101]. This suggests that some welders may not have used the ventilation system effectively, as all participants were exposed to the same type of welding fumes and were working on the same tasks. In addition, when looking at 8 h OELs for respirable particles (3 mg/m^3) for 8 h, some participants

were close to exceeding the limit based on gravimetric analysis [101]. Concentrations were compared to respirable limits even though we collected total dust, because studies have determined that majority of particles in welding fumes consist of respirable particles [18, 20, 23, 24]. This indicates that welding apprentices at NAIT are possibly overexposed to respirable particles as the 90% upper confidence limit for sampling days 1, 7, and 50 was 5.538 mg/m^3 compared to the 8 h OEL of 3 mg/m^3. Also, since professional welders work 10 h shifts in AB, they are likely overexposed not only to respirable particles but also to Mn if no proper ventilation is present.

Significant differences for all measured metals in air samples were found between baseline levels on day 0 and other sampling days (day 1, 7, and 50) as expected, for participants exposed to fumes. Welders learned GMAW and oxyacetylene cutting on mild steel during all sampling days. High concentrations of Fe and Mn were present for sampling days 1, 7, and 50 in welders (Figure 3.5). This is expected, as the main components often found in mild steel GMAW are Fe and Mn as observed in previous studies [17]. Baseline concentrations (day 0) in welders, and all sampling days for controls had negligible exposure for all metals tested. In comparison welder subjects were exposed to higher metal concentrations from the fumes generated during their welding labs. Originally it was hypothesized that this early exposure would lead to detectable alterations in urinary metals. However, only minimal differences were observed using our highly sensitive, validated ICP-MS approach.

4.3 Urinary metal concentrations

Overall, no differences in urinary metals were found over time in welding apprentices, except for V, which increased in initial sampling days (1 and 7) to day 50. There were also initial differences in metal concentrations on day 0 with increases in controls compared to welders in As, Fe, and Mo. In addition, control values exhibited a wide range of urinary metal concentrations, whereas urinary metal concentrations in welders were often quite similar across the dates of sample collection. Significantly higher levels of Cd, Fe, and Mo were found in controls compared to welding subjects on day 50 using independent *t*-tests. In contrast, V was found to be increased in welding subjects on day 50 compared to controls using an

independent *t*-test. As training was only 8 weeks long, it is possible that urinary metals may become more prominent over increased exposure as high exposures to metals is often required to detect environmental differences in urine.

As V was found to increase in welders on day 50 using univariate analysis (RM-ANOVA and independent *t*-test), and was indicated in machine learning models and multivariate analysis (PLS-DA), it is a potential metal of interest for detection of welding fume exposure in urine. An impurity often found in mild and stainless steel, V can be found naturally in the environment, but is strongly associated with industrial sources [8, 102, 104]. One study has recently looked at occupational exposure to V and its increases in biofluid concentrations [102]. Ellingsen *et al*. (2017) reported significantly higher concentrations of V in urine and serum in welders, who have been employed for at least 6 months, compared to a control group [102]. In addition, they found a correlation between increased exposure and increased V concentrations in biofluids, supporting our finding of increased V on day 50 in welding subjects, and the correlation between dose exposure and urinary concentrations. Furthermore, we did see an increase of V on day 0 in welders compared to controls, this could be related to V half-life in the body (Figure 3.9). It is reported that V is released in three half-lives, the first being after an hour of exposure, the second after a day or two of exposure and finally ten days after exposure [102]. As most of the welding apprentices were welding until beginning at NAIT, this increase in V could be from their previous exposure prior to enrolment in our study.

Our results do not resolve the confusion in the literature regarding other urinary metal concentrations as a form of exposure assessment. Significantly higher concentrations of urinary Cr, Mn, and Ni levels have been reported in occupationally exposed welders when compared to unexposed populations [15]. However, Mergler *et al*. (1994) reported no metal differences in urinary measurements of occupationally exposed individuals [46]. When looking at urinary Mn, substantial variation over different periods of time have been reported in the same occupationally-exposed individuals [44]. This suggests that even using baseline data from non-exposed individuals for comparison, urinary metal concentrations may vary too greatly to be used as a reliable predictor for early exposure. Variations may be related to environmental exposures, such as pollution, however this is unlikely in Edmonton as pollution is relatively low

[105]. In addition, controls may not have been appropriately used, causing mixed results across the literature.

Although metal increases in urine due to welding fume exposure has presented some conflicting evidence, some studies looking at other biofluids have found increases of metal concentrations in welders compared to control populations. Wultsch *et al.* (2014) found higher metal concentrations in welders for serum and whole blood [4]. Others have reported that Mn exposure can be detected using hair or Mn/Fe ratios in blood to detect occupational exposures [47, 56]. This could be related to the way the body processes heavy metals. Metals are required by the body to aid in certain bodily processes and are often absorbed [106]. Overexposure to metals can lead to bioaccumulation in certain organs and they are not always excreted [46, 106, 107]. When metals are excreted, they can leave through a variety of metabolic pathways, such as urine or fecal matter [106]. Urinary metal concentrations may be inconclusive and show contradicting results in the literature due to accumulation and multiple excretion paths.

No differences in Cu and Zn, the remaining reliably measured metals, were found over time or between control and welding subjects. As previously mentioned, many urinary metal concentrations (Al, Be, Co, Cr, Mn, Ni, and Pb) were too low to be reliably measured in either subject group and were removed from further analysis. This could be attributable to exposed subjects having minimal accumulation of detectable metals in their urine after only 8 weeks' exposure. Cena *et al.* (2014) found that air exposure levels of Ni and Cr for GMAW of mild steel were below their respective LODs [24]. Although our air exposure samples detected metal concentration above LODs, they may not have been at high enough levels to result in elevated urinary concentrations. Another possibility is that exposure may be limited, with the use of the ventilation system at NAIT, and respirators. However, with previous conflicting evidence, we conclude that urine may not be an ideal biofluid for detecting metals from early occupational exposures, and another biofluid, such as serum, may be more useful for detection of metals from short-term welding fume exposure. This study also shows that controlling blank levels is paramount to obtaining good results, since urine samples contain low levels of metal concentrations close to detection limits.

4.4 Urinary metabolite concentrations

4.4.1 Quality control analysis of urine samples in NMR

Many metabolomics studies have identified significant differences and metabolic fingerprints when comparing case and control groups for diseases [65, 71, 76]. However, inadequate standardization and QCs have been reported in metabolomics studies [63, 72]. Currently, no methods are in place to efficiently determine accurate quantification. To address this, we used our own pooled QC sample. A strength of this study was the use of a pooled QC urine sample that was measured every 8 samples (*n* = 33). The RSD (%) was calculated to determine metabolites that were precisely fit and quantified consistently with the algorithm. This is crucial in biomarker determination if the goal is to apply metabolomics as a possible exposure tool [65].

4.4.2 Identified metabolites in welders and controls

Despite welding fume exposure, limited changes in urinary metabolites were detected. This was found over time, and when comparing welders to a non-exposed group. Based on participant information the control and welder groups were similar, with limited differences (Table 3.1). Multivariate models, PCA and PLS-DA, supported that our groups were well-matched as limited variation was detected between groups. Differences detected in metabolites 2-hydroxyisobutyrate, u11, u185, and u362 support the initial hypothesis that exposure to ultrafine particles in welding fumes results in detectable changes in urine samples. Changes only began to appear on day 50 and were found in only a few metabolites, suggesting that 8 weeks may not be long enough for exposure, or NMR technology may be insensitive to early changes. Further, as our sample size was small (*n* = 20), observed changes may be a coincidence. Samples were collected the day following exposure and metabolites may have returned baseline levels, whereas post-shift samples may have increased metabolic changes.

Urinary metabolite concentrations of passed metabolites, were compared to healthy normal levels as described in the human metabolome database (HMDB) and values found in the literature, as summarized by Bouatra *et al*. (2013) in Table 4.1 [53]. Overall, previously reported passed metabolites fall in the healthy or average range. Metabolite concentrations of controls

and welders remained separate for comparison because analysis did report preliminary differences. Three metabolites - 5-aminolevulinate, malonate, and β-alanine - report higher concentrations in both control and welder groups than those previously reported in the literature. Population differences or spectral fitting methods are possible explanations for this discrepancy.

A comparison of urinary metabolite concentrations that demonstrated changes between welders and controls on each sampling day to normal levels in the HMDB was conducted (Table 4.2). Taurine, trigonelline, and cis-aconitate were indicated in VIP plots as influencing the variance in the first two components (Figures 3.17 and 3.19). In addition, metabolites found to have significant differences in long-term welders, glycine, hippurate, taurine, and trigonelline [34]. All metabolite concentrations fall in the normal, healthy range.

When looking at welders with years of experience and exposure, Kuo *et al.* (2012) reported significant increases in acetone, betaine, creatinine, gluconate, glycine, hippurate, serine, S-sulfocysteine, and taurine, and decreased creatine, suggesting a correlation between welding fume exposure and oxidative stress as these metabolites have been suggested to be important in inflammatory processes [34]. Only four of the indicated metabolites passed according to our QC analysis (< 20% RSD): creatinine (2%), glycine (3%), hippurate (4%), and taurine (9%), where the RSD for creatinine was calculated from non-normalized data. The remaining 5 metabolites that indicated significant increases in welders all failed our QC (> 20% RSD) or were not fit to the spectra because of spectral interference or absence from the Chenomx library: acetone (26%), betaine (91%), gluconate (22%), serine and S-sulfocysteine. A decrease in creatine was also reported by Kuo *et al*. (2012), which was hypothesized to be the result of welding fumes causing an increase in reactive oxygen species in response to oxidative stress, which inhibits creatine kinase activity and decreases the amount of creatine produced [34]. It is important to note that creatine and creatine phosphate are difficult to distinguish in NMR spectra because of overlaps with their peaks, resulting in unreliable quantification. In the data presented here, when both creatine and creatine phosphate were fit to the spectra, this resulted in metabolites having > 20% RSD (31% and 78%, respectively). Therefore, they were

not considered reliable for comparison, and any fluctuations in creatine due to welding fume exposure in this study could not be assessed.

Studies have reported increases in the metabolite hippurate in response to inflammation, and has been recorded in occupationally exposed populations [34, 69, 108]. Increases in urinary hippurate levels have been correlated with environmental exposure to toluene, commonly present in spray-painting environments [109]. While hippurate was precisely detected (RSD = 4%) there were no differences found over time for welders or between welders and controls on any of the sampling days. However, as exposure time increases hippurate concentrations may slowly increase over time because of respiratory inflammation.

This study did find preliminary differences in 4 metabolites between controls and welders on day 50. Three of these metabolites are unknown and provide little insight into metabolic responses to welding fume exposure. The fourth metabolite, 2-hydroxyisobutyrate, was found to decrease in welding participants on day 50. The metabolite 2-hydroxyisobutyrate is a known metabolite of methyl *tert*-butyl ether, an additive in gasoline, and is the major excretory product for environmental exposure to gasoline [110]. Studies in mice and rats have found increases of 2-hydroxyisobutyrate in response to increases of gasoline exposure, which can be detectable for up to 72 h [111, 112]. Welders are not generally exposed to high concentrations of gasoline over the normal population, and a decrease of 2-hydroxyisobutyrate was found in welding participants compared to controls, demonstrating that it is most likely not due to environmental exposure. Interestingly, 2-hydroxyisobutyrate in humans has recently been associated with different diseases, such as gastric cancer, obesity, and inflammatory bowel disease, and was reported to decrease in urine in these conditions [70, 113, 114]. Therefore, the decrease observed in 2-hydroxyisobutyrate in welders may be caused by metabolic changes in response to the occupational exposure. As this is a preliminary study, more research will be required to validate decreases in 2-hydroxyisobutyrate in response to welding fume exposure, and if this has the potential to serve as a biomarker.

Table 4.1 Comparison of selected passed metabolite concentrations (µM/mM creatinine) from the HMDB, other studies, and welder and controls from current study. Values for comparison were collected from the HMDB and from a review by Bouatra et al. (2013) that looked at normal metabolite values in urine across multiple studies, some values were missing and are not presented in the following table as consequence [53]. Data represents the mean (range) or mean ± SD. The concentration of control and welder urine sample is the average over all sampling days.

Metabolite	HMDB Reported	Literature Values	Control Urine Samples	Welder Urine Samples
1-Methylnicotinamide	5.8 (1.2-15.0)	6.1 (0.2-12.0)	5.7 (1.1-36.6)	5.4 (0.9-21.5)
2-Furoylglycine	4.0 (0.9-8.4)	9.95 (2.0-18.66)	13.4 (4.4-59.7)	13.8 (3.6-65.9)
3-Aminoisobutyrate	26.0 (2.2-140.0)	(2.91-116.43)	12.6 (4.3-74.3)	11.7 (4.2-62.7)
3-Hydroxybutyrate	3.6 (1.3-6.4)	1.4 ± 1.3	8.2 (3.3-43.2)	7.8 (2.6-40.7)
3-Hydroxyisovalerate	6.8 (3.2-21.8)	8.5 ± 3.2	5.7 (1.9-17.2)	5.2 (2.4-13.2)
5-Aminolevulinate	2.9 (1.2-4.4)	1.45 ± 0.72	24.2 (9.5-44.2)	23.3 (9.2-55.3)
Azelate	2.8 (1.8-4.8)	4.8 (1.3-15.0)	6.1 (1.3-18.8)	6.5 (1.3-19.0)
Cis-Aconitate	20.9 (3.8-95.3)	13.0 (2.7-44.0)	29.6 (7.5-155)	37.2 (7.4-330.5)
Citrate	203 (49-600)	242.0 ± 129.6	144.4 (31.8-384)	107.9 (32.9-336)
Dimethylamine	30.8 (20.3-59.2)	39.3 ± 8.3	27.4 (20.3-35.4)	27.2 (21.5-33.6)
Ethanol	3.1	(5-500)	15.7 (7-234)	13.9 (5.7-141)
Ethanolamine	37.0 (24.8-56.2)	21.4 (6.6-36.2)	48.8 (28.1-73)	46.7 (23.5-73)
Formate	26.8 (6.9-120.9)	20.39 ± 11.84	11.4 (2.2-26.6)	10.2 (2.3-27.2)
Glycine	106 (44-300)	151 (233-248)	80.8 (28.2-442)	68.1 (9.9-283)
Hippurate	229 (19-622)	257 (20-770)	173.6 (12.8-658)	114.5 (9.8-470)
Hypoxanthine	7.2 (1.8-24.1)	4.67 (2.80-6.38)	9.3 (1.9-90.5)	6.9 (1.5-59.6)
Malonate	2.9 (2.0-3.5)	1 (0-2)	23.5 (7.4-73.2)	24.1 (5.1-53.2)
Mannitol	32.4 (5.2-85.1)	10.26 ± 9.14	15.8 (2.8-72)	17.3 (3.3-212)
Methylguanidine	2.7 (1.2-6.0)	1.25 ± 0.72	7.9 (5.1-12.6)	8.6 (5.2-88.6)
Pantothenate	1.9 (0.6-4.4)	2.7 ± 0.9	2.3 (1.0-9.7)	2.4 (0.7-5.3)
Propylene Glycol	6.7 (1.4-44.3)	N/A	15.7 (1.9-87.3)	9.5 (1.2-66.5)
Pseudouridine	28.9 (13.3-41.3)	26.02 ± 4.62	10.9 (2.2-16.8)	10 (2.8-19.8)
Pyroglutamate	20.7 (10.2-32.6)	28.8 (3.4-54.2)	23.4 (13.2-41.8)	22.9 (7.7-37)
Sarcosine	2.9 (0.5-5.4)	2.8 (0.0-5.6)	3.0 (1.0-25.8)	2.6 (1.2-6.9)
Taurine	81 (13-251)	(4.00-159.98)	70.4 (5.9-622.5)	76.1 (4.4-309)
Trigonelline	31.1 (5.5-109.3)	16.08	12.5 (0.8-56.9)	8.5 (0.2-52)
Valine	1.6 (0.6-3.3)	1.0 (0.0-2.5)	2.8 (4.4-1.5)	2.6 (1.4-4.3)
β-Alanine	5.9 (3.4-13.0)	5 (0-10)	21.5 (5.7-382.5)	14.9 (8.1-24.6)

Table 4.2 Comparison of passed metabolite concentrations (µM/mM creatinine) that indicated preliminary differences in this study and in Kuo et al. (2012) from HMDB, and welders and controls from current study. Values for comparison were collected from the HMDB, as reported in Bouatra et al. (2013) [34, 53]. Concentrations from controls and welders are separated by group and sampling day as metabolites had a strong influence on PLS-DA. Data represents mean (range).

Metabolite	HMDB Reported	Controls				Welders			
		0	1	7	50	0	1	7	50
Cis-Aconitate	20.9 (3.8-95)	30 (8.9-106)	26.3 (9.1-103)	34.7 (8.1-155)	27.2 (7.5-102)	27.4 (8.1-81)	27.1 (7.4-74)	46.7 (11-190)	49 (13-331)
Glycine	106 (44-300)	70.9 (29-199)	80.6 (34-249)	86 (31-442)	85.6 (28-233)	67 (19-256)	73.5 (26-283)	61.3 (9.9-150)	69.9 (23-195)
Hippurate	229 (19-622)	205 (32-658)	198.8 (20-549)	143.3 (13-327)	146.2 (14-424)	106.3 (13-407)	117.2 (17-315)	123.8 (15-344)	112 (9.8-470)
Taurine	81 (13-251)	62.5 (15-179)	71.2 (9.8-358)	66.3 (5.9-410)	82.7 (13-623)	72.3 (7.6-225)	65.3 (10-227)	86.1 (12-300)	82 (4.4-309)
Trigonelline	31.1 (5.5-109)	11.7 (1.4-57)	11.9 (2.5-35)	11.4 (1.6-32)	15.4 (0.8-56.3)	6.5 (0.2-26)	10.2 (1.3-52)	9.7 (1.1-41)	7.7 (1.3-25)

4.5 Multivariate models with combined data

Application of PCA, an unsupervised model, found very little variation between the two groups, with the first two components explaining 28.94% of the variance. However, when supervised methods such as PLS-DA were applied to distinguish between welding and control groups, the models explained 95-100% of the variation. It is important to note that these models rely heavily on air exposure concentration values and not urinary metal and metabolite values in the combined data analysis. While a large portion of variance was explained, the PLS-DA model was not robust, as it fit the data feebly and had poor predictive power. In addition, overfitting is quite common in PLS-DA models and as a supervised method should be considered with caution as it will maximize differences between groups. Similar results were found when comparing baseline values (day 0) in welders to the final collection day (day 50). This demonstrates that the measured urinary values had very little influence in distinguishing between welder and control groups and the most reliable method to determine occupational exposure is through air sampling. Air sampling does not allow early detection of respiratory problems that can arise from occupational exposure to welding fumes. These results stress the importance of surveillance programs and until reliable biomarkers for exposure are discovered, air sampling and workplace assessments continue to offer exceptional value in establishing health and safe working conditions for employees.

4.6 Strengths

A major strength of this study was the use of randomized sample analysis that occurred in a blinded manner [86]. In addition, we used a pooled urine sample as QC for NMR analysis (4.4.1). The addition of field blanks for urinary metal analysis was also an important strength in this study. By subtracting field blank averages from urine samples, it allowed us to remove values associated with background contamination resulting from transportation and processing of samples. The field blanks were crucial to achieve reliable results, as metal concentrations are very low in urine samples. Finally, the longitudinal design of the study was a strength as it allowed us to see if any changes occurred over time, with increased exposure to welding fumes in apprentices, while building metabolomic personal trajectory profiles.

4.7 Limitations

A few road blocks, or limitations, were encountered throughout this project. As this was a pilot study to determine initial changes and develop a baseline, the questionnaire used was not validated, so it is possible that it did not accurately report participant background information. However, as the questionnaire's primary purpose was to ensure well-matched control and welder populations, the questionnaire design used should have little effect on the overall results. Moreover, as unsupervised multivariate models showed limited variation, our welder and control groups were well-matched.

As this study uses human subjects, compliance can be a limitation. As mentioned, NAIT has local exhaust ventilation systems for their welding students. However, as demonstrated by the spread of exposure values, these systems are not always effectively, or consistently, being used by apprentices. Respirators were worn by only a few students (n = 7), and were not used correctly if subjects had facial hair, and both impact fume exposure. Further, some students would remove pumps during their coffee break during welding labs, which prevented an accurate and true measurement of exposure. When reapplying pumps, welders would not always accurately replace these in their breathing zones. Another limitation regarding compliance was failure to follow the 12 h fast rule prior to urine collection. Although all participants were asked to refrain from eating for 12 h, and any alcohol and drug consumption for at least 48 h and 10 days, respectively, this was not always followed. To account for this, prior to urine collections, subjects were asked if they had consumed anything in the past 12 h, and any infractions were recorded, with the urine sample excluded from analysis.

Samples were only collected over an 8-week period. During this 8-week period, welding apprentices were only exposed to fumes for approximately 3 h a day, overall this may not have been long enough for cumulative exposure to cause changes. In addition, as the majority of welding participants reported previous welding fume exposures, this study did not observe a true baseline prior to any exposures, which is a limitation.

Metal analysis with ICP-MS is a highly sensitive technique, and sample contamination can be a concern. As our laboratory is filled with potential contaminants, urine samples were at

risk. To overcome this, samples were sent to the SWAMP laboratory, a metal-free zone, to be diluted and processed prior to analysis to ensure quality results.

4.8 Conclusions

Welders were exposed to significantly (* $p < 0.05$) higher concentrations of metals and particulates from welding fumes than control participants on days 1, 7, and 50. Some urinary metabolites were different between welders and controls, indicating early metabolic changes detectable in urinary molecules. As our study occurred over only 8 weeks, increased exposure and time may reveal more differences between the two groups at the individual metabolite level and in multivariate analysis. Overall this study provides a strong baseline of urinary measurements for individuals beginning their career with occupational exposure to welding fumes and can be used to compare to data from those who have been exposed for years to begin developing an understanding of when and how health problems associated with welding fume exposure begin to develop, and if there are biomarkers that indicate hazardous welding fume exposure.

Chapter 5

Future Directions

5.0 Future Directions

The following chapter focuses on the next steps for this project. This entails continuing to work on the analysis of the current data by trying a different peak fitting algorithm, along with further sample collection, such as smoking apprentice welders and welders who have been employed in the industry for an extended period. If possible, following-up with our current participants to observe changes as their exposure increases with employment. Finally, the following section proposes trying alternative methods with increased sensitivity compared to NMR.

5.1 Monte Carlo algorithm for fitting NMR spectra

Metabolite concentrations in this thesis were determined using PSRR, a peak-fitting algorithm developed by Pascal Mercier (NANUC, University of Alberta). However, Dr. Mercier has recently established a new computational approach based on a Monte Carlo simulation algorithm that automatically fits NMR spectra. Initial analysis of QC samples showed 59 metabolites that pass using Monte Carlo according to < 20% RSD. The next step would be to apply multivariate analysis on the passed metabolites fit by the Monte Carlo algorithm to compare control and welding subjects.

5.2 Effects of smoking on metabolic profiles of apprentices

Studies have found that smoking can influence the metabolic phenotype, and have suggested that smoking may interact with welding fume exposure magnifying the effect [26, 34]. An important step in furthering this research would be to test if tobacco smoking impacts urinary metabolic fingerprints and if any interactions between cigarette smoking and exposure to welding fumes is observed. To test this, 20 smoking, male apprentice welders and controls were recruited from NAIT to undergo the same sample collection and analysis as described in this thesis. This will allow comparisons to be made between control and welder smoking groups, and between the smoking and non-smoking groups.

5.3 Additional methods with increased sensitivity

As ICP-MS and NMR spectroscopy found preliminary differences in welders over the eight-week period, or between welders and controls, it would be potentially valuable to look at alternative techniques with increased sensitivity. Additional metabolomic techniques, such as GC-MS and LC-MS present the opportunity to identify additional metabolites, as mass spectrometry is often used complementary to NMR [59]. A pilot study has been initiated with LC-MS at Dr. Liang Li's laboratory (Department of Chemistry, University of Alberta) comparing five random control and welder samples from day 50 and indicated initial early differences between the two groups, which were not significant. It would be valuable to increase the sample size and continue LC-MS analysis comparing control and welder groups, along with considering GC-MS analysis. In addition, field flow fractionation coupled with ICP-MS, presents a unique opportunity to detect differences between metals found in the two groups that were not found using ICP-MS.

5.4 Follow-up of current subjects

The eight-week period of exposure may be insufficient for observable differences in urinary metabolite concentrations to be found. A follow-up of our current apprentice welders would enrich our understanding and further develop personalized profile trajectories as their careers continue. A profile following apprentices from their early training into employment would allow changes that occur over time and increased exposure to be created, providing valuable insight into how the body responds to occupational exposure over time.

5.5 Welders employed in the industry

In addition to following current participants as they begin working in industry, it is imperative to enhance the understanding of those who have been exposed to welding fumes for a large portion of their career. For this reason, an important next step would be collecting samples from career welders, who have been employed for 5, 10, and 15+ years to compare metabolomic profiles to those who are just at the beginning of their careers and have limited exposure.

Overall, this study provides a strong baseline for individuals at the start of their careers and initial exposure to welding fumes. This will need to be built upon, using current and additional techniques, to deepen our understanding of the exposure of welding fumes and how over time, it may alter and impact an individual's health. Through this increased understanding, better surveillance techniques can be put in place, ensuring the health and safety of those exposed to welding fumes.

References

1. *Welding-overview of types and hazards*. 2010 [cited 2017 March 14]; Available from: https://www.ccohs.ca/oshanswers/safety_haz/welding/overview.html.
2. *Welding Basics*. Welding Information Center 2004 [cited 2017 March 14]; Available from: http://www.weldinginfocenter.com/basics/ba_02.html.
3. Antonini, J.M., *Pulmonary responses to welding fumes: Role of metal constituents.* Toxicological Sciences, 2003. **72**: p. 233-249.
4. Wultsch, G., Nersesyan, A., Kundi, M., Jakse, R., Beham, A., Wagner, K.H., and Knasmueller, S., *The sensitivity of biomarkers for genotoxicity and acute cytotoxicity in nasal and buccal cells of welders.* International Journal of Hygiene and Environmental Health, 2014. **217**(4-5): p. 492-498.
5. *OCCinfo: Occupations and Educational Programs: Welder*. 2015 [cited 2017 March 18]; Available from: http://occinfo.alis.alberta.ca/occinfopreview/info/browse-occupations/occupation-profile.html?id=71003108.
6. Nait. *Welder*. [cited 2017 April 29]; Available from: http://www.nait.ca/program_home_81442.htm.
7. Clancy, C., *Declining interest in welding apprenticeships at NAIT parallels economic slump*, in *Edmonton Journal*. 2016.
8. *Welding-fumes and gases*. 2010 [cited 2017 March 14]; Available from: http://www.ccohs.ca/oshanswers/safety_haz/welding/fumes.html.
9. Bor-Cheng Han, I.-J.L., Hsiao-Chi Chuang, Chih-Hong Pan, Kai-Jen Chuang, *Effect of welding fume on heart rate variability among workers with respirators in a shipyard.* Scientific Reports, Nature, 2016. p.1-6.
10. Blunt, J. and Balchin, N.C., *Health and Safety in Welding and Allied Processes*. 5 ed. 2002, Cambridge, England: Woodhead Publishing, Limited.
11. Jorgensen, R.B., Buhagen, M., and Foreland, S., *Personal exposure to ultrafine particles from PVC welding and concrete work during tunnel rehabilitation.* Occupational and Environmental Medicine, 2016. **73**(7): p. 467-473.

12. Spellman, F.R., *Industrial Hygiene Simplified: A Guide to Anticipation, Recognition, Evaluation, and Control of Workplace Hazards*. 2006, Lanham, Maryland Government Institutes, an imprint of The Scarecrow Press, Inc.
13. Beach, J.R., Dennis, J.H., Avery, A.J., Bromly, C.L., Ward, R.J., Walters, E.H., Stenton, S.C., and Hendrick, D.J., *An epidemiologic investigation of asthma in welders.* American Journal of Respiratory and Critical Care Medicine, 1996. **154**(5): p. 1394-1400.
14. American Welding Society Project Committee on Fumes and Gases, *Method for Sampling Airborne Particulates Generated by Welding and Allied Processes*, in *An American National Standard*, AWS Committee on Safety and Health. 1999, American Welding Society: 550 N.W. LeJeune Road, Miami Florida.
15. Persoons, R., Arnoux, D., Monssu, T., Culie, O., Roche, G., Duffaud, B., Chalaye, D., and Maitre, A., *Determinants of occupational exposure to metals by gas metal arc welding and risk management measures: A biomonitoring study.* Toxicology Letters, 2014. **231**(2): p. 135-141.
16. Antonini, J.M., Roberts, J.R., Stone, S., Chen, B.T., Schwegler-Berry, D., Chapman, R., Zeidler-Erdely, P.C., Andrews, R.N., and Frazer, D.G., *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.
17. Hoffmeyer, F., Raulf-Heimsoth, M., Weiss, T., Lehnert, M., Gawrych, K., Kendzia, B., Harth, V., Henry, J., Pesch, B., Bruning, T., and Weldox Study Group, *Relation between biomarkers in exhaled breath condensate and internal exposure to metals from gas metal arc welding.* Journal of Breath Research, 2012. **6**(2). p. 1-8.
18. Brand, P., Lenz, K., Reisgen, U., and Kraus, T., *Number Size Distribution of Fine and Ultrafine Fume Particles From Various Welding Processes.* Annals of Occupational Hygiene, 2013. **57**(3): p. 305-313.
19. Couch, J., Debia, M., Weichenthal, S., and Dufresne, A., *Case Study Ultrafine Particles Exposure in Apprentice Welders.* Journal of Occupational and Environmental Hygiene, 2014. **11**(1): p. D1-D9.

20. Lehnert, M., Pesch, B., Lotz, A., Pelzer, J., Kendzia, B., Gawrych, K., Heinze, E., Van Gelder, R., Punkenburg, E., Weiss, T., Mattenklott, M., Hahn, J.U., Mohlmann, C., Berges, M., Hartwig, A., Bruning, T., and The Weldox Study Group, *Exposure to Inhalable, Respirable, and Ultrafine Particles in Welding Fume.* Annals of Occupational Hygiene, 2012. **56**(5): p. 557-567.
21. Pfefferkorn, F.E., Bello, D., Haddad, G., Park, J.Y., Powell, M., Mccarthy, J., Bunker, K.L., Fehrenbacher, A., Jeon, Y., Virji, M.A., Gruetzmacher, G., and Hoover, M.D., *Characterization of Exposures to Airborne Nanoscale Particles During Friction Stir Welding of Aluminum.* Annals of Occupational Hygiene, 2010. **54**(5): p. 486-503.
22. Lehnert, M., Hoffmeyer, F., Gawrych, K., Lotz, A., Heinze, E., Berresheim, H., Merget, R., Harth, V., Van Gelder, R., Hahn, J.U., Hartwig, A., Weiss, T., Pesch, B., Bruning, T., and Group, W.S., *Effects of Exposure to Welding Fume on Lung Function: Results from the German WELDOX Study.* Adv Exp Med Biol, 2015. **834**: p. 1-13.
23. Harris, M.K., Ewing, W.M., Longo, W., Depasquale, C., Mount, M.D., Hatfield, R., and Stapleton, R., *Manganese exposures during shielded metal arc welding (SMAW) in an enclosed space.* J Occup Environ Hyg, 2005. **2**(8): p. 375-382.
24. Cena, L.G., Chisholm, W.P., Keane, M.J., Cumpston, A., and Chen, B.T., *Size Distribution and Estimated Respiratory Deposition of Total Chromium, Hexavalent Chromium, Manganese, and Nickel in Gas Metal Arc Welding Fume Aerosols.* Aerosol Sci Technol, 2014. **48**(12): p. 1254-1263.
25. Donaldson, K., Stone, V., Clouter, A., Renwick, L., and Macnee, W., *Ultrafine particles.* Occupational and Environmental Medicine, 2001. **58**(3): p. 211-216.
26. Thaon, I., Demange, V., Herin, F., Touranchet, A., and Paris, C., *Increased Lung Function Decline in Blue-collar Workers Exposed to Welding Fumes.* Chest, 2012. **142**(1): p. 192-199.
27. Blanc, P.D., Boushey, H.A., Wong, H., Wintermeyer, S.F., and Bernstein, M.S., *Cytokines in metal fume fever.* American Review of Respiratory Disease, 1993. **147**(1): p. 134-138.
28. *Siderosis: Causes, Symptoms, Diagnosis, Treatment, Prevention*. 2016 [cited 2017 March 15]; Available from: https://www.epainassist.com/chest-pain/lungs/siderosis.

29. Emmanouil, C., *Mechanisms of metal fume fever and its provocation by selected metals.* Fresenius Environmental Bulletin, 2012. **21**(8B): p. 2310-2315.
30. Coggon, D., Inskip, H., Winter, P., and Pannett, B., *Lobar pneumonia - an occupational-disease in welders.* Lancet, 1994. **344**(8914): p. 41-43.
31. Buerke, U., Schneider, J., Rosler, J., and Woitowitz, H.J., *Interstitial pulmonary fibrosis after severe exposure to welding fumes.* American Journal of Industrial Medicine, 2002. **41**(4): p. 259-268.
32. Sardas, S., Omurtag, G.Z., Tozan, A., Gul, H., and Beyoglu, D., *Evaluation of DNA damage in construction-site workers occupationally exposed to welding fumes and solvent-based paints in Turkey.* Toxicology and Industrial Health, 2010. **26**(9): p. 601-608.
33. Guha, N., Loomis, D., Guyton, K.Z., Grosse, Y., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Vilahur, N., Muller, K., Straif, K., and International Agency for Research on Cancer Monograph Working Group, *Carcinogenicity of welding, molybdenum trioxide, and indium tin oxide.* Lancet Oncol, 2017. **18**(5): p. 581-582.
34. Wang, K.C., Kuo, C.H., Tian, T.F., Tsai, M.H., Chiung, Y.M., Hsiech, C.M., Tsai, S.J., Wang, S.Y., Tsai, D.M., Huang, C.C., and Tseng, Y.J., *Metabolomic characterization of laborers exposed to welding fumes.* Chem Res Toxicol, 2012. **25**(3): p. 676-686.
35. Antonini, J.M., Lawryk, N.J., Murthy, G.G.K., and Brain, J.D., *Effect of welding fume solubility on lung macrophage viability and function in vitro.* Journal of Toxicology and Environmental Health-Part A, 1999. **58**(6): p. 343-363.
36. Antonini, J.M., Roberts, J.R., Schwegler-Berry, D., and Mercer, R.R., *Comparative Microscopic Study of Human and Rat Lungs After Overexposure to Welding Fume.* Annals of Occupational Hygiene, 2013. **57**(9): p. 1167-1179.
37. Charr, R., *Respiratory Disorders among Welders.* Jama-Journal of the American Medical Association, 1953. **152**(16): p. 1520-1522.
38. Harding, H.E., Mclaughlin, A.I.G., and Doig, A.T., *Clinical, radiographic, and pathological studies of the lungs of electric-arc and oxyacetylene welders.* Lancet, 1958. **2**: p. 394-398.
39. Tuschl, H., Weber, E., and Kovac, R., *Investigations on immune parameters in welders.* Journal of Applied Toxicology, 1997. **17**(6): p. 377-383.

40. Gube, M., Ebel, J., Brand, P., Goen, T., Holzinger, K., Reisgen, U., and Kraus, T., *Biological effect markers in exhaled breath condensate and biomonitoring in welders: impact of smoking and protection equipment.* International Archives of Occupational and Environmental Health, 2010. **83**(7): p. 803-811.
41. Boudia, N., Halley, R., Kennedy, G., Lambert, J., Gareau, L., and Zayed, J., *Manganese concentrations in the air of the Montreal (Canada) subway in relation to surface automobile traffic density.* Science of the Total Environment, 2006. **366**(1): p. 143-147.
42. Grass, D.S., Ross, J.M., Family, F., Barbour, J., Simpson, H.J., Coulibaly, D., Hernandez, J., Chen, Y.D., Slavkovich, V., Li, Y.L., Graziano, J., Santella, R.M., Brandt-Rauf, P., and Chillrud, S.N., *Airborne particulate metals in the New York City subway: A pilot study to assess the potential for health impacts.* Environmental Research, 2010. **110**(1): p. 1-11.
43. Aschner, M., Erikson, K.M., and Dorman, D.C., *Manganese dosimetry: Species differences and implications for neurotoxicity.* Critical Reviews in Toxicology, 2005. **35**(1): p. 1-32.
44. Baker, M.G., Simpson, C.D., Sheppard, L., Stover, B., Morton, J., Cocker, J., and Seixas, N., *Variance components of short-term biomarkers of manganese exposure man inception cohort of welding trainees.* Journal of Trace Elements in Medicine and Biology, 2015. **29**: p. 123-129.
45. Baker, M.G., Stover, B., Simpson, C.D., Sheppard, L., and Seixas, N.S., *Using exposure windows to explore an elusive biomarker: blood manganese.* International Archives of Occupational and Environmental Health, 2016. **89**(4): p. 679-687.
46. Mergler, D., Huel, G., Bowler, R., Iregren, A., Belanger, S., Baldwin, M., Tardif, R., Smargiassi, A., and Martin, L., *Nervous-system dysfunction among workers with long-term exposure to manganese.* Environmental Research, 1994. **64**(2): p. 151-180.
47. Cowan, D.M., Fan, Q.Y., Zou, Y., Shi, X.J., Chen, J., Aschner, M., Rosenthal, F.S., and Zheng, W., *Manganese exposure among smelting workers: blood manganese-iron ratio as a novel tool for manganese exposure assessment.* Biomarkers, 2009. **14**(1): p. 3-16.
48. Kim, J.Y., Chen, J.C., Kim, J.Y., and Christiani, D.C., *Exposure to welding fumes is associated with acute systemic inflammatory responses.* Occupational and Environmental Medicine, 2005. **62**(3): p. 157-163.

49. Mocevic, E., Kristiansen, P., and Bonde, J.P., *Risk of ischemic heart disease following occupational exposure to welding fumes: a systematic review with meta-analysis.* Int Arch Occup Environ Health, 2015. **88**(3): p. 259-272.

50. Hjollund, N.H.I., Bonde, J.P.E., Jensen, T.K., Ernst, E., Henriksen, T.B., Kolstad, H.A., Giwercman, A., Skakkebaek, N.E., and Olsen, J., *Semen quality and sex hormones with reference to metal welding.* Reproductive Toxicology, 1998. **12**(2): p. 91-95.

51. Stacey, P., Revell, G., and Tylee, B., *Accuracy and repeatability of weighing for occupational hygiene measurements: Results from an inter-laboratory comparison.* Annals of Occupational Hygiene, 2002. **46**(8): p. 693-699.

52. Miller Welds, *Inverter Technology Helps Alberta Technical Institutions Tackle Welder Shortage*. [cited 2017 April 27]; Available from: https://www.millerwelds.com/resources/article-library/inverter-technology-helps-alberta-technical-institutions-tackle-welder-shortage.

53. Bouatra, S., Aziat, F., Mandal, R., Guo, A.C., Wilson, M.R., Knox, C., Bjorndahl, T.C., Krishnamurthy, R., Saleem, F., Liu, P., Dame, Z.T., Poelzer, J., Huynh, J., Yallou, F.S., Psychogios, N., Dong, E., Bogumil, R., Roehring, C., and Wishart, D.S., *The human urine metabolome.* PLOS ONE, 2013. **8**(9): p. 1-28.

54. Morton, J., Tan, E., Leese, E., and Cocker, J., *Determination of 61 elements in urine samples collected from a non-occupationally exposed UK adult population.* Toxicol Lett, 2014. **231**(2): p. 179-193.

55. Morton, J., Leese, E., Cotton, R., Warren, N., and Cocker, J., *Beryllium in urine by ICP-MS: a comparison of low level exposed workers and unexposed persons.* International Archives of Occupational and Environmental Health, 2011. **84**(6): p. 697-704.

56. Reiss, B., Simpson, C.D., Baker, M.G., Stover, B., Sheppard, L., and Seixas, N.S., *Hair Manganese as an Exposure Biomarker among Welders.* Annals of Occupational Hygiene, 2016. **60**(2): p. 139-149.

57. Nuernberg, A.M., Boyce, P.D., Cavallari, J.M., Fang, S.C., Eisen, E.A., and Christiani, D.C., *Urinary 8-isoprostane and 8-OHdG concentrations in boilermakers with welding exposure.* J Occup Environ Med, 2008. **50**(2): p. 182-189.

58. Llorach, R., Garcia-Aloy, M., Tulipani, S., Vazquez-Fresno, R., and Andres-Lacueva, C., *Nutrimetabolomic Strategies To Develop New Biomarkers of Intake and Health Effects.* Journal of Agricultural and Food Chemistry, 2012. **60**(36): p. 8797-8808.

59. Mercier, P., Lewis, M.J., Chang, D., Baker, D., and Wishart, D.S., *Towards automatic metabolomic profiling of high-resolution one-dimensional proton NMR spectra.* J Biomol NMR, 2011. **49**(3-4): p. 307-323.

60. Meetcolab. *ICP-MS Diagram*. 2017 [cited 2017 May 16]; Available from: http://meetcolab.com/Diagram/icp-ms-diagram/.

61. Beckonert, O., Keun, H.C., Ebbels, T.M.D., Bundy, J., Holmes, E., Lindon, J.C., and Nicholson, J.K., *Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts.* Nature Protocols, 2007. **2**(11): p. 2692-2702.

62. Budde, K., Gok, O.N., Pietzner, M., Meisinger, C., Leitzmann, M., Nauck, M., Kottgen, A., and Friedrich, N., *Quality assurance in the pre-analytical phase of human urine samples by 1H-NMR spectroscopy.* Archives of Biochemistry and Biophysics, 2016. **589**: p. 10-17.

63. Emwas, A.H., Luchinat, C., Turano, P., Tenori, L., Roy, R., Salek, R.M., Ryan, D., Merzaban, J.S., Kaddurah-Daouk, R., Zeri, A.C., Gowda, G.a.N., Raftery, D., Wang, Y., Brennan, L., and Wishart, D.S., *Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review.* Metabolomics, 2015. **11**: p. 872-894.

64. Stringer, K.A., Mckay, R.T., Karnovsky, A., Quemerais, B., and Lacy, P., *Metabolomics and Its Application to Acute Lung Diseases.* Front Immunol, 2016. **7**: p. 1-22.

65. De Laurentiis, G., Paris, D., Melck, D., Montuschi, P., Maniscalco, M., Bianco, A., Sofia, M., and Motta, A., *Separating smoking-related diseases using NMR-based metabolomics of exhaled breath condensate.* J Proteome Res, 2013. **12**(3): p. 1502-1511.

66. Jiang, L., Huang, J., Wang, Y., and Tang, H., *Eliminating the dication-induced intersample chemical-shift variations for NMR-based biofluid metabonomic analysis.* Analyst, 2012. **137**(18): p. 4209-4219.

67. Dona, A.C., Jimenez, B., Schafer, H., Humpfer, E., Spraul, M., Lewis, M.R., Pearce, J.T., Holmes, E., Lindon, J.C., and Nicholson, J.K., *Precision high-throughput proton NMR spectroscopy of human urine, serum, and plasma for large-scale metabolic phenotyping.* Anal Chem, 2014. **86**(19): p. 9887-9894.

68. Lacy, P., Mckay, R., Finkel, M., Karnovsky, A., Woehler, S., Lewis, M.J., Chang, D., and Stringer, K.A., *Signal intensities derived from different NMR probes and parameters contribute to variations in quantifications of metabolites.* PLOS ONE, 2014. **9**(1): p. 1-10.

69. Zhang, J., Shen, H., Xu, W., Xia, Y., Barr, D.B., Mu, X., Wang, X., Liu, L., Huang, Q., and Tian, M., *Urinary metabolomics revealed arsenic internal dose-related metabolic alterations: a proof-of-concept study in a Chinese male cohort.* Environ Sci Technol, 2014. **48**(20): p. 12265-12274.

70. Chan, A.W., Mercier, P., Schiller, D., Bailey, R., Robbins, S., Eurich, D.T., Sawyer, M.B., and Broadhurst, D., *H-1-NMR urinary metabolomic profiling for diagnosis of gastric cancer.* British Journal of Cancer, 2016. **114**(1): p. 59-62.

71. Pan, Z., Gu, H., Talaty, N., Chen, H., Shanaiah, N., Hainline, B.E., Cooks, R.G., and Raftery, D., *Principal component analysis of urine metabolites detected by NMR and DESI-MS in patients with inborn errors of metabolism.* Anal Bioanal Chem, 2007. **387**(2): p. 539-549.

72. Ravanbakhsh, S., Liu, P., Bjorndahl, T.C., Mandal, R., Grant, J.R., Wilson, M., Eisner, R., Sinelnikov, I., Hu, X., Luchinat, C., Greiner, R., and Wishart, D.S., *Accurate, fully-automated NMR spectral profiling for metabolomics.* PLOS ONE, 2015. **10**(5): p. 1-15.

73. Keun, H.C., Ebbels, T.M.D., Antti, H., Bollard, M.E., Beckonert, O., Schlotterbeck, G., Senn, H., Niederhauser, U., Holmes, E., Lindon, J.C., and Nicholson, J.K., *Analytical Reproducibility in1H NMR-Based Metabonomic Urinalysis.* Chemical Research in Toxicology, 2002. **15**(11): p. 1380-1386.

74. Worley, B. and Powers, R., *Multivariate Analysis in Metabolomics.* Curr Metabolomics, 2013. **1**(1): p. 92-107.

75. Wehrens, R., *Chemometrics with R: Multivariate Data Analysis in the Natural and Life Sciences*, ed. R. Gentleman, K. Hornik, and G. Parmigiani. 2011, New York: Springer.

76. Allalou, A., Nalla, A., Prentice, K.J., Liu, Y., Zhang, M., Dai, F.F., Ning, X., Osborne, L.R., Cox, B.J., Gunderson, E.P., and Wheeler, M.B., *A Predictive Metabolic Signature for the Transition From Gestational Diabetes Mellitus to Type 2 Diabetes.* Diabetes, 2016. **65**(9): p. 2529-2539.

77. Wang, L., Ko, E.R., Gilchrist, J.J., Pittman, K.J., Rautanen, A., Pirinen, M., Thompson, J.W., Dubois, L.G., Langley, R.J., Jaslow, S.L., Salinas, R.E., Rouse, D.C., Moseley, M.A., Mwarumba, S., Njuguna, P., Mturi, N., Williams, T.N., Scott, J.a.G., Hill, A.V.S., Woods, C.W., Ginsburg, G.S., Tsalik, E.L., Ko, D.C., Wellcome Trust Case, C., and Kenyan Bacteraemia Study Group, *Human genetic and metabolite variation reveals that methylthioadenosine is a prognostic biomarker and an inflammatory regulator in sepsis.* Science Advances, 2017. **3**(3): p. 1-14.

78. Du, X. *Statistical analysis of metabolomics data*. 2014 [cited 2017 June 22]; Available from: https://www.uab.edu/proteomics/metabolomics/workshop/2013/videos_dec/data%20analysis%20and%20interpretation.pdf.

79. *Research Ethics Office*. 2017 [cited 2017 June 19]; Available from: http://www.reo.ualberta.ca/.

80. *Research Ethics Board*. 2017 [cited 2017 June 19]; Available from: http://www.nait.ca/95291.htm.

81. Jhangri, G., Associate Professor, Biostatistics, School of Public Health, University of Alberta, Edmonton, Alberta.Personal Communication with. June 2017.

82. Manoni, F., Gessoni, G., Alessio, M.G., Caleffi, A., Saccani, G., Silvestri, M.G., Poz, D., Ercolin, M., Tinello, A., Valverde, S., Ottomano, C., and Lippi, G., *Mid-stream vs. first-voided urine collection by using automated analyzers for particle examination in healthy subjects: an Italian multicenter study.* Clinical Chemistry and Laboratory Medicine, 2012. **50**(4): p. 679-684.

83. *Calculating Exposure Doses*, in *Public Health Assessment Guidance Manual*. 2005, Agency for Toxic Substances and Disease Registry, U.S. Department of Health & Human Services: Atlanta, GA, US.

84. Krachler, M., Mohl, C., Emons, H., and Shotyk, W., *Influence of digestion procedures on the determination of rare earth elements in peat and plant samples by USN-ICP-MS.* Journal of Analytical Atomic Spectrometry, 2002. **17**(8): p. 844-851.

85. Matusiewicz, H., *Wet digestion methods*, in *Sample preparation for trace element analysis*. 2003, Elsevier Science.

86. Gotzsche, P.C., *Blinding during data analysis and writing of manuscripts.* Controlled Clinical Trials, 1996. **17**(4): p. 285-290.

87. Cassiede, M., Nair, S., Dueck, M., Mino, J., Mckay, R., Mercier, P., Quemerais, B., and Lacy, P., *Assessment of 1H NMR-based metabolomics analysis for normalization of urinary metals against creatinine.* Clin Chim Acta, 2017. **464**: p. 37-43.

88. Xia, J. and Wishart, D.S., *Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis.* Current Protocols in Bioinformatics, 2016. **55**(14.10): p. 1-14.

89. Bonsnes, R.W. and Taussky, H.H., *On the colorimetric determination of creatinine by the Jaffe reaction.* Journal of Biological Chemistry, 1945. **158**(3): p. 581-591.

90. Husdan, H. and Rapoport, A., *Estimation of creatinine by Jaffe reaction - a comparison of 3 methods.* Clinical Chemistry, 1968. **14**(3): p. 222-238.

91. *Report on Human Biomonitoring of Environmental Chemicals in Canada: Results of the Canadian Health Measures Survey Cycle 1 (2007-2009)*, Minsiter of Health. 2010: Ottawa.

92. Fisher, M.J., Marshall, A.P., and Mitchell, M., *Testing differences in proportions.* Australian Critical Care, 2011. **24**(2): p. 133-138.

93. Quemerais, B., Mino, J., Amin, M.R., Golshahi, H., Izadi, H., and Iop, *Detailed characterization of welding fumes in personal exposure samples*, in *4th International Conference on Safe Production and Use of Nanomaterials*. 2015, Iop Publishing Ltd: Bristol.

94. U.S. EPA, *Exposure Factors Handbook (1997 Final Report)*. 1997, Washington, D.C. : U.S. Environmental Protection Agency.

95. Exposure Assessment Solutions, I. *IHData Analyst*. [cited 2017 April 14]; Available from: http://www.easinc.co/ihda-software/.

96. Croghan, C.W. and Egeghy, P.P., *Methods of dealing with values below the limit of detection using SAS.* EPA Science Inventory, 2003: p. 1-5.
97. Witten, I.H., *Data Mining with WEKA*, in *Data Mining with WEKA*, I.H. Witten, Editor., The University of Waikato.
98. *Classification Methods*. University of Minnesota Duluth: Duluth, Minnesota.
99. Bradley, A.P., *The use of the area under the ROC curve in the evaluation of machine learning algorithms.* Pattern Recognition, 1997. **30**(7): p. 1145-1159.
100. Di Eugenio, B. and Glass, M., *Squibs and discussions - The kappa statistic: A second look.* Computational Linguistics, 2004. **30**(1): p. 95-101.
101. Alberta Government, *Occupational Health and Safety Code*, Occupational Health and Safety Act. 2009, Alberta Queen's Printer: Edmonton, Alberta.
102. Ellingsen, D.G., Chashchin, M., Berlinger, B., Fedorov, V., Chashchin, V., and Thomassen, Y., *Biological monitoring of welders' exposure to chromium, molybdenum, tungsten and vanadium.* Journal of Trace Elements in Medicine and Biology, 2017. **41**: p. 99-106.
103. Wu, Y. and Li, L., *Sample normalization methods in quantitative metabolomics.* J Chromatogr A, 2016. **1430**: p. 80-95.
104. Division of Toxicoloyg and Human Health Services, *Public Healthy Statement: Vanadium*. 2012, Agency for Toxic Substances and Disease Registry.
105. Bari, M.A. and Kindzierski, W.B., *Concentrations, sources and human health risk of inhalation exposure to air toxics in Edmonton, Canada.* Chemosphere, 2017. **173**: p. 160-171.
106. Collins, J.F., Prohaska, J.R., and Knutson, M.D., *Metabolic crossroads of iron and copper.* Nutrition Reviews, 2010. **68**(3): p. 133-147.
107. Lucchini, R., Selis, L., Folli, D., Apostoli, P., Mutti, A., Vanoni, O., Iregren, A., and Alessio, L., *Neurobehavioral effects of manganese in workers from a ferroalloy plant after temporary cessation of exposure* Scandinavian Journal of Work Environment & Health, 1995. **21**(2): p. 143-149.
108. Mcclay, J.L., Adkins, D.E., Isern, N.G., O'connell, T.M., Wooten, J.B., Zedler, B.K., Dasika, M.S., Webb, B.T., Webb-Robertson, B., Pounds, J.G., Murrelle, E.L., Leppert, M.F., and

Van Den Oord, E.J.C.G., *1H Nuclear Magnetic Resonance Metabolomics analysis identifies novel urinary biomarkers for lung function.* Journal of Proteome Research, 2010. **9**: p. 3083-3090.

109. Amorim, L.C.A. and Alvarezleite, E.M., *Determination of o-cresol by gas chromatography and comparison with hippuric acid levels in urine samples of individuals exposed to toluene.* Journal of Toxicology and Environmental Health, 1997. **50**(4): p. 401-407.
110. Pechlivanis, A., Papaioannou, K.G., Tsalis, G., Saraslanidis, P., Mougios, V., and Theodoridis, G.A., *Monitoring the Response of the Human Urinary Metabolome to Brief Maximal Exercise by a Combination of RP-UPLC-MS and H-1 NMR Spectroscopy.* Journal of Proteome Research, 2015. **14**(11): p. 4610-4622.
111. Benson, J.M., Tibbetts, B.M., and Barr, E.B., *The uptake, distribution, metabolism, and excretion of methyl tertiary-butyl ether inhaled alone and in combination with gasoline vapor.* Journal of Toxicology and Environmental Health-Part A, 2003. **66**(11): p. 1029-1052.
112. US Department of Health and Human Services, *NTP technical report on the toxicology and carcinogenesis studies of isobutene (cas no. 115-11-7) in F344/N rats and B6C3F mice (inhalation studies)*. 1998, National Institutes of Health.
113. Dawiskiba, T., Deja, S., Mulak, A., Zabek, A., Jawien, E., Pawelka, D., Banasik, M., Mastalerz-Migas, A., Balcerzak, W., Kaliszewski, K., Skora, J., Barc, P., Korta, K., Pormanczuk, K., Szyber, P., Litarski, A., and Mlynarz, P., *Serum and urine metabolomic fingerprinting in diagnostics of inflammatory bowel diseases.* World Journal of Gastroenterology, 2014. **20**(1): p. 163-174.
114. Calvani, R., Miccheli, A., Capuani, G., Miccheli, A.T., Puccetti, C., Delfini, M., Iaconelli, A., Nanni, G., and Mingrone, G., *Gut microbiome-derived metabolites characterize a peculiar obese urinary metabotype.* International Journal of Obesity, 2010. **34**(6): p. 1095-1098.

Appendix A: Questionnaire

University of Alberta
Pulmonary Research Group
Room 559 HMRC
Pre-Screening Questionnaire

Before you begin to complete this form, determine if the volunteer is 18 years of age or older. All information is kept confidential.

Demographic Information

First Name: _____ Last Name: _____ Initial: _____

Address: _____

_____

_____

Home #: _____ Work #: _____

Cellular #: _____ Email Address: _____

Ethnicity: _____

Subject Code: _____

Date of Birth: ____ / _____ / _____ (Day / Month / Year)	Gender: ☐ Male ☐ Female	Smoker? ☐ Yes How many/day? _____ ☐ No
Height: _____ ☐ ft/in ☐ cm	Weight: _____ ☐ lbs ☐ kg	Frame Size: ☐ Small ☐ Medium ☐ Large
Office Use Only	BMI: (kg/m²) = _____	Subject Code: _____

Miscellaneous Information

1. Have you ever been in a research study before? ☐ Yes ☐ No
   If yes, date _____:
2. This study will involve urine collection sampling, we need to know if you are comfortable with this.
   Do you mind providing a urine sample? ☐ Yes ☐ No
3. Do you drink alcohol? ☐ Yes ☐ No
4. Do you take drugs, either prescription or others? ☐ Yes ☐ No

Medical History

1. Do you have high blood pressure? ☐ Yes ☐ No
2. Do you have low blood pressure? ☐ Yes ☐ No
3. Do you have diabetes? ☐ Yes ☐ No
4. Do you have any heart problems? ☐ Yes ☐ No
5. Have you ever had hepatitis? ☐ Yes ☐ No
6. Have you ever had kidney problems? ☐ Yes ☐ No
7. Have you ever had liver problems? ☐ Yes ☐ No
8. Do you have any breathing problems or asthma? ☐ Yes ☐ No
9. Are you seeing a doctor now for any reason? ☐ Yes ☐ No
   If yes, describe:
   _____

Current Medications and OTC Products	
1. Are you currently taking any prescription medications? If so, what are they?	
2. Are you regularly taking over-the-counter medications (e.g., Tylenol, aspirin)? If so, what are they?	
3. Are you currently taking any vitamins, minerals or natural health products? If so, what are they? You can opt to say "prefer not to say."	
4. Have you done welding or been exposed to welding fumes in the past 3 months? If so, when was the last time you were exposed to welding fumes? Was this full time work?	
Wrap Up	
Any additional information to note (please remember that you need to avoid all alcohol for 48 hours and other drugs for at least 10 days prior to each sample collection – if you do consume some, you will need to provide information regarding when these were taken and how much was consumed. No strenuous exercise the day before and a 12 hour fast):	
Completed by:	Date:

Appendix B: Consent form

UNIVERSITY OF ALBERTA

CONSENT FORM

Title of Study: Metabolomics Analysis of Apprentice Welders

Principal Investigator(s): Paige Lacy and Bernadette Quémerais
Phone Number(s): Department of Medicine, 780-492-3457 or 780-492-6085
Emails: paige.lacy@ualberta.ca, bernadette.quemerais@ualberta.ca

	Yes	No
Do you understand that you have been asked to be in a research study?	☐	☐
Have you read and received a copy of the attached Information Sheet?	☐	☐
Do you understand the benefits and risks involved in taking part in this research study?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Do you understand that you are free to leave the study at any time, without having to give a reason?	☐	☐
Has the issue of confidentiality been explained to you?	☐	☐
Do you understand who will have access to your records, including personally identifiable health information?	☐	☐
Who explained this study to you?		

I agree to take part in this study:

_____ _____
Printed Name Signature of Research Participant

Date: _____

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate. If you become ill or injured as a result of being in this study, you will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s) and/or institution(s) from their legal and professional responsibilities.

Signature of Investigator or Designee _____ Date _____

THE INFORMATION SHEET MUST BE ATTACHED TO THIS CONSENT FORM AND A COPY GIVEN TO THE RESEARCH PARTICIPANT

Appendix C: Participant information sheet

UNIVERSITY OF ALBERTA

INFORMATION SHEET

Title of Project: **Metabolomics Analysis of Apprentice Welders**

Investigators: **Paige Lacy (paige.lacy@ualberta.ca) and Bernadette Quémerais (bernadette.quemerais@ualberta.ca)**

Background and Purpose of this Study: We are principal investigators from the University of Alberta interested in understanding whether a new approach for measuring urine samples may help us to monitor exposure to welding fumes. A better understanding of this approach, called metabolomics, may be beneficial for preventing lung diseases in welders. In order to evaluate this new approach, we are asking if you would be willing to provide at least four (4) samples of urine for this research. We are monitoring samples from two groups of individuals at NAIT for this study: (1) apprentice welders enrolled in the welding program, and (2) students enrolled in the Powerline Technician program. We require samples from students in the Powerline Technician program to provide "control" samples from an environment that does not have welding fumes. We will also ask that welding participants carry a sampling pump for several hours so that we can assess your environmental exposure to welding fumes in correlation with urine analysis.

What will I be asked to do? If you are a **welder**, you will be asked to carry a personal sampling pump connected to a filter holder that contains a filter for trapping airborne particles. The pump will be fixed to your belt. The filter holder will be located on your shoulder to collect particles in your breathing zone. Each sampling pump weighs approximately 2 lbs. This is only required from welding participants, and not the control group.

For all participants (**welders** and **controls**), you will be asked to provide four (4) **fasting** urine samples. The first one will be obtained at the start of the welding program, the second after one day of the welding program, the third after one week of the welding program, and a final sample at the completion of the welding program (7-8 weeks after the start). Each urine sample should be a minimum of 25-50 ml (or about 2-4 tablespoons).

You will need to ensure that you are not taking any alcohol or drugs for up to 48 hours prior to sample collection, and that you are fasting for a minimum of 12 hours prior to sample collection in the morning before class. Your urine will be collected, transported, stored, and then subjected to measurement on an instrument known as a nuclear magnetic resonance (NMR) spectrometer, which is essentially a giant supercooled magnet that can identify and quantify chemicals in your urine sample. The study is designed for the collection of four urine samples from each participant so that we can compare the levels of urinary metabolites (chemicals) before and after exposure to welding fumes.

Pulmonary Research Group, Room 559 HMRC
University of Alberta, Edmonton, AB Canada T6G 2S2
Phone: (780) 492-3457; Fax: (780) 492-5329

What type of personal information will be collected? Should you agree to participate, you will be asked to provide your gender, age, and academic major. Any personal information gathered for the research project will be protected and used in compliance with Alberta's Freedom of Information and Protection of Privacy Act. We will also be asking for some of your medical history, alcohol consumption, and drug/medication exposure in the 2 days prior to each sample collection. This is done to ensure that there are no confounding factors which could complicate the measurement of metabolites in your urine sample.

Your name will be associated with a code that we assign to it to ensure confidentiality in our laboratory records. We will never report personal information and the results from this study will not be associated with your name.

Are there risks or benefits if I participate?

Benefits: You may receive a direct benefit from participating in this study. This research will provide feedback regarding the possibility that exposure to welding fumes may be monitored using this new approach. Once the study is complete, the results will be sent to NAIT and you will be able to learn whether airborne particles from welding fumes may be monitored through urine sampling. With the results of the study, we will be better able to provide health and safety information and recommendations.

Risks: Participation in this research study poses minimal risk for research participants. This means that the potential harm you may encounter through participation in this study is no greater than the possible harm you might encounter in any other aspect of your everyday life. The only risk of wearing the sampling pump is physical discomfort due to the weight of the pump, which is around 1 lb. However, pumps are designed for personal sampling, and the belt and pouches used should make the equipment reasonably comfortable to wear. There are no known health risks associated with the provision of urine samples.

What happens to the information I provide?

Confidentiality: Participation is completely voluntary, anonymous, and confidential. You are free to discontinue participation at any time during the study. No one except the researchers and their team will be allowed to see any of the answers to the questionnaire. Only group information will be summarized for any presentation or publication of results. We will keep track of basic personal information such as age, sex, and date of sample collection in a confidential file. All study information, including the questionnaire, will be kept in a locked cabinet in our laboratory that is only accessible by the researchers in this study. The anonymous data will be stored on a password-protected computer at the University of Alberta. Your identity will be kept completely confidential during our analysis of your urine samples, and each sample will be coded upon its receipt in the laboratory. The study data will be stored for a minimum of 5 years at the offices of the principal investigators.

For our research studies, it is important that the data we get is accurate. For this reason, the study information, including your name, may be looked at by the study sponsor, the Health Research Ethics Board and the University of Alberta. By signing this form you are giving permission to the researchers to collect, use and disclose information about you as described above.

Voluntary Participation and Freedom to Withdraw: You do not have to agree to participate in this study. Even if you agree, you are free to withdraw from the research study at any time, and we will not contact you about future contributions. You do not have to give a reason for withdrawing.

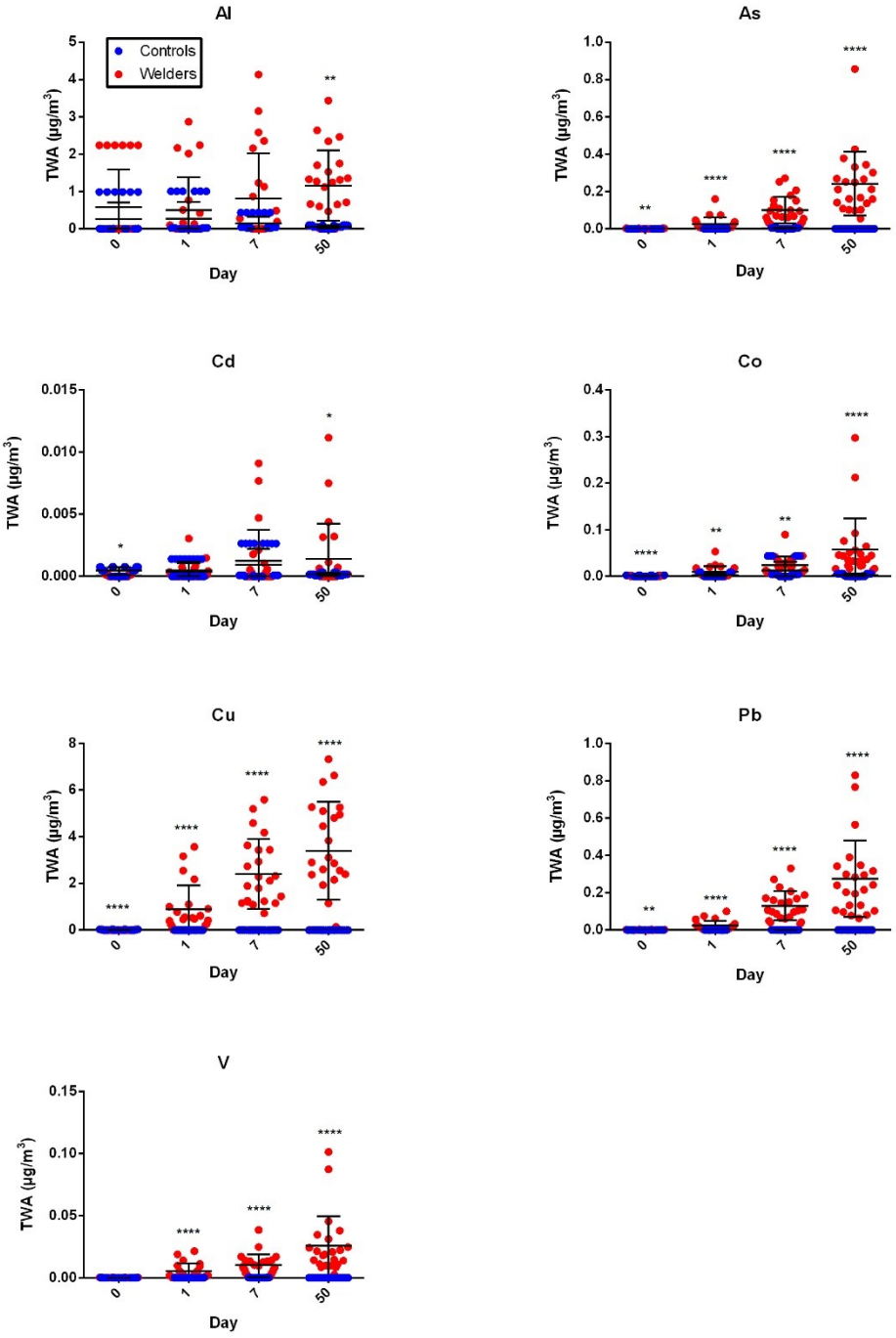
Costs: The study-related tests and procedures will be provided to you at no cost to you or your insurance company. You will be compensated for parking fees and additional expenses.

Contact Information: If you have concerns about your participation in this study, you may contact any of the study investigators at 780-492-3457 or 780-492-6085. You may also contact the University of Alberta Research Ethics Board Office at 780-492-2615 if you have any concerns about your rights as a study participant. If you have concerns about the way you've been treated as a participant and would like to communicate with NAIT officials, please contact the NAIT Research Ethics Board Chair, Dr. Melissa Dobson, NAIT, at 780-378-5185, email: mdobson@nait.ca.

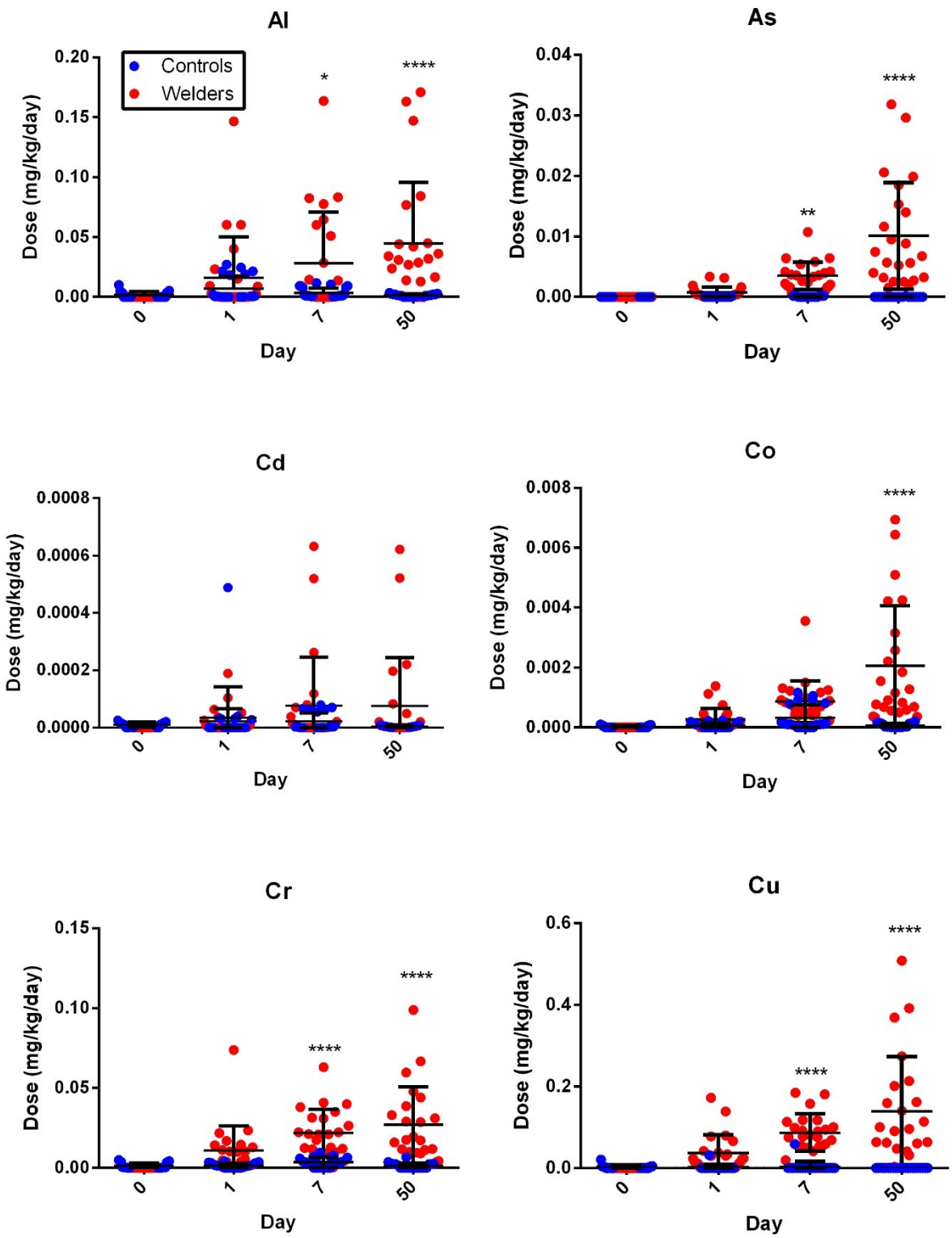
Funding for this study is supported by a grant from Work Safe Alberta, Occupational Health and Safety, Government of Alberta (http://work.alberta.ca/occupational-health-safety.html).

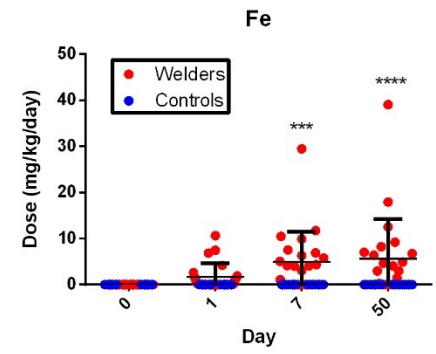
Appendix D: Summary of air exposure results

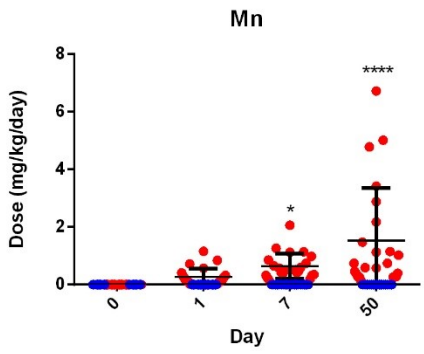
TWA in µg/m^3

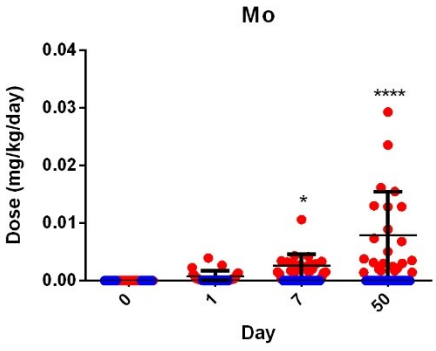


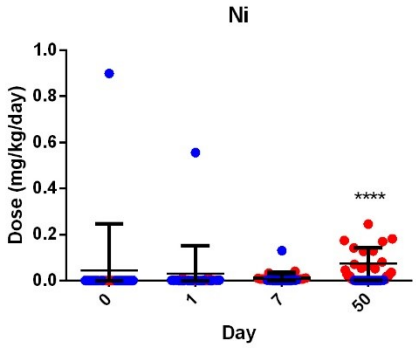
Dose concentrations (in mg/kg/day)

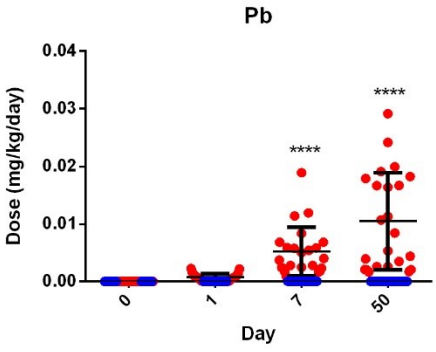


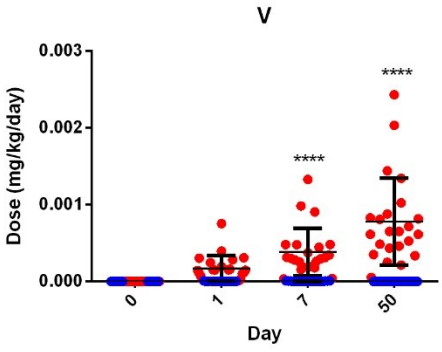

Fe
Welders
Controls
Dose (mg/kg/day)
50
40
30
20
10
0
***
****
0
1
7
50
Day

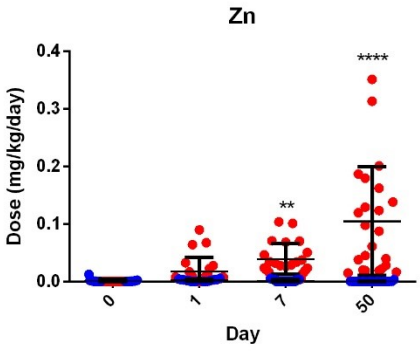

Mn
Dose (mg/kg/day)
8
6
4
2
0
*
****
0
1
7
50
Day


Mo
Dose (mg/kg/day)
0.04
0.03
0.02
0.01
0.00
*
****
0
1
7
50
Day

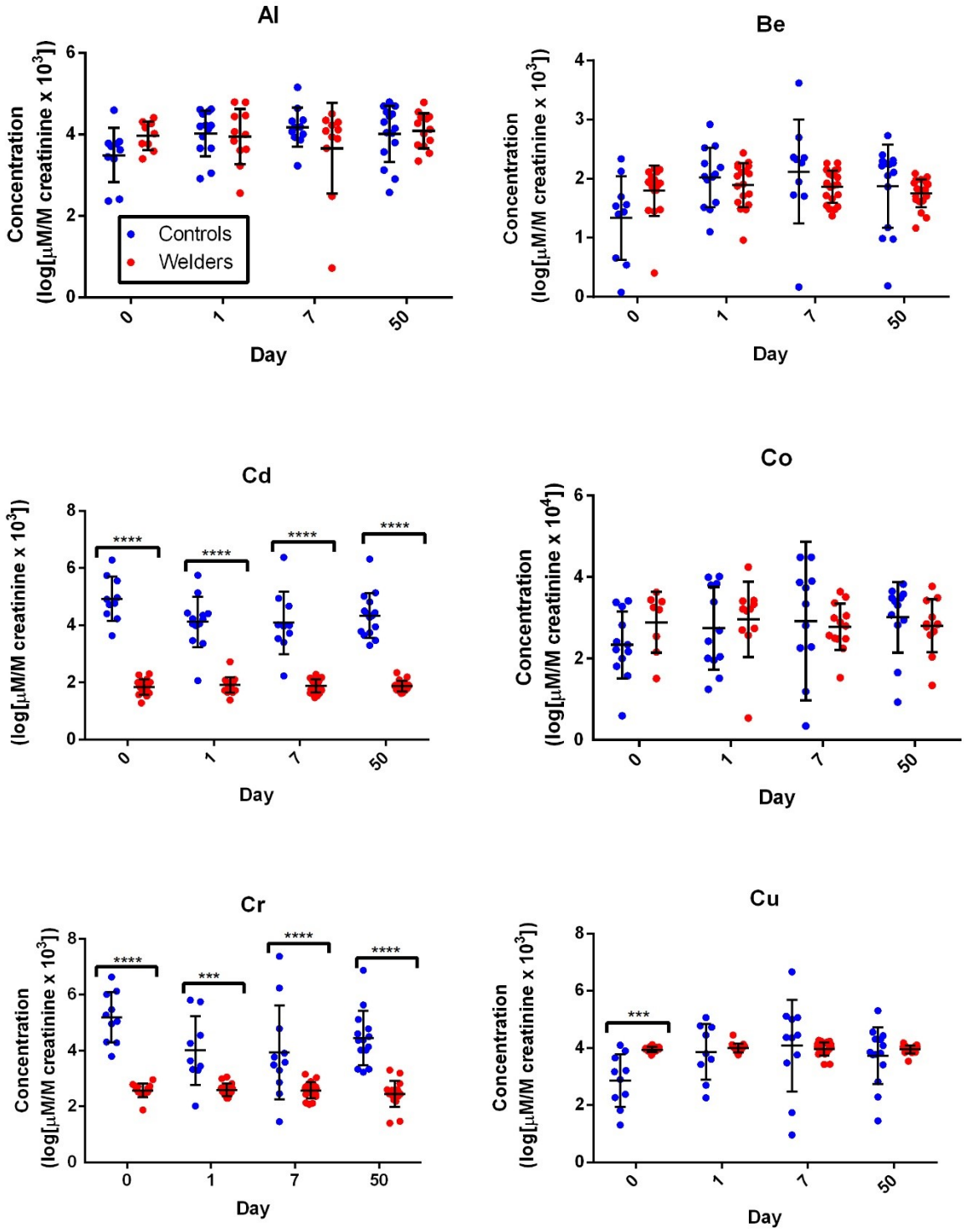

Ni
Dose (mg/kg/day)
1.0
0.8
0.6
0.4
0.2
0.0
****
0
1
7
50
Day

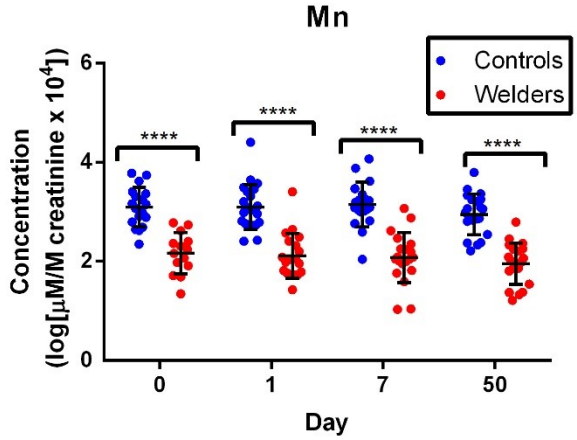

Pb
Dose (mg/kg/day)
0.04
0.03
0.02
0.01
0.00
****
****
0
1
7
50
Day

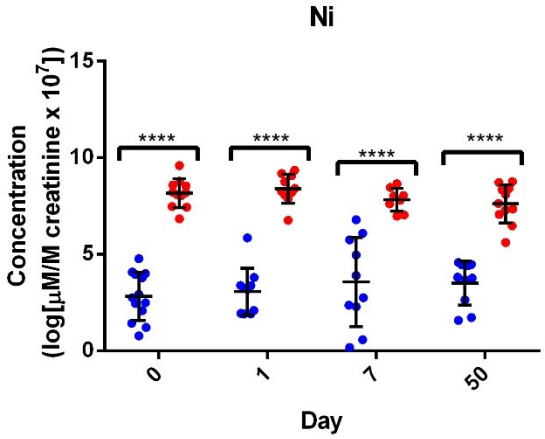

V
Dose (mg/kg/day)
0.003
0.002
0.001
0.000
****
****
0
1
7
50
Day

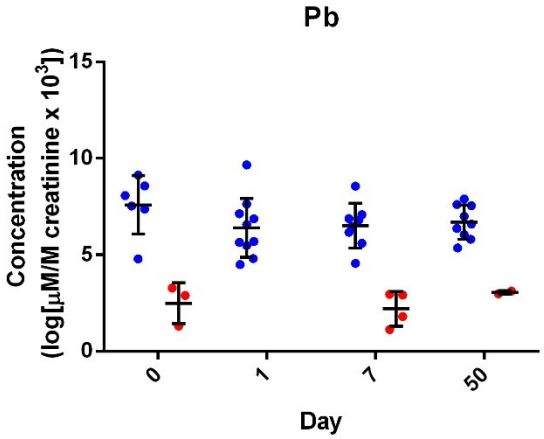

Zn
Dose (mg/kg/day)
0.4
0.3
0.2
0.1
0.0
**
****
0
1
7
50
Day

Appendix E: Summary of urinary metal results




Mn
Controls
Welders
Concentration (log[µM/M creatinine x 10^4])
****
0
1
7
50
Day


Ni
Concentration (log[µM/M creatinine x 10^7])
****
0
1
7
50
Day


Pb
Concentration (log[µM/M creatinine x 10^3])
0
1
7
50
Day

Appendix F: Summary of QC metabolites

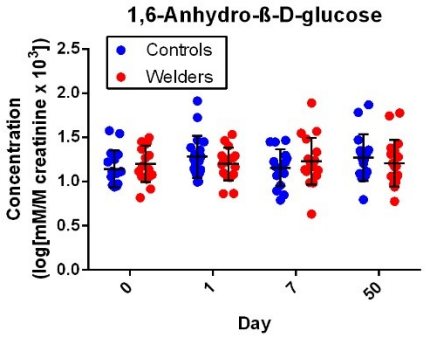
Normalized urinary metabolites mean (log [mM/M creatinine x 10^3]), SD and (%) RSD are summarized below for 33 QC samples. They are in order from the lowest to highest RSD (%). A total of 149 metabolites are shown as creatinine was used for normalization, DSS was used as an internal standard. Passed metabolites are in **bold**. ND represents not detectable.

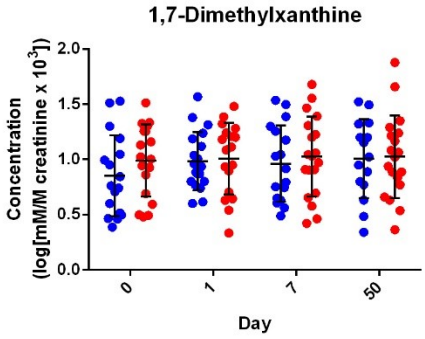
Metabolite	Mean	SD	RSD (%)	Metabolite	Mean	SD	RSD (%)
Dimethylamine	**$4.7x10^{-1}$**	**$3.5x10^{-3}$**	**1**	**3-Hydroxybutyrate**	**$1.1x10^{-1}$**	**$1.1x10^{-2}$**	**11**
Citrate	**1.8**	**$3.8x10^{-2}$**	**2**	**Carnitine**	**$2.3x10^{-1}$**	**$2.5x10^{-2}$**	**11**
u11	**3.2**	**$6.4x10^{-2}$**	**2**	**Ibuprofen**	**$3.1x10^{-2}$**	**$3.5x10^{-3}$**	**11**
u122	**3.3**	**$7.0x10^{-2}$**	**2**	**3-Hydroxy-3-Methylglutarate**	**$4.3x10^{-2}$**	**$5.2x10^{-3}$**	**12**
2-Hydroxyisobutyrate	**$7.5x10^{-2}$**	**$2.4x10^{-3}$**	**3**	**Azelate**	**$9.4x10^{-2}$**	**$1.1x10^{-2}$**	**12**
3-Hydroxyisovalerate	**$9.7x10^{-2}$**	**$3.1x10^{-3}$**	**3**	**Indole-3-Acetate**	**$2.4x10^{-1}$**	**$2.8x10^{-2}$**	**12**
Alanine	**$2.9x10^{-1}$**	**$9.6x10^{-3}$**	**3**	**Methylguanidine**	**$1.1x10^{-1}$**	**$1.3x10^{-2}$**	**12**
Glycine	**1.3**	**$3.7x10^{-2}$**	**3**	**Pseudouridine**	**$1.8x10^{-1}$**	**$2.2x10^{-2}$**	**12**
u233	**$7.1x10^{-1}$**	**$2.3x10^{-2}$**	**3**	**Cis-Aconitate**	**$5.9x10^{-1}$**	**$7.2x10^{-2}$**	**12**
Hippurate	**1.3**	**$5.7x10^{-2}$**	**4**	**β-Alanine**	**$2.5x10^{-1}$**	**$2.9x10^{-2}$**	**12**
Pyroglutamate	**$3.8x10^{-1}$**	**$1.7x10^{-2}$**	**4**	**2-Aminoadipate**	**$1.7x10^{-1}$**	**$2.3x10^{-2}$**	**13**
3-Hydroxyisobutyrate	**$1.3x10^{-1}$**	**$6.8x10^{-3}$**	**5**	**Propylene Glycol**	**$1.5x10^{-1}$**	**$1.9x10^{-2}$**	**13**
N-Acetylglutamine Derivative	**1.2**	**$5.6x10^{-2}$**	**5**	**u185**	**$1.2x10^{-2}$**	**$1.6x10^{-3}$**	**13**
Formate	**$1.5x10^{-1}$**	**$8.6x10^{-3}$**	**6**	**1,7-Dimethylxanthine**	**$2.5x10^{-1}$**	**$3.6x10^{-2}$**	**14**
N-Acetylornithine	**$1.6x10^{-1}$**	**$9.3x10^{-3}$**	**6**	**1-Methylnicotinamide**	**$7.4x10^{-2}$**	**$1.0x10^{-2}$**	**14**
Proline	**$6.8x10^{-1}$**	**$4.3x10^{-2}$**	**6**	**Xylose**	**$4.4x10^{-1}$**	**$6.0x10^{-2}$**	**14**
u380Large	**$2.1x10^{-1}$**	**$1.2x10^{-2}$**	**6**	**u362**	**$1.0x10^{-1}$**	**$1.5x10^{-2}$**	**14**
N-Acetylglutamine	**$1.5x10^{-1}$**	**$1.0x10^{-2}$**	**7**	**u217**	**$2.7x10^{-1}$**	**$4.0x10^{-2}$**	**15**
Glutamine	**$4.7x10^{-1}$**	**$4.0x10^{-2}$**	**8**	**1,6-Anhydro-β-Glucose**	**$2.7x10^{-1}$**	**$4.3x10^{-2}$**	**16**
Valine	**$4.7x10^{-2}$**	**$3.8x10^{-3}$**	**8**	**Hypoxanthine**	**$7.0x10^{-2}$**	**$1.2x10^{-2}$**	**17**
u122Triplet	**$1.3x10^{-1}$**	**$1.0x10^{-2}$**	**8**	**Mannitol**	**$3.5x10^{-1}$**	**$5.9x10^{-2}$**	**17**
π-Methylhistidine	**$9.6x10^{-1}$**	**$8.1x10^{-2}$**	**8**	**Phenylalanine**	**$3.1x10^{-1}$**	**$5.3x10^{-2}$**	**17**
τ-Methylhistidine	**$3.6x10^{-1}$**	**$2.9x10^{-2}$**	**8**	**Sarcosine**	**$4.0x10^{-2}$**	**$6.6x10^{-3}$**	**17**
Chlorogenate	**$2.3x10^{-2}$**	**$2.1x10^{-3}$**	**9**	**2-Oxoglutarate**	**$1.7x10^{-1}$**	**$3.0x10^{-3}$**	**18**
Ethanol	**$1.8x10^{-1}$**	**$1.7x10^{-2}$**	**9**	**Leucine**	**$2.4x10^{-2}$**	**$4.2x10^{-3}$**	**18**
Ethanolamine	**$7.9x10^{-1}$**	**$6.8x10^{-2}$**	**9**	**Pantothenate**	**$3.3x10^{-2}$**	**$5.8x10^{-3}$**	**18**
Taurine	**1.0**	**$9.7x10^{-2}$**	**9**	**2-Furoylglycine**	**$2.1x10^{-1}$**	**$4.0x10^{-2}$**	**19**
3-Aminoisobutyrate	**$1.7x10^{-1}$**	**$1.7x10^{-2}$**	**10**	**5-Aminolevulinate**	**$3.8x10^{-1}$**	**$7.3x10^{-2}$**	**19**
Trigonelline	**$1.1x10^{-1}$**	**$1.1x10^{-2}$**	**10**	**Malonate**	**$3.2x10^{-1}$**	**$6.0x10^{-2}$**	**19**

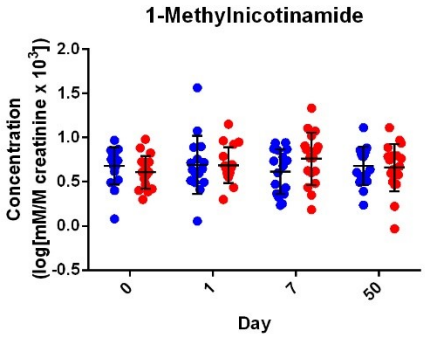
Methionine	**$6.5x10^{-2}$**	**$1.2x10^{-2}$**	**19**	N-Acetyltyrosine	$2.7x10^{-2}$	$7.2x10^{-3}$	27
N-Phenylacetylglycine	**$2.3x10^{-1}$**	**$4.2x10^{-2}$**	**19**	Sucrose	$1.7x10^{-1}$	$4.5x10^{-2}$	27
Butanone	$4.5x10^{-2}$	$8.8x10^{-3}$	20	Lactose	$4.7x10^{-1}$	$1.3x10^{-1}$	28
Tyrosine	$1.5x10^{-1}$	$3.0x10^{-2}$	20	Lactulose	$2.6x10^{-1}$	$7.4x10^{-2}$	29
Anserine	$1.0x10^{-1}$	$2.1x10^{-2}$	21	N-Methylhydantoin	$1.3x10^{-1}$	$3.6x10^{-2}$	29
Arginine	$2.3x10^{-1}$	$4.9x10^{-2}$	21	u87	$7.3x10^{-2}$	$2.1x10^{-2}$	29
Histamine	$2.2x10^{-1}$	$4.5x10^{-2}$	21	Acetate	$4.0x10^{-2}$	$1.2x10^{-2}$	30
Trimethylamine	$3.9x10^{-2}$	$8.1x10^{-3}$	21	Homovanillate	$6.5x10^{-2}$	$1.9x10^{-2}$	30
u433	$2.8x10^{-1}$	$5.7x10^{-2}$	21	Indole-3-Lactate	$1.4x10^{-1}$	$4.1x10^{-2}$	30
3-Hydroxymandelate	$5.2x10^{-2}$	$1.1x10^{-2}$	22	ATP	$2.8x10^{-2}$	$8.8x10^{-3}$	31
Fumarate	$3.1x10^{-3}$	$6.9x10^{-4}$	22	Creatine	$5.7x10^{-1}$	$1.8x10^{-1}$	31
Gluconate	$3.7x10^{-1}$	$8.3x10^{-2}$	22	4-Hydroxy-3-Methoxymandelate	$7.6x10^{-2}$	$2.4x10^{-2}$	32
Histidine	$6.5x10^{-1}$	$1.4x10^{-1}$	22	Acetaminophen	$2.6x10^{-2}$	$8.2x10^{-3}$	32
Isoleucine	$3.0x10^{-2}$	$6.8x10^{-3}$	22	Ascorbate	$2.1x10^{-1}$	$6.8x10^{-2}$	32
u144	1.1	$2.5x10^{-1}$	22	Glucose-6-Phosphate	$4.7x10^{-1}$	$1.5x10^{-1}$	32
Acetoacetate	$4.4x10^{-2}$	$1.0x10^{-2}$	23	O-Acetylcarnitine	$6.4x10^{-2}$	$2.0x10^{-2}$	32
Galactose	$3.9x10^{-1}$	$1.1x10^{-1}$	23	1,3-Dimethylurate	$4.5x10^{-2}$	$1.5x10^{-2}$	33
Tryptophan	$1.8x10^{-1}$	$4.3x10^{-2}$	23	Carnosine	$3.4x10^{-1}$	$1.1x10^{-1}$	33
u43	$1.0x10^{-2}$	$2.3x10^{-3}$	23	Fructose	$3.6x10^{-1}$	$1.2x10^{-1}$	33
Adipate	$3.0x10^{-2}$	$7.2x10^{-3}$	24	Nicotinic Acid Adenine Derivative	$1.9x10^{-2}$	$6.5x10^{-3}$	33
3-Indoxylsulfate	$1.8x10^{-1}$	$4.4x10^{-2}$	25	Nicotinate	$2.4x10^{-2}$	$8.2x10^{-3}$	34
4-Aminohippurate	$7.1x10^{-2}$	$1.8x10^{-2}$	25	Trimethylamine N-Oxide	$4.4x10^{-1}$	$1.6x10^{-1}$	36
Caffeine	$6.5x10^{-2}$	$1.6x10^{-2}$	25	u14Doublet	1.7	$6.3x10^{-1}$	36
Galactarate	$1.5x10^{-1}$	$3.8x10^{-2}$	25	Phenylacetate	$1.1x10^{-1}$	$4.1x10^{-2}$	37
Tropate	$2.5x10^{-2}$	$6.3x10^{-2}$	25	Pyruvate	$2.6x10^{-2}$	$9.5x10^{-3}$	37
Trans-Aconitate	$6.2x10^{-2}$	$1.5x10^{-2}$	25	Threonine	$1.7x10^{-1}$	$6.5x10^{-2}$	38
uarm1	$1.3x10^{-2}$	$3.3x10^{-3}$	25	2-Aminobutyrate	$2.5x10^{-2}$	$9.9x10^{-3}$	40
Acetone	$1.0x10^{-2}$	$2.6x10^{-3}$	26	Glycolate	$5.2x10^{-1}$	$2.1x10^{-1}$	40
Choline	$7.0x10^{-2}$	$1.9x10^{-2}$	26	2-Hydroxyphenylacetate	$6.2x10^{-2}$	$2.6x10^{-2}$	41
Glucitol	$4.0x10^{-1}$	$1.1x10^{-1}$	26	4-Hydroyphenylacetate	$5.4x10^{-2}$	$2.5x10^{-2}$	41
Glucose	$3.9x10^{-1}$	$1.0x10^{-1}$	26	Oxypurinol	$3.3x10^{-1}$	$1.4x10^{-1}$	41
Glycylproline	$3.9x10^{-1}$	$9.9x10^{-2}$	26	5-Hydroxytryptophan	$1.7x10^{-1}$	$7.1x10^{-2}$	42
Pyridoxine	$2.7x10^{-2}$	$7.2x10^{-3}$	26	N-Acetylserotonin	$2.9x10^{-2}$	$1.2x10^{-2}$	43
u361	$5.4x10^{-2}$	$1.4x10^{-2}$	26	O-Phosphocholine	$4.3x10^{-2}$	$1.9x10^{-2}$	44
uarm2	$1.1x10^{-2}$	$2.7x10^{-2}$	26	Serotonin	$2.0x10^{-1}$	$8.9x10^{-2}$	45
4-Hydroxynenzoate	$7.2x10^{-1}$	$1.9x10^{-2}$	27	Succinate	$1.7x10^{-2}$	$7.8x10^{-3}$	45
Asparagine	$3.2x10^{-1}$	$8.7x10^{-2}$	27	u14	$5.5x10^{-2}$	$2.5x10^{-2}$	46
Maltose	$4.4x10^{-1}$	$1.2x10^{-1}$	27	4-Hydroxyphenyllactate	$7.0x10^{-2}$	$3.3x10^{-2}$	47
N,N-Dimethylglycine	$8.2x10^{-2}$	$2.2x10^{-2}$	27	6-Hydroxynicotinate	$1.2x10^{-1}$	$5.8x10^{-2}$	50

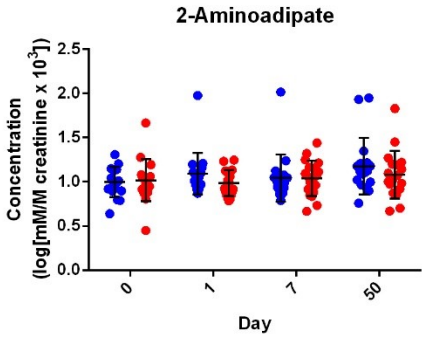
u072	$1.9x10^{-1}$	$1.1x10^{-1}$	58	Xanthine	$2.7x10^{-1}$	$2.6x10^{-1}$	97
Lactate	$1.1x10^{-1}$	$6.7x10^{-2}$	61	u1125	$5.4x10^{-2}$	$5.9x10^{-2}$	110
u075	$3.0x10^{-1}$	$2.0x10^{-1}$	68	3-Hydroxyphenylacetate	ND	ND	ND
Methanol	$4.3x10^{-2}$	$3.0x10^{-2}$	70	Mandelate	ND	ND	ND
Creatine Phosphate	$2.8x10^{-1}$	$2.2x10^{-1}$	78	N-Phenylacetylphenylalanine	ND	ND	ND
Lysine	$1.5x10^{-1}$	$1.2x10^{-1}$	78	Urocanate	ND	ND	ND
Betaine	$2.2x10^{-1}$	$2.0x10^{-1}$	91				

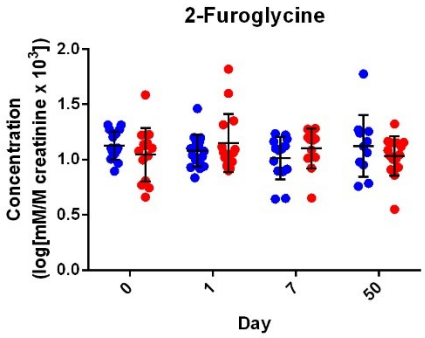
Appendix G: Summary of passed urinary metabolite results

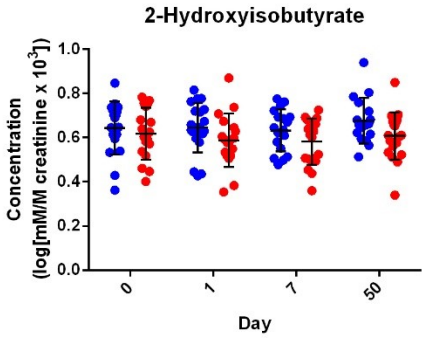


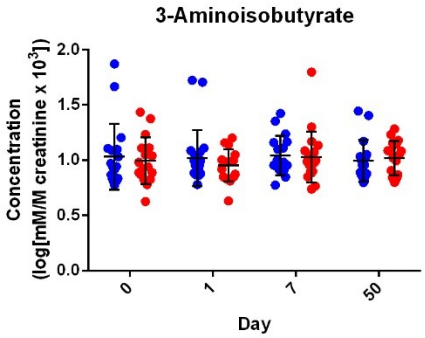


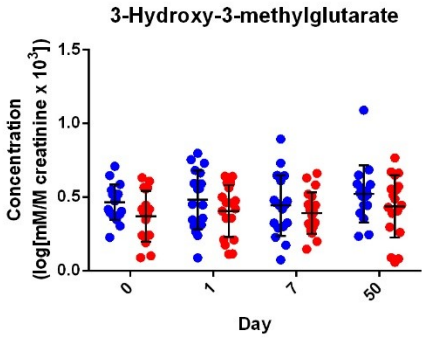


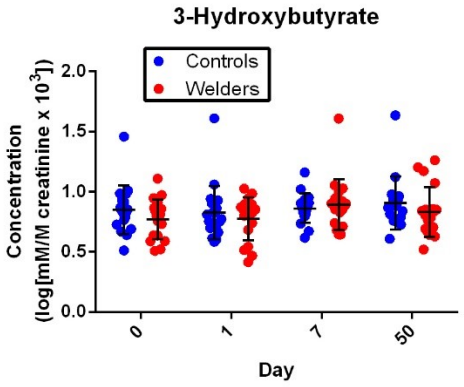


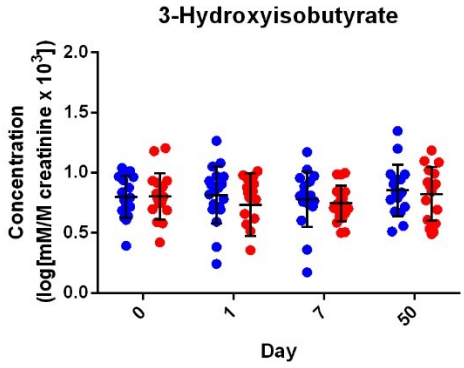


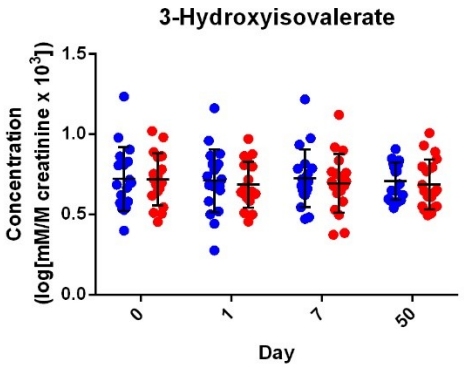


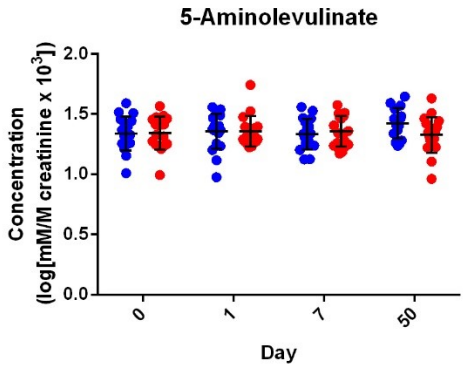


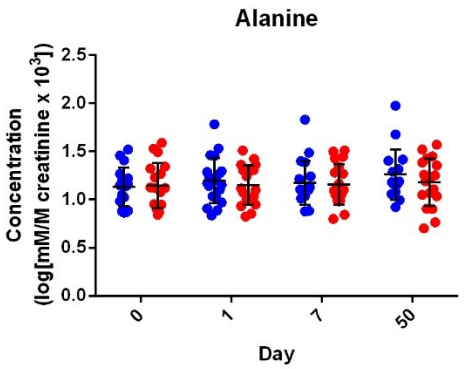


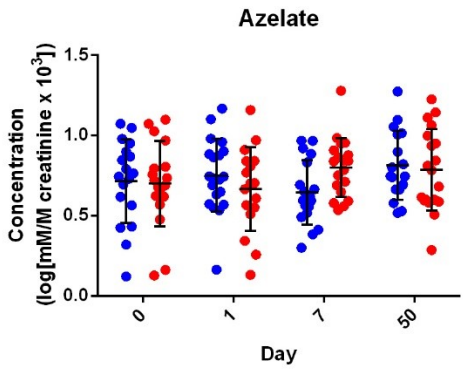

3-Hydroxybutyrate
Controls
Welders
Concentration (log[mM/M creatinine x 10^3])
Day

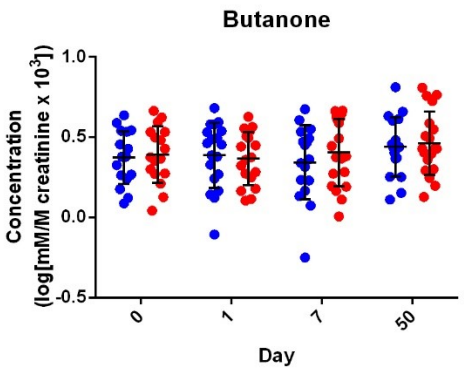

3-Hydroxyisobutyrate
Concentration (log[mM/M creatinine x 10^3])
Day

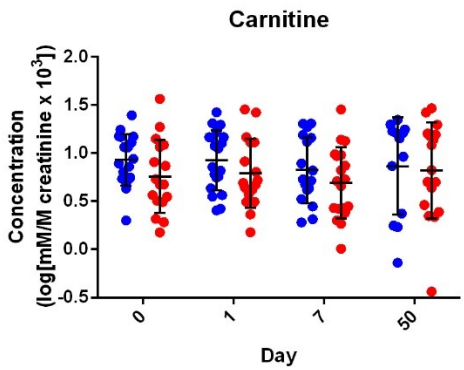

3-Hydroxyisovalerate
Concentration (log[mM/M creatinine x 10^3])
Day

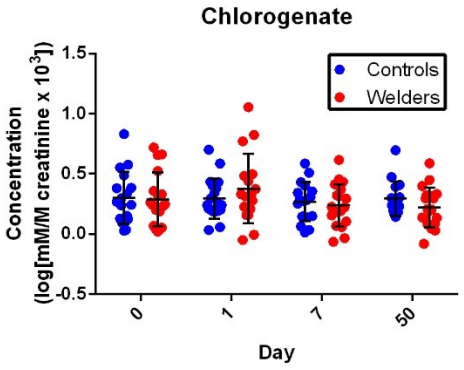

5-Aminolevulinate
Concentration (log[mM/M creatinine x 10^3])
Day

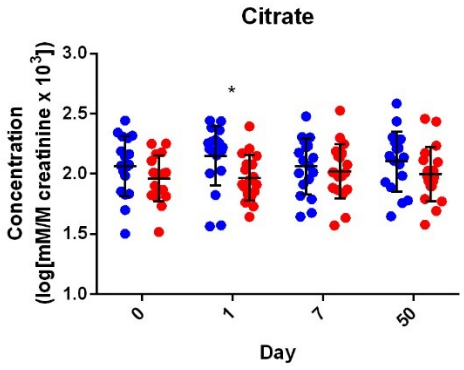

Alanine
Concentration (log[mM/M creatinine x 10^3])
Day

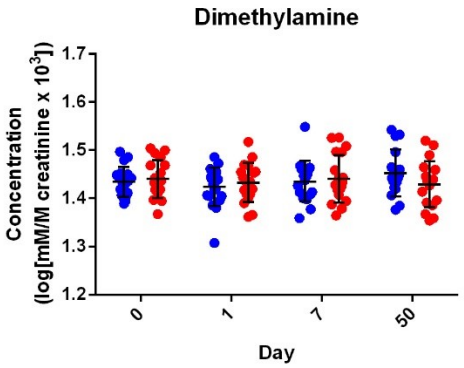

Azelate
Concentration (log[mM/M creatinine x 10^3])
Day

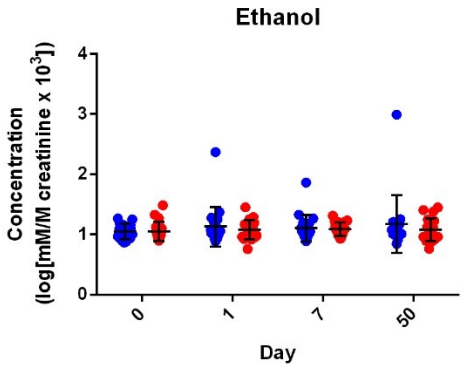

Butanone
Concentration (log[mM/M creatinine x 10^3])
Day

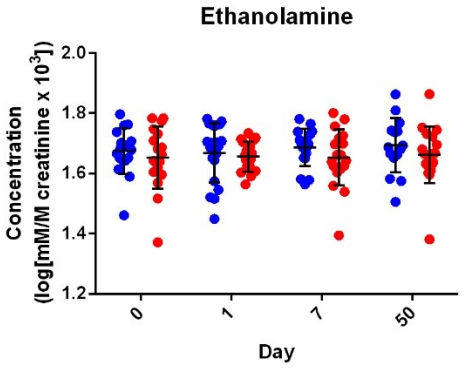

Carnitine
Concentration (log[mM/M creatinine x 10^3])
Day

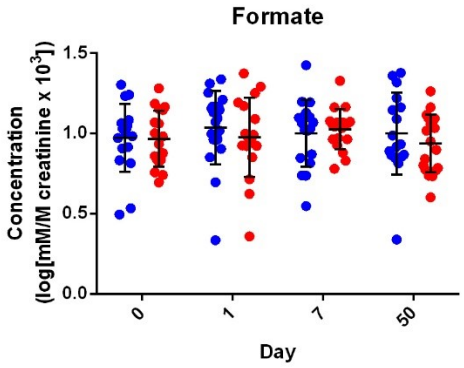

Chlorogenate
Controls
Welders
Concentration (log[mM/M creatinine x 10^3])
1.5
1.0
0.5
0.0
-0.5
0
1
7
50
Day

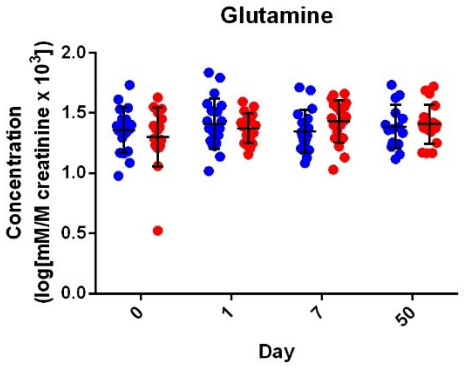

Citrate
*
Concentration (log[mM/M creatinine x 10^3])
3.0
2.5
2.0
1.5
1.0
0
1
7
50
Day

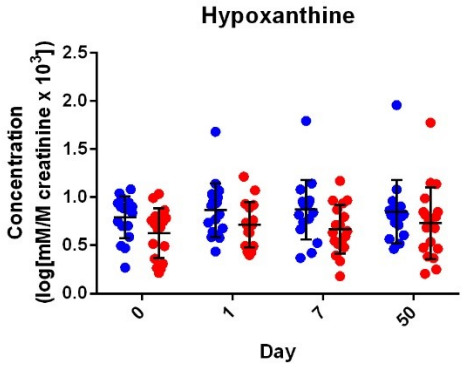

Dimethylamine
Concentration (log[mM/M creatinine x 10^3])
1.7
1.6
1.5
1.4
1.3
1.2
0
1
7
50
Day

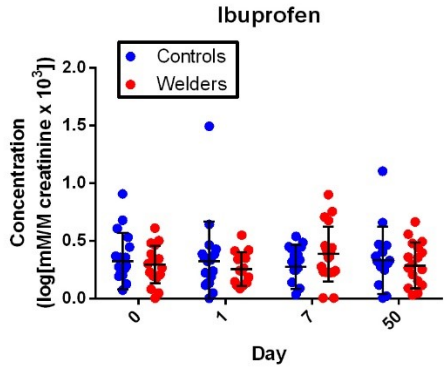

Ethanol
Concentration (log[mM/M creatinine x 10^3])
4
3
2
1
0
0
1
7
50
Day

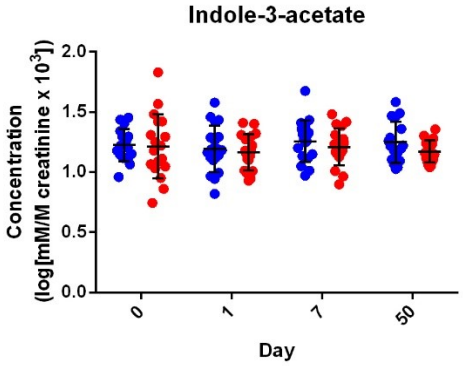

Ethanolamine
Concentration (log[mM/M creatinine x 10^3])
2.0
1.8
1.6
1.4
1.2
0
1
7
50
Day

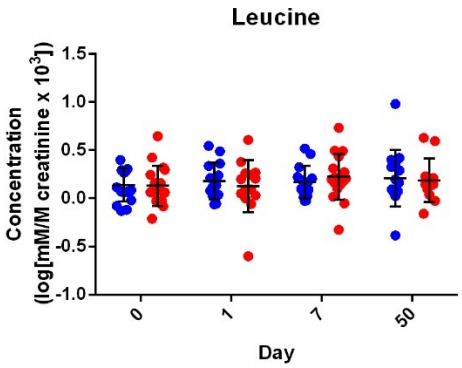

Formate
Concentration (log[mM/M creatinine x 10^3])
1.5
1.0
0.5
0.0
0
1
7
50
Day

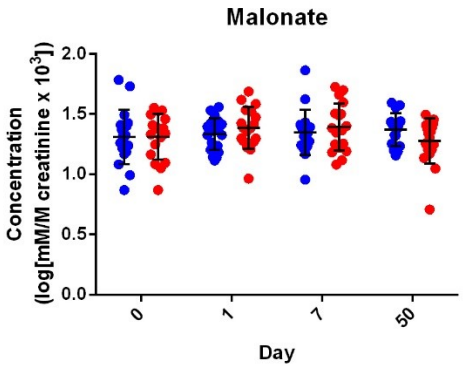

Glutamine
Concentration (log[mM/M creatinine x 10^3])
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

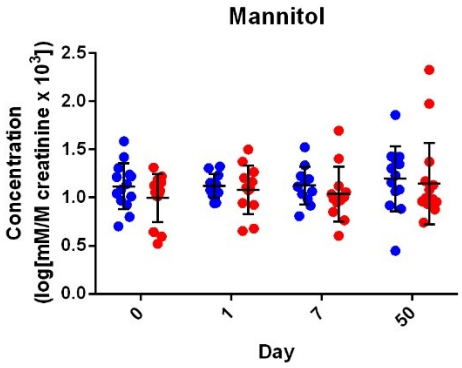

Hypoxanthine
Concentration (log[mM/M creatinine x 10^3])
2.5
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

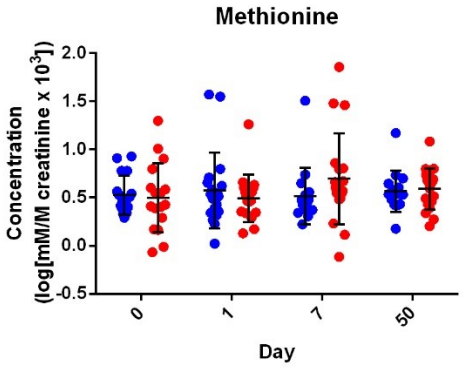

Ibuprofen
Controls
Welders
Concentration (log[mM/M creatinine x 10^3])
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

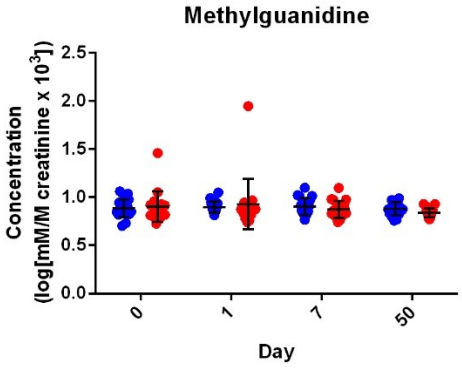

Indole-3-acetate
Concentration (log[mM/M creatinine x 10^3])
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

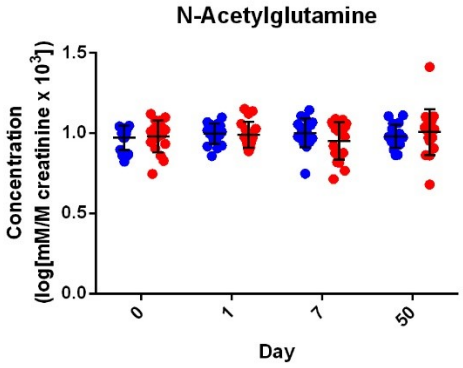

Leucine
Concentration (log[mM/M creatinine x 10^3])
1.5
1.0
0.5
0.0
-0.5
-1.0
0
1
7
50
Day

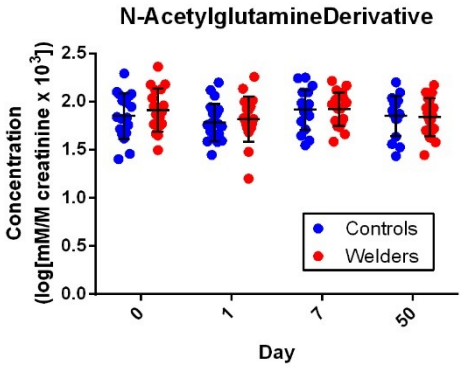

Malonate
Concentration (log[mM/M creatinine x 10^3])
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

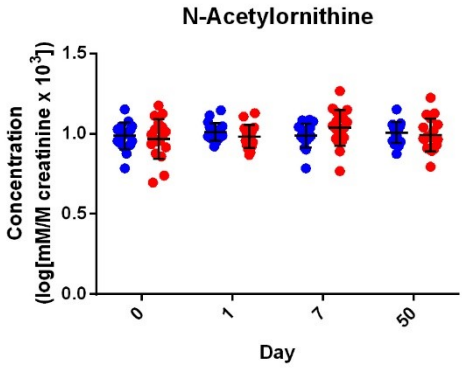

Mannitol
Concentration (log[mM/M creatinine x 10^3])
2.5
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

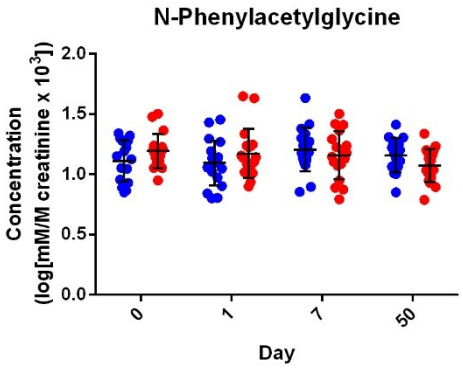

Methionine
Concentration (log[mM/M creatinine x 10^3])
2.0
1.5
1.0
0.5
0.0
-0.5
0
1
7
50
Day

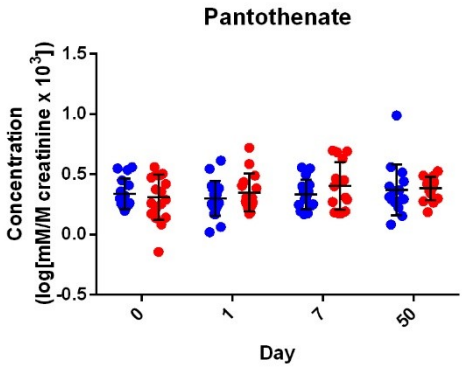

Methylguanidine
Concentration (log[mM/M creatinine x 10^3])
2.5
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

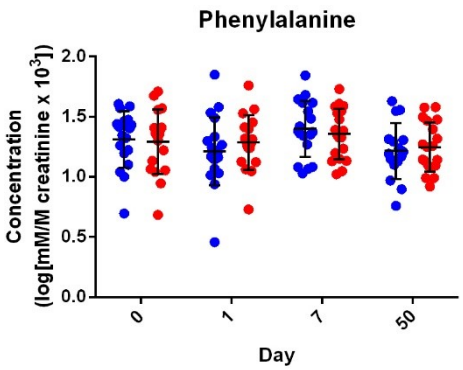

N-Acetylglutamine
Concentration (log[mM/M creatinine x 10^3])
1.5
1.0
0.5
0.0
0
1
7
50
Day

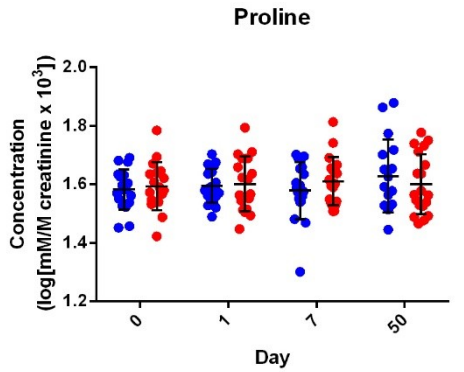

N-AcetylglutamineDerivative
Concentration (log[mM/M creatinine x 10^3])
Controls
Welders
Day

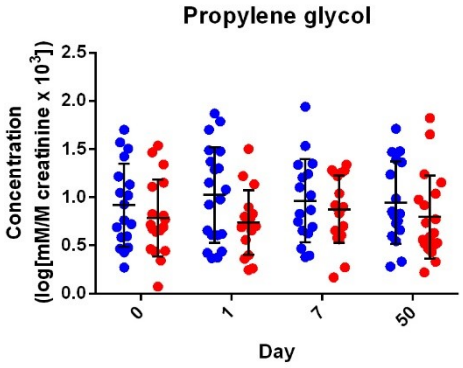

N-Acetylornithine
Concentration (log[mM/M creatinine x 10^3])
Day

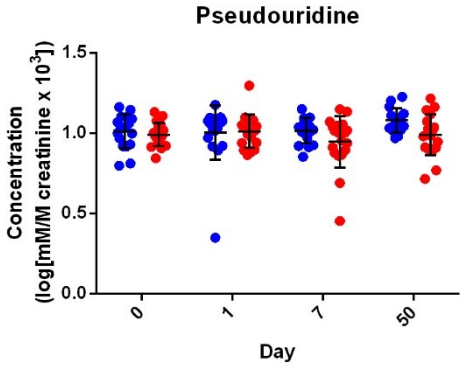

N-Phenylacetylglycine
Concentration (log[mM/M creatinine x 10^3])
Day

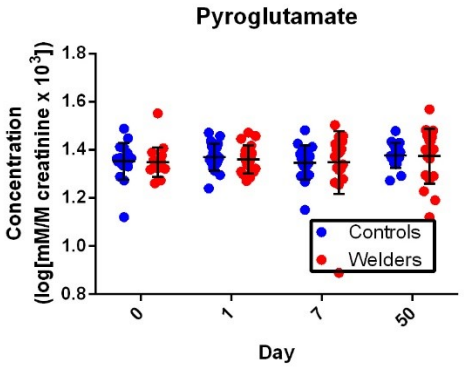

Pantothenate
Concentration (log[mM/M creatinine x 10^3])
Day

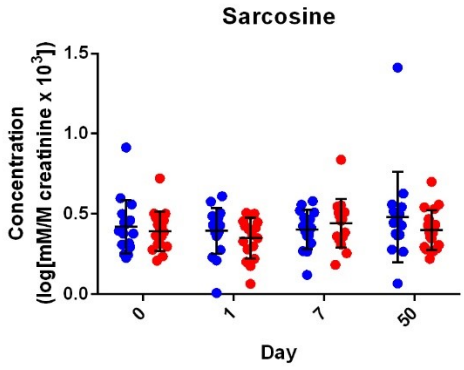

Phenylalanine
Concentration (log[mM/M creatinine x 10^3])
Day

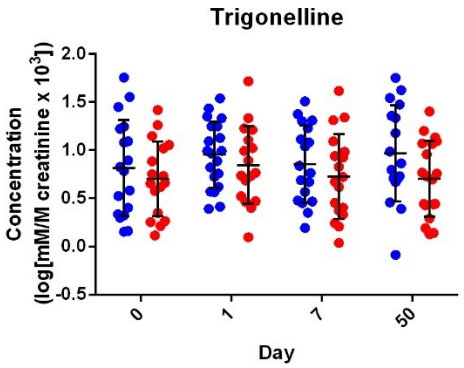

Proline
Concentration (log[mM/M creatinine x 10^3])
Day

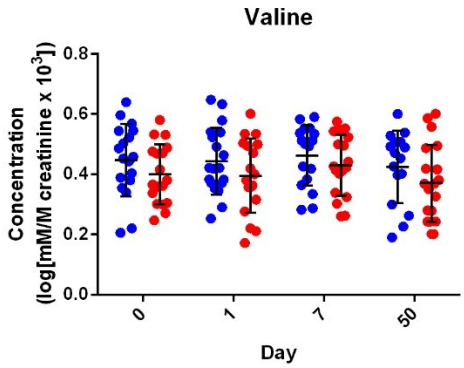

Propylene glycol
Concentration (log[mM/M creatinine x 10^3])
Day

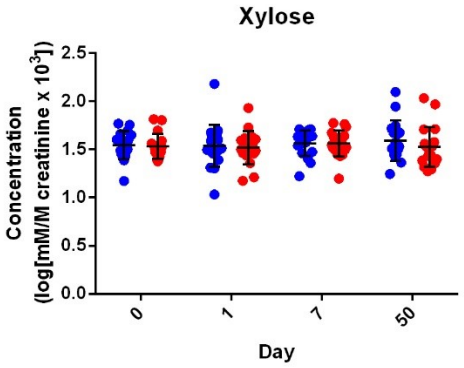

Pseudouridine
Concentration (log[mM/M creatinine x 10^3])
Day

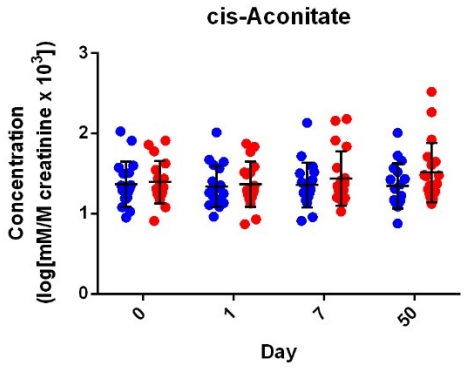

Pyroglutamate
Concentration (log[mM/M creatinine x 10^3])
1.8
1.6
1.4
1.2
1.0
0.8
Controls
Welders
0
1
7
50
Day

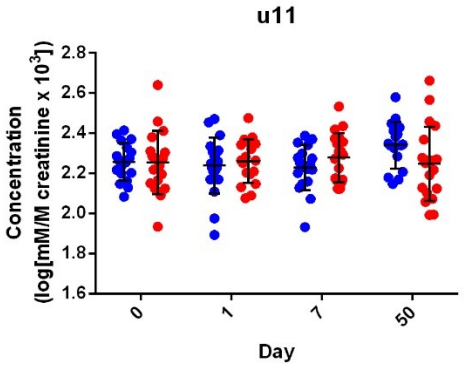

Sarcosine
Concentration (log[mM/M creatinine x 10^3])
1.5
1.0
0.5
0.0
0
1
7
50
Day

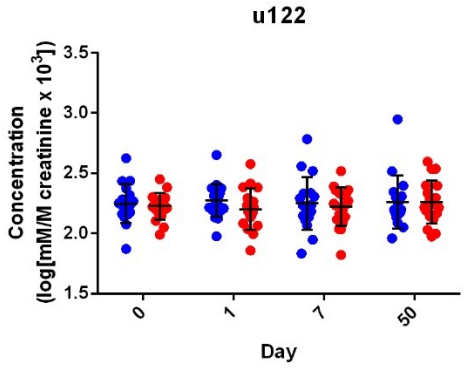

Trigonelline
Concentration (log[mM/M creatinine x 10^3])
2.0
1.5
1.0
0.5
0.0
-0.5
0
1
7
50
Day

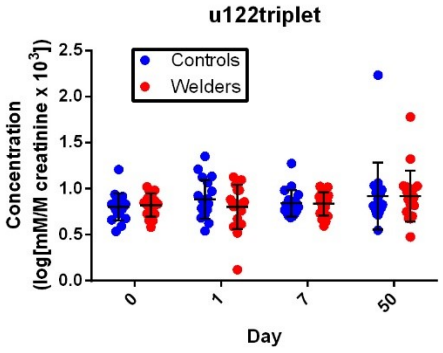

Valine
Concentration (log[mM/M creatinine x 10^3])
0.8
0.6
0.4
0.2
0.0
0
1
7
50
Day

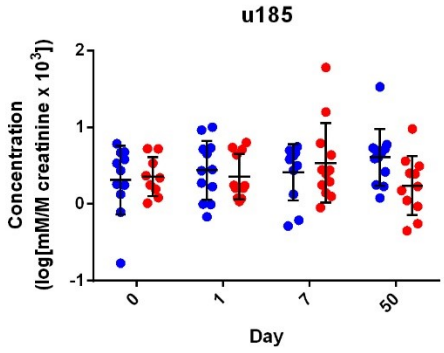

Xylose
Concentration (log[mM/M creatinine x 10^3])
2.5
2.0
1.5
1.0
0.5
0.0
0
1
7
50
Day

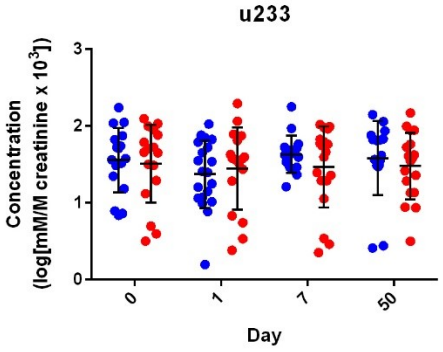

cis-Aconitate
Concentration (log[mM/M creatinine x 10^3])
3
2
1
0
0
1
7
50
Day

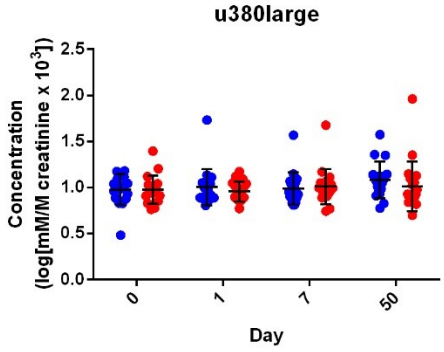

u11
Concentration (log[mM/M creatinine x 10^3])
2.8
2.6
2.4
2.2
2.0
1.8
1.6
0
1
7
50
Day

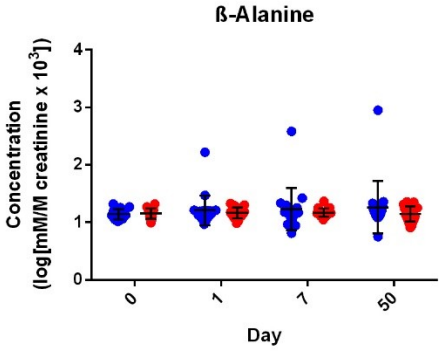

u122
Concentration (log[mM/M creatinine x 10^3])
3.5
3.0
2.5
2.0
1.5
0
1
7
50
Day

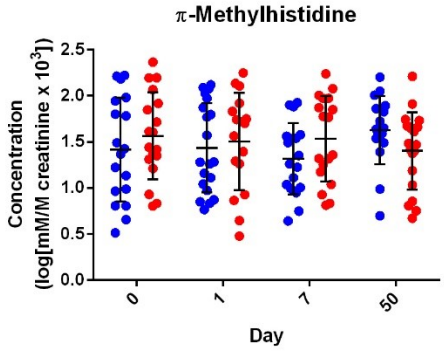

u122triplet
Controls
Welders
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50

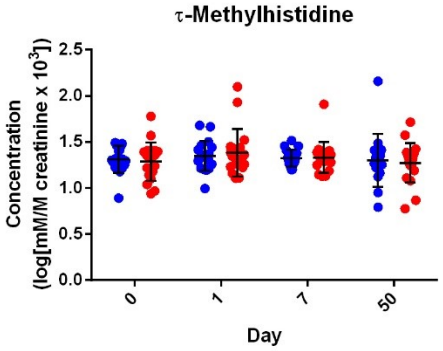

u185
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50


u233
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50


u380large
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50


ß-Alanine
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50


π-Methylhistidine
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50


τ-Methylhistidine
Concentration (log[mM/M creatinine x 10^3])
Day
0
1
7
50