Assessing the Applicability of Methods to Analyze Metals in Toenails

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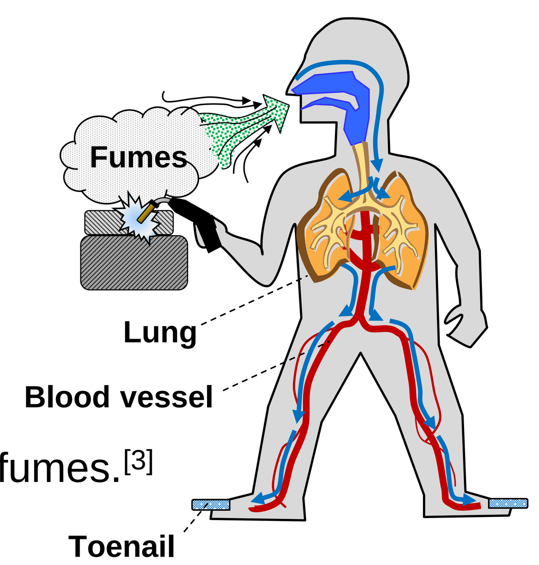
Background

➢ **Welding fumes**

- 574,000 employees in welding, soldering, and brazing occupations in US
- The majority are welders who are exposed to welding fumes.
- Hazardous metals in welding fumes[1]
  - Manganese (Mn): Parkinson's-like disorder
  - Iron (Fe): pneumoconiosis
  - Zinc (Zn): metal fume fever
  - Chromium (Cr): lung cancer (Cr-VI)



➢ **Metals in toenail as biomarkers**

- Inhaled metals deposit in toenails and other body parts.[2]
- Mn concentration in toenails was proposed as a biomarker of chronic exposure to welding fumes.[3]

➢ **Toenail metal analysis**

- No standard methods
- Inductively coupled plasma (ICP)−mass spectrometry (−MS) is widely used.
- ICP−optical emission spectrometry (−OES) or X−ray fluorescence (XRF) can be more appropriate for toenail metal analysis.

Methods	Limit of detection	Cost, time, & labor effectiveness	Sample destructiveness
ICP-MS	+++	+	Destructive
ICP-OES	++	++	Destructive
XRF	+	+++	Non-destructive

➢ **Relevance to National Occupational Research Agenda (NORA)**

- Sector programs of "Manufacturing" and "Construction"
- Core and Specialty Program of "Exposure Assessment"
- Cross-sector program of "Cancer, Reproductive, Cardiovascular and Other Chronic Disease Prevention"

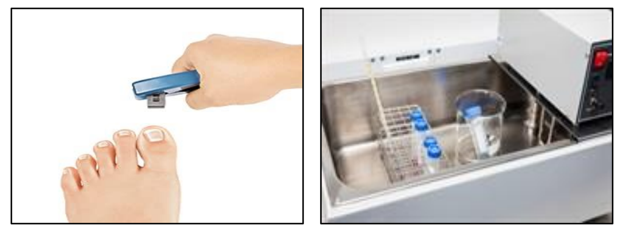
Objectives

➢ **Explore the applicability of ICP−OES and XRF for analyzing metals in toenails in comparison with ICP−MS.**

➢ **Hypothesis: ICP−OES and XRF can measure toenail metal concentrations as accurately as ICP−MS.**

- Specific Aim 1: Measure metal concentrations in toenails using ICP−OES and compare the results to ICP−MS measurements
- Specific Aim 2: Measure metal concentrations in toenails using XRF and compare the results to ICP−MS measurements

Experimental design



Collection Cleaning

➢ **Step 1: Sample collection**

- Clip toenails of subjects
- 20 welders and 20 non-welders

➢ **Step 2: Sample cleaning and weighing**

- Wash toenails in Triton X−100 (non-ionic surfactant, 1% in deionized water) using ultra-sonication for 1 hour
- Rinse with deionized water (ASTM II) 3 times
- Dry in an oven for > 24 hours and weigh using a microscale

➢ **Step 3: XRF measurement**

- Measure toenail phantom (95% of polyester resin, 5% of salt) containing known concentrations of metals
- Create calibration curves of metals
- Measure toenail samples using both portable−XRF (p−XRF, Niton XL3t 955 Ultra, Fisher Scientific) and benchtop−XRF (b−XRF, Epsilon 4, Malvern Panalytical)
- Normalize the measurement using Compton Scattering Ag anode peak
- Calculate metal concentrations in toenails using calibration curves and XRF measurements

➢ **Step 4: Sample digestion**

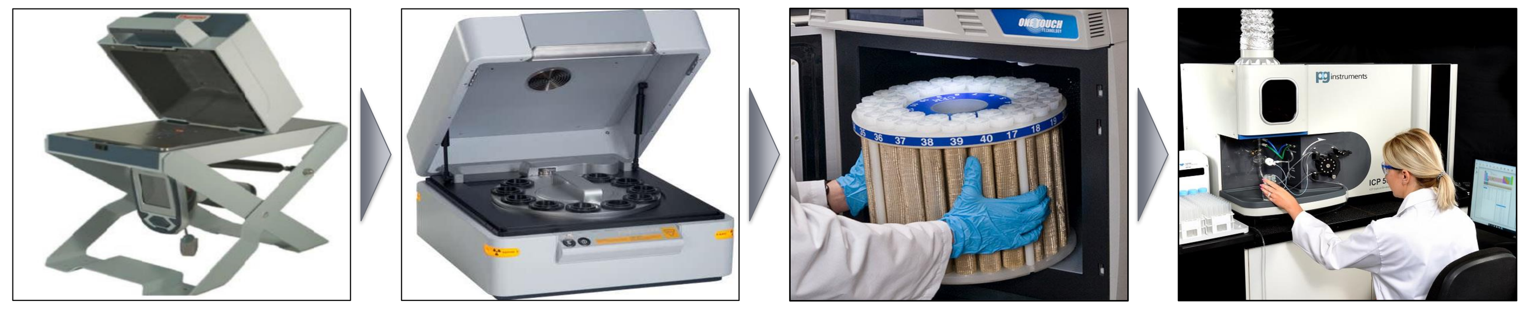
- Pre-digest toenail samples using 70% HNO_3 for 10 minutes
- Digest in HNO_3 using microwave digestion system (MARS 6, CEM co.) for 15 minutes at 200ºC
- Dilute 35-fold so that the HNO3 concentration is 2%

➢ **Step 5: ICP−OES and ICP−MS measurement**

- Measure the metal concentrations in toenails using ICP−OES and ICP−MS
- Analyze eight metals (Mn, Fe, Zn, Cr, aluminum (Al), copper (Cu), nickel (Ni), and cadmium (Cd))

➢ **Data analysis**

- Calculate toenail metal concentrations by dividing the metal mass obtained using XRFs and ICPs by toenail mass
- Calculate Pearson's correlation coefficients of ICP−OES, p−XRF, and b−XRF against ICP−MS
- Regression analysis to compare the results



p-XRF b-XRF Digestion ICP-OES & -MS

Expected results

➢ **Comprehensive data**

- Toenail metal level data of welders and non-welders
- Correlation factors of ICP-OES and XRFs with ICP-MS
- Potential correction factors to convert ICP-OES or XRF measurements to match ICP-MS measurements

➢ **Impact of results**

- Suggestion of appropriate methods for toenail metal analysis based on exposure levels, budget, and manpower
- Benefit for welders and other metal workers by providing exposure assessment through toenail metal analysis
- Potential to improve workplace safety and promote better health outcomes for workers

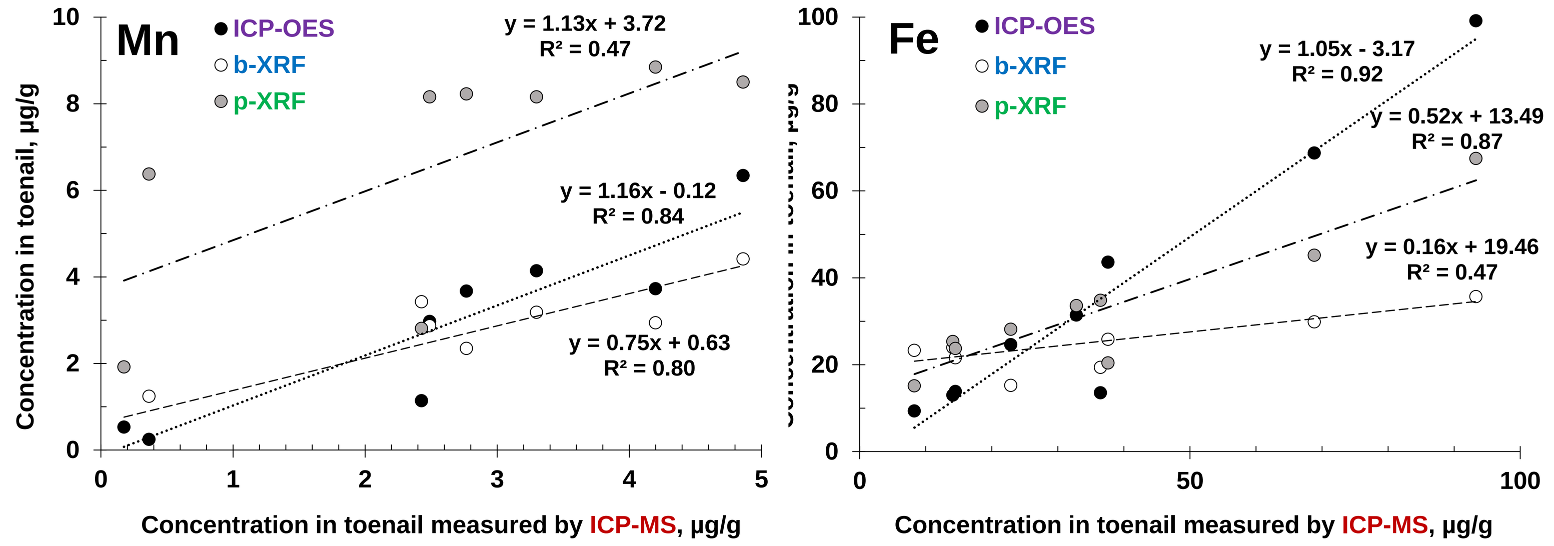
➢ **Research to practice**

- Provide a rapid, easy-to-use, inexpensive, and non-invasive method to analyze toenail as a biomarker
- Provide direct means to quantitatively evaluate the extent of exposure to metals
- Guideline to select an appropriate method to analyze toenail metals

Preliminary data

➢ **Comparison of ICP−OES, b−XRF, and p−XRF against ICP−MS (welders: n = 6, non-welders: n = 4)**

Method	Metal concentration in toenails, μg/g						
	Mn	Fe	Zn	Al	Cr	Cu	Ni
ICP-MS	2.6±1.7	36.5±28.0	94.7±27.9	28.2±37.6	1.0±2.6	5.4±1.8	0.1±0.3
ICP-OES	2.8±2.1	35.3±30.6	81.0±29.3	16.0±32.0	2.2±1.9	6.3±3.0	0.2±0.6
Pearson's r	**1.00**	**0.96**	**0.79**	**0.99**	0.22	-0.15	0.19
b-XRF	2.6±1.4	25.4±6.6	113.2±14.3	0.4±1.9	1.4±0.6	25.8±9.5	0.5±0.5
Pearson's r	**1.00**	**0.68**	0.18	0.3	-0.09	-0.24	-0.10
p-XRF	6.6±2.7	32.6±15.8	72.2±35.5	N/A	1.4±0.5	4.9±1.8	0.1±0.0
Pearson's r	**0.83**	**0.93**	**0.72**	N/A	0.51	0.15	0.52



- **ICP-OES** showed strong correlation with ICP-MS in **Mn, Fe, Zn, and Al** (r = 1.00, 0.96, 0.79, and 0.99, respectively).
- **b-XRF** showed strong correlation with ICP-MS in **Mn, and Fe** (r = 1.00, and 0.68).
- **p-XRF** showed strong correlation with ICP-MS in **Mn, Fe, and Zn** (r = 0.83, 0.93, and 0.72, respectively).

Future funding plan

➢ **Future funding plan**

- Investigation of the relationship between toenail metal concentrations and health outcomes (target: National Institute for Occupational Safety and Health (NIOSH) R21 or R01 grant)
- Development of evidence-based policies and regulations for protecting workers and the general population using toenail metal analysis as a tool for exposure monitoring (target: grant from National Institutes of Health (NIH), NIH Health Sciences (NIEHS), or Environmental Protection Agency (EPA)

References

[1] Antonini et al. 2003. *Critical Reviews in Toxicology* 33 (1): 61–103

[2] Hopps et al. 1977. *Science of the Total Environment* 7 (1): 71–89

[3] Ward et al. 2018. *Annals of Work Exposures and Health* 62 (1): 101–11

Acknowledgements

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