COMPARISON OF BIOLOGICAL SPECIMENS FOR MANGANESE DETERMINATION AMONG HIGHLY EXPOSED WELDERS

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Abstract. This research aimed to determine if less invasive biological specimens (other than blood), such as feces and clipped toenails could be used to determine manganese concentrations among occupationally exposed human subjects. In addition to blood samples, which have routinely been used in determining manganese concentration, specimens were collected from welders working at the Electricity Generating Authority of Thailand, Mae Moh Thermal Power Plant, Lampang Province. Manganese concentrations in these three biological samples were determined by atomic absorption spectrophotometer. Correlations of manganese concentrations among these three biological samples were measured, and found to be rather poor (Pearson's $r < \pm 0.2$, $p > 0.1$ for any pair-wise comparisons). Blood remains the recommended material for biomonitoring manganese concentrations in occupationally exposed subjects.

INTRODUCTION

Manganese is one of the most abundant elements in the earth's crust, found in soil, sediment, rock, water, and biological materials (Parmeggiani, 1983). It is widely used in various industries, such as in alloys, steel, dry cell batteries, production of potassium permanganate, as well as being an oxidizing agent in the chemical industry. As a result, it causes a health risk to workers, known as manganese poisoning. There have been many reports concerning manganese toxicity, both acutely and chronically (ATSDR, 1997; Sato *et al*, 2000; Gerber *et al*, 2002).

A blood sample is the most commonly used specimen for manganese determination since the volume required is low and sensitivity is adequate (Buchet *et al*, 1976). On the other hand, feces and nail clippings are increasingly being used in trace element analysis. It was reported that measuring manganese levels in feces may serve as a useful guide for exposure (WHO, 1981). Determination of manganese in stool has been recommended as a group test for the evaluation of the level of occupational exposure to manganese (Herber and Stoeppler, 1994). Regarding nail clippings, in addition to their usage in studies on the proper balance of trace elements, analysis of toenail clippings from workers exposed to manganese has shown that the level detected gave information on the rate of exposure (Zaprianov *et al*, 1989).

To avoid inconvenience and the invasive approach of blood collection, together with the reasons outlined above, this study proposed the non-invasive use of feces and toenail clippings as alternative biological samples to determine manganese concentrations in occupationally-exposed subjects. Should the potential prove to be feasible, the expense of using such biological samples to determine manganese concentrations would be taken into account.

MATERIALS AND METHODS

The volunteers in this study were welders at the Electricity Generating Authority of Thailand (EGAT), Mae Moh Thermal Power Plant, Lampang Province. Inclusion criteria for the study

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subjects were: welders in direct contact with manganese, working in the welding section of the thermal plant for at least six hours a day, and granting consent. Those excluded from the study were welders with rheumatoid arthritis and anemia. For specimen collection, a blood sample was drawn by health personnel of the Mae Moh Thermal Power Plant's Health Facility as part of its annual examination. Three ml of blood sample was used to determine manganese concentration. The collection time was in the morning of a weekday towards the weekend. On the same day of blood collection, the welders were asked to bring their feces and toenail clippings, which were collected in the containers provided. These containers were previously soaked in 20% nitric acid, rinsed with deionized water and dried in a hot air oven at low temperature (<40ºC). Blood, fecal, and toenail specimens were stored in iceboxes and transported to the Faculty of Tropical Medicine, Mahidol University for manganese determination. Blood samples were stored at 4ºC and analyzed within four days. Feces and toenail clippings were also kept at 4ºC until analyzed.

In addition to specimen collection, welders were asked to fill in the self-administered questionnaire collecting their working information, *ie*, types of work prior to joining EGAT, how long they had been working with EGAT, types of job, personal protective equipment used, etc. Risk behaviors that might contribute to manganese concentration, such as smoking, tea and alcohol consumption, were included in the questionnaire. Moreover, welders were asked whether they had rheumatoid arthritis or anemia, since these two diseases interfere with the manganese concentration in the body (Cotzias *et al*, 1968; Mena *et al*, 1969). They were also asked if they possessed any symptoms of manganese poisoning, *eg* irritability, difficulty in walking, speech disturbances, and a mask-like face.

Whole blood analysis

The protocol for determining manganese in whole blood was slightly modified from that recommended by the Hitachi Company (Hitachi, 1988). The modification, based on preliminary testing, was applied in order to verify the interference and to produce a final reading in the range between two levels of seronorm used. Briefly, 50 µl of whole blood specimen was transferred to acid-washed polyethylene tube and 450 µl diluent (0.2% ammonium dihydrogenphosphate + 0.1% Triton X-100 solution) was added. The sample was then made homogeneous by inversion and analyzed for manganese concentration by graphite furnace atomic absorption spectrophotometer (Z-8200). The accuracy of the method was ascertained by comparison with known samples of Seronorm Trace Elements (Sero AS – whole blood 3, Sero, Asker, Norway). Operational parameters and settings were as recommended by Hitachi (1988).

Fecal analysis

Fecal analysis followed the wet ash method of Osis *et al* (1972), with minor modification. A step that was added to the protocol was the removal of water from the sample before digesting with the same reagent used by Osis *et al* (1972). Approximately two grams of feces was dried in a hot air oven of 85ºC, until constant weight was obtained. It was then transferred to a 250-ml Erlenmeyer flask. One ml of concentrated sulfuric acid, five ml of concentrated nitric acid and five ml of 30% hydrogen peroxide were subsequently added. The sample was heated on a hot plate (250ºC) for two hours until it became clear and weak yellow in color. It was cooled down to room temperature, transferred to a 50 ml volumetric flask and diluted to 50 ml with deionized water. A blank sample was prepared in the same manner. Twenty microliters of digested and blank samples were introduced into a graphite furnace atomic absorption spectrophotometer (Z-8200) for manganese determination. The operational settings for graphite furnace atomic absorption spectrophotometry in determining manganese in feces followed those of Hitachi (1988).

Toenail clipping analysis

The procedure of clipping analysis was modified from the method of Sohler *et al* (1976). A large amount of clipping was used due to the fact that subjects with fairly low manganese exposure tended to accumulate less manganese in their nails. Prior to analysis, organic materials on toenail clippings were removed by washing with acetone twice on a mechanical shaker, for 20 minutes each. Afterward, the clippings were dried in

hot air oven at 85ºC for 20 minutes. Approximately 20-30 mg of clippings was weighed out, put into a test tube and one ml of concentrated nitric acid was added. The test tube was placed in a block heater and heated to 100ºC for one hour. This temperature ensured complete digestion in one hour. After cooling down to room temperature, the solution was diluted to a volume of 10 ml with deionized water. The blank sample was prepared by the same protocol. Twenty microliters of the digested and blank samples were introduced into the graphite furnace atomic absorption spectrophotometer (Z-8200) and the manganese concentration determined under the same operational settings as the fecal sample.

Data analysis

Descriptive statistics were used to present manganese concentrations in whole blood, feces, and toenail clippings. Pearson's correlation coefficient was used to determine the relationships of manganese concentrations among these three biological samples. In addition, a standard cut point of manganese concentration in blood samples, 1.01 μg/dl, was employed to classify subjects into two groups: low- and high- manganese exposure concentrations. Both manganese concentrations in feces and toenail clippings were then compared between the two groups to identify whether the standard cut-point was applicable in these samples. Moreover, comparisons of potential risk factors and clinical symptoms between the two groups were investigated.

RESULTS

There was a total of 182 welders at the Mae Moh Thermal Power Plant. Subjects with rheumatoid arthritis and iron deficiency anemia were excluded from the study, due to the fact that they would have elevated levels of manganese in their whole blood (Cotzias *et al*, 1968), and were referred for proper care. Two subjects with either high or low outlying manganese in their feces, and two with outlying manganese in their toenail clippings, were also excluded. The remaining 135 subjects were taken for analysis. All were males working on average 31.3 (± 12.5) hours per week. The manganese concentrations in whole blood, feces and toenail clippings of these welders are shown in Table 1.

The correlation of manganese concentrations among the three biological samples is presented in Table 2. It was found that the correlation of manganese concentrations among these three biological samples was very poor (Pearson's $r < \pm 0.2$, $p > 0.1$ for any pair-wise comparisons). This seems to indicate that, from our data, feces and toenail clippings could not replace blood specimens in monitoring manganese levels in exposed

Table 1
Manganese concentrations in different biological samples.

Biological sample	N	Range	$\bar{X}$ ± SD
Whole blood (μg/dl)	135	0.38-2.37	1.20 ± 0.39
Feces (μg/g)	135	0.07-15.86	5.75 ± 3.28
Toenail clippings (μg/mg)	135	0.02-14.68	4.86 ± 3.13

Table 2
Correlation coefficients of manganese concentrations among whole blood, feces, and toenail clippings.

Biological sample	Whole blood	Feces	Toenail clippings
Whole blood	-		
Feces	-0.14		
	(p = 0.1)	-	
Toenail clippings	0.05	-0.11	
	(p = 0.6)	(p = 0.2)	-

subjects.

To confirm that the correlation did not exist, another approach was utilized to determine whether welders with high manganese levels in whole blood would likewise have elevated manganese levels in their feces and toenail clippings. According to baseline information from the Central Equipment Unit, Faculty of Tropical Medicine, Mahidol University, the normal concentration of manganese in human blood is 1.01 μg/dl. With this cut-point, welders in this study were divided into two categories, namely low- and high-blood manganese groups. Descriptive statistics for these two groups are shown in Table 3. Our data indicate that volunteers with high blood manganese do not necessarily have high manganese concentrations in their feces or toenail clippings.

Risk behaviors potentially accounting for high concentrations of manganese in exposed subjects were considered in both high- and low-concentration groups. These included smoking, number of cigarettes smoked per day, alcohol consumption, tea consumption, and types of personal protective equipment (PPE) used. These behaviors are summarized in Table 4.

The distribution of smoking, number of cigarettes smoked per day, alcohol consumption, tea consumption, and type of PPE used did not seem to play a role in manganese concentrations in the biological samples obtained from the welders in this study. Subjects with high blood manganese were similar to those in the low-blood manga-

Table 3
Descriptive statistics of subjects categorized as low- and high-blood manganese groups.

Biological sample	N	$\bar{X}$ ± SD
Feces		
Low-blood manganese	49	6.10 ± 2.92
High-blood manganese	86	5.58 ± 3.47
Toenail clippings		
Low-blood manganese	49	4.71 ± 2.72
High-blood manganese	86	4.95 ± 3.36

Table 4
Comparison of potential risk behaviors between low- and high-blood manganese groups.

Risk behavior	Grouping		Chi-square
	Low-blood manganese Number (% within group)	High-blood manganese Number (% within group)	
Smoking			0.789
Never	11 (22.4)	25 (29.1)	
Ex-smoker	23 (46.9)	35 (40.7)	
Yes, and regularly	15 (30.6)	26 (30.2)	
No. of cigarettes smoked per day			2.311
None	34 (70.8)	60 (70.6)	
Less than 10	8 (16.7)	20 (23.5)	
10 and more	6 (12.5)	5 (5.9)	
Alcohol consumption			2.219
Never	4 (8.2)	13 (15.3)	
Ex-consumer	40 (81.6)	60 (70.6)	
Yes and regularly	5 (10.2)	12 (14.1)	
Tea consumption			0.194
Never	27 (57.4)	44 (53.7)	
Ex-consumer	16 (34.0)	31 (37.8)	
Yes and regularly	4 (8.5)	7 (8.5)	
Type of PPE used			2.195
All available	2 (4.1)	10 (11.6)	
Either glove or mask	47 (95.9)	76 (88.4)	

nese group in terms of potential risk behaviors. Smoking and alcohol consumption were chosen as risk behaviors since they act synergistically with manganese in causing toxic effects (WHO, 1981). However, according to the responses to the questionnaire, none of the subjects showed any sign of clinical manganese poisoning. On the other hand, this is probably not the case with tea consumption. Even though the consumption of tea may add substantially to the daily intake of manganese (De, 1949; Schroeder *et al*, 1966; Meranger and Smith, 1972), only 11 subjects in this study consumed tea regularly. Of these, four (8.5%) of the 47 subjects were in the low-blood manganese group and seven (8.5%) of the 82 subjects were in the high-blood manganese group. The type of PPE used was not associated with manganese concentration either. Subjects who used either gloves or masks, or even all PPE available, had fair amounts of manganese in their blood.

Although it was not the purpose of this study to investigate manganese poisoning in these welders, they were asked whether they had any signs or symptoms of manganese poisoning. None of the volunteers reported any signs and symptoms of manganese poisoning. The most common symptoms found in the subjects of this study were back and muscle pain (86.7%), which might be the result of ergonomic working conditions. These symptoms appeared in both groups of welders, regardless of the manganese concentrations in their blood.

It was found that two symptoms were significantly different, between the high- and the low-blood manganese subjects. They were apathy (30.6% *vs* 8.2%, $p < 0.1$) and disturbance of libido (17.9% *vs* 39.6%, $p = 0.02$).

DISCUSSION

The manganese concentrations in whole blood revealed in this study were not higher than the maximum allowable manganese concentration of 8 μg/dl, as set by US National Institute for Occupational Safety and Health - NIOSH (Eller, 1996). One of the reasons could be the use of personal protective equipment (PPE). According to the responses to the questionnaire, all workers used PPE at all times while welding. It is also believed that specific welding techniques and the materials to be welded play an important role in manganese uptake (Elias *et al*, 1989).

Although manganese levels in feces may serve as a useful guide to exposure (WHO, 1981), the result of this study showed great fluctuations in manganese concentrations (5.75 ± 3.28 μg/g). Therefore, feces might not be an appropriate biological sample for biomonitoring occupational exposure. This is in accordance with Bergert *et al* (1982), who reported that there was no significant difference in manganese concentration in feces between controls and exposed workers.

This study also showed great variation in manganese concentrations in the toenail clippings of the subjects (4.86 ± 3.13 μg/mg), leading to the presumption that toenail clippings might also not be good biological material for biomonitoring manganese. This is supported by Kasperek *et al* (1982) who demonstrated that the elemental composition of nails is influenced by age, sex, and geographical location. Therefore, using nails as a diagnostic index for trace elements can be equivocal, given susceptibility of nails to external contamination.

In this study, years of working as a welder were not considered as seriously as they should have been despite being included in the questionnaire. This is because the half time for disappearance of manganese from the whole body is about 37 days (Mena *et al*, 1969).

Apathy and disturbance of libido were two common symptoms reported as being significantly different between the low- and high-blood manganese subjects. The association of these two symptoms is self-explained. It is also noted that these are classified as subclinical symptoms, according to Klaassen (1996).

In spite of the evidence that blood samples are routinely used in determining manganese concentrations in exposed subjects, an attempt has been made to investigate the possibility that less invasive biological specimens, such as feces and toenail clippings, be employed. Welders were included in the study. It was shown that there was no statistically significant correlation between manganese concentrations in the three biological

samples collected from the subjects. Therefore, blood is still the recommended biological material for biomonitoring manganese concentrations in occupationally exposed subjects. In terms of risk behaviors, it was found that smoking, number of cigarettes smoked per day, alcohol consumption, tea consumption, and type of PPE used did not play an important role in the manganese levels of these subjects. The most common symptoms were back and muscle pain which, appeared in both low- (<1.01 μg/dl) and high- (>1.01 μg/dl) blood manganese groups. In addition, high-blood manganese tended to have apathy and reduced libido, which have been classified as subclinical symptoms of manganese poisoning. No other signs, especially for chronic manganese poisoning, were prominently reported. In all, blood is still the recommended specimen for determining manganese concentrations in occupationally exposed subjects.

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