Toenail as Non-invasive Biomarker in Metal Toxicity Measurement of Welding Fumes Exposure – A Review

S F Z Bakri[1, 2], A Hariri[1], N F Ma'arop[2] and N S A W Hussin[2]

[1]Universiti Tun Hussein Onn Malaysia, Johor, Malaysia

[2]DRB-HICOM University of Automotive Malaysia, Pahang, Malaysia

E-mail: farhana@dhu.edu.my

Abstract. Workers are exposed to a variety of heavy metal pollutants that are released into the environment as a consequence of workplace activities. This chemical pollutants are incorporated into the human by varies of routes entry and can then be stored and distributed in different tissues, consequently have a potential to lead an adverse health effects and/or diseases. As to minimize the impact, a control measures should be taken to avoid these effects and human biological marker is a very effective tool in the assessment of occupational exposure and potential related risk as the results is normally accurate and reproducible. Toenail is the ideal matrix for most common heavy metals due to its reliability and practicality compared to other biological samples as well as it is a non-invasive and this appears as a huge advantage of toenail as a biomarker. This paper reviews studies that measure the heavy metals concentration in toenail as non-invasive matrix which later may adapt in the investigation of metal fume emitted from welding process. The development of new methodology and modern analytical techniques has allowed the use of toenail as non-invasive approach. The presence of a heavy metal in this matrix reflects an exposure but the correlations between heavy metal levels in the toenail must be established to ensure that these levels are related to the total body burden. These findings suggest that further studies on interactions of these heavy metals in metal fumes utilizing toenail biomarker endpoints are highly warranted especially among welders.

1. Introduction

Biological marker or biomarker is widely known as a one of the alternative method used to measure and observed on the outcome of the disease and medical state as the results is accurate and reproducible [1]. Mayeux, (2004) had categorised biomarker into two major groups: biomarker of exposure which are used in risk prediction, and biomarker of disease, used to screen, diagnose and monitor disease progression [2]. The markers of human tissue and excreta can be obtained by invasive and non-invasive methods where non-invasive is much preferred due to greater acceptability [3]. The applications of biomarker in assessing welder's health have been conducted in a few researches especially in investigating the impact of workplace setting to health. In recent years, a few authors have begun to measure heavy metals concentration emits from welding fumes by using a biomarker. However, the scope of biomarker used is limited to a specific method. Previous study has reported that the exposure to welding fumes especially manganese has significantly higher than controls and led to altered status of serum ferritin and transferrin receptor, TfR [4]. The findings had shown that serum ferritin and TfR have a similar adequate sensitivity and were associated with age-related changes [5]. However, the results is biased as serum ferritin is an acute-phase reactant and this lead to gender differences where the results normally lower in women and this make ferritin less ideal [6], [7]. Furthermore, the issues of timing, persistence, dose and storage site need to be considered for this banked serum [2]. From the current research, Baker et al., (2015) found out that manganese influences the magnetic resonance properties in surrounding tissues since manganese is part of a paramagnetic element [8]. Despite the fact that MRI is highly sophisticated and may presented high accuracy data, but this approach also require a proficient and knowledgeable operator to operate machine, time consuming and require a special software prior to automated analysis [9].

Data from several sources have identified the used of urine as biomarker in tracing toxic metals [10]–[12]. Along the same line, there is a research revealed that the metal concentration exposed from metal fumes appeared positively in urine samples among welders with higher readings of manganese and chromium in female welders [13]. This view is supported by Golbabaei et al., (2012) who writes that the concentration of metals in welder's urine is greater for chromium, cadmium and nickel respectively [14]. The proteins and polypeptides are quite stable in urine samples anyhow exosomes in urine represent a less stability due to changes of the temperature on sample storage [15], [16].

An assessment of occupational exposure and potential related risk then had been widely explored including implementation of blood as biomarker. In 1999, Bader et al. published a paper in which they described that blood is better than urine and/or hair in identifying manganese since blood is a highly complex tissue [17],[18]. In contrast to Bader, a number of studies argue that blood is not the recommended biomarker due to the different toxicokinetic profile where bloods represent recent exposures [19], [20]. Likewise, from the experiment of assessing metal fumes exposure among welders by using blood, urine and toenail; the researchers holds a view that toenail is preferred as a biomarker for long-term exposure assessment than blood and urine [21], [22].

Studies of biomarkers show the importance of invasive and non-invasive approach for a particular clinical study [23]. This approach offers some important insights into the observation of end points in a continuum of effects prominent from exposure of environmental agents to diseases; due to sensitivity, it is able to identify risk factors for the disease outcome and provide vast value to an environmental carcinogen; and non-invasive methods are fast becoming a key instrument in assessing metal toxicity compared to invasive methods because of greater acceptability [3]. This approaches provides immense mean values in identify risk factors for a disease due to its sensitivity and particularly beneficial in measuring the progressive diseases based on manifest symptoms due to prolong exposure to welding fumes. Therefore, the ability of biomarkers to detect medical state and heavy metals constituents in welders is promising. Biomarkers used to assist in extrapolation of available data to obtain the distinct data. Biomarkers may be classified into five categories: molecular lesions, metabolized exogenous agents, endogenously produced biomolecules, cellular/tissue changes and unchanged exogenous agents [3].

Since non-invasive is preferable than invasive method, thus, the study of metals due to welding fumes exposure by using biomarkers had undergo an extensive research. In view of all that has been mentioned so far, these studies provide important insight into the exploration of toenail as an assessment tools in evaluation of long-term occupational health impact due to metal fumes exposure towards welder.

2. Objective

This paper attempts to review on crucial sources of information on application of toenail as biomarker to identify heavy metal concentration in which later may adapt in the investigation of metal fume emitted from welding process. The reliability, validity, collection and sampling of metals in toenail due to welding activities were also assessed in this study. The purpose of this paper is to review research on toenail used as a biomarker to be incorporated in welding-metal fume studies. An overall brush up of welding fume biomarkers is crucially needed to give a clear picture and direction of future innovative and exploration of welding fume studies with potential health risks among welders.

3. Methods

This review of the world literature on metal concentration and toenail as biomarkers due to exposure to welding included a search of the online electronic databases published in English language in PubMed, Science Direct, and Scopus. The relevant bibliographies in identified paper were also taken into considerations and these efforts were not limited to put into use of Google and reference manager programme as a further confirmatory search tools. The literatures searched in the reference manager were published from 2010 to 2016 and these were focused on toenail as biomarker of heavy metal only. Each database was searched through April to June 2016. A general key words used to make the full review more focused in the search are: toenail, nail, heavy metal, welding fume, metal fume, exposure to metal, health risk, welder, metal toxicity and bio-monitoring. In obtaining a specific focused on reliability and validity of toenail, the search have been made through search engine of Science Direct, PubMed and Scopus by using term "toenail AND heavy metal AND air" within year 2010 to 2016 with the search details are ("nails"[MeSH Terms] OR "nails"[All Fields] OR "toenail"[All Fields]) AND ("metals, heavy"[MeSH Terms] OR ("metals"[All Fields] AND "heavy"[All Fields]) OR "heavy metals"[All Fields] OR ("heavy"[All Fields] AND "metal"[All Fields]) OR "heavy metal"[All Fields]) AND ("air"[MeSH Terms] OR "air"[All Fields]) AND ("2010/01/01"[PubDate] : "2016/12/31"[PubDate]). Science Direct had listed 20 articles journal and only 3 articles were significant with the research scope. Whilst, PubMed had listed 180 articles with only 6 articles were found relevant where one of the articles is the same articles as listed in the Scopus search engine.

4. Results

4.1. Toenail: Biomonitoring Human Exposure to Metals

There is a growing interest in the health assessment of heavy metal exposure as these elements may initiate an adverse health effect and have a potential to induce multiple organ damage, even at a lower level of exposure since it is considered as systemic toxicants [24]. Air, surface water and soil are the medium of heavy metals emissions to the environment and the existence is derived from a wide range of processes and pathways [25]. The toxicity of this heavy metals is depends on several factors including dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals [26]. The evaluation of metal exposure and prediction of human health risks is important and needed for decision maker as this have potential to minimize exposure risks [27]. Apart of metal exposure along any route of entry, it has long been recognized that, in occupational settings, a significant exposure to metal either in form of dust or fume can occur among welder through inhalation and may lead to adverse health effects.

Therefore, in assessing the subsequent biological effect and risk of metal toxicity in health of workers, the biomarkers is one of the best approaches to provide important information [3]. The variety of biomarkers for heavy metals is not focusing into one method only but the availability is largely different. Blood, hair, nails, and bone or urine specimens were used previously to assess heavy metals [28]. Heavy metal in welding fumes studies which have incorporated biomarkers to examine the heavy metal exposure via inhalation of welding metal fumes were carried out in Malaysia, Germany, France, Denmark, Saudi Arabia, and the United States [29]–[38]. In the literature on biomarker of heavy metals, the relative importance of toenail has been subject to considerable decision. This is by reason of toenail samples are non-invasive yet convenient to collect and store, grow more slowly compared to hair, not susceptible from external contaminants, and represent longer-term exposures than blood or urine [39]. This research has been supported by a previous study on the use of toenail as an attractive diagnostic tool in assessing heavy metals as it is an economical method, protected from infections and contaminations [40]. In investigating a concentration of heavy metals among workers, toenail is used to provide a better insight into biological marker of welding fumes. It is belief that a diversified heavy metals constitute in fine particulate matter were arise from welding fumes and the level of these metals in toenail is corresponded with concurrent environmental exposure [41]. Therefore, all research adopted toenail as a specimen used as metal biomarker were included in this reviewed paper. A non-invasive approach of toenail concentrations is preferred and suggested as a useful biomarkers because it provide a time-integrated measure of intake in body and represent a long exposure time frame due to relatively slow growth rate [28], [42], [43]. A finding on the presences of heavy metals in the toenail from a different medium of exposure in a various sample subjects worldwide has been presented in Table 1. The results shows that toenail have a capacity to be a reliable biomarker for heavy metal tracing and this would suggest that it may be a promise tools in determine the existence of metal fumes in welders.

Table 1. Application of toenail as biomonitoring tools in assessing heavy metal concentration.

Study Area	Medium of Exposure	Subject	Heavy Metals	Findings	References
South Korea	Food: consumption of fish and seafood in dietary intakes.	Middle-aged adults	Hg	Higher toenail levels of mercury were associated with a higher risk of dysregulation of lipids.	[44]
Pakistan	Air and food: Dust exposure, drinking water and food.	Adults and children	Cr, Mn, Co, Ni, Cu, Zn, Cd, Pb.	The bioaccumulation ratios (BAF) shows that nail is an effective tools to fingerprint long term trace metals exposure.	[45]
Cambodia	Water: drinking water from groundwater.	Residential community.	Mo, Co, Pb, Ni, Cr, As, Cu, Ba, Mn, Zn, Fe.	The levels of metals accumulated in toenail were similar for hair and corresponded with the levels of metals in the groundwater.	[46]
Zambia	Air: inhalation and exposure at mining industry.	Exposed and non-exposed community adults.	Cd, Co, Cu, Pb, Ni, Se, Zn.	Metal concentrations were found higher in toenail.	[47]
Qatar	Air, ingestion of food and water contamination.	Immigrant farm workers	As, Ba, Cd, Cu, Mn, Mo, Pb, Se, U	Toenail likewise accumulates both toxic and essential elements in farm workers but there is no association of metals was found between toenail and urine.	[48]
United States	Air: metal fumes exposure	Welder	Pb, Mn, Cd, Ni, As	All toenail metal concentrations were significantly correlated with one another.	[29]
Iraq	Severe contamination of water, soil, and air due to bombardment.	Adults and children (Fallujah families).	Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Pb, Th, U	The metal concentration level has been represented in toenails but hair contained significant higher amount than toenails.	[49]
United States	Air: metal fumes exposure	Welder	Mn	Manganese in the toenail appeared to have well correlated with 7 – 12 months exposure than blood or urine.	[22]

United Kingdom	Food: ingestion of contaminated soil and dust.	Residential community of the former mine site.	As	Total As concentrations in toenails from the exposed group were significantly elevated and lead to chronic exposure to high environmental contamination.	[50]
Kenya	Air: industrial and vehicle air pollution sources.	School age children	Pb, Cd, Zn, Fe, Ca	The levels of heavy metal in the nail were significantly influenced by both environmental and nutritional factors.	[40]
Northern Japan	Food: fish	Adults (women)	Hg	Toenail was significantly correlated with the estimated daily mercury intake.	[51]
Italy	Food: diet and other sources	Hospitalize male patients	Cd	Suggest that nails and toenails are a biomarker of environmental cadmium exposure.	[52]
Finland, Germany, Israel, Netherland, Norway, Russia, UK, Switzerland, Spain	Air: inhalation of atmospheric soil dust.	Adults (men)	Ce	A metal concentration in toenail level was associated with an increased risk of acute myocardial infarction (AMI).	[53]

4.2. Reliability and Validity of Toenails as Biomarker of Metal Fumes

Park et al., (2012) suggested that nail has a potential to act as a biomarker and might be a favourable tools to monitor the xenobiotic exposure and adverse health effects to human's anatomy [54]. Similar to welding fumes, tobacco smoke contained toxic materials and considerable amount of heavy metals such as cadmium, chromium, lead, nickel, aluminium, arsenic, copper, manganese, selenium and zinc [55]–[57]. Along these lines, based on research of tobacco smoke, it was decided that toenail is the best non-invasive biomarker to adopt for heavy metal investigation due to few factors: rapid growth, less exposure to external contamination, adequate sample availability and fusion of elements in the tissue [58], [59].

Finding from Sanders, Miller, Nguyen, Kotch, & Fry (2014) shows a positive results that seem to be consistent with other research which revealed a strong significant relation via Spearman's correlation of metal in toenail notably manganese, chromium, lead, cadmium and arsenic among Vietnamese children's lived in smelting craft village [60]. Although hair also is classified as non-invasive biomarkers alike toenails, by contrast, the exposure towards external contamination such as shampoo, dust, bleaching, dyeing and permanent waving may influenced the results and therefore, this is the main limitation of this biomarkers [23], [61]–[65]. Since, there is a limited study on the research of heavy metal concentration outlined in welders toenail, therefore, this review is focused on the correlation of heavy metal found by the utilization of toenail as non-invasive biomarker. The inter-comparison or the consideration of similar researches with regards to the heavy metal concentration traced in toenails via inhalation are discussed and has been presented in Table 2 and is intended to provide a clarification regarding the ability and capability of toenails as an effective tool to analyse heavy metal content in the human body. Indirectly, this comparison can reinforce a research and serve as a benchmark for measuring the use of toenails as an effective tool in the study and details the content of heavy metal in humans.

Apart of contaminated food and water, dust exposure may contribute to the burden of heavy metals in human body and approximately 50% of trace metal that exceed Agency for Toxic Substances and Disease Registry (ATSDR) is found in the Punjab's industrial areas, thus confirming on the health threat to human [45]. The studies presented thus far provide evidence that the accumulation of heavy metals found in the toenail samples is higher compared to other biomarkers and this are associated with industrial sites that had an elevated level of metals in dust which may be affected by demographic characteristics, sources contamination and a difference of exposure routes [66]. In a research of toenail concentration in mining industry, Ndilila et al., (2014) had mentioned that the metal concentration of cadmium, cobalt, copper, lead, nickel, selenium and zinc were greater where the toenail copper concentration marked 85% of the validity; and admitting the small sample size and one form of biomarker used are the limitation in this study, yet authors have been quantified the significance of toenail as long-term metal exposure [47]. Similarly, Sanders et al., (2014) found that six types of metal concentrations of cadmium, manganese, lead, chromium, mercury and arsenic had detected in the toenail of every participant and has suggested that heavy metal levels in the blood have reflect recent exposures on the order of days, whereas in the toenail the levels represent longer-term exposures till a month onwards [60].

To better understand the mechanisms of toenail and its validity, Grashow et al., (2013) had analysed the metal level in the toenail among construction workers that was exposed to metal fumes in welding hours [29]. Notably, the lead, manganese, cadmium, nickel and arsenic were detectable in the toenail clippings and there were a high correlation between toenail metals in each participant especially between manganese and cadmium. Realizing that the growth rate or clipping length of toenail is varies across individuals and may considered as a constraint in this study, this study had proven that variation would not affect the validity of the study results as it is not correlated with hours welded [29]. Thus, this outcome had supported the ability of toenail as biomarker since this biological sample may capture an internal exposure over specific time intervals and have a capability to reflect a longer term exposure. This view is supported a findings of Mordukhovich et al., (2012) who writes on the potency of toenail as biomarker in a study of a cohort of elderly men and a positive association between toenail arsenic with the blood pressure levels have been discovered [39]. Once confirmed, a little evidence of association of toenail cadmium, mercury and lead with blood pressure may give an important impact in the United States since there are no investigations have assessed relations between blood pressure with arsenic, cadmium, mercury, manganese, or lead by using toenails as biomarkers. In the same year, Amaral et al., (2012) had revealed the high relation of exocrine pancreatic cancer (EPC) risk and toenail concentrations of lead, selenium, nickel, cadmium and arsenic since this is first epidemiologic study conducted [67]. Despite the study design is a retrospective with a small and difference of the recruitment period of sample size and appears as a weakness of the study, the authors also highlight on the matching on residence area; equal age distributions; the simultaneous quantification of the trace elements in the same laboratory and under the same quality control procedures as the important strength in the research and this had present toenail as a reliable approaches in assessing past exposures.

In another major study, Al-Sabbak et al., (2012) found that most metals of aluminium, manganese, cobalt, copper, zinc, molybdenum, lead, thorium and uranium had contained a significant amount in the toenail [7]. The studies presented thus far provide evidence that toenail is able to detect a level of metal concentrations in human body with a longer exposure window. A research executed by Laohaudomchok et al., (2011) in the population recruited from welding school observed that toenail is more practical than fingernail and hair as both of samples are more frequently open to the external environment thus this subject to contamination of samples [8].

The wide range of process had influence the occurrence of heavy metals in environment for a long time and currently, due to the rapid growth of modernisation and technology, the exposure of heavy metals is increasing in some areas in the workplace. Air, water, soil and food are the pathway of environmental exposure and this normally require in-situ monitoring to evaluate the existence of heavy metals in environment. The common pathway for heavy metals in occupational exposure is via inhalation. Therefore, in assessing of heavy metals among worker due to occupational exposure, the implementation of toenail as biomarker requires a personal sampling at work area to ensure the data gain thru biomarker is conclusive.

Table 2. The inter-comparison of trace heavy metal concentrations (µg/g) reported worldwide in toenails for year 2010 – 2016.

Study Design	Element (µg/g)										Instruments	Authors
	Cd	Cu	Fe	Mn	Ni	Zn	Pb	Cr	As	Co		
Population based study	0.005 – 1.00	0.20 – 88.0	–	0.20 – 24.0	0.10 – 19.0	8.00 – 382.0	0.30 – 34.0	0.02 – 2.00	–	0.00 – 0.3	ICP - MS	[45]
Population based study	–	–	–	–	–	–	–	–	1.68	–	ICP – MS	[66]
Cross-sectional study	0.37 – 35.5	32.5 – 2225	–	–	0.37 – 33.8	62.0 – 599.0	0.8 – 158.0	–	<DL – 1.00	0.40 – 11.5	ICP – AES	[47]
Cross-sectional pilot study	1.07	–	–	15.6	–	–	957	2.09	1.09	–	ICP – MS	[60]
Retrospective study	0.02	–	–	0.81	2.19	–	0.35	–	0.17	–	DRC – ICP – MS	[29]
Cross-sectional study	1.97	–	–	8.82	–	–	14.70	–	1.68	–	ICP – MS	[39]
Retrospective study	–	–	–	0.05 – 10.41	–	–	–	–	–	–	ICP – MS	[22]
Case-control study	0.008 – 0.029	–	–	–	0.186 – 0.885	–	0.253 – 0.975	–	0.052 – 0.106	–	ICP – MS	[67]
Epidemiological case study	0.32	3.86	63.54	1.04	2.03	116.4	0.598	1.299	0.102	0.043	ICP – MS	[49]

ICP-MS = Inductively Coupled Plasma Mass Spectrometry, DRC-ICP-MS = Dynamic Reaction Cell-Inductively Coupled Plasma Mass Spectrometry, ICP-AES = Inductively Coupled Plasma Atomic Emission Spectrometry.

4.3. Strength and Limitation of Toenail

From the past study, it has conclusively been shown that dietary selenium from food intake did not influence the concentration of selenium in the toenail [68]. In the same way, the correlation research was consistent and supported by other study on traced manganese from toenail [22], [69]. On the other hand, there is a correlation observed between intakes of arsenic in dietary with arsenic concentration in toenail among subjects in Bangladesh [70]. The most likely causes of this connection are due to presence of high concentration of arsenic in staples food in Bangladesh diet: rice and vegetables. Regardless of Kile et al., (2007) findings, a few researchers had acknowledged that in order to monitor the occupational exposure, trace elements or heavy metals in nails to be widely accepted and analysed [40], [71], [72]. In the 1970s, Hopps pointed to some of the ways in which he concluded the trace element analysis from nail samples is precise, accurate and reproducible as well as the specimen is easy to obtain and store [73].

A considerable amount of literature has been published on the adoption of both fingernails and toenails as biomarkers to trace specific metal concentrations in human body and each represent particular strength and limitation [74]–[77]. There are a number of important differences between fingernail and toenail. Compare to toenail, fingernail growth faster which this conditions is accessible for a frequent sampling among subject [78]. Nonetheless, fingernail is commonly exposed to exogenous chemicals (i.e. medication and nail polish) therefore this might leads to contamination and may affect results such as overestimation of endogenous chemical amount in body [22], [28]. In spite of this constraint, such circumstance may turn into an opportunity to assess the impact of the toxic chemicals handling.

However, unlike fingernail, toenail is more prevalent and preserved from any exogenous exposure and this provide a better option to measure any relevant metal concentration from industrial setting. This technique is scrutinize as a reliable and convenient tool in assessment of exposure because less exposure to water than other non-invasive biomarker particularly fingernail, skin and hair [79]. Moreover, toenails are easier to collect and store and this indicate a practical advantage on this approach instead of blood and urine [22], [43]. The reliability of toenail as a long-term biomarker for metal exposure has been discussed and considered and provide a more stable measure for trace elements [20], [80]–[82]. The analysis of toenail expresses a long-term exposure which reflects 2 – 12 months before collection of specimen whereas urine and blood reflects current exposure of 3 – 4 days and 2 – 3 days respectively [65], [79], [83]. Hence, the toenail clipping for heavy metal assessment in target population is required to be done at least 2 months before so that the reading of heavy metal is meticulous [75]. According to Hinwood et al., (2003), the metabolic activities in the body will be isolated from the time when nails detached from skin which causes the accumulation of heavy metals concentration in toenail is reliable [84].

Sriram et al., (2012) stated that the slow growth rate of toenail compared to fingernail is the advantage of this approach over any biological fluid i.e. blood or serum because it's allow for continuous and chronological bio-monitoring of adverse exposure and may prove an advantageous in monitoring of long-term exposure and reflects any burden in human body and/or target organ [85]. Yet, the application of toenail as biomarker have been the subject of questioned by Angerer, Ewers, & Wilhelm (2007) which claimed to be hampered due to no standard operating procedure (SOPs) have been evaluated and published. The absence of SOPs for this approach is considered as limitation for this method [86].

4.4. Toenail as Non-Invasive Method: Collecting and Sampling

Toenail is the main non-invasive method used to determine a metal concentration in human body. The toenail from all toes of each subject were clipped and stored in small envelopes together with indication of toenail clipping date. This compound was prepared by adapting the procedure used by Laohaudomchok et al., (2011) [22]. The collection method of nail specimen is one of the more practical ways of retrieve and analyses of metals concentrations in epidemiological studies even the specimen has been stored for a months or years [87], [88]. According to Kile et al., (2005) and Sanders et al., (2014), a sufficient mass of toenail from all toes were collected in pooled by a registered nurse using a clean clipper and it is best if the metal concentration in the toenail can be analysed in authorized inter-laboratory nail testing to ensure an adequate quality assessment as the certified references material is insufficient [60], [70], [77]. Briefly, a modified version of Method 3050B, USEPA was used to extract the toenail [60]. Then, each sample was spiked with 10 μL of 1000 ppm gold stock solution in order to retain mercury for measurement. Next, 0.5 mL nitric acid (HNO_3), 0.1 mL hydrochloric acid (HCl), and 1 mL deionized water were added to the samples. The samples were heated at 95°C loosely capped to allow reflux for one hour. The samples were cooled, 1 mL of 30% hydrogen peroxide was added, and the samples were heated at 95°C for 15 min. The samples were cooled, 0.4 mL of HCl was added, and the samples were heated at 95°C for an additional 15 min. The samples were brought to a final volume of 10 mL using deionized water and vortex mixed. Samples were analysed using a Thermo X-Series II ICP- MS equipped with a dual gas collision cell. The limit of detection (LOD) among toenail metals varied for each individual because the available mass differed by sample, and a higher sample mass resulted in a lower LOD.

In order to remove external contamination from nails, Kile et al., (2005) stated the sonicating samples in a 1% Triton X-100 solution is used for 20 minutes [70]. After that, toenails were then rinsed repeatedly in Milli-Q water, dried, weighed, and digested in nitric acid at room temperature [22], [39], [60], [65]. Inductively coupled plasma–mass spectrometry (ICP-MS) were used in analysed heavy metals in specimens as described by Goulle et al., (2009) after the resultant solution was diluted to 8% HNO_3 [77]. In the same vein, other past researchers however suggested that apart of accessing protein from nails for diagnosis and molecular epidemiological research, DNA in toenail also can be analysed in this biomarkers and the quality of DNA remained constant even though it has been stored for a long period [89], [90]. These statement has been proven by Nakashima et al., (2008) that found out the total called rate for DNA in old nails was 95% or higher which mean the preserved nail clipping for a prolong time were equivalent with fresh clipping nails [91].

5. Conclusions

This systematic review of a few published studies highlights the importance of biological monitoring of heavy metals by using toenail. Based on the reviewed done, the findings of this research provide insights for application of toenail as a useful tool of metal toxicity measurement and this can be administered to welder in order to minimize the exposure of heavy metals and adverse health effect. According to the studies that had been done, the results suggest that toenail may be a useful alternative matrix and attractive biomarker for the detection of heavy metal in human body.

Conflict of Interest

The authors firmly declare that there are no conflicts of interest related to this work.

Acknowledgements

No funding was received.

References

[1] K. Strimbu and J. A. Tavel, ‘What are biomarkers?’, *Curr. Opin*. HIV AIDS, vol. 5, no. **6**, pp. 463–466, Nov. 2010.

[2] R. Mayeux, ‘Biomarkers: Potential uses and limitations’, *NeuroRX*, vol. 1, no. **2**, pp. 182–188, Apr. 2004.

[3] P. Kakkar and F. N. Jaffery, ‘Biological markers for metal toxicity’, vol. 19, no. January 2004, pp. 335–349, 2005.

[4] L. Lu, L. Zhang, G. J. Li, W. Guo, W. Liang, and W. Zheng, ‘Alteration of Serum Concentrations of Manganese, Iron, Ferritin, and Transferrin Receptor Following Exposure to Welding Fumes Among Career Welders’, *Neurotoxicology*, vol. 26, no. **2**, pp. 257–265, Mar. 2005.

[5] M. Olivares, T. Walter, J. D. Cook, E. Hertrampf, and F. Pizarro, ‘Usefulness of serum transferrin receptor and serum ferritin in diagnosis of iron deficiency in infancy.’, *Am. J. Clin. Nutr.*, vol. 72, no. **5**, pp. 1191–5, Nov. 2000.

[6] J. B. Wish, ‘Assessing Iron Status: Beyond Serum Ferritin and Transferrin Saturation’, *Clin. J. Am. Soc. Nephrol.*, vol. 1, no. Supplement 1, pp. S4–S8, Sep. 2006.

[7] WHO, ‘Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitamin and Mineral Nutrition Information System.’, Who, pp. 1–5, 2011.

[8] M. G. Baker, S. R. Criswell, B. A. Racette, C. D. Simpson, L. Sheppard, H. Checkoway, and L.

Sheppard, ‘Neurological outcomes associated with low-level manganese exposure in an inception cohort of asymptomatic welding trainees’, *Scand. J. Work. Environ. Heal.*, vol. 41, no. **1**, pp. 94–101, 2015.

[9] P. Mathuranath and P. Wattamwar, ‘An overview of biomarkers in Alzheimer′s disease’, *Ann. Indian Acad. Neurol*., vol. 13, no. **6**, p. 116, 2010.

[10] K. E. Pharr and B. T. Jones, ‘Extraction of Cadmium from Urine: A Brief Review’, *Appl. Spectrosc. Rev.*, vol. 42, no. **6**, pp. 563–572, Nov. 2007.

[11] G. F. Nordberg, ‘Biomarkers of exposure, effects and susceptibility in humans and their application in studies of interactions among metals in China’, *Toxicol. Lett*., vol. 192, no. **1**, pp. 45–49, Jan. 2010.

[12] M. Tellez-Plaza, E. Guallar, B. V Howard, J. G. Umans, K. a Francesconi, W. Goessler, E. K. Silbergeld, R. B. Devereux, and A. Navas-Acien, ‘Cadmium Exposure and Incident Cardiovascular Disease’, *Epidemiology*, vol. 24, no. **3**, pp. 421–429, May 2013.

[13] V. H. Arrandale, J. Beach, G. S. Cembrowski, and N. M. Cherry, ‘Urinary Metal Concentrations Among Female Welders’, *Ann. Occup. Hyg.,* vol. 59, no. **1**, pp. 52–61, Jan. 2015.

[14] F. Golbabaei, M. Seyedsomea, A. Ghahri, H. Shirkhanloo, M. Khadem, H. Hassani, N. Sadeghi, and B. Dinari, ‘Assessment of welders exposure to carcinogen metals from manual metal arc welding in gas transmission pipelines, Iran.’, *Iran. J. Public Health*, vol. 41, no. **8**, pp. 61–70, 2012.

[15] J. Wu, Y. Chen, and W. Gu, ‘Urinary proteomics as a novel tool for biomarker discovery in kidney diseases’, *J. Zhejiang Univ. Sci. B*, vol. 11, no. **4**, pp. 227–237, Apr. 2010.

[16] H. Zhou, P. S. T. Yuen, T. Pisitkun, P. A. Gonzales, H. Yasuda, J. W. Dear, P. Gross, M. A. Knepper, and R. A. Star, ‘Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery’, *Kidney Int*., vol. 69, no. **8**, pp. 1471–1476, Apr. 2006.

[17] M. Bader, M. C. Dietz, A. Ihrig, and G. Triebig, ‘Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries.’, *Int. Arch. Occup. Environ. Health*, vol. 72, no. **8**, pp. 521–7, Nov. 1999.

[18] M. Thambisetty and S. Lovestone, ‘Blood-based biomarkers of Alzheimer’s disease: challenging but feasible’, *Biomark. Med*., vol. 4, no. **1**, pp. 65–79, Feb. 2010.

[19] P. Olmedo, A. Pla, A. F. Hernández, O. López-Guarnido, L. Rodrigo, and F. Gil, ‘Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in human whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry’, *Anal. Chim. Acta,* vol. 659, no. **1–2**, pp. 60–67, Feb. 2010.

[20] S. L. O’Neal and W. Zheng, ‘Manganese Toxicity Upon Overexposure: a Decade in Review’, *Curr. Environ. Heal*. Reports, vol. 2, no. **3**, pp. 315–328, Sep. 2015.

[21] H. Hassani, F. Golbabaei, H. Shirkhanloo, and M. Tehrani-doust, ‘Relations of biomarkers of manganese exposure and neuropsychological effects among welders and ferroalloy smelters’, 2016.

[22] W. Laohaudomchok, X. Lin, R. F. Herrick, S. C. Fang, J. M. Cavallari, D. C. Christiani, and M. G. Weisskopf, ‘Toenail, Blood, and Urine as Biomarkers of Manganese Exposure’, *J. Occup. Environ. Med.,* vol. 53, no. **5**, pp. 506–510, May 2011.

[23] C. Jurado, P. Kintz, M. Menéndez, and M. Repetto, ‘Influence of the cosmetic treatment of hair on drug testing’, *Int. J. Legal Med*., vol. 110, no. **3**, pp. 159–163, Apr. 1997.

[24] J.-L. C. M. Dorne, G. E. N. Kass, L. R. Bordajandi, B. Amzal, U. Bertelsen, A. F. Castoldi, C. Heppner, M. Eskola, S. Fabiansson, P. Ferrari, E. Scaravelli, E. Dogliotti, P. Fuerst, A. R. Boobis, and P. Verger, ‘Human risk assessment of heavy metals: principles and applications.’, *Met. Ions Life Sci.*, vol. 8, pp. 27–60, 2011.

[25] L. Järup, ‘Hazards of heavy metal contamination.’, *Br. Med. Bull*., vol. 68, pp. 167–82, 2003.

[26] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, Heavy Metal Toxicity and the Environment, vol. 101. 2012.

[27] B. Yousaf, Amina, G. Liu, R. Wang, M. Imtiaz, M. S. Rizwan, M. Zia-ur-Rehman, A. Qadir, and Y. Si, 'The importance of evaluating metal exposure and predicting human health risks in urban–periurban environments influenced by emerging industry', *Chemosphere*, vol. 150, pp. 79–89, May 2016.
[28] K. He, 'Trace elements in nails as biomarkers in clinical research', *European Journal of Clinical Investigation*, vol. 41, no. **1**. pp. 98–102, 2011.
[29] R. Grashow, J. Zhang, S. C. Fang, M. G. Weisskopf, D. C. Christiani, and J. M. Cavallari, 'Toenail metal concentration as a biomarker of occupational welding fume exposure', *J. Occup. Environ. Hyg.*, vol. 11, no. **6**, p. 131227104636009, Dec. 2013.
[30] T. Weiss, B. Pesch, A. Lotz, E. Gutwinski, R. Van Gelder, E. Punkenburg, B. Kendzia, K. Gawrych, M. Lehnert, E. Heinze, A. Hartwig, H. U. Käfferlein, J.-U. Hahn, and T. Brüning, 'Levels and predictors of airborne and internal exposure to chromium and nickel among welders—Results of the WELDOX study', *Int. J. Hyg. Environ. Health*, vol. 216, no. **2**, pp. 175–183, Mar. 2013.
[31] M. G. Baker, C. D. Simpson, B. Stover, L. Sheppard, H. Checkoway, B. A. Racette, and N. S. Seixas, 'Blood Manganese as an Exposure Biomarker: State of the Evidence', *J. Occup. Environ. Hyg.*, vol. 11, no. **4**, pp. 210–217, Apr. 2014.
[32] A. Erdely, R. Salmen-Muniz, A. Liston, T. Hulderman, P. C. Zeidler-Erdely, J. M. Antonini, and P. P. Simeonova, 'Relationship between pulmonary and systemic markers of exposure to multiple types of welding particulate matter', *Toxicology*, vol. 287, no. **1–3**, pp. 153–159, 2011.
[33] S. Hulo, N. Chérot-Kornobis, M. Howsam, S. Crucq, V. de Broucker, A. Sobaszek, and J. L. Edme, 'Manganese in exhaled breath condensate: A new marker of exposure to welding fumes', *Toxicol. Lett.*, vol. 226, no. **1**, pp. 63–69, 2014.
[34] J. P. Bond, K. S. Hansen, and R. J. Levine, 'Fertility among Danish male welders', *Scand. J. Work. Environ. Heal.*, vol. 16, no. **5**, pp. 315–322, 1990.
[35] A. R. Sørensen, A. M. Thulstrup, J. Hansen, C. H. Ramlau-Hansen, A. Meersohn, A. Skytthe, and J. P. Bonde, 'Risk of lung cancer according to mild steel and stainless steel welding', *Scand. J. Work. Environ. Health*, vol. 33, no. **5**, pp. 379–386, Oct. 2007.
[36] N. Abdull, N. Wahida, M. Hassan, and A. R. Ismail, 'Heavy metal emitting from welding fumes in automotive industry', *Int. J. Curr. Res. Acad. Rev.*, vol. 2, no. **2**, pp. 148–156, 2015.
[37] M. A. Balkhyour and M. K. Goknil, 'Total fume and metal concentrations during welding in selected factories in Jeddah, Saudi Arabia', *Int. J. Environ. Res. Public Health*, vol. 7, no. **7**, pp. 2978–2987, 2010.
[38] R. Persoons, D. Arnoux, T. Monssu, O. Culi, G. Roche, B. Duffaud, D. Chalaye, and A. Maitre, 'Determinants of occupational exposure to metals by gas metal arc welding and risk management measures: A biomonitoring study', *Toxicol. Lett.*, vol. 231, no. **2**, pp. 135–141, 2014.
[39] I. Mordukhovich, R. O. Wright, H. Hu, C. Amarasiriwardena, A. Baccarelli, A. Litonjua, D. Sparrow, P. Vokonas, and J. Schwartz, 'Associations of toenail arsenic, cadmium, mercury, manganese, and lead with blood pressure in the Normative aging study', *Environ. Health Perspect.*, vol. 120, no. **1**, pp. 98–104, 2012.
[40] F. Hussein Were, W. Njue, J. Murungi, and R. Wanjau, 'Use of human nails as bio-indicators of heavy metals environmental exposure among school age children in Kenya', *Sci. Total Environ.*, vol. 393, no. **2–3**, pp. 376–384, Apr. 2008.
[41] J. Y. Y. Wong, S. C. Fang, R. Grashow, T. Fan, and D. C. Christiani, 'The Relationship Between Occupational Metal Exposure and Arterial Compliance', *J. Occup. Environ. Med.*, vol. 57, no. **4**, pp. 355–360, Apr. 2015.
[42] M. Garland, J. S. Morris, B. A. Rosner, M. J. Stampfer, V. L. Spate, C. J. Baskett, W. C. Willett, and D. J. Hunter, 'Toenail trace element levels as biomarkers: reproducibility over a 6-year period.', *Cancer Epidemiol. Biomarkers Prev.*, vol. 2, no. **5**, pp. 493–7, 1993.

[43] F. I. Abdulrahman, J. C. Akan, Z. M. Chellube, and M. Waziri, 'Levels of Heavy Metals in Human Hair and Nail Samples from Maiduguri Metropolis, Borno State, Nigeria', *World Environ.*, vol. 2, no. **4**, pp. 81–89, Aug. 2012.

[44] K. Park and E. Seo, 'Toenail mercury and dyslipidemia: Interaction with selenium', *J. Trace Elem. Med. Biol.*, vol. 39, pp. 43–49, Jan. 2017.

[45] J. Mohmand, S. A. M. A. S. Eqani, M. Fasola, A. Alamdar, I. Mustafa, N. Ali, L. Liu, S. Peng, and H. Shen, 'Human exposure to toxic metals via contaminated dust: Bio-accumulation trends and their potential risk estimation', *Chemosphere*, vol. 132, pp. 142–151, Aug. 2015.

[46] P. Chanpiwat, S. Himeno, and S. Sthiannopkao, 'Arsenic and Other Metals' Presence in Biomarkers of Cambodians in Arsenic Contaminated Areas', *Int. J. Environ. Res. Public Health*, vol. 12, no. **11**, pp. 14285–14300, Nov. 2015.

[47] W. Ndilila, A. C. Callan, L. A. McGregor, R. M. Kalin, and A. L. Hinwood, 'Environmental and toenail metals concentrations in copper mining and non mining communities in Zambia', *Int. J. Hyg. Environ. Health*, vol. 217, no. **1**, pp. 62–69, Jan. 2014.

[48] N. Kuiper, C. Rowell, J. Nriagu, and B. Shomar, 'What do the trace metal contents of urine and toenail samples from Qatar′s farm workers bioindicate?', *Environ. Res.*, vol. 131, pp. 86–94, May 2014.

[49] M. Al-Sabbak, S. S. Ali, O. Savabi, G. Savabi, S. Dastgiri, and M. Savabieasfahani, 'Metal contamination and the epidemic of congenital birth defects in Iraqi cities', *Bull. Environ. Contam. Toxicol.*, vol. 89, no. **5**, pp. 937–944, 2012.

[50] M. Button, G. R. T. Jenkin, C. F. Harrington, and M. J. Watts, 'Human toenails as a biomarker of exposure to elevated environmental arsenic', *J. Environ. Monit.*, vol. 11, no. **3**, p. 610, 2009.

[51] T. Ohno, M. Sakamoto, T. Kurosawa, M. Dakeishi, T. Iwata, and K. Murata, 'Total mercury levels in hair, toenail, and urine among women free from occupational exposure and their relations to renal tubular function', *Environ. Res.*, vol. 103, no. **2**, pp. 191–197, Feb. 2007.

[52] M. Vinceti, M. Venturelli, C. Sighinolfi, P. Trerotoli, F. Bonvicini, A. Ferrari, G. Bianchi, G. Serio, M. Bergomi, and G. Vivoli, 'Case-control study of toenail cadmium and prostate cancer risk in Italy', *Sci. Total Environ.*, vol. 373, no. **1**, pp. 77–81, Feb. 2007.

[53] J. Gómez-Aracena, R. A. Riemersma, M. Gutiérrez-Bedmar, P. Bode, J. D. Kark, A. Garcia-Rodríguez, L. Gorgojo, P. van't Veer, J. Fernández-Crehuet, F. J. Kok, and J. M. Martin-Moreno, 'Toenail cerium levels and risk of a first acute myocardial infarction: The EURAMIC and heavy metals study', *Chemosphere*, vol. 64, no. **1**, pp. 112–120, Jun. 2006.

[54] J. Park, D. Liang, J. W. Kim, Y. Luo, T. Huang, S. Y. Kim, and S. S. Chang, 'Nail DNA and possible biomarkers: A pilot study', *J. Prev. Med. Public Heal.*, vol. 45, no. **4**, pp. 235–243, 2012.

[55] M. Chiba and R. Masironi, 'Toxic and trace elements in tobacco and tobacco smoke.', *Bull. World Health Organ.*, vol. 70, no. **2**, pp. 269–275, 1992.

[56] M. W. Ashraf, 'Levels of heavy metals in popular cigarette brands and exposure to these metals via smoking.', *ScientificWorldJournal.*, vol. 2012, p. 729430, 2012.

[57] R. Caruso, R. O'Connor, W. Stephens, K. Cummings, and G. Fong, 'Toxic Metal Concentrations in Cigarettes Obtained from U.S. Smokers in 2009: Results from the International Tobacco Control (ITC) United States Survey Cohort', *Int. J. Environ. Res. Public Health*, vol. 11, no. **1**, pp. 202–217, Dec. 2013.

[58] W. K. Al-Delaimy and W. C. Willett, 'Toenail nicotine level as a novel biomarker for lung cancer risk', *Am. J. Epidemiol.*, vol. 173, no. **7**, pp. 822–828, 2011.

[59] J. A. Satia, I. B. King, J. S. Morris, K. Stratton, and E. White, 'Toenail and plasma levels as biomarkers of selenium exposure', *Ann. Epidemiol.*, vol. 16, no. **1**, pp. 53–58, 2006.

[60] A. P. Sanders, S. K. Miller, V. Nguyen, J. B. Kotch, and R. C. Fry, 'Toxic metal levels in children residing in a smelting craft village in Vietnam: a pilot biomonitoring study.', *BMC Public Health*, vol. 14, no. **1**, p. 114, 2014.

[61] R. R. Eastman, T. P. Jursa, C. Benedetti, R. G. Lucchini, and D. R. Smith, 'Hair as a biomarker of environmental manganese exposure', *Environ. Sci. Technol.*, vol. 47, no. **3**, pp. 1629–1637, 2013.

[62] T. Wang, J. Fu, Y. Wang, C. Liao, Y. Tao, and G. Jiang, 'Use of scalp hair as indicator of human exposure to heavy metals in an electronic waste recycling area', *Environ. Pollut.*, vol. 157, no. **8–9**, pp. 2445–2451, 2009.

[63] V. Bencko, 'Use of human hair as a biomarker in the assessment of exposure to pollutants in occupational and environmental settings', *Toxicology*, vol. 101, no. **1–2**, pp. 29–39, 1995.

[64] D. K. Harkins and A. S. Susten, 'Hair analysis: Exploring the state of the science', in *Environmental Health Perspectives*, 2003, vol. 111, no. **4**, pp. 576–578.

[65] G. Samanta, R. Sharma, T. Roychowdhury, and D. Chakraborti, 'Arsenic and other elements in hair, nails, and skin-scales of arsenic victims in West Bengal, India', *Sci. Total Environ.*, vol. 326, no. **1–3**, pp. 33–47, Jun. 2004.

[66] M. Subhani, I. Mustafa, A. Alamdar, I. A. Katsoyiannis, N. Ali, Q. Huang, S. Peng, H. Shen, and S. A. M. A. S. Eqani, 'Arsenic levels from different land-use settings in Pakistan: Bio-accumulation and estimation of potential human health risk via dust exposure', *Ecotoxicol. Environ. Saf.*, vol. 115, pp. 187–194, May 2015.

[67] A. F. S. Amaral, M. Porta, D. T. Silverman, R. L. Milne, M. Kogevinas, N. Rothman, K. P. Cantor, B. P. Jackson, J. A. Pumarega, T. López, A. Carrato, L. Guarner, F. X. Real, and N. Malats, 'Pancreatic cancer risk and levels of trace elements', *Gut*, vol. 61, no. **1110**, pp. 1583–1588, 2012.

[68] M. P. Longnecker, M. J. Stampfer, J. Stephen Morris, V. Spate, C. Baskett, M. Mason, and W. C. Willett, 'A 1-y trial of the effect of high-selenium bread on selenium concentrations in blood and toenails', *Am. J. Clin. Nutr.*, vol. 57, no. **3**, pp. 408–413, 1993.

[69] J. M. Guthrie, J. D. Brockman, J. S. Morris, and J. D. Robertson, 'The "one source" cohort - Evaluating the suitability of the human toenail as a manganese biomonitor', in *Journal of Radioanalytical and Nuclear Chemistry*, 2008, vol. 276, no. **1**, pp. 41–47.

[70] M. L. Kile, E. A. Houseman, E. Rodrigues, T. J. Smith, Q. Quamruzzaman, M. Rahman, G. Mahiuddin, L. Su, and D. C. Christiani, 'Toenail arsenic concentrations, GSTT1 gene polymorphisms, and arsenic exposure from drinking water', *Cancer Epidemiol. Biomarkers Prev.*, vol. 14, no. **10**, pp. 2419–2426, 2005.

[71] M. L. Kile, E. A. Houseman, C. V Breton, Q. Quamruzzaman, M. Rahman, G. Mahiuddin, and D. C. Christiani, 'Association between total ingested arsenic and toenail arsenic concentrations.', *J. Environ. Sci. Health. A. Tox. Hazard. Subst. Environ. Eng.*, vol. 42, no. **12**, pp. 1827–34, 2007.

[72] B. Nowak and H. Kozłowski, 'Heavy metals in human hair and teeth', *Biol. Trace Elem. Res.*, vol. 62, no. **3**, pp. 213–228, Jun. 1998.

[73] H. C. Hopps, 'The biologic bases for using hair and nail for analyses of trace elements', *Sci. Total Environ.*, vol. 7, no. **1**, pp. 71–89, Jan. 1977.

[74] M. Sakamoto, H. M. Chan, J. L. Domingo, R. B. Oliveira, S. Kawakami, and K. Murata, 'Significance of fingernail and toenail mercury concentrations as biomarkers for prenatal methylmercury exposure in relation to segmental hair mercury concentrations', *Environ. Res.*, vol. 136, pp. 289–294, 2015.

[75] M. J. Slotnick and J. O. Nriagu, 'Validity of human nails as a biomarker of arsenic and selenium exposure: A review', *Environ. Res.*, vol. 102, no. **1**, pp. 125–139, Sep. 2006.

[76] M. A. Davis, Z. Li, D. Gilbert-Diamond, T. A. Mackenzie, K. L. Cottingham, B. P. Jackson, J. S. Lee, E. R. Baker, C. J. Marsit, and M. R. Karagas, 'Infant toenails as a biomarker of in utero arsenic exposure', *J Expo Sci Env. Epidemiol*, vol. 24, no. **5**, pp. 467–473, 2014.

[77] J. P. Goulle, E. Saussereau, L. Mahieu, D. Bouige, S. Groenwont, M. Guerbet, and C. Lacroix, 'Application of Inductively Coupled Plasma Mass Spectrometry Multielement Analysis in Fingernail and Toenail as a Biomarker of Metal Exposure', *J. Anal. Toxicol.*, vol. 33, no. **2**,

pp. 92–98, Mar. 2009.

[78] S. Yaemsiri, N. Hou, M. M. Slining, and K. He, ‘Growth rate of human fingernails and toenails in healthy American young adults’, *J. Eur. Acad. Dermatology Venereol.*, vol. 24, no. **4**, pp. 420–423, 2010.

[79] B. M. Adair, E. E. Hudgens, M. T. Schmitt, R. L. Calderon, and D. J. Thomas, ‘Total arsenic concentrations in toenails quantified by two techniques provide a useful biomarker of chronic arsenic exposure in drinking water’, *Environ. Res.*, vol. 101, no. **2**, pp. 213–220, 2006.

[80] M. R. Karagas, ‘Measurement of Low Levels of Arsenic Exposure: A Comparison of Water and Toenail Concentrations’, *Am. J. Epidemiol.*, vol. 152, no. **1**, pp. 84–90, Jul. 2000.

[81] M. Hashemian, H. Poustchi, A. Pourshams, M. Khoshnia, J. D. Brockman, A. Hekmatdoost, C. C. Abnet, and R. Malekzadeh, ‘The Nail as a Biomonitor of Trace Element Status in Golestan Cohort Study’, *Middle East J. Dig. Dis.*, vol. 8, no. **1**, pp. 19–23, Sep. 2015.

[82] M. G. O’Doherty, C. C. Abnet, L. J. Murray, J. V. Woodside, L. A. Anderson, J. D. Brockman, and M. M. Cantwell, ‘Iron intake and markers of iron status and risk of Barrett’s esophagus and esophageal adenocarcinoma’, *Cancer Causes Control*, vol. 21, no. **12**, pp. 2269–2279, Dec. 2010.

[83] K. Phan, S. Sthiannopkao, and K.-W. Kim, ‘Surveillance on chronic arsenic exposure in the Mekong River basin of Cambodia using different biomarkers’, *Int. J. Hyg. Environ. Health*, vol. 215, no. **1**, pp. 51–58, Dec. 2011.

[84] A. L. Hinwood, M. R. Sim, D. Jolley, N. de Klerk, E. B. Bastone, J. Gerostamoulos, and O. H. Drummer, ‘Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations’, *Environ. Health Perspect.*, vol. 111, no. **2**, pp. 187–193, 2003.

[85] K. Sriram, G. X. Lin, A. M. Jefferson, J. R. Roberts, R. N. Andrews, M. L. Kashon, and J. M. Antonini, ‘Manganese accumulation in nail clippings as a biomarker of welding fume exposure and neurotoxicity’, *Toxicology*, vol. 291, no. **1–3**, pp. 73–82, Jan. 2012.

[86] J. Angerer, U. Ewers, and M. Wilhelm, ‘Human biomonitoring: State of the art’, *Int. J. Hyg. Environ. Health*, vol. 210, no. **3–4**, pp. 201–228, May 2007.

[87] P. Xun, K. Liu, J. S. Morris, M. L. Daviglus, and K. He, ‘Longitudinal association between toenail selenium levels and measures of subclinical atherosclerosis: The CARDIA trace element study’, *Atherosclerosis*, vol. 210, no. **2**, pp. 662–667, Jun. 2010.

[88] P. Xun, K. Liu, J. Steven Morris, M. L. Daviglus, J. Stevens, D. R. Jacobs, and K. He, ‘Associations of toenail selenium levels with inflammatory biomarkers of fibrinogen, high-sensitivity C-reactive protein, and interleukin-6’, *Am. J. Epidemiol.*, vol. 171, no. **7**, pp. 793–800, 2010.

[89] T. D. Anderson, J. P. Ross, R. K. Roby, D. A. Lee, and M. M. Holland, ‘A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework’, *J. Forensic Sci.*, vol. 44, no. **5**, pp. 1053–1056, 1999.

[90] S. G. Van Breda, J. G. Hogervorst, L. J. Schouten, A. M. Knaapen, J. H. van Delft, R. A. Goldbohm, F. J. van Schooten, and P. A. van den Brandt, ‘Toenails: An Easily Accessible and Long-Term Stable Source of DNA for Genetic Analyses in Large-Scale Epidemiological Studies’, *Clin. Chem.*, vol. 53, no. **6**, pp. 1168–1170, Apr. 2007.

[91] M. Nakashima, M. Tsuda, A. Kinoshita, T. Kishino, S. Kondo, O. Shimokawa, N. Niikawa, and K. -i. Yoshiura, ‘Precision of High-Throughput Single-Nucleotide Polymorphism Genotyping with Fingernail DNA: Comparison with Blood DNA’, *Clin. Chem.*, vol. 54, no. **10**, pp. 1746–1748, Aug. 2008.