# *STUDIES IN THE USE OF HAIR AS A BIOPSY MATERIAL FOR ESTIMATING LEAD EXPOSURE.*

*A Thesis*

*Submitted in Fulfilment of the Requirement for the Degree of*

*Master of Philosophy in Chemistry*

*of*

*The University of The West Indies*

*by*

*GARVIN WILLIAMS*

*1993*

**Department of Chemistry**

**Faculty of Natural Sciences**

**St. Augustine.**

## ABSTRACT

**Studies In The Use of Hair As A Biopsy Material For Estimating Lead Exposure.**

**Garvin Williams**

This dissertation deals with the investigation of the use of hair as a biopsy material for estimating lead exposure. The Trinidadian population was thought to be suitable for this study because: (1) being a multiracial society, comparative studies of lead levels among the various racial groups could be assessed. Many of the studies reported in the literature were done on populations comprising a single race. (2) Trinidad uses leaded gasoline with a lead concentration of approximately 0.79g/L. Studies have shown that lead in gasoline causes enhanced lead levels in the environment which often result in increased blood and hair lead levels in individuals residing in areas close to highways and busy intersections.

The sample population consisted of one hundred and eighty nine volunteers belonging to four races: East Indian (N=91), African (N=49), Chinese (N=11), and Mixed (N=41). Also sampled were traffic police personnel (N=29) and battery workers (N=22). The hair samples were taken from the nape of the neck by cutting 2-3cm closest to the scalp and were washed using a washing procedure developed in our laboratory. The effectiveness of the

washing procedure was partly determined using scanning electron microscope analysis of the hair samples. Both hair and blood samples were acid digested using nitric/perchloric acid mixture and nitric/trichloroacetic acid mixture respectively and analyzed for lead using flame atomic absorption spectroscopy. The sulphur and carbon:sulphur ratios of the scalp hair sample were determined by elemental analysis.

The results of the investigation into scalp hair lead levels among the African, East Indian and Chinese racial groups indicated significant differences among the three races. As a result separate upper limits have to be set for each race. No significant differences were found for either pubic hair lead levels or blood lead levels among the three races. In each of the East Indian and Mixed racial groups, scalp hair, pubic hair and blood lead values between males and females were significantly different. However, no significant difference was found in the African group. No significant correlation was found between scalp hair lead and blood lead values of the general population but a difference was found between the scalp hair lead levels and the pubic hair lead levels. No significant correlation was found between the time spent in "traffic jams" or the time spent on the road in motor vehicles and scalp hair lead levels. Traffic police and battery plant operators sampled had a much higher average scalp hair and pubic hair lead levels than the non-occupationally exposed general population. No significant difference was found between the blood lead values of these workers and the general population. The percentage sulphur or the carbon:sulphur ratios in scalp hair did not

influence the scalp hair lead levels among the general population. A significant difference in the carbon:sulphur ratios was observed between African and East Indian racial groups.

Many problems associated with the use of hair as a bioindictor for detecting lead exposure still exist. Once these problems have been resolved, hair analysis is likely to gain more international recognition as a bioindicator for determining lead levels.

# *ACKNOWLEDGEMENTS*

*The author wishes to express his utmost appreciation to Dr. L. Hall and Dr. J. Addae, both of whom had made this work possible through their close and careful supervision, and their expert suggestions. He also extends his appreciation to Ms. Rachel Williams for her vital assistance in the statistical treatment of the data in this project.*

# DEDICATION

*TO RHONDA*

# *TABLE OF CONTENTS*

Page:

## *LIST OF FIGURES*

## *LIST OF TABLES*

CHAPTER ONE:

LITERATURE REVIEW

# CHAPTER ONE:

# LITERATURE REVIEW

## 1:1 HISTORY OF LEAD POISONING

Historically, the use of lead by human beings began about 9000 years ago[1]. A number of biblical references to lead have been made, some of which dealt with the refining of precious metals from lead containing ores. In addition, ancient ruins containing metallic lead artifacts dating back to the fifth and sixth millenia B.C., have been discovered in Turkey, Iraq and Egypt[1].

Despite the fact that lead was used in ancient times for making a variety of objects and utensils, its use did not attain industrial proportions until Roman times. The Romans developed an impressive lead technology after they began utilizing lead pipes for the transport of water. This resulted in an increase in the risk of lead poisoning associated with the use of lead and lead plumbing[1]. It was during this period that the first case of lead poisoning was reported. Also, at that time, a concentrated grape syrup, supa, was used for making wine and as a sweetening agent. This additive used in wine preparation contained significant amounts of lead which originated from the utensils used in its production and added to the problem of lead contamination. According to authentic Roman recipes, the lead used as a flavouring agent in wine was present at a level between 15 to 30 mg Pb/L[1]. Due to their consumption of large quantities of wine, the Roman aristocracy on average ingested between 160 to 1520 ug Pb/day compared to only 35 to 320 ug Pb/day for an average Roman citizen[1].

A significant number of cases of lead poisoning were reported during the Middle Ages as well, mostly due to the contamination of beverages with lead. Even as late as the eighteenth century alcoholic beverages were still being distilled using lead containers[1].

Many lead workers were known to be exposed to lead in the period of history known as the Industrial Revolution. Lead production rose during this period, increasing from about 100,000 tonnes per year, 300 years before, to one million tonnes per year. Lead production prior to the Industrial Revolution, had peaked at approximately 80,000 tonnes per year at the height of the Roman Empire and then declined[1]. During the Industrial Revolution era, a higher than normal incidence of sterility, abortion, stillbirth and premature delivery occurred in female lead workers and wives of male lead workers. Mental retardation, failure to thrive, convulsion and congenital lead poisoning were experienced by their offspring[1].

The first documented case of lead poisoning from contaminated paint occurred in Australia in 1890 while the first case in the United States was reported in 1914[1]. However, the severity of the poisoning problem from lead-contaminated paint was not recognized until the middle 1950's. For example, a report from a study done in Baltimore, USA, in 1956 revealed that 90% of the infants studied had blood lead concentrations in excess of 30 ug/dL which was higher than the presently accepted critical limit in children of 10 ug/dL proposed by the Centres for Disease Control (CDC) in the U.S.A.[2] This was attributed to lead found

in wall paint in the low-income area studied. Blood lead concentrations in excess of 60 ug/dL were found in 26% of these infants.

There was also great concern in the United States that a number of inner-city children had abnormally elevated blood lead levels (greater than 40ug/dL) during the period 1965-1970. Many researchers studying the blood-lead levels in children during this period found that a significant percentage of children had levels greater than this value. For example in 1965, Bradley[3] reported that 40% of the 333 children screened in a low income area of Baltimore had blood lead levels greater than 50ug/dL. Also in a New York city study, done in 1969, 45% of the children had blood lead levels greater than 40ug/dL. The number of children studied (2468) was considered to be a fair representation of the entire population. In all three studies, elevated blood lead levels in children were attributed to ingestion of lead-containing paints.

However, in 1976, Billick[4] studied blood lead levels in a sample of New York city children and found that there had been a consistent decline in blood lead levels from 36ug/100mL in 1970 to 22ug/100mL at the time the study was done. Also, a research project done in the United States by The Second National Health and Nutrition Survey (NHANES II) revealed that the mean blood lead values of 675,000 children (between 6 months- 5 years) sampled for the period 1976-1980 was 30ug/dL which represented a decrease from 1970[5]. Billick[4] had shown in his study that the blood lead levels in children correlated with gasoline lead levels. It should be noted that gasoline consumption started to decrease in 1970.

Therefore, the decreased lead levels in blood was most likely due to the decrease in gasoline consumption. Other factors responsible for the decrease in lead in the American environment and the associated decrease in blood lead levels were:

(1) The change in buying habits of the American automobile consumers. They started buying cars with low compression engines which used regular gasoline with a lower lead content.

(2) The phase-down in the allowable lead in gasoline called for by The Environmental Protection Agency (EPA) regulations.

(3) The increased activities related to lead pollution by other Federal Agencies. New regulations governing lead exposure of the population in general had been implemented.

Today, approximately three million tonnes of lead are produced worldwide each year. Due to poor antipollution practices, a significant amount of lead pollution seems to occur in third world countries. For example, it is reported that Nigeria is significantly affected by lead pollution. There are many lead smelters and mines in Nigeria and environ

mental safety precautions are virtually nonexistent. In addition, in 1991 gasoline sold in Nigeria contained 7ug/L of lead (The European Communities' upper limit is 1.5ug/L)[6].

The results of all these practices are:

1. street dust lead concentrations of 7000ug/g; one of the highest reported in the world and

2. a water lead concentration of 2ug/L. Countries like Glasgow in England and Boston, Massachusetts, U.S.A consider a water lead level of 0.5ug/L critical.

The high lead levels in soil, street dust and water is reflected in the ingested lead and blood lead concentrations of people living in Nigeria. Children in Lagos, Nigeria ingest between 150-1200ug/day of lead and have blood lead levels higher than that defined (25ug/dL) as critical by The Centers for Disease Control in the United States of America[6].

As in biblical times, the problem of lead pollution still exists in the present day environment. However, there is now a greater awareness of the problem coined by many as the 'silent epidemic'. As such, a number of countries have reduced the concentration of lead used in gasoline or have altogether started using unleaded gasoline. Also, industries are implementing preventative measures in factories in order to reduce the environmental levels of lead contamination.

## 1:2 LEAD IN THE LOCAL ENVIRONMENT

A major source of lead in Trinidad is from automobile exhaust emissions. It is estimated that approximately 6.84 tonnes of lead per month are emitted into the Trinidad environment by this means[7]. Further, it has been suggested that about 80% of the particulate lead emitted from gasoline combustion is less than 0.9 um in mean diameter, and fifty percent of these particulates can be absorbed by inhalation. The local population is therefore exposed to approximately 2.7 tonnes per month of these lead particles that can be absorbed via respiration[7].

There are also other major sources of lead contamination in Trinidad. These include (1) three battery manufacturing plants; (2) paint manufacturers; (3) printing enterprises; (4) ceramic industries and (5) many battery repair operations. These sources of lead may together represent greater health hazards than automobile exhaust emissions[7]. This is because lead emitted by these sources tend to be localized, resulting in high concentrations whereas lead from automobile exhaust emissions becomes delocalized due to diffusion along the roads and highways.

## 1:3 MAJOR PROJECT OBJECTIVE

Although the use of hair as a biopsy material is increasing, blood is still the most widely used indicator of human exposure to inhaled and ingested lead. The major objective behind this project was to further investigate the viability of replacing blood by hair as an indicator of lead exposure. The rationale for testing this alternative is now presented:

(1) Hair samples are more easily obtained and easier to manipulate than blood samples. Hair is also a stable material and can be stored for longer periods and at lower cost than blood. For third world countries like Trinidad and Tobago, the cost of analytical procedures used in assessing any environmental problem in general, and exposure to lead in particular, is of paramount importance due to existing economic problems.

(2) In spite of the fact that attempts have already been made to use hair as a biopsy material, convincing proof has to be forthcoming in order that acceptance by the recognized World Health Organizations is to be achieved. Therefore, this project attempted to gain additional evidence which might have been useful in fulfilling this goal.

Other related objectives were as follows:

(1) To investigate any significant differences between scalp hair and pubic hair lead levels, in order to determine the effects of contamination of scalp hair by exogenous lead. Pubic hair is not normally exposed to exogenous lead and it was assumed that comparisons of lead levels would help to shed further light on this problem.

(2) To determine whether there are any significant differences in scalp lead levels among the racial groups since. If differences were shown to exist, separate upper limits for normal lead exposure would then have to be established for each race in multiracial societies.

(3) To determine any correlation between hair texture and lead levels in scalp hair. Different races tend to have different hair textures which might be related to the carbon/sulphur ratios. Since absorption of lead by hair is apparently affected by the sulphur content it was decided to investigate whether any correlation existed.

(4) To study special groups such as industrially exposed persons and police personnel involved in traffic control, in order to investigate the relationships between hair and blood lead levels and occupational exposure.

(5) To compare our findings with respect to well-researched factors like sex and hair colour with findings in the literature.

(6) To investigate any relationship between blood and hair lead levels and any medical symptoms associated with enhanced lead levels in the human body.

## 1:4 ASSESSMENT OF INDICATORS OF LEAD EXPOSURE

There are many types of tissues in the human body that can be used for lead analysis. However some of them such as bone, kidney, brain and liver are not accessible from living individuals. Specimens readily available for analysis include blood, hair, teeth, urine and nails[8]. There are disadvantages associated with the use of urine, teeth and nails that make them unpopular, especially for routine testing. Lead levels from urine samples reflect the amount of lead that is being excreted from the body but not the amount of lead the body actually contains[8]. Healthy teeth are difficult to obtain and little information about nails is available.

Thus, hair and blood remain the most appropriate specimens for lead analysis. Their worth as bioindicators is dependent on their capacity to store trace elements. In the case of blood, trace elements spend a maximum of a few days after which they are deposited in other parts of the body. Hair, however, is a storage organ and retains trace elements over an extended period of time[8]. Hence, hair can provide information on the lead intake of the body over several months while blood lead levels only indicate the extent of recent exposure. It must be emphasized that these two indicators do not represent different means of assessing the same information but reflect the body's trace element status for different time periods.

In spite of the fact that researchers and medical practitioners traditionally use blood as a diagnostic tool there are instances where it has been proven to be an inadequate medium. Although lead in blood is still the most commonly used index for measuring exposure to lead it does not appear to be the ideal indicator for the following reasons[8]:-

(1) Lead in blood has a half-life of about one month after which the lead is deposited in the bones and hair. Hence, blood lead levels may not give a true indication of exposure when such exposure is on an intermittent basis.

(2) Lead can be quite suddenly released from bones. This results in a person's blood lead concentration increasing significantly when such conditions exist compared to concentrations prior to the release.

(3) The sensitivity of blood to numerous influences results in many constraints as to its use as a bioindicator. Blood is a complex and heterogenous medium where all the parts exhibit different concentrations of trace elements. It is not certain which of the following - whole blood or its components plasma, serum, leucocytes, or erythrocytes - offer a reliable reflection of the body status of trace elements. Trace elements in the blood are not fixed to any of these components but are constantly transferred from one component to another. The parts are in equilibrium with each other[8]. In addition, there appears

to be a wide range of factors involved in the binding of lead to the haemoglobin which is a component of the erythrocytes. The protein in blood provides a matrix onto which metals are strongly bound, resulting in difficulty in determining these protein-bound metals directly[9]. Many metals are present as complexes in blood but there is still much uncertainty about the mechanism of interaction between blood and metals. In order to remove trace elements from such complexes, two methods are generally employed. Chelating agents can be used to form a complex with the lead, or acid digestion of the blood can be used to decompose the blood-lead complex. Many of these procedures, however, have either a significant risk of contamination or a considerable decomposition time.



Given the difficulties with the use of blood as a specimen, hair deserves special consideration as an alternative since hair offers a number of advantages[8]:-

(1) Short term variations are averaged out in hair, when hair representing a few weeks of growth is sampled and its trace element concentration measured. From this, one can obtain an average value for a certain period of time. This can be done by cutting the hair into similar sections (each section representing a particular time period) and analyzing each separately or by taking samples

periodically eg. monthly. This allows for a better assessment of the normal trace element concentrations in the body.

(2) Hair is inert, chemically homogeneous and forms a permanent structure. Once trace element atoms are incorporated into it, they become fixed. In addition, there are no problems caused by deterioration during storage prior to analysis.

(3) Hair-lead concentrations are comparatively higher than the values obtained from other tissue specimens and therefore measurements are less subject to error.

(4) Compared to blood, urine and other tissue specimens, hair can be collected quickly, easily and without pain or embarrassment to the donors. In addition, collection requires no special training or equipment.

(5) Samples may be taken retrospectively, since hair provides a record of past as well as present trace element levels. For example, if a pregnant woman has been diagnosed for lead poisoning, a sample can be taken to discover her condition prior to conception.

Although these advantages clearly make hair an attractive alternative for trace element testing, a number of criticisms have been made which require consideration.

Firstly, there exists some variation in the trace element concentrations of the hair across the scalp. However, these variations tend to be small when compared to the variations found between individuals. These small diversities in the hair-lead values obtained from one patient may be due to hair samples of differing lengths being taken from different locations on the head. This problem can be resolved, by sampling from only one specific region of the head. The nape of the neck is usually regarded as the most appropriate place since it is least exposed to exogenous contaminants[10].

Secondly, there is the problem of exogenous contamination of the hair with trace elements. For example, hair treatment effects must be considered. Mild hair preparations, such as conditioners and hair sprays do not leach out trace elements but bleaching and dyeing do[11]. In order to avoid incorrect results, account must be taken, where necessary, of the use of hair treatments. As a result of exogenous contamination it is difficult to always differentiate between systemic trace elements and non-systemic trace elements in hair. However, research has shown that the least amount of diffusion occurs in hair closest to the root[10]. Thus this segment gives a better exposure index than other segments and provides, at least, a partial solution to the problem.

Another alternative for reducing the problem of exogenous contamination associated with scalp hair is to use pubic hair since it is not usually subjected to the application of cosmetics and shampoos that may contain heavy metals. However, it grows more slowly than scalp hair and therefore when comparing pubic hair with scalp hair the growth rate of each would have to be taken into consideration[10].

Thirdly, choosing an effective washing procedure poses problems. In the literature there is a variety of washing procedures, ranging from methods involving distilled water to complicated procedures involving organic solvents. The acetone washing procedure is accepted by The International Atomic Energy Agency[12]. However, this washing procedure has been criticized because acetone removes less exogenous trace elements than ether, EDTA or detergent washing procedures and thus acetone-washed samples do not give as good an indication of systemic lead as samples washed using the ether, EDTA or detergent washing procedures[13].

At the present time it is not possible to deduce whether some trace elements are extracted from the hair by the washing methods employed. As a result, it is difficult to determine which is the most suitable washing procedure. However, comparisons can be made between washing procedures where the effects are shown to be similar and such comparisons should then be reasonably valid[14].

Finally, it must be remembered that the time scale for blood and hair is different and therefore one should not expect any clear-cut correlation between the concentrations in the hair and blood[11].

Since lead must enter the blood in order to produce detrimental changes to the body, it is important to know how much lead is in the blood. But, in terms of assessing exposure and health problems, lead in the hair seems to be a better indicator. Thus, a number of studies have shifted to using lead levels found in hair[12,13,14].

Nowadays, some international recognition is being given to scalp hair for the monitoring of heavy metal toxicity. For example, in Japan, Iraq and the United States, hair has been used to estimate the body burden of mercury[15]. More recently, similar reports on the use of hair as a bioindicator have been published. One study has used hair zinc analysis as one of the criteria for identifying children with increased body burdens[16]. Another has reported the scalp hair lead levels from children suffering from chronic plumbism, mostly from pica[17], while yet another study has compared the lead levels in antique and contemporary hair samples from both children and adults[18].

As cited in the literature, hair is of significant importance as a bioindicator for trace metal analysis. Hair therefore offers a possible alternative to blood as a bioindicator. In

many instances when trace element concentrations in the body are to be determined, hair can be the more appropriate bioindicator.

## 1:5 FACTORS AFFECTING TRACE ELEMENT CONCENTRATIONS IN HAIR

The major factors that affect the trace element concentrations in hair are[19]:

(1) the distance from the scalp from which the hair sample was taken;

(2) the anatomical location of the hair sample;

(3) the colour of the hair sample;

(4) the geographical location of the donor;

(5) dietary supplements and medications consumed by the donor and

(6) age, race and sex

### 1:5.a Length of Hair

A study done by Hambidge revealed that the length of the hair influenced trace metal concentrations. Hair samples used were from subjects who did not use treatments such as dyes or bleaches and each sample was separated into a proximal and a distal section. Analysis of these samples for copper showed that there was an increasing concentration gradient in progressing from the proximal to the distal end of the hair[19]. It was concluded

that since the distal region of the hair was exposed to the environment for a longer period of time, the contamination of this region was greater and hence the reason for the higher copper concentration.Other independent studies which showed similar trends were done by Grandjean[20], Fergusson et al[21], and by Renshaw et al[22]. Exogenous contaminants such as, dirt, sweat, and sebum were shown to be the cause of these differences.

### 1:5.b Anatomical Location

In humans, hair is distributed unevenly over the body and can be categorized into the following four groups-: pubic, scalp, facial and axillary hair. Many researchers have compared the effectiveness of hair obtained from these anatomical sites for the purpose of determining extent of exposure to lead.

Pubic and pectoral hair are not normally exposed to the environment and should be less affected by exogenous contaminants compared to hair obtained from other regions of the body. The uncertainty associated with exogenous contamination could be removed by using pubic or pectoral hair as a bio-indicator instead of scalp hair. However, it must be noted that pubic and pectoral hair grow more slowly than scalp hair and are more liable to be contaminated by sweat and sebum[19].

In addition to environmental contaminants, hair from different parts of the body are also exposed to different external conditions with respect to toiletries and medications. However, there is at present no experimental evidence to support the possibility that these factors contribute to the lead body burden.

One investigation compared the concentrations of zinc, cadmium, lead and copper in facial and scalp hair samples taken from the same individuals[19]. It was found that the scalp hair concentrations were from 2 to 20 times greater than those for the facial hair. Similar investigations showed mean scalp hair values to be significantly higher than those for pubic hair[19]. However, in a more recent study, the scalp and pubic hair from 15 healthy non-pregnant women showed no significant difference between their copper, zinc and manganese concentrations[19].

Since at present there is no means of differentiating between trace elements of exogenous and endogenous origin, one must be careful when lumping together data acquired from hair belonging to different anatomical sites.

### 1:5.c Hair Colour

Hair colour is another variable which can affect the concentration of trace elements in hair. The colour of one's hair is influenced by the concentration of melanin pigment in the cortex. Blonde or white hair has less melanin than dark hair. Red hair contains an additional pigment called siderin. It was suggested that since the siderin pigments contain iron and since copper is used as a cofactor for the enzyme tyrosinase in the biosynthesis of

the melanin pigment, there may be a relationship between iron and copper concentrations and colour. Black or blonde hair was reported to have a lower iron content compared to red hair[19].

Although dark hair tends to have a higher level of lead than white hair, a study done by Grandjean[20] showed no significant difference between dark hair and white hair among occupationally exposed workers. However work done by Shroeder and Nason[23] showed that hair lead levels decrease in the order, brown, blonde, black, and red.

### 1:5.d Geographical Habitat

Geographical location appears to contribute to the concentration of trace elements in scalp hair. In some cases, these differences are due to variations in environmental pollution from one location to another. For example residents of Port Arthur[19], Texas, U.S.A., were found to have scalp hair lead levels 36% greater than the residents of Hanover, New Hampshire, U.S.A. It was proposed that the higher hair lead concentrations were as a result of exposure to airborne lead from the gasoline production center at Port Arthur. Differences

may also be due to genetic and/or dietary factors for races who often tend to inhabit specific geographical locations across the globe.

### 1:5.e Dietary Supplements and Medicines

Distinct differences in trace element concentrations in scalp hair have been found between vegetarians and non-vegetarians by some investigators[19]. In addition, it has been reported that scalp hair zinc concentrations for the subjects studied increased after a period of zinc supplementation[19]. The consumption of a highly refined diet can also result in an increase in scalp hair zinc level[19].

### 1:5.f Age, Race and Sex

Many examples have appeared in the literature which suggest that age, race and sex do influence trace element concentrations. A number of researchers have found an association between trace element concentration and age. In one study the scalp hair concentrations of copper, lead and cadmium of donors under the age of 30 were found to be significantly different from donors over the age of 40[23]. In another study done by Petering and Yeager, lead and cadmium concentrations were also found to be dependent on age[24].

Among age dependent subgroups, variations in trace element concentrations exist[19]. This could be attributed to differences in dietary habits and nutritional consumption.

Racial/ethnic groups often have unique and characteristic dietary habits associated with their culture. This may give rise to the variations in trace element concentrations seen among these groups.

Race was also thought to influence trace element concentrations in the body. Hung et al[25] studied 20 Vietnamese students and their Polish counterparts, all of whom lived under identical conditions in a hostel in Krakow, Poland. The study involved comparing the iron:zinc, copper:zinc and calcium:zinc ratios in both groups of students. Both the iron:zinc and the calcium:zinc ratios of the Vietnamese were approximately half that of the Polish, while the copper:zinc ratio was the same for both groups. A study done by Hall et al[26] on a sample of 115 Trinidadians showed that there was a significant difference in the lead concentration among the East Indian, African, Chinese and Mixed race groups studied. This was attributed to differences in hair texture. In another study done in Santo Amaro, Brazil the ethnic group 'Dark', had a higher lead concentration compared to the ethnic groups 'Medium' and 'Light'[27]. The classification was based on the degree of the negroid phenotype. Hair colour, type and conformation of nose and lip, and skin colour were the criteria used in phenotyping. It must be noted that differences found among ethnic/racial groups may also be affected by environmental and geographical factors.

Whether or not a significant difference exists in trace element concentrations of scalp hair between males and females, is still a subject of controversy. One investigator found that

at the 90% confidence level there is no significant difference in the mean lead in the scalp hair between sexes[28]. Another reported that the lead content of hair from females was significantly higher than that from males[29]. In yet another study it was found that, in females, the calcium and magnesium levels were twice that of males while the nickel levels were four times that of males. Petering and Yeager[24] also found that lead and cadmium concentrations in hair are dependent on sex. It has been postulated that differences in hormonal activity may be responsible for variations in trace element concentrations found between sexes.

As mentioned before in this section, many factors affect the trace element concentrations in hair. As such researchers would have to pay attention to these factors when pooling data of trace elements in hair and when comparing their research results with those present in the literature.

## 1:6 LEAD IN THE ENVIRONMENT:-NATURAL OCCURRENCES

### 1:6.a Rocks

Rocks that make up the earth's crust have lead concentrations that range between 10-20ug/g[30]. There are areas, such as lead ore deposits for example, where the concentrations are higher. Igneous and metamorphic rocks characteristically contain lead, having concentrations ranging between 10-20mg/kg[30]. Sedimentary rocks also tend to have concentrations of lead between 10-20mg/kg. Sandstone and the carbonaceous shales from both the United States and Europe have lead concentrations ranging between 10-70mg/kg[30]. Phosphate rocks tend to have even higher concentrations that exceed 100mg/kg in certain instances[31].

### 1:6.b Soils

Surface soils are in contact with the external environment and hence are most likely to be polluted by man's activities. Therefore, one has to distinguish between soils that contain naturally occurring lead and those that are polluted by man. Levels of naturally occurring lead in soils have a range between 2-200mg/kg and a mean concentration of 16mg-/kg[32]. These values are extremely low compared to industrially polluted soil. One investigator

found the lead concentration in soil near a lead mining area to have a value of 20,000mg/K-g[32].

With respect to naturally occurring lead, alkaline soils tend to have a higher content than acidic soils. The lead content in alkaline soil is influenced by the nature of the organic matter present. Those rich in chelating agents tend to have high levels of bound lead . This results in either movement of lead out of the soil or fixing of the metal in the soil, depending on the solubility properties of the lead complexes[33].

Lead analyses done on surficial soil along a major highway in Trinidad (The Churchill Roosevelt Highway), showed that the lead levels increased over a one year period. In July of 1977 the lead levels in soil found close to this highway was recorded as being 90ug/g. Soil samples taken at the same location in February, 1978 contained 172ug/g of lead and in August in the same year, 238ug/g of lead[34].

**1:6.c Water**

Many investigators have carried out studies of the lead concentrations in natural surface water. Friberg et al. have found the lead concentrations in water to be in the range of 7-10ug/L in rivers of the Midi-Pyrenees of France[32]. Livingstone et al.[35] have estimated that a range of 1-10ug/L of lead is considered to be the global range for rivers and lakes.

This is considered to be a reasonable estimate although it includes man-made pollution. The self-cleaning mechanism of water flowing through the ecological system allows for this accommodation.

Research has shown that freshwater has a higher lead concentration than seawater. With respect to seawater, surface seawater tends to have higher lead concentrations than deep seawater. For example surface seawater around the coast of California was reported to have lead concentrations in the range of 0.08-0.40 ug/L while deep waters around the coast of Bermuda contained an average of 0.07ug/L of lead[36]. It has been estimated that surface ocean waters have a lead concentration range of approximately 0.30ug/L while deep ocean waters have a concentration of about 0.10-0.22ug/L[37].

A survey done in 1980 by the Water and Sewerage Authority in Trinidad showed that the concentration of lead in freshwater lay between 4.1-8.1ug/L[34]. At that time these values were considered too low to be of any significance. They were actually lower than the present standard for potable water in the USA which is set at 50ug/L. More recent figures are unavailable.

**1:6.d Air**

In the most remote areas of the world, for example Greenland, the concentration of lead in air ranges between 0.0001-0.001ug/$m^3$ [38]. The concentration of naturally occurring lead in air using geochemical data was estimated to be approximately 0.0006ug/$m^3$ [39]. However, in a study done in 1972 in the remote Southern California mountains, in the U.S.A., the concentration of lead in air was found to be 0.008ug/$m^3$ [40]. It seems therefore that the air lead concentrations even in remote uninhabited areas have been affected by man-made pollution. Other examples are the North-Central Pacific Ocean and Southern Indian Ocean, where the mean air lead concentration is 0.001ug/$m^3$. On the contrary, a typical urban environment has ambient air lead concentrations in the range of 280ug/$m^3$ - 3000ug/$m^3$ in the vicinity of heavy traffic[41].

The eight-hour daily average lead levels in air along the single lane section of a busy highway in Trinidad (The Solomon Hochoy Highway) measured over a three month period in 1988 ranged between 0.83-12.65ug/$m^3$. The United States Environmental Protection Agency has set an ambient air quality standard of 1500ug Pb/$m^3$ for eight-hour averages. It is clear that the study done in Trinidad showed lead in air values below this limit[42].

### 1:6.e Plants

Plants also accumulate lead naturally from the environment. Leaves and twigs of woody plants have normal lead concentrations of 2.5ug/kg (dry weight) while vegetables and cereals have concentrations ranging between 0.1-1.0mg/kg[43].

Grasses that grow in the vicinity of roads prone to heavy traffic, may have very high lead concentrations. One such study showed a lead value of 250mg/kg in grasses at roadsides[32], this being attributed to environmental exposure. In a study conducted in 1979[34], it was shown that vegetables grown close to the Churchill Rosevelt Highway in Trinidad contained about 1-5ug/g of lead by dry weight for parts usually consumed. It was also demonstrated that much of the lead deposited on vegetables was removed during rainfall or by washing.

Lead occurs naturally in all areas of the environment. It is present in air, water, soil, rock and even in plants. The lead concentrations that occur naturally in components of the global environment are way below the levels which pose a threat to man and his environment. However, due to man's activities lead levels have increased significantly throughout the ages. Therefore, the severe environmental problems which face man today with respect to lead pollution, is as a result of his disregard for the well-being of the environment.

## 1:7 GENERAL CONSUMPTION AND USES OF LEAD AND ITS COMPOUNDS AND THE RESULTING POTENTIAL FOR POLLUTION.

### 1:7.a Storage Battery Industry

The largest consumption of industrial lead normally results from the manufacture of electric storage batteries. Lead, both in the form of lead-antimony alloys and lead oxide is used by this industry in almost equal proportions. While the oxides litharge (PbO), red lead ($Pb_3O_4$) and grey oxide ($PbO_2$) are used in the active material that is pasted on the plates, the metallic lead is used in grids and lugs. Much of the lead used in the battery industry comes from secondary lead production. It has been established that about 80% of the lead in storage batteries is recovered lead, produced at secondary smelters[44].

### 1:7.b Alkyllead Fuel Additives

Using alkyllead compounds as additives has been the most economical way of achieving required octane levels in commercial gasolines. By far, the most commonly used lead alkyls are tetraethyllead (TEL) and tetramethyllead (TML) which were discovered in the early 1920's[45]. The use of alkyllead additives is now the major contributor to lead pollution worldwide as it is in Trinidad and Tobago.

### 1:7.c Chemical Industry

About 40% of the lead produced worldwide is incorporated into chemical compounds. Lead salts form the principal ingredient in the manufacture of some paints and pigments. White pigments are produced from lead carbonate and lead sulphate. Lead chromate paints (chrome yellow and chrome red) are used for painting steel structures. Other chemicals containing lead are lead arsenate (insecticide), lead sulphate (rubber compounding) and lead naphthenate (dryer in oil paints)[46].

The paint industry in Trinidad and Tobago uses lead compounds in the manufacture of some paint. The lead compounds are used as:

(1) dryers in oil paint;

(2) anti-corrosive agents in primer for steel work;

(3) components in certain car paints and

(4) antifouling agents in boat-bottom paint to prevent barnacle formation[7].

### 1:7.d Miscellaneous

The surface of lead oxidizes readily to form a layer that is very resistant to corrosion. As a result of this property, the motor vehicle industries coat motor vehicles and motor vehicles spare parts which can become carroded with a layer of lead. Such industries account for a substantial proportion of the total lead consumption. Lead sheet is used for roofing and other flashings, wall cladding and sound insulation in the building and construction industries. Solder, bearing metals, brasses, type metal and collapsible tubes are all made from alloys of which lead is a component[46].

### 1:7.e Pollution by the Use and Consumption of Lead

Automotive exhaust is the major contributor to lead emissions in most cities. Other minor sources include paint and factory emissions. In Trinidad and Tobago, the gasoline sold contains approximately 2.75mL of tetraethyllead (TEL) per US gallon (4.5L) and together with other additives the total concentration of lead is about 0.5g/L. The average annual consumption of gasoline in Trinidad and Tobago is 517,972 kilolitres. Therefore, gasoline containing at least 259 metric tonnes of lead is utilised by automobiles in Trinidad and Tobago every year[7]. In Australia it has been estimated that about ninety eight percent of lead emissions come from lead in petrol, though the lead added to petrol only represents fourteen percent of the total usage[47].

There are also other sources of lead that contribute to environmental pollution. Eye cosmetics used by women and children in India, Bangladesh, the Middle East, Pakistan and North Africa contain lead. It is called kohl or surma and contains lead sulphide[48]. Many earthenware containers used at present contain lead in the form of lead oxide, lead carbonate and lead monosilicate. These lead compounds are leached out of the containers during use[49]. Mexican children and adults use a folk medicine called azarcon, which contains 86 to 90 percent lead tetraoxide, to relieve abdominal symptoms[50]. Toys, furniture, lead plumbing and leaded glass artwork also contribute to environmental pollution[51].

## 1:8 ENVIRONMENTAL TRANSPORT AND DISTRIBUTION OF LEAD

The mechanism by which lead is transported and distributed in the environment, whether it is from a stationary or mobile source, is mainly through the atmosphere. Large discharges may also occur directly into natural waters and onto the land. But in such cases, lead tends to be localized near points of discharge owing to very low solubilities of the compounds that are formed upon contact with soil and water[52]. The mechanism by which lead is removed from air and transferred to other media is not fully understood. The most efficient cleaning method is probably rain. There are however data which suggest that sedimentation contributes significantly to the proportion of lead that is removed from air[53]. The high affinity of organic matter for lead results in lead being rapidly removed from water as it passes through soil and bottom sediment. Thus, lead concentrations in both natural water and water supplies are generally low[54].

Motor vehicle exhaust systems emit lead compounds into the atmosphere in a number of forms. Lead can be emitted as volatile lead compounds such as lead halides, unburnt tetraethyllead and as particulates such as lead oxides. Approximately 70% of these particulates have a size of less than 0.1 micron which make them easily dispersed over large distances, and extremely hazardous to humans, since they are small enough to pass into the lower part of the lungs[47].

In Trinidad and Tobago a great proportion of the lead in the atmosphere comes from automobile exhaust emissions. Most of this lead emitted is deposited in the region of roads and highways[7]. Thus the roadside vegetable vendors and the vegetables sold by them become contaminated by this lead deposit. However, most of these vegetables are washed prior to human consumption and as such pose little threat with respect to the ingestion of lead by humans.

## 1:9 EFFECTS OF LEAD ON HUMAN BEINGS

As expected from a poison which damages the nervous system, with acute lead poisoning there are physiological and behavioural effects. Since most neurotoxins show disturbance of behaviour and/or intelligence it is no surprise that behavioural effects result from lead intake at even low concentrations.

### 1:9.a Hyperactivity and psychological effects in Children

Chronic lead exposure has been linked to hyperactivity in children. One definition of hyperactivity is "a sustained and excessive level of motor activity which gives rise to significant complaints at home and at school" [55]. The symptoms of hyperactivity include restlessness, difficulty in concentrating, excessive talking, poor tolerance to frustration, rejection of authority and poor control of impulses.

The most comprehensive study so far done on the effects of subclinical lead intoxication in children has come from the Harvard Medical Center in Boston, Massachusetts, U.S.A.[56] In this study, social and environmental factors were eliminated through the statistical technique of analysis of variance. This technique compensates for the effects of aggregate socioeconomic background factors. In this study done by Needleman[56], the sample population consisted of 2000 children. A Teacher's Behavioural Rating Scale assessment of

each child was done by his/her teacher and each child was also given a standard psychological performance test. A shed milk tooth was the indicator used to determine the concentration of lead in each subject.

The main conclusions from the study were:

1. Higher-lead groups had an Intelligence Quotient (I.Q.) of about five points lower than lower lead groups.

2. Higher-lead groups had an attention span test performance of about 14% below that of the control group.

3. Reaction times for picture completion and some verbal processing were delayed by about 16%.

4. Teacher assessment showed a number of characteristics associated with high lead level groups, such as:

   (a) lack of independence

   (b) lack of organization

   (c) hyperactivity

(d) lack of persistence

(e) distractibility

The Needleman study showed that there is no clear-cut "no effect" threshold at which the problem could be considered safe. The number of behavioural problems increased in a dose-related fashion with dentine lead levels.

### 1:9.b Social Deviance in Children

According to researchers there have been suggestions of direct links between the concentration of lead in the blood and a range of socially deviant behaviour[57]. Skepticism initially greeted this idea. However, there have now been studies showing possible relationships. A link between aggressive to violent behaviour and high blood lead levels has been suggested by one study[58]. In Sydney, Australia the lead content of the blood and hair of school children was studied as well as the lead content of the surrounding air. This study investigated the effecs of lead on the behavioural characteristics in children. A strong association was found between lead levels in blood and behavioural characteristics[47].

### 1:9.c Brain Structure and Biochemical Effects

The level of uncertainty in the behavioral studies, due to the possibility of other social causes, was reduced considerably when it was found that complementary studies[55] showed

that lead alters the brain structure and the biochemistry of the nervous system in the following ways:

(i) Biochemical changes

Lead inhibits many enzymes in the brain and thus affects several biosynthetic pathways. Lead also competes with calcium in a range of other biochemical processes. A study[59] showed that interference with calcium by lead results in changes to:

(a) brain maturation processes;

(b) the conduction of nervous impulses resulting in alteration of anxiety levels;

(c) the regulation of energy supplies to the brain and

(d) the ability of the brain to mediate sensory inputs and behaviour outputs.

(ii) Brain Structure Changes

Lead exposure also produces physiological changes in addition to biochemical changes. A 23% reduction in the number of synapses in rats[60] with significant alteration in brain tissue[50] and a 13% reduction in brain weight have been found at high lead levels. It

was also found that at lower levels - equivalent to normal urban exposure - lead concentrated in regions of the brain believed to control behaviour resulted in these regions exhibiting changes, particularly in cell thickness[60]. Needleman[56] demonstrated that children with high dentine lead levels also showed significantly different electrical activity in the brain. This study showing how behavioural changes can be induced by low lead level exposure is considered one of the most significant pieces of work done in this area.

### 1:9.d Other Effects of Lead

(i) Development of the Foetus

Studies have shown that the foetus can concentrate lead in the brain which can cross the placental membrane from the mother to the developing foetus[61]. For about fifty years it has been known that the foetus can be exposed to lead via the mother's blood. Also known was the fact that women exposed to lead had a higher risk of spontaneous abortions and still-born babies.

Males can also contribute to deformities and behavioural problems in the developing foetus of the children, if they were exposed to lead. This is because lead which accumulates in the sperms causes the sperms to change their normal shape resulting in unfavourable mutations[62].

(ii) Cancer

Strong correlations have been found between exposure to lead and cancer mortality among lead workers and those exposed to vehicle exhaust fumes[47]. Well below the human exposure limit (4ug/100mL for blood) lead has been found to cause chromosomal damage. Lead binds to organic chemicals such as benzpyrene very easily. This could therefore be the means by which benzpyrene can be transported into the body and could help to explain the increased levels of cancer found near busy roads.

(iii) Decreased Resistance

Research has revealed that at doses below clinical lead toxicity levels, lead interferes with the body's immune system resulting in viral infections[47].

(iv) Interference With Drugs

Lead reacts with a variety of organic compounds and since most drugs are organic by nature it can possibly alter the pharmocological effects of these drugs. This is particularly important in cases where the drugs are chelating agents which could cause the release of lead stored in bones or organs and its subsequent redeposition into more susceptable areas such as the brain[47].

## 1:10 ANALYTICAL METHODS

Introduction:

The choice of a method for the determination of lead in a particular laboratory depends on factors such as:

(1) the purpose of the analysis;

(2) the availability of equipment; and

(3) the number of samples to be analyzed per day.

The analysis of samples for low levels of lead is carried out by three main methods:

(1) colorimetric analysis;

(2) polarographic analysis; and

(3) atomic absorption spectroscopy (AAS).

There are also several non-routine methods for the analysis of lead in a variety of matrices. These methods include electron microprobe analysis, X-ray fluorescence

spectroscopy, neutron activation analysis and mass spectroscopy. All require the use of expensive facilities and a high degree of analyst expertise.

### 1:10:a Colorimetric Analysis[63]

Of all the reagents which form coloured complexes with lead, dithizone is considered the best reagent for the determination of trace quantities of that metal. The results obtained are comparable to those obtained by Atomic Absorption Spectroscopy. However dithizone forms coloured complexes with seventeen metals including mercury, bismuth, copper, gold, thallium and cadmium. The latter two, not masked by cyanide, are co-extracted with lead. Since a colorimeter cannot differentiate between the dithizonates of lead, thallium and cadmium the accuracy of lead determination can be seriously affected in the presence of thallium and cadmium. Many modifications of the dithizone method for lead analysis can be found in the literature, but sources of error in the dithizone method still exsist, especially when analyzing clinical samples.

Other complexing agents can also be utilised in the colorimetric determination of lead. For example, lead can be quantitatively extracted as a benzoyl acetonate over a pH range of 7-10 while hydroxyquinoline forms a complex extractable over the pH range of 6-10. At pH 5, lead can be extracted as a thenoyltrifluoroacetonate complex. Cupferron extracts lead completely over pH range 3-9. Beta-naphthylthiocarbazone is employed in the analysis of

lead in biological material. The beta-naphthylthiocarbazone complex formed is completely extractable at pH 9.8. Ammonium pyrollidine dithiocarbamate (APDC) is a fairly effective lead chelate over a narrow pH range of 2.2-2.8[63]. Once extracted with the specific complexing agent, the lead concentration is then determined colorimetrically at wavelengths appropriate to the respective complex.

### 1:10.b Polarography[64]

Isolation of lead by its electro-deposition from a solution of decomposed biological material was recommended as early as 1941. Microgram quantities of lead could be detected by this method. Iron and copper were found to interfere but their effects could apparently be minimized by the addition of potassium cyanide.

The technique, using anodic stripping voltametry (ASV), has become a widely accepted method for lead analysis. In this method ionic lead in samples is first reduced to elemental lead by a negative potential applied to a working mercury electrode. After a pre-selected time the working electrode potential is moved towards more positive values, thereby causing re-oxidation of the lead. The resulting anodic current from this oxidation, which is proportional to the concentration of lead in the sample, is measured[64].

### 1:10.c Atomic Absorption Spectrometry[65]

Atomic absorption spectrometry appears to have a number of advantages over methods such as polarography and atomic emission spectroscopy for trace metal determination. It is still one of the cheapest methods of metal analysis. In addition, a large number of samples can be rapidly analyzed using autosamplers. Another distinct advantage is that interference problems are either minor or non-existent, and where such problems exist, simple solutions are readily available.

The most widely used technique for analysis of heavy metals in recent times is atomic absorption spectrometry. It is based on the absorption of radiation by free ground state atoms. Basically it is a method which interposes metal atoms in the pathway of a beam of radiation emitted by the same atomic species and measures the amount of radiation which is absorbed. This is compared with standard solutions of the same metal for the determination of the concentration of the metal in the solution being studied. Since radiation is emitted and absorbed at a characteristic line peculiar to that element, the presence of large excesses of the other atoms is not normally a serious problem, although matrix affects can lead to errors of analysis.

The sample, liquid in the case of flame atomic absorption, is aspirated into a flame in which the solvent is evaporated and the molecules are decomposed into atoms. The radiation from the hollow cathode lamp (HCL) or electrodeless discharge lamp (EDL), made

of the element to be determined, is directed through the flame and is received on a detector (a photo cell or photomultiplier). The radiation is decreased when solutions of the species are aspirated, and the reduction in intensity of the radiation received is recorded or displayed as the signal. The recent development of the carbon tube atomizer, which is electrically heated, has enabled the use of smaller volumes of liquid samples and allowed the direct analysis of solid samples.

There are few spectral interferences in atomic absorption spectroscopy, since the resonance line in the spectrum of the element being determined is used. In the determination of trace metals by atomic absorption spectroscopy the main interference problems are due to:

(1) scattering of radiation by particles in the flame,

(2) matrix effects due to large excess of other ions,

(3) flame instability, and

(4) background absorption by other molecules in the flame.

Most of these interferences can be adequately corrected for, through the use of deuterium background correction. At very low levels, flame noise becomes predominant and

limits the sensitivity. One other source of error which cannot easily be corrected is the effect on the flame of the constituents of the sample solution, since this is unknown.

The advantages of atomic absorption spectrometry:

(1) high specificity,

(2) high sensitivity,

(3) many elements can be determined in one solution,

(4) relatively low sample preparaton and analysis time, and

(5) versatility with regards to sample types and concentration ranges.

### 1:10.d Carbon/Sulphur Analysis-The Elemental Analyzer

The modern analytical techniques for carbon and sulphur determination are still based on The Dumas-Pregl method[66]. The Perkin elmer 240B Elemental Analyzer is based on this method.

The 240B Elemental Analyzer

In the 240B model, the weighed sample is combusted in a pure static oxygen atmosphere. This has been shown to be the most efficient means of combusting even the most volatile sample material. The process is finished with a dynamic combustion (rapid flow) to ensure complete combustion of even the most stable materials. The products of combustion are passed over suitable selective materials to ensure complete oxidation and removal of undesirable by-products such as phosphorus and halogen gases. The combustion products are then passed through the reduction tube, where oxides of nitrogen are converted to molecular nitrogen.

The carbon dioxide, sulphur dioxide and nitrogen are then flushed by helium into a mixing chamber where they are thoroughly homogenized at a precise temperature of 1000°C and pressure of 30 atm. This homogenous mixture is then analyzed by passage through a series of three high precision thermal conductivity detectors (glass coated platinum thermal filaments), each detector consisting of two sensing cells.

For sulphur determination, between the first pair of T-cells, there is a tube containing a tungstic oxide packing plus a dehydrating agent in the cool zone of this same tube. The T-cells are the combustion units in the elemental analyser. There is a bridge area packed with silver oxide to absorb sulphur dioxide, the product of combustion. The sulphur from the sample is oxidized to form sulphur dioxide and the water formed is removed in the combustion tube. Between the first pair of T-cells is an absorption trap containing a

dehydrating material, magnesium perchlorate. As the gas passes through, water is removed from the stream. The differential signal obtained before and after the trap, as read on the potentiometric recorder, reflects the sulphur dioxide concentration and therefore the amount of sulphur in the original sample. A similar measurement is made with a second pair of conductivity cells between which there is a trap that removes carbon dioxide. The remaining gas now consists of only helium and nitrogen. This gas passes through a conductivity cell, the output of which is compared to that of the reference cell, through which pure helium flows, to give the nitrogen concentration. The main detection systems used in modern instruments utilise thermal conductivity and infra-red detectors.

## 1:11 DIGESTION PROCEDURES

Quantitative extraction procedures are dependent on the effectiveness of the decomposition steps. Thus the decomposition procedure is one of the most important steps in the analysis of a sample. Several different acid mixtures can be used for the extraction of heavy metals from biological samples of which nitric-sulphuric-perchloric, nitric-perchloric and nitric-sulphuric acid mixtures are the most popular combinations. However, it is important to avoid utilising troublesome and possibly dangerous reagents for such procedures. Simple and safe methods are desirable[67].

The explosive nature of perchloric acid makes it unattractive for routine digestion methods. However, although perchloric acid is a very potent oxidant, only relatively small volumes are required for the digestion of hair samples and therefore its use could not have been ignored in this project. The decomposition procedure involving perchloric acid is simple, economical, relatively safe and provides an effective means of lead determination in routine monitoring of a large number of samples. In addition, since it involves the use of only small amounts of reagents the blank value is normally kept low.

# CHAPTER TWO:

# EXPERIMENTAL

## 2:1 INSTRUMENTS AND REAGENTS

Instruments Used

Atomic Absorption Spectrophotometer (Pye Unicam SP-9, England)

Elemental Analyzer (Perkin Elmer 240 B, United States)

Scanning Electron Microscope (JEOL JSM-35CF, England)

Centrifuge (DAMON/IEC Division CU-5000, France)

Ultrasonic Cleaner (CHIMICA OMNIA, Italy)

Microbalance (Perkin Elmer Ultra-Microbalance Model AD-6, England)



Reagents

The major reagents nitric acid, methanol, perchloric acid, trichloroacetic acid and all other chemicals were of AnalaR grade and were used as obtained from the British Drug Houses (BDH), Poole, England. The liquid detergent Kleenol® is a product of Superchem, Trinidad and Tobago Limited.

## 2:2 SAMPLING

The sample population consisted of one hundred and eighty nine volunteers from four different races-: African, East Indian, Chinese and persons of Mixed parentage. Classification was based on phenotype and as claimed by the participants. The sample population consisted of 46 Africans, 91 East Indians, 11 Chinese and 41 Mixed (ie. persons of mixed parentage). Also included were occupationally exposed groups which would normally have a greater risk of being exposed to lead contamination compared to the general population. These consisted of 22 battery plant workers and 29 traffic police personnel. All participants were given a questionnaire designed to obtain information on social and environmental factors that are known to have an impact on lead contamination as well as their health status.

As recommended[17], hair growing 2-3 cm closest to the scalp was cut from the nape of the neck, using a pair of stainless steel scissors washed with ethanol. The hair samples were kept sealed in plastic bags prior to analysis. Venous samples of blood were taken from each subject's arm and stored in heparinized lead-free containers prior to analysis. All blood lead analyses were done within twelve hours of obtaining the samples.

## 2:3 HAIR WASHING PROCEDURE

No washing procedure has yet been developed which removes all the exogenous particulates from the surface of the hair. Therefore, several washing procedures were compared in order to select the one which removes most of the exogenous particulates. All washing procedures except the Kleenol®/methanol wash were recommended literature procedures used in other studies.

(i) Ether Wash[68]

The hair samples (~0.30g) were placed, without prerinsing, into the thimble of a soxhlet apparatus. Both the thimble and the reflux condenser were washed with dilute nitric acid prior to the insertion of the hair samples. The hair samples were refluxed for six hours at an average rate of five minutes per cycle, using diethyl ether. The samples of hair were then removed and allowed to stand overnight at room temperature. The dry samples were then stored in a sealed plastic bag.

(ii) EDTA Wash[68]

All glassware used in this washing procedure was rinsed with dilute nitric acid and then thoroughly washed with deionized water. The average sample weights used were 0.5g.

The washing procedure was carried out as follows:

(1) The hair samples were each placed in 1L of deionized water in a glass beaker and then stirred with a glass rod to effect wetting and

help separate heavier particulate matter adhering to the hair strands. The deionized water was drained and the procedure repeated. Each of the drained hair samples was then placed in 1L of 10% Kleenol® detergent solution.

(2) The detergent solution containing hair was then heated, with frequent stirring, to incipient boiling. The hair was again drained and rinsed with 1L of deionized water. The deionized water was then drained from the hair sample. The entire process was repeated, with the exception that two deionized water rinses were used.

(3) The drained hair from (2) was soaked in 200mL of AR acetone, drained again and this process repeated.

(4) The drained hair from (3) was soaked in 1L of a saturated solution of EDTA and heated to incipient boiling. The EDTA solution containing the hair was stirred frequently and allowed to stand for 5 minutes (when permitted to stand for longer than five minutes, the saturated solution, upon cooling, precipitated fine EDTA crystals unto the hair.

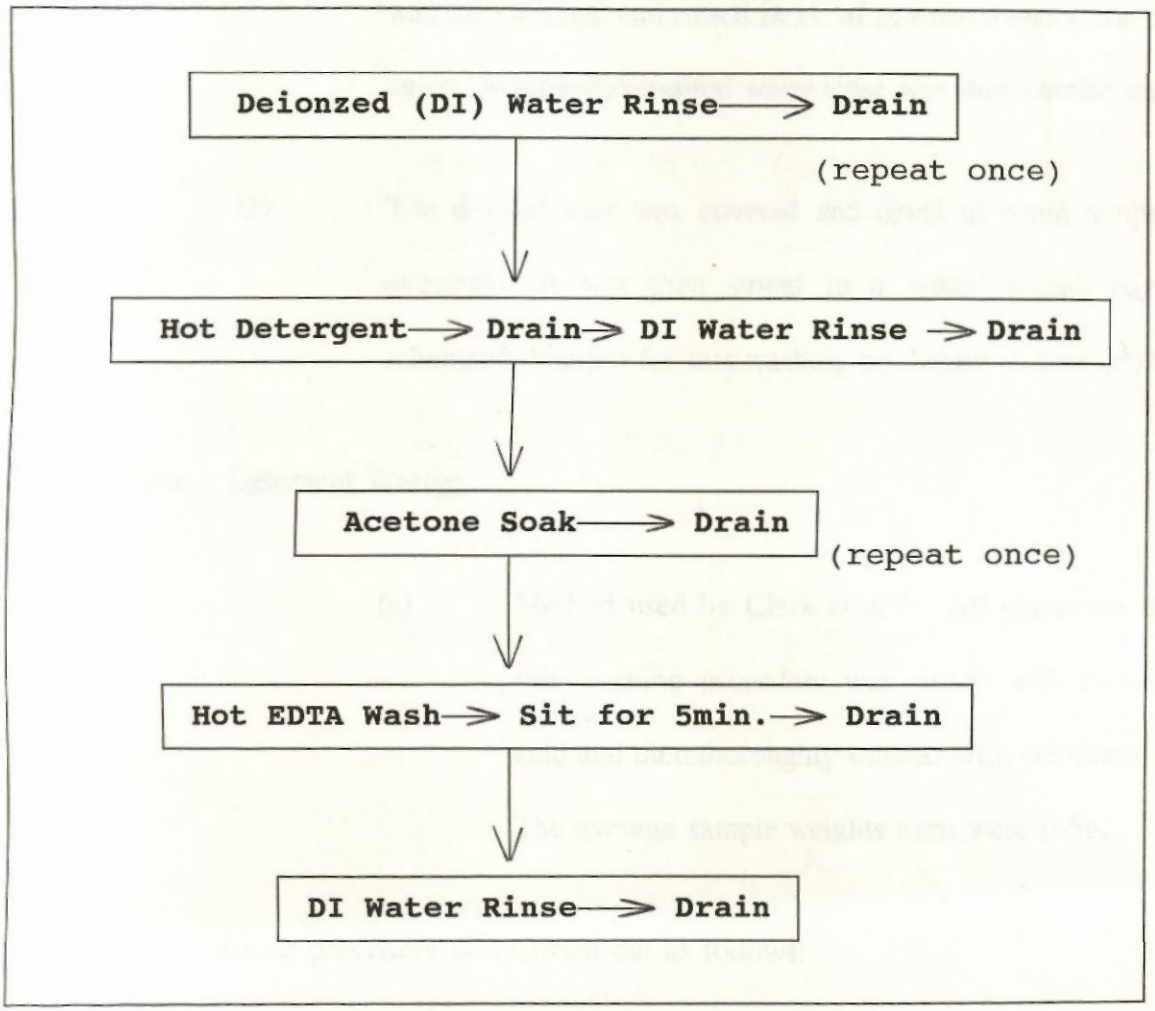


Fig.1: Schematic for EDTA Washing Procedure.

These proved difficult to remove in the remaining steps.) The hair was then drained and rinsed in 1L of deionized water and drained again. A second deionised water rinse was then carried out.

(5) The drained hair was covered and dried at room temperature overnight. It was then stored in a sealed plastic bag. The schematic diagram for this washing procedure is seen in Fig. 1.

(iii) Detergent Washes

(a) Method used by Clark et al[68]: All glassware used in this washing procedure was rinsed with dilute nitric acid and then thoroughly washed with deionized water. The average sample weights used were 0.5g.

The washing procedure was carried out as follows:

(1) The hair samples were each placed in 1L of deionized water in a beaker and then stirred with a glass rod to effect wetting and help separate heavier particulate matter suspended in the hair strands. The deionized water was drained and the procedure repeated. Each of the drained hair samples was then placed in 1L of 10% Kleenol® detergent solution.

(2) The detergent solution containing the hair was then heated, with frequent stirring, to incipient boiling. The hair was again drained and rinsed in 1L of deionized water. The deionized water was then drained from the hair sample. The entire process was then repeated, with the exception that 2 deionized water rinses were used.

(3) The drained hair from (2) was soaked in 200mL of AR acetone, drained again and this process repeated.

(4) The drained hair from (3) was then soaked in 1L of deionized water, drained and this process repeated.

(5) The drained hair was covered and dried at room temperature overnight. It was then stored in a sealed plastic bag. The schematic diagram for this washing procedure is given in Fig. 2.

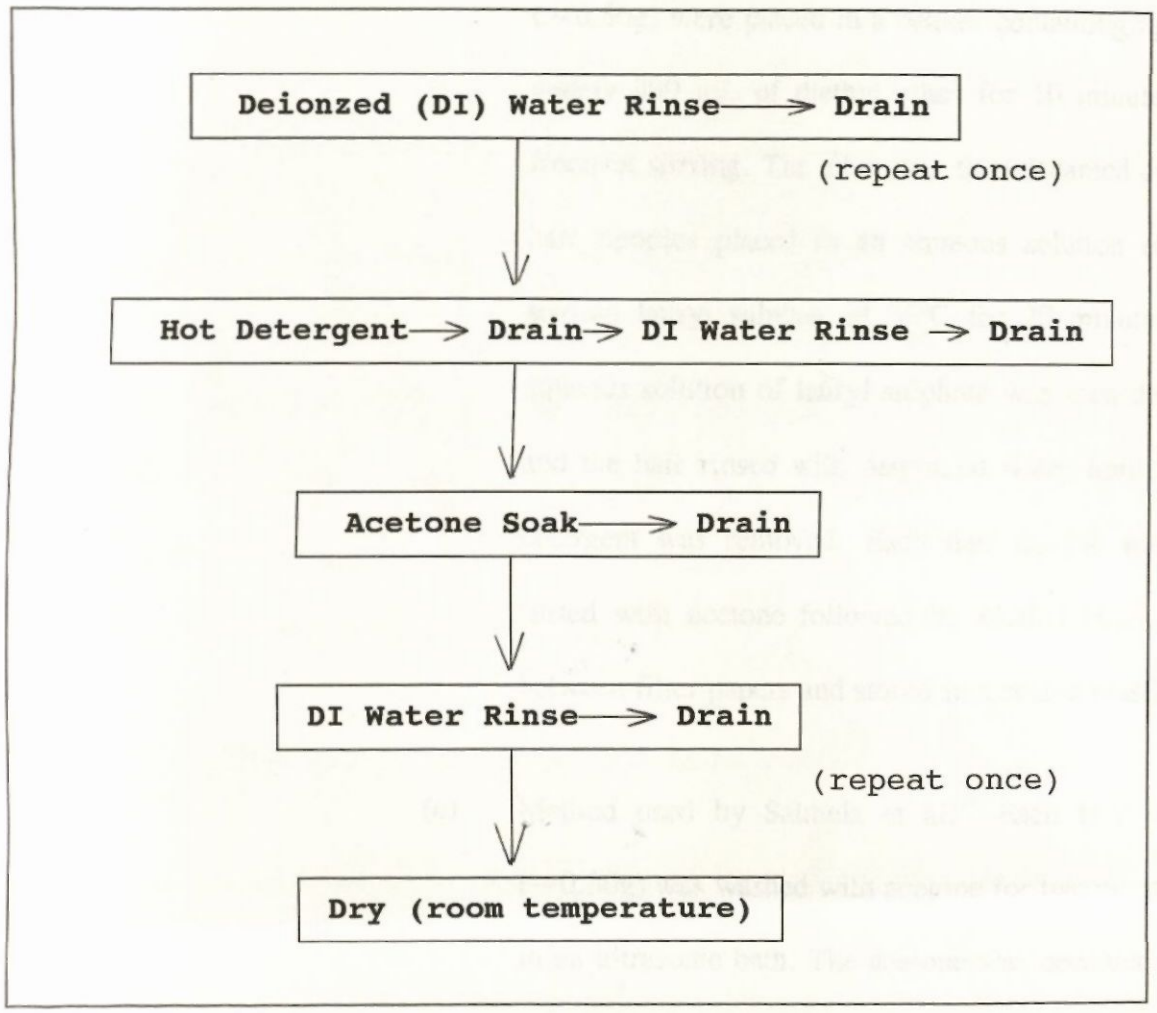


Fig.2: Schematic of Detergent Washing Procedure

(b) Method used by Creason et al[69]: The hair samples (~0.30g) were placed in a beaker containing approximately 200 mL of diethyl ether for 10 minutes with frequent stirring. The ether was then decanted and the hair samples placed in an aqueous solution of 50% sodium lauryl sulphate at 35°C for 20 minutes. The aqueous solution of lauryl sulphate was then decanted and the hair rinsed with deionized water until all the detergent was removed. Each hair sample was then rinsed with acetone followed by diethyl ether, dried between filter papers and stored in a sealed plastic bag.

(c) Method used by Salmela et al:[13] Each hair sample (~0.30g) was washed with acetone for twenty minutes in an ultrasonic bath. The acetone was decanted and a 2mL aliquot of a 1% Triton X-100 solution added to each hair sample. Each sample was placed again in the ultrasonic bath for two minutes. This was followed by the decanting of the detergent solution and the rinsing of the hair with deionized water and acetone sequentially. The hair samples were then placed in an oven to dry at approximately 100°C for 30 minutes.

Each hair sample was then stored in a sealed plastic bag.

(iv) Acetone Wash (used by Hambidge et al):[14]

Each hair sample (approx. 0.1g) was placed in a beaker containing 125mL of acetone and stirred using a magnetic stirrer for 10 minutes. The acetone was decanted and the hair sample washed twice with 125mL aliquots of deionized water. Upon decanting of the deionized water, the hair was again washed with 125mL of acetone, then placed between filter papers to remove excess acetone and air dried at room temperature for 24hours. The hair was then stored in a sealed plastic bag.

(v) (Kleenol®/Methanol Wash (used in this project)

Hair samples were washed sequentially with 50mL of aqueous Kleenol® solution (10% V/V) and 50mL of methanol for approximately ten minutes. These washings were accompanied by frequent stirring to remove grease and superficial particulates. The hair samples were dried in an oven (approx. 100°C) for 10 minutes. Each hair sample was then stored in a sealed plastic bag prior to analysis.

## 2:4 SCANNING ELECTRON MICROSCOPE ANALYSIS

The efficiencies of the washing procedures carried out were determined by comparing the scanning electron microscope (SEM) photographs of hair samples washed by the different procedures. These photographs were taken at x 1500 magnification. The scanning electron microscope analyses were done by the Caribbean Industrial Research Institute, Mocoya, Trinidad, West Indies.

## 2:5 LEAD DETERMINATION IN HAIR AND BLOOD

(i) Hair-Lead Determination

Hair samples between 0.2 - 0.3g were wet digested with 12mL of a 5:1 mixture of concentrated nitric acid and concentrated perchloric acid for approximately twenty minutes[26]. The digested samples were then filtered into 10mL volumetric flasks and made up to 10mL. The lead concentrations were determined directly on the digests using flame atomic absorption spectrophotometry. Suitable blanks were determined along with the samples. Estimations were carried out at least twice. If the range of duplicates exceeded 10% of the mean, further aliquots were analyzed.

In all analyses, a hollow cathode lamp was used as the source of radiation and acetylene as the fuel. Lamp current and position, slit width, wavelength and air/fuel ratio of the spectrophotometer were optimized prior to each determination. The principal line of 217nm with automatic deuterium continuium background correction was used.

Standards were made by serial dilution of a stock solution (1000 ug $mL^{-1}$) of lead nitrate. Concentrations of the standard solutions were chosen such that the linear range for the element was not exceeded. Appropriate sample dilutions were made in instances where this condition was not fulfilled. All stock solutions were prepared according to the procedures recommended by the Pye Unicam handbook[68].

(ii) Blood-Lead Determination

Blood lead was estimated following acid digestion. Ten mL. of whole blood was digested using 4mL of 20% V/V concentrated nitric acid and 4mL of 7% m/v trichloroacetic acid. Samples were homogenized using a vortex mixer and centrifuged at 2500g for 20 minutes[70]. A known volume of the supernatant was then analyzed by flame atomic absorption spectrophotometry.

The Pye Unicam atomic absorption spectrophotometer was used, and determinations done at a wavelength of 217 nm with automatic deuterium continuium background correction. Suitable blanks were analyzed along with the samples. Estimations were carried out at least twice. If the range of duplicates exceeded 10% of the mean, further aliquots were analyzed.

Standard material was prepared by adding known amounts of lead to a human blood pool which had a known low lead concentration. This was done in order to compensate for the matrix effect of blood.

## 2:6 CARBON AND SULPHUR ANALYSIS

(i) Carbon and Sulphur Determination

Hair samples were chipped into very fine segments using a pair of stainless steel scissors. Roughly 2-3 mg of each sample was placed in a platinum boat and weighed on a microbalance.

All elemental analyses were done by a Perkin-Elmer 240B elemental analyzer. For carbon and sulphur analysis, an auxiliary temperature of 820°C, combustion temperature of 1000°C, oxygen pressure of 35 psi and helium pressure of 30 psi, were used as recommended by the Perkin-Elmer 240B elemental analyzer handbook. The elemental analyzer was standardized using high purity thiourea.

# CHAPTER THREE

# RESULTS AND DISCUSSION

## 3:1 SELECTION OF HAIR WASHING PROCEDURE

The scanning electron micrographs (SEMS) of the unwashed hair samples showed that the hair shafts were contaminated with particulate material which possibly included lead. Therefore the samples needed to be washed before analysis in order to attempt to eliminate these exogenous contaminants (Fig. 3a).

The washing method that removes the most exogenous particulate material from hair, as evidenced by SEM analysis, has traditionally been selected by researchers as the method of choice. For example, Clarke et al[68] chose an EDTA washing procedure over a detergent wash or an ether wash because the SEMS of the hair samples subjected to the detergent washing procedure or the ether washing procedure showed a greater concentration of particulates on the hair surfaces than the SEMS of the hair from the EDTA wash. In addition, the lead levels in hair samples subjected to the EDTA wash were lower than those hair samples subjected to the detergent wash or the ether wash. Grandjean[20] found that the lowest concentration of lead in a scalp hair sample was obtained after washing the hair using an ultrasonic method compared to washing procedures involving Freon, diethyl ether and sodium lauryl sulphate respectively.

**TABLE 1 COMPARISON OF WASHING PROCEDURES FOR HAIR CONCENTRATION OF PB UG/G (MEAN ± SD) IN 4 SAMPLES**

| Washing Procedures | Samples | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Ether | 32.39 ± 0.52 | 21.21 ± 0.72 | 11.64 ± 0.14 | 10.64 ± 0.54 |
| EDTA | 23.55 ± 0.38 | 19.61 ± 2.06 | 10.60 ± 0.43 | 10.35 ± 0.59 |
| Detergent | 28.94 ± 0.06 | 25.64 ± 1.08 | 26.22 ± 1.99 | 15.59 ± 0.03 |
| Ultrasonic | 33.66 ± 1.16 | 21.96 ± 0.94 | 11.55 ± 0.15 | 12.52 ± 0.72 |
| Acetone | 37.69 ± 0.25 | 31.10 ± 0.14 | 21.44 ± 0.09 | 20.62 ± 0.16 |
| Sodium Lauryl Sulphate | 34.84 ± 1.25 | 37.40 ± 0.36 | 11.60 ± 0.16 | 15.52 ± 0.32 |
| Kleenol® | 15.03 ± 0.50 | 18.64 ± 0.35 | 8.64 ±1.45 | 9.92 ±1.15 |

The results in Table 1 show that the lowest concentration of lead found in the acid digests from the four different hair samples occurred in the digests from the samples subjected to the Kleenol® washing procedure. In addition, the Scanning Electron micrographs (SEMS) suggested that the Kleenol® wash removed more particulates from the surface of the hair than other methods (Fig. 3b-3h). This method was therefore selected as the washing procedure for this investigation since the results indicated that it gives the cleanest wash.

The higher lead level values found in the digests by the other washing procedures were probably due to the failure of these procedures to remove as much exogenous lead contamination as the Kleenol® procedure resulting in elevated lead levels in these digests.

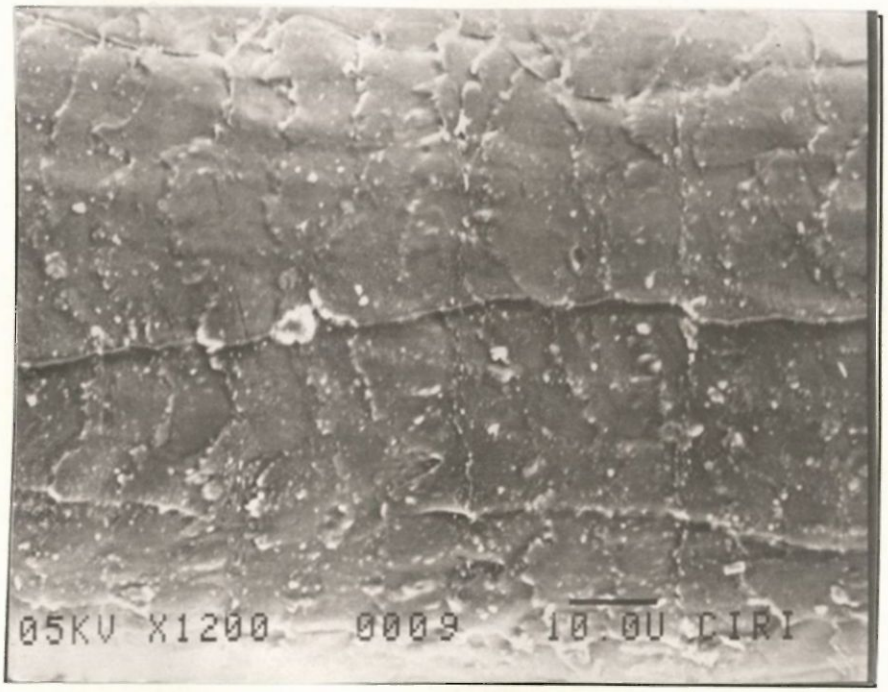


Fig. 3:a. UNWASHED HAIR SAMPLE

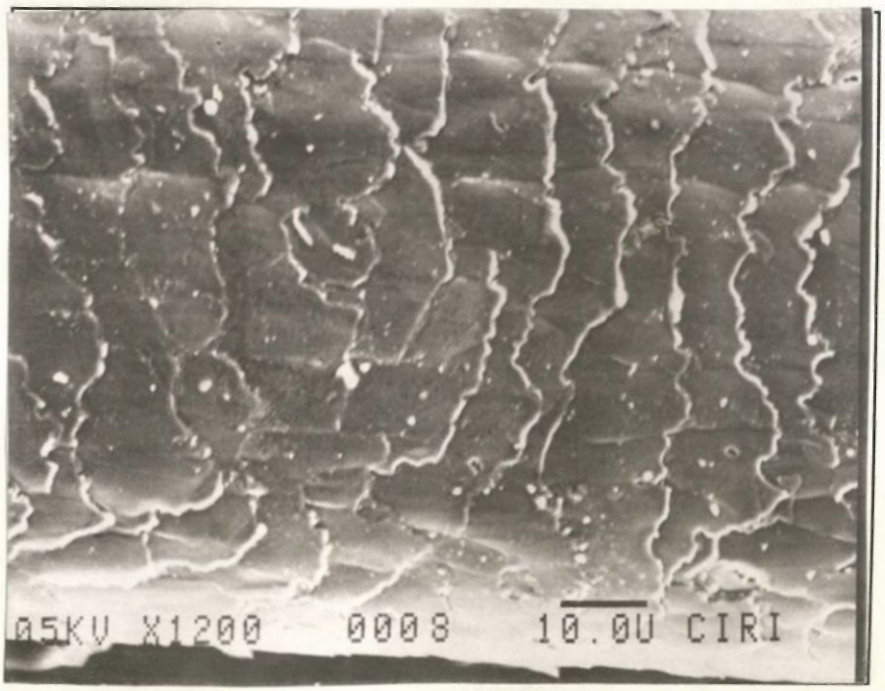


Fig. 3:b. ETHER WASH

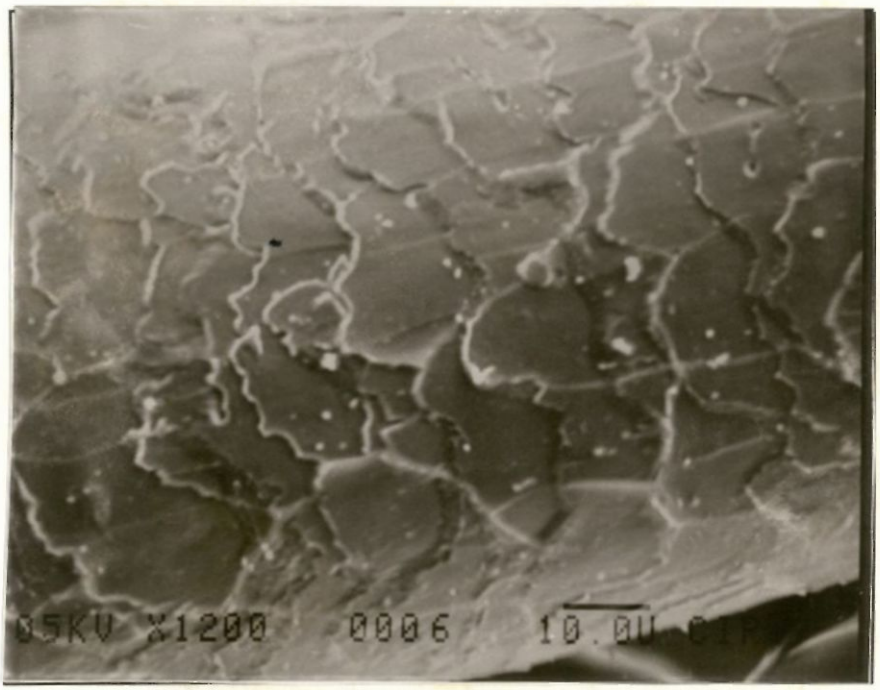


Fig:3c. EDTA WASH

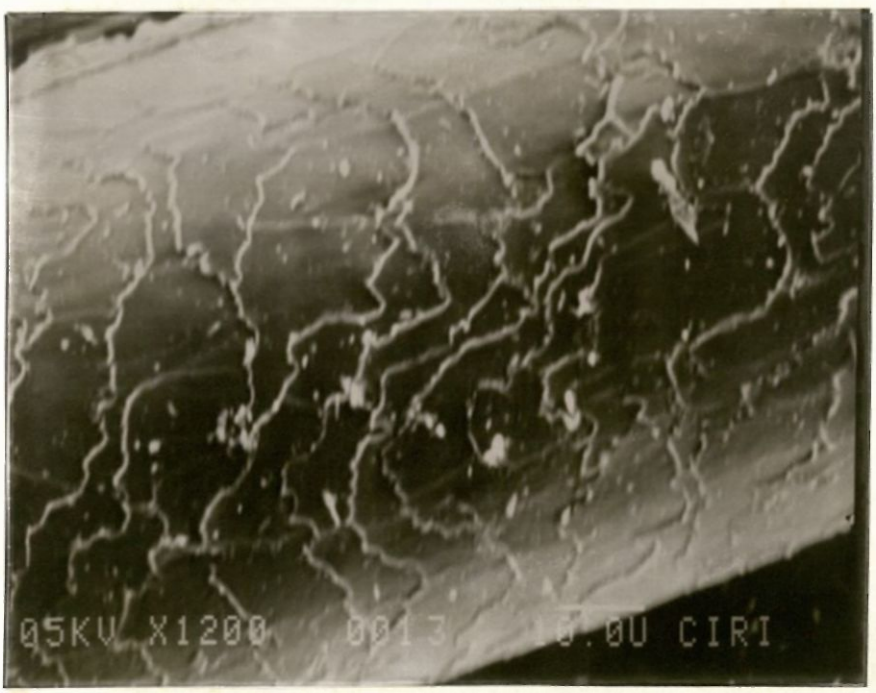


Fig. 3:d. DETERGENT WASH

Fig. 3:e. SODIUM LAURYL SULPHATE WASH

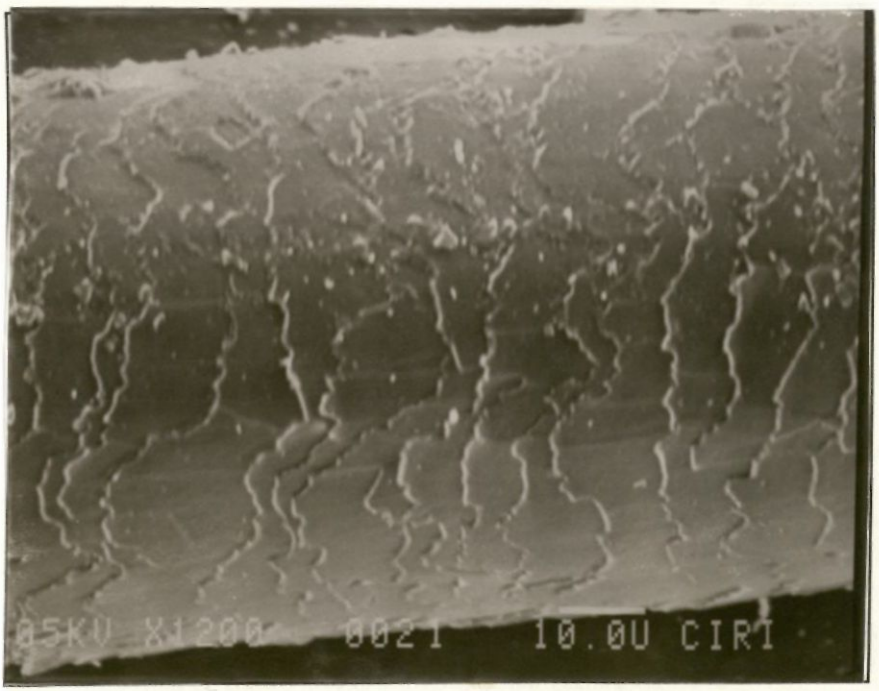


Fig. 3:f. ULTRASONIC WASH

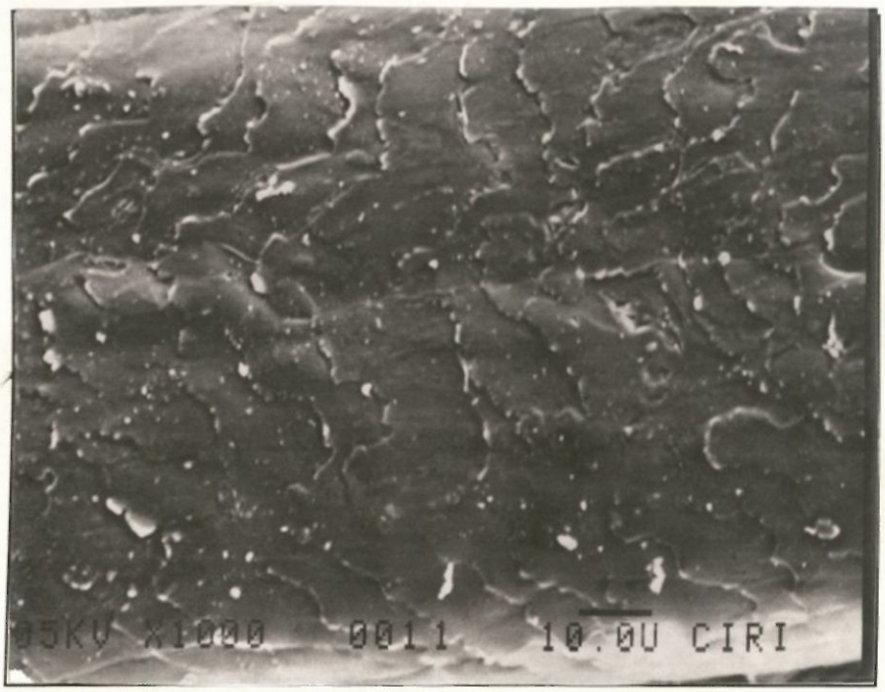


Fig. 3:g. ACETONE WASH

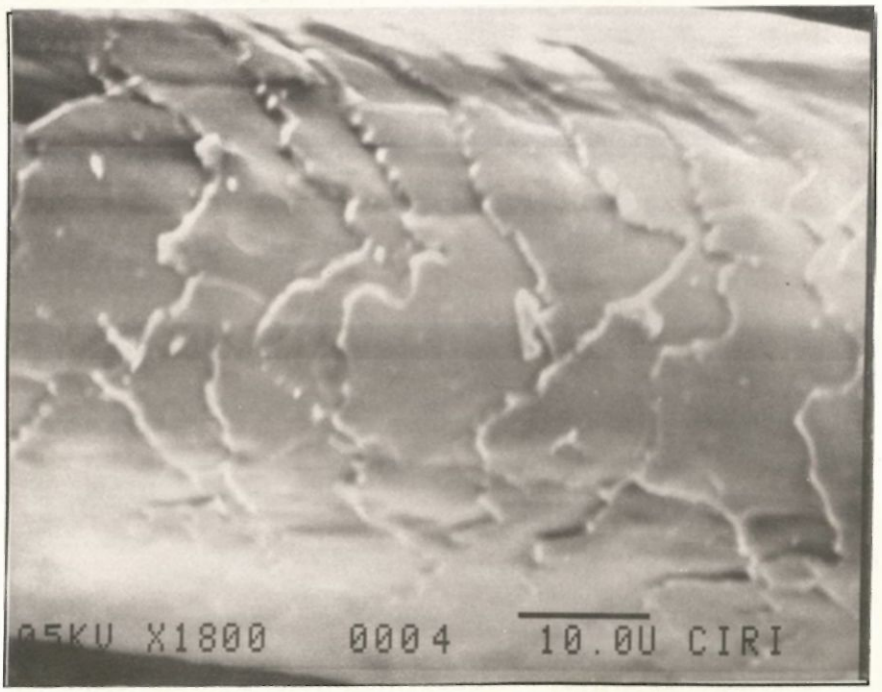


Fig. 3:h. KLEENOL WASH

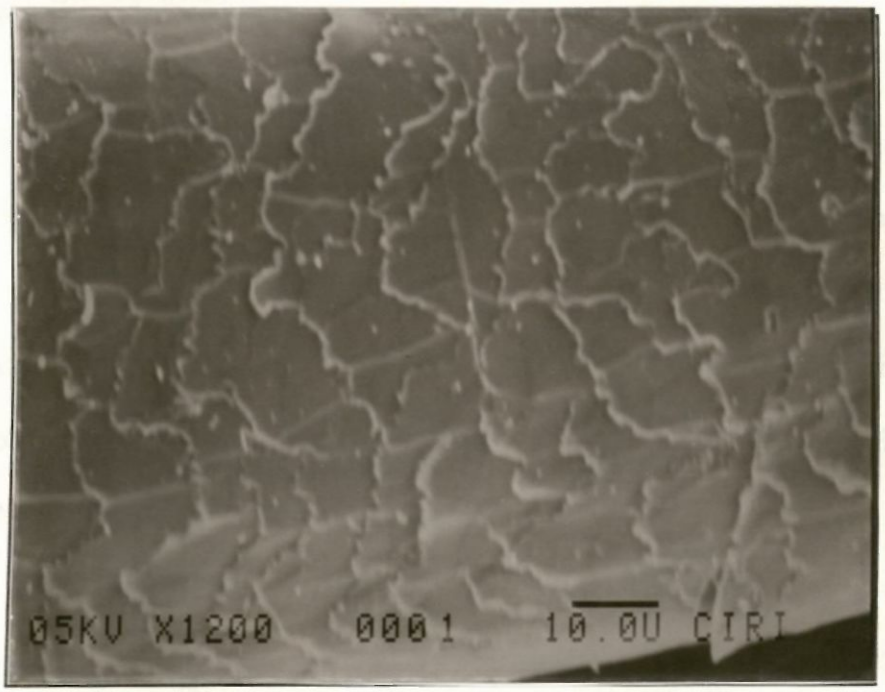


Fig. 3:i. KLEENOL WASH OF A BATTERY WORKER HAIR STRAND

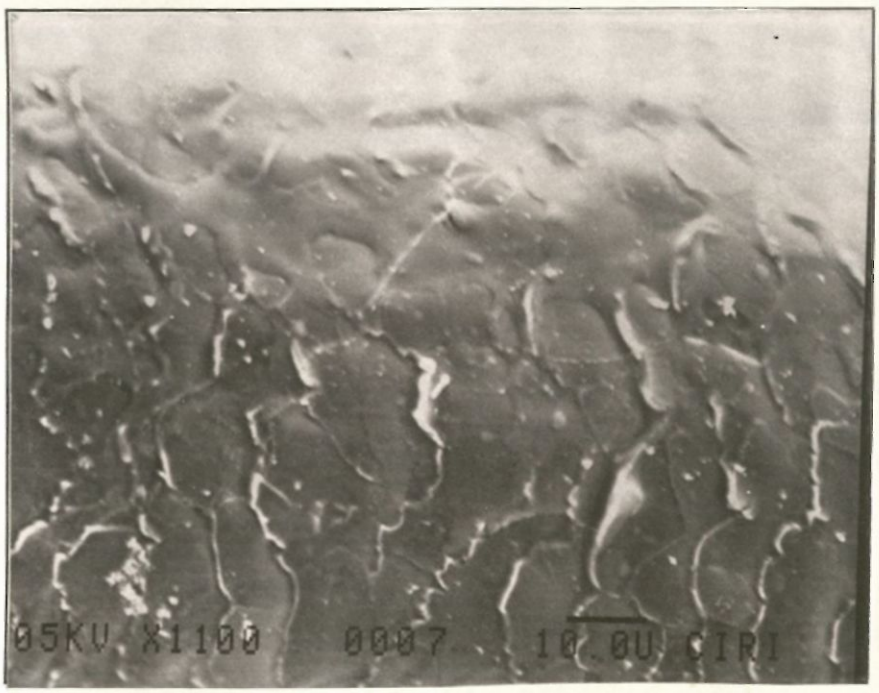


Fig. 3:j. KLEENOL WASH OF A TRAFFIC POLICE HAIR STRAND

## 3:2 GENERAL DISCUSSION

The data were acquired on one hundred and eighty nine (189) non-occupationally-exposed persons and are presented in terms of, (1) race, (2) sex, (3) total time spent in traffic, (4) time spent in traffic jams, and (5) the %C, %S and C:S ratio in scalp hair samples. Age was not considered because most of the sample population belonged to the adult age group (20-35 years). The four racial groups studied were African, East Indian, Chinese and Mixed (that is, of mixed parentage). The non-occupationally exposed group was made up of volunteers, and phenotyping was used to categorize the individuals into racial groups. The sample population consisted of 46 Africans, 91 East Indians, 11 Chinese and 41 persons of Mixed parentage. The individuals selected all had non-processed hair (that is, not chemically treated except for washing with soap and/ or shampoos and conditioners). Two occupationally exposed groups, which consisted of 27 traffic police personnel and 22 battery workers were also sampled.These occupationally exposed groups were also made up of volunteers. Sampling was done between 10.00am and 12.00 noon, that is, a few hours after the volunteers completed their morning commute to their places of work.

Statistical analysis suggested that the distribution of the data obtained in this project was skewed (Figs.4-12). In order to normalise the distribution the data were log transformed such that parametric analysis could be used (Figs. 13-21).

SCALP HAIR LEAD LEVELS
GENERAL POPULATION

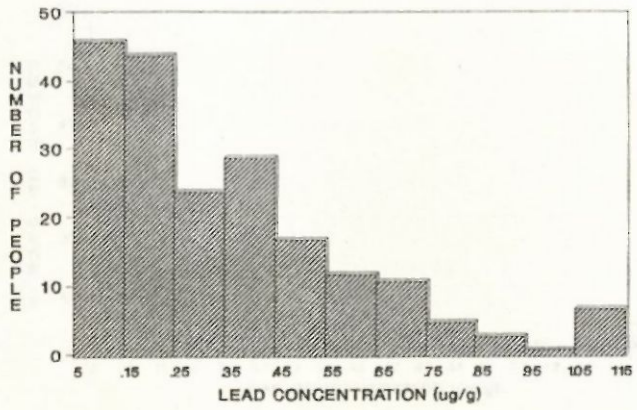


Fig.4

SCALP HAIR LEAD LEVELS
TRAFFIC POLICE

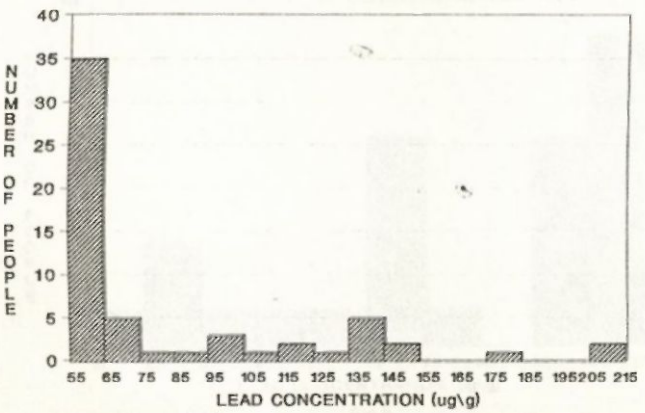


Fig.5

SCALP HAIR LEAD LEVELS
BATTERY WORKERS

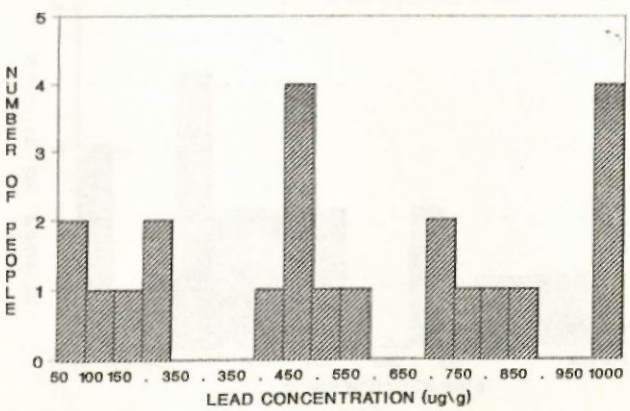


Fig.6

PUBIC HAIR LEAD LEVELS
GENERAL POPULATION

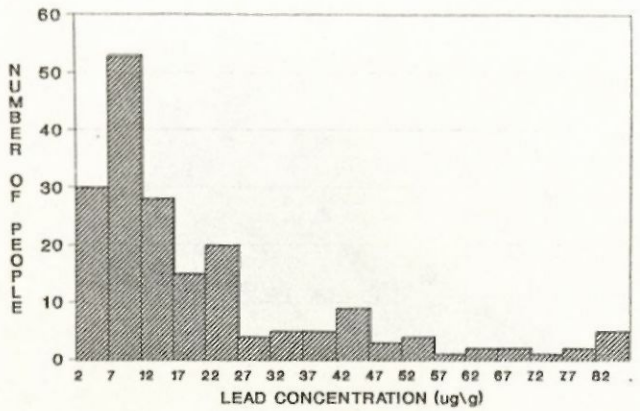


Fig.7

PUBIC HAIR LEAD LEVELS
TRAFFIC POLICE

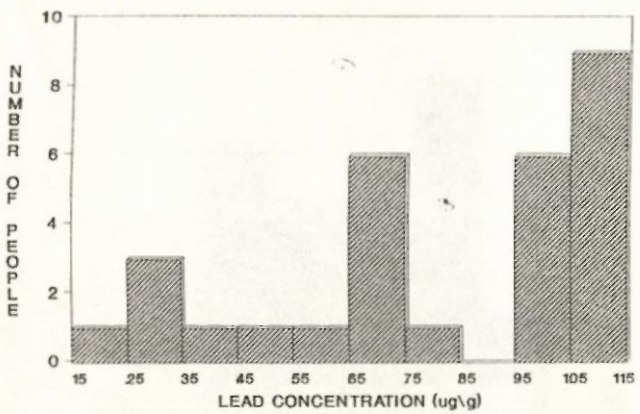


Fig.8

PUBIC HAIR LEAD LEVELS
BATTERY WORKERS

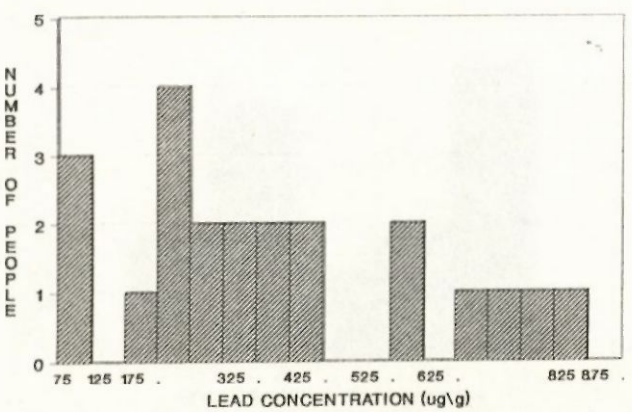


Fig.9

BLOOD LEAD LEVELS
GENERAL POPULATION

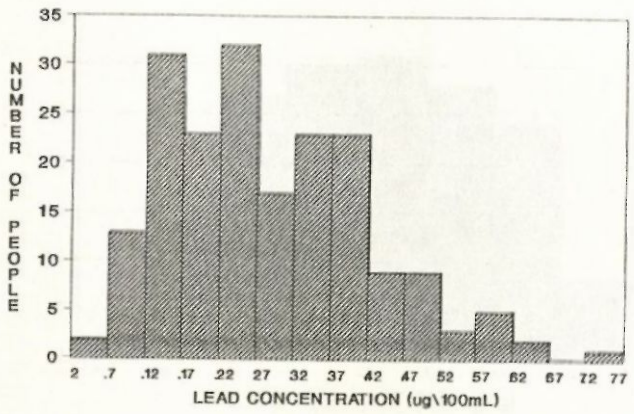


Fig.10

BLOOD LEAD LEVELS
TRAFFIC POLICE

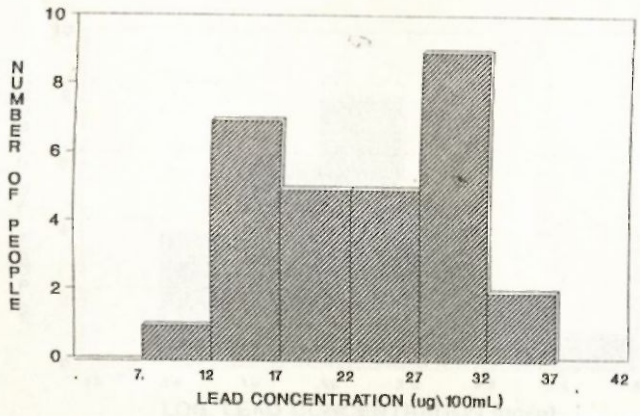


Fig.11

BLOOD LEAD LEVELS
BATTERY WORKERS

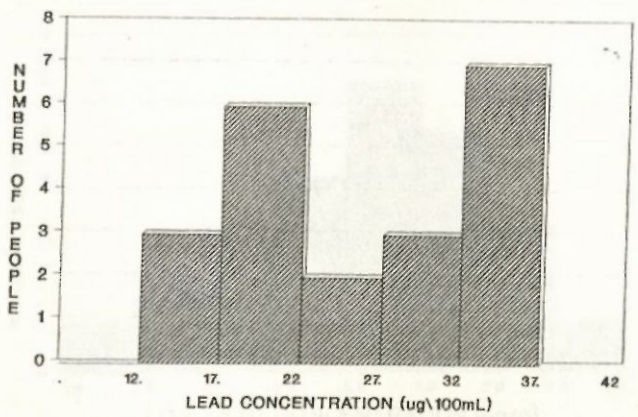


Fig.12

SCALP HAIR LEAD
GENERAL POPULATION

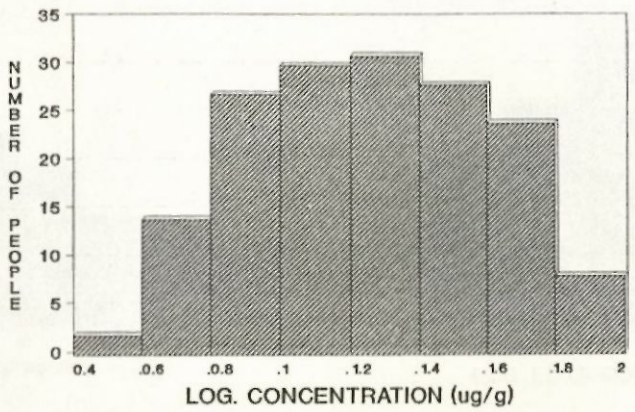


Fig.13

SCALP HAIR LEAD
TRAFFIC POLICE

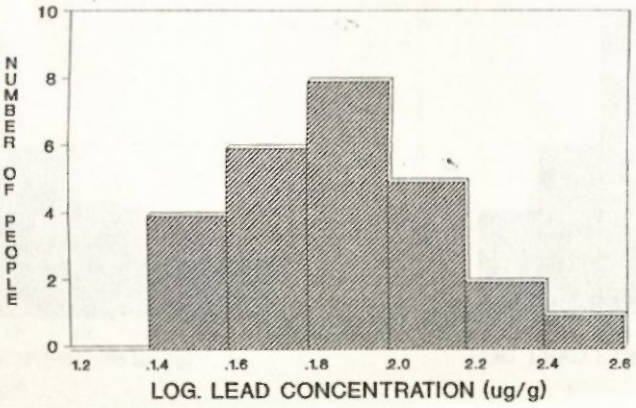


Fig.14

SCALP HAIR LEAD
BATTERY WORKERS

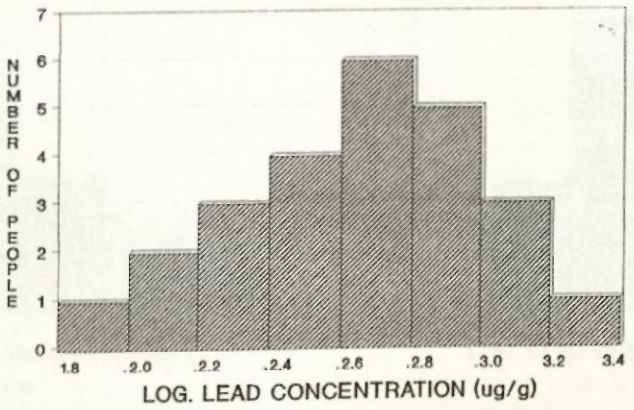


Fig.15

PUBIC HAIR LEAD
GENERAL POPULATION

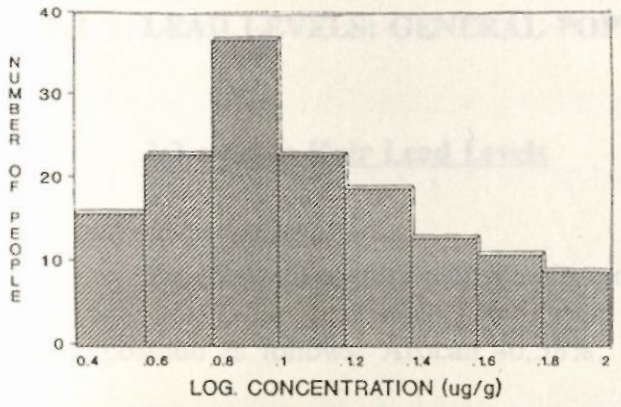


Fig.16

BLOOD LEAD
GENERAL POPULATION

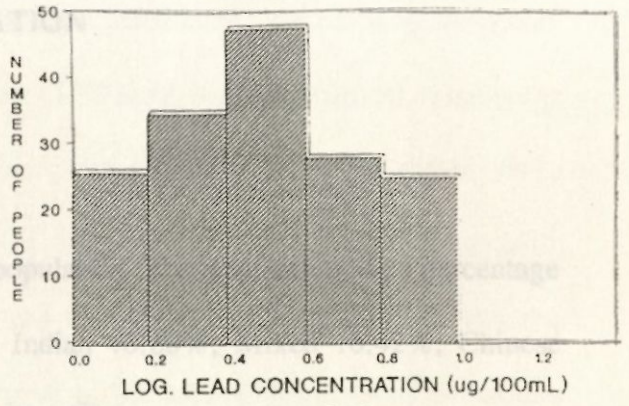


Fig.19

PUBIC HAIR LEAD
TRAFFIC POLICE

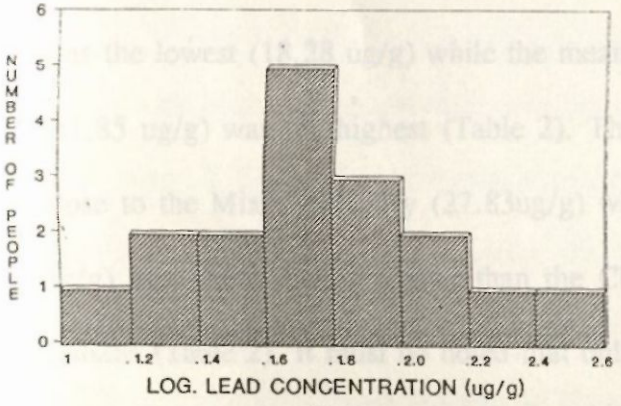


Fig.17

BLOOD LEAD
TRAFFIC POLICE

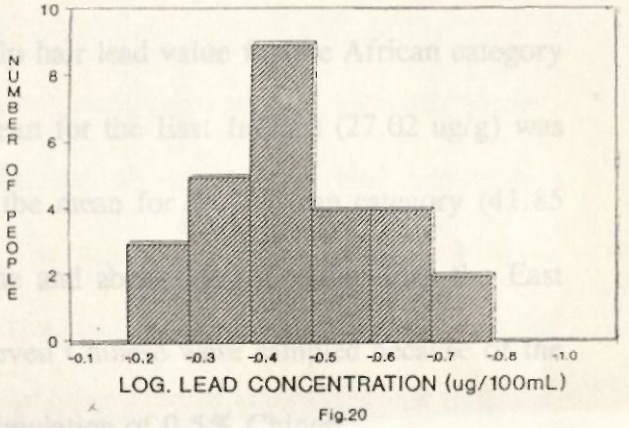


Fig.20

PUBIC HAIR LEAD
BATTERY WORKERS

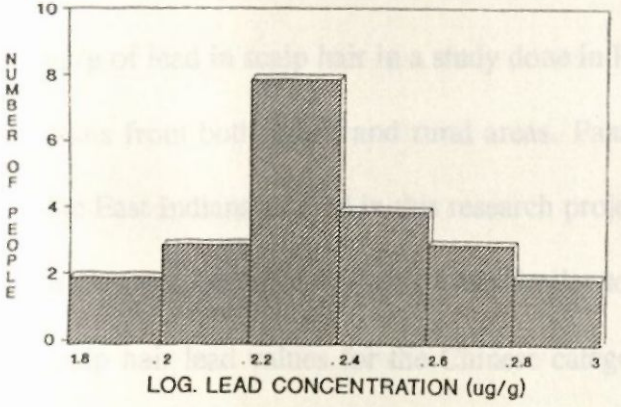


Fig.18

BLOOD LEAD
BATTERY WORKERS

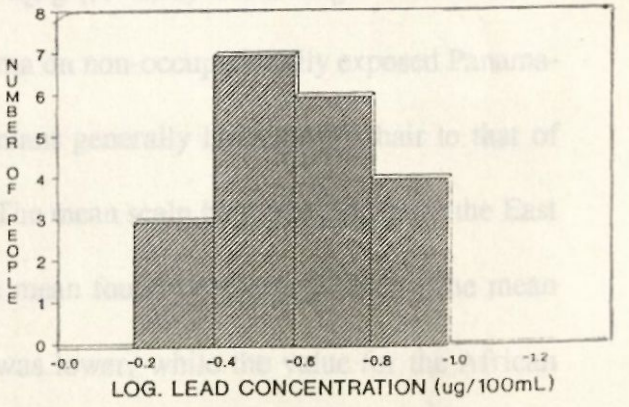


Fig.21

## 3:3 LEAD LEVELS: GENERAL POPULATION

### 3:3.a Scalp Hair Lead Levels

Trinidad is a country with a multi-racial population. The population has a percentage composition as follows: African 40.58%; East Indian 40.80%; Mixed 16.42%; Chinese 0.50%; and Others 1.70% (figures from 1980 census)[71].

Of the four racial categories studied the mean scalp hair lead level for the Chinese was the lowest (18.28 ug/g) while the mean scalp hair lead value for the African category (41.85 ug/g) was the highest (Table 2). The mean for the East Indians (27.02 ug/g) was close to the Mixed category (27.83ug/g) while the mean for the African category (41.85 ug/g) was about 23ug/g higher than the Chinese and about 14ug/g higher than the East Indians (Table 2). It must be noted that only eleven Chinese were sampled because of the difficulty in obtaining Chinese volunteers in a population of 0.5% Chinese[17].

Klevay[72] obtained a mean value of 30.23 ug/g (N=426) and a range between 30-40 ug/g of lead in scalp hair in a study done in Panama on non-occupationally exposed Panamanians from both urban and rural areas. Panamanians generally have similar hair to that of the East Indians studied in this research project. The mean scalp hair lead values for the East Indian and Mixed categories were similar to the mean found in Klevay's study. The mean scalp hair lead values for the Chinese category was lower, while the value for the African category was higher than that obtained by Klevay[72]. Grandjean[20] obtained median values between 3.70 ug/g and 7.00 ug/g for dark hair, blond hair and grey hair individuals respec-

tively (N=79) (no arithmetic mean was given) for a population of caucasians. The median values obtained in the Trinidad study ranged from 15-34 ug/g, with the highest value being that for the African group and the lowest being that of the Chinese group. The difference in the scalp hair lead levels of the African category compared to Klevay's or Grandjean's studies could be that the scalp hair lead levels of the African sample population represented a different position on the normal distribution curve of the total (international) population when compared to the other studies mentioned. No test of significance could be done because neither Klevay nor Grandjean reported standard deviations for their samples.

One obvious physical difference between the racial groups studied is the hair texture. Since one of the major problems with scalp hair as an indicator of lead exposure is exogenous contamination, it is possible that the difference in scalp hair lead values between the Panamanians studied by Klevay[72] and the African and Chinese categories in the Trinidad study may be due to the differences in the extent of adsorption of exogenous lead particles. The East Indian category has similar scalp hair lead values to the subjects of Klevay's study possibly because of similar hair characteristics (straight and thick) of both groups. The Chinese have fine straight hair which should not be as susceptible to exogenous contamination as persons of East Indian descent and hence the lower scalp hair lead values than those obtained in Klevay's study. On the other hand, persons of African descent have coarse-grained, curly hair which should retain larger amounts of exogenous particles for longer periods resulting in a greater chance of being chemisorbed and eventually becoming endogenous lead. This may be a possible reason why African lead levels are higher than that of the Chinese and East Indians. Also, it may be that the difference between the levels

obtained in Klevay's study and those obtained for the African and Chinese categories in the Trinidad may be due to differences in environmental factors.

The average scalp hair lead values found in this study were comparable to those found in a previous study done by Hall et al.[26] in which average scalp hair lead values obtained for the different racial group were as follows: African (40.10ug/g, N=35), East Indian (26.82ug/g, N=38), Chinese (18.83ug/g, N=6) and Mixed (47.87ug/g, N=36). However, the values for the Mixed category in both studies differed markedly. It is possible that the variation in racial combinations of the mixed category in these two studies were sufficiently different to cause the observed disparity between the average scalp hair lead values. The sample population used by Hall et al[26] was similar to the one used in this project with respect to age, gender and geographical location.

**TABLE 2 SCALP HAIR LEAD LEVELS FOR THE FOUR GROUPS**

| GROUPS | NUMBER SAMPLED | MEAN±SD (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|---|
| EAST INDIAN | 91 | 27.02 ±0.52 | 17.23 | 2.80-107.05 |
| AFRICAN | 46 | 41.85 ±0.38 | 33.86 | 5.76-155.36 |
| CHINESE | 11 | 18.28 ±0.25 | 15.70 | 1.00- 45.62 |
| MIXED | 41 | 27.83 ±0.50 | 20.70 | 3.41-126.93 |

The ranges for the different racial groups studied in the general population in this project (Table 2) were much wider than those obtained in the other investigations mentioned. The scalp hair lead values obtained in Grandjean's study ranged between 37.00 - 48.00ug/g while the ranges in this study were 2.80 - 107.05ug/g for the East Indian group and 5.76 - 155.36ug/g for the African group. A probable reason for this observation is that persons chosen in our study came from diverse geographical locations where the level of lead contamination they were exposed to varied to a greater extent than those persons studied by Grandjean[20]. The difference found between the mean and median scalp hair lead values in the Trinidad study (Table 2) was due to some individuals having significantly different values from the rest of the sample population.

In order to establish whether there was a significant difference in scalp hair lead among the East Indian, African and Chinese racial groups in the Trinidad study an unweighted one-way analysis of variance (ANOVA) was carried out. The data indicated that the interracial differences observed were real (Table 3). The Mixed category was not used in this statistical analysis because of the differences in the type and extent of mixing in this category. Therefore, if lead in hair is accepted as a suitable bioindicator it is likely that separate upper limits for different racial groups would have to be set for non-occupational exposure to lead, since each racial group appears to be affected differently even when they inhabit similar environments. Already upper limits have been proposed by

**TABLE 3 ANALYSIS OF VARIANCE OF LEAD LEVELS IN THE SCALP HAIR AMONG THE EAST INDIAN, AFRICAN AND CHINESE RACIAL GROUPS**

| SOURCE | DEGREE OF FREEDOM | SUMS OF SQUARES | MEAN SQUARES | F-RATIO | P VALUE |
|---|---|---|---|---|---|
| TOTAL | 191 | 28.89 | | | |
| TREATMENTS | 2 | 1.81 | 0.60 | 4.21 | <0.05 |
| ERROR | 189 | 27.08 | 0.14 | | |

other workers. In one study done by Grandjean[20] on caucasians, an upper limit of 70ug/g was set while an upper limit of 110ug/g was set for Japanese in a study done by Suzuki[73]. In the Trinidad study the upper limits for each racial group were established by using the mean scalp hair lead values plus two standard deviations. This method of selecting the upper limits was found to give a reliable minimum value which is considered to be critical with respect to lead toxicity[74]. The upper limit for the East Indians was 28.06ug/g, for the Africans was 42.62ug/g, for the Chinese was 18.75ug/g and the Mixed was 27.83ug/g. The methods for selecting the upper limits in Grandjean[20] and Suzuki[73] studies were not known and therefore cannot be compared with the Trinidad study. Having established that there was also a significant difference in scalp hair lead among the three racial groups in this present study, a forward multiple regression analysis was carried out on East Indian, African and Mixed racial groups in order to determine what possible factors could have influenced such a result. Scalp hair lead was used as the dependant variable and the four independent variables used were time spent in vehicular traffic on the road, time spent in "traffic jams" (as defined in the following section), sex and blood. None of the variables had statistically significant association with scalp hair lead concentration for any racial group (Table 4). There was insufficient data to do a forward multiple regression analysis for the Chinese group.

**TABLE 4 STEPWISE REGRESSION ANALYSIS OF SCALP HAIR LEAD FOR ETHNIC GROUPS IN RELATION TO GIVEN VARIABLES SETS**

| ETHNIC GROUPS | $R^2$ | | | | C/S RATIO |
|---|---|---|---|---|---|
| | TIME SPENT ON ROAD | TIME SPENT IN TRAFFIC | SEX | BLOOD | |
| EAST INDIAN | 0.02 | 0.003 | 0.09 | 0.11 | 0.02 |
| AFRICAN | 0.05 | 0.03 | 0.01 | 0.39 | 0.25 |
| MIXED | 0.05 | 0.03 | 0.01 | 0.01 | 0.26 |

n.s.

A study done on a sample of the Trinidad population by Hall et al[26] in 1990 also found a significant difference in scalp hair lead levels among the four racial groups studied. In order to account for the differences among racial groups in Hall's study a multiple regression model was used, with scalp hair lead as the dependant variable and total time spent in traffic, time spent in "traffic jams" and sex as independent variables. A "traffic jam" was defined as a condition in which, because of traffic congestion, vehicular movement was slow (0-24 kph) and sporadic. The Chinese population in this study was too small to allow multiple regression to be done. However, the highest coefficient of determination ($R^2$ = 0.51) was obtained for Africans, and for this category, the regression coefficients for all variables were significant, thus total traffic exposure time, "traffic jam" exposure and sex all appeared to have contributed to the level of lead in the hair of people of African descent. In the case of the East Indian category, however, it was found that none of the regressors in the model used was significant.

It is generally expected that total time spent in vehicular traffic on the road and time spent in traffic jams should have some impact on scalp hair lead levels, since lead emitted from gasoline can be inhaled and then transported in the blood to binding sites in the hair or deposited on the surface of hair and become chemisorbed into the hair. However, the results did not show any correlation between scalp hair lead levels and any of the parameters mentioned. This is possibly due, at least partly, to two factors namely: (1) the times given by persons studied may have been inaccurate since they were estimates made by the participants and were not measured accurately with a timing device, (2) maybe the lead particles

emitted from the motor vehicles did not circulate in the atmosphere at heights where they could have been inhaled or entrapped in the hair of the individuals sampled.

It must be emphasized too, that the non-significance of a particular regression coefficient in general does not imply that the independent variable concerned does not affect the dependant variable. It implies that at the 5% level of significance adopted, given the variability of the results, the confidence interval for the estimate of the regression includes zero as a possible value. Thus it cannot be discounted that the time spent on the road and time spent in "traffic jams", actually contributed to scalp hair lead levels. It may be concluded then, that further work is required to define the relationship more precisely.

### 3:3.b Pubic Hair Lead Levels

Of the four racial groups, the mean pubic hair lead value for the Chinese group was the lowest (14.89ug/g) while the mean values for the African group (20.45ug/g), East Indian group (20.45ug/g) and Mixed group (18.12ug/g) were all close to each other (Table 5).

**TABLE 5 PUBIC HAIR LEAD LEVELS FOR THE FOUR GROUPS**

| GROUPS | NUMBER SAMPLED | MEAN±SD (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|---|
| EAST INDIAN | 91 | 20.50 ±0.42 | 10.77 | 5.62-167.42 |
| AFRICAN | 46 | 20.45 ±0.37 | 16.57 | 2.85-73.59 |
| CHINESE | 11 | 14.84 ±0.84 | 8.76 | 2.06-57.78 |
| MIXED | 41 | 14.89 ±0.57 | 8.76 | 2.06-57.78 |

**TABLE 6 ANALYSIS OF VARIANCE OF LEAD LEVELS IN THE PUBIC HAIR AMONG THE EAST INDIAN, AFRICAN AND CHINESE RACIAL GROUPS**

| SOURCE | DEGREE OF FREEDOM | SUMS OF SQUARES | MEAN SQUARES | F-RATIO | P VALUE |
|---|---|---|---|---|---|
| TOTAL | 188 | 41.10 | | | |
| TREATMENTS | 2 | 0.60 | 0.20 | 0.92 | >0.05 |
| ERROR | 186 | 40.51 | 0.22 | | |

Pubic hair lead levels showed no significant difference among the East Indian, African and Chinese racial groups studied. This was revealed by the statistical test of unweighted one-way analysis of variance (ANOVA) at the 95% confidence level (Table 6). As mentioned previously, pubic hair is not normally exposed to the environment and therefore the problem of exogenous lead contamination may not be as great as in the case of scalp hair. As a result the effect of hair texture for different racial groups may not be an important factor when pubic hair is involved.

The median values for the four racial groups studied were East Indian (16.57ug/g); African (10.77ug/g); Chinese (8.76ug/g) and Mixed (9.63ug/g). The difference found between the mean and median pubic hair lead values for the different racial groups in the Trinidad study (Table 5) was due to some individuals having significantly different values from the rest of the sample population. The mean and median values for pubic hair lead were significantly lower than those of scalp hair lead for any given racial group. One explanation for this is that exogenous contamination is greater in scalp hair than pubic hair since the latter is not normally exposed to the environment. Although all hair samples were washed before hair analysis was carried out, no washing procedure has been shown to completely remove exogenous material. The scanning electron micrographs showed that none of the washing procedures employed completely removed all external contaminants. Thus it is possible that the greater quantity of exogenous lead on scalp hair accounts for the higher lead concentration in scalp hair compared to pubic hair. This difference in lead levels between the two bioindicators can also be explained in terms of the growth rate of both types of hair. Pubic hair grows more slowly than scalp hair and therefore lead accumulation in pubic hair

would be less than that of scalp hair[75]. As pubic hair grows more slowly than scalp hair, it may be that less binding sites (disulphide groups) of the hair proteins are available for the deposition of lead from the blood at any particular time period, compared to scalp hair. This could have resulted in the lead concentration in scalp hair being significantly higher than in pubic hair[75]. It is proposed that pubic hair is more suitable than scalp hair because it is not usually subjected to the application of cosmetic preparations or shampoos that may contain lead[75].

### 3:3.c Blood Lead Levels

The four racial groups mentioned in this study have blood lead values of the same order of magnitude (Table 7). The mean values for the four groups studied were East Indian (0.26ug/mL); African (0.34ug/mL); Chinese (0.27ug/mL) and Mixed (0.21ug/mL). In order to establish whether there was a significant difference in blood lead levels among the East Indian, African and Chinese racial groups an unweighted one-way analysis of variance (ANOVA) was carried out. The statistical test indicated no significant difference in blood lead levels among these groups (Table 8). A study done by Singal[76] in the United Kingdom among caucasian and ethnic minorities also showed that race is not a factor affecting lead deposition in other organs[8]. It may be that only when lead is deposited in other tissues (for example scalp hair) that the effect of race becomes pronounced.

In a study done on a group of caucasians by Hunt et al[77], the mean and range of blood lead values found were 0.30ug/mL and 0.18-0.44ug/mL respectively, while Mahaffey

et al[78] obtained mean and median values of 0.14ug/mL and 0.13ug/mL (N=1041) respectively in a study done to estimate the national blood lead levels in the United States. The sample population contained both Caucasians and African-Americans which belonged to the age group 25-34. The median values in the Trinidad study were East Indian (0.24ug/-mL); African (0.30ug/mL); Chinese (0.21ug/mL) and Mixed (0.20ug/mL). The similarity found between the mean and median blood lead values for the different racial groups in the Trinidad study (Table 7) was due to the fact that all the individuals in the sample population had values which fell within a narrow range.

All the mean and median values in this study were similar to those found by Hunt et al[77] but different to those obtained in the Mahaffey et al[78] study. The similarity in blood lead values in the former case with that found in this study could have been due to the fact that both sample populations were from urban and suburban districts, while Mahaffey et al[78] also included subjects from rural districts in his study. Urban and suburban areas normally have more motor vehicles and greater vehicular congestion compared to rural

**TABLE 7 BLOOD LEAD LEVELS FOR THE FOUR GROUPS**

| GROUPS | NUMBER SAMPLED | MEAN±SD (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|---|
| EAST INDIAN | 91 | 0.26 ±0.02 | 0.24 | 0.05-0.82 |
| AFRICAN | 46 | 0.34 ±0.04 | 0.30 | 0.23-0.66 |
| CHINESE | 11 | 0.27 ±0.05 | 0.21 | 0.07-0.91 |
| MIXED | 41 | 0.21 ±0.03 | 0.20 | 0.08-0.41 |

**TABLE 8 ANALYSIS OF VARIANCE OF LEAD LEVELS IN THE BLOOD BETWEEN THE EAST INDIAN, AFRICAN AND CHINESE RACIAL GROUPS**

| SOURCE | DEGREE OF FREEDOM | SUM OF SQUARES | MEAN SQUARES | F-RATIO | P VALUE |
|---|---|---|---|---|---|
| TOTAL | 188 | 15.12 | | | |
| TREATMENTS | 2 | 0.77 | 0.26 | 3.33 | >0.05 |
| ERROR | 186 | 14.35 | $7.75X10^{-2}$ | | |

n.s

areas. As a result it is expected that there would be a greater amount of lead emissions from gasoline in these areas than in rural areas. The lead particles emitted from gasoline can be deposited on food and in water which can enter the body by ingestion. This is conceivable, since ingestion of lead is thought to account for most of the lead that enters the human body[26]. The sample population in Mahaffey's et al[78] study consisted mainly of caucasians. His study showed that Caucasians had lower blood lead values than the African-Americans. No caucasians were sampled in the Trinidad study because of the difficulty in obtaining volunteers. Caucasians make up a very small fraction of the total population in Trinidad.

### 3:3.d Analysis of Lead Levels with Respect to Sex

In order to establish whether there was a significant difference in scalp hair lead levels, pubic hair lead levels and blood lead levels between sexes in the general population, unpaired t-tests were carried out. No significant difference in scalp hair lead levels was found between males and females belonging to the general population (Table 9). In addition no significant differences were found in pubic hair lead levels and blood lead levels between males and females belonging to the general population. The data were then categorized on the basis of racial groups to see if there was any difference in lead levels between males and females belonging to a particular racial group (Tables 10-12). The data showed that scalp

**TABLE 9 SCALP HAIR LEAD LEVELS BETWEEN SEXES**

| SEX | MEAN (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|
| MALE | 30.40 | 20.01 | 1.00-38.10 |
| FEMALE | 28.49 | 26.31 | 2.80-86.43 |

**TABLE 10 ANALYSIS OF SCALP HAIR LEAD WITH RESPECT TO SEX**

| RACIAL GROUPS | MEAN LEAD LEVELS(ug/g) (MALES) | MEAN LEAD LEVELS(ug/g) (FEMALES) | UNPAIRED t-TEST VALUES | P VALUE |
|---|---|---|---|---|
| EAST INDIAN | 25.60 | 19.80 | t=2.33 (N=91) | <0.05 |
| AFRICAN | 34.28 | 23.82 | t=1.17 (N=29) | >0.05 |
| MIXED | 22.29 | 15.52 | t=1.84 (N=30) | <0.05 |

**TABLE 11 ANALYSIS OF PUBIC HAIR LEAD WITH RESPECT TO SEX**

| RACIAL GROUPS | MEAN LEAD LEVELS(ug/g) (MALES) | MEAN LEAD LEVELS(ug/g) (FEMALES) | UNPAIRED t-TEST VALUES | P VALUE |
|---|---|---|---|---|
| EAST INDIAN | 20.45 | 11.23 | t=2.40 (N=91) | <0.05 |
| AFRICAN | 20.63 | 14.44 | t=1.51 (N=32) | >0.05 |
| MIXED | 9.43 | 18.71 | t=2.01 (N=31) | <0.05 |

**TABLE 12 ANALYSIS OF BLOOD LEAD WITH RESPECT TO SEX**

| RACIAL GROUPS | MEAN LEAD LEVELS(ug/mL) (MALES) | MEAN LEAD LEVELS(ug/mL) (FEMALES) | UNPAIRED t-TEST VALUES | P VALUE |
|---|---|---|---|---|
| EAST INDIAN | 0.26 | 0.40 | t=2.34 (N=91) | <0.05 |
| AFRICAN | 0.31 | 0.37 | t=0.95 (N=33) | >0.05 |
| MIXED | 0.19 | 0.23 | t=2.86 (N=35) | <0.05 |

hair, pubic hair and blood lead values between males and females belonging to the Mixed category were significantly different. Significant differences in scalp hair, pubic hair and blood lead values, between male and females were also found in the East Indian category. It may be that differences in hormonal activity between males and females in these two groups influence blood , scalp and pubic lead levels to different extents resulting in significant differences. It is also possible that the difference in hair growth rate between males and females can influence the scalp and pubic hair values to different extents. In addition, the discharge of blood during the menstruation in females may lower blood lead levels, resulting in a significant difference when compared to male blood lead values.

The African category however showed no significant differences in scalp hair lead values blood lead values and pubic hair lead values between males and females. The lack of any significant differences in the lead levels between males and females for the African category could be probably due to some other factor which influences both sexes to the same extent and which is also more influential to lead accumulation than hormonal activity. The data for the Chinese group was to few to carry out any meaningful statistical analysis.

### 3:3.e Blood Lead-Hair Lead Correlation

The scalp hair lead and blood lead data for the general population were subjected to a correlation analysis. The association of lead in blood and scalp hair of the general population studied indicated an exponential distribution with values ranging from 1-110ug/g in hair and from 0.05-0.81ug/mL in blood. The correlation coefficient obtained (R=0.31)

was found to be statistically insignificant, indicating that scalp hair lead did not correlate well with the blood lead levels in the present study (Fig. 22). The data were best fitted by the regression equation log $Y=0.1X+0.38$ where Y and X are scalp hair and blood lead concentrations, respectively.

There was no statistically significant correlation between scalp hair lead concentrations and blood lead concentrations by race for the four racial groups studied (Chinese, $r=0.04$; East Indian, $r=-0.32$; African, $r=-0.63$; and Mixed, $r=-0.10$.) In addition, no significant correlations were found between pubic hair lead concentrations and blood lead concentrations with respect to race for the racial groups studied except for Chinese (Chinese, $r=0.63$; East Indian, $r=0.10$; African, $r=-0.07$; and Mixed, $r=-0.10$).

This was similar to the observation made by Laker[8], and also by Ahmed et al[79] that one should not expect a correlation between lead contents of these two body tissues. Lead in blood is constantly being transferred from one components to another. Lead binds to the sulphur atom of the amino-acid cysteine, which is incorporated in proteins found in blood[80]. Lead concentrations in blood are time-dependent, reflecting their intake in the previous hours or days. In contrast, hair, being metabolically inert, fixes trace elements, and there provides a lasting record of intake over the previous several months. The disulphides of the hair protein have been proposed as the binding sites for lead. The amino groups may also be involved[80]. The binding of endogenous lead in the hair is assumed to be irreversible[80]. Thus, hair shows a cumulative concentration which cannot be compared with the transient concentrations in blood[80].

However, the lack of correlation between hair lead levels and blood lead levels should not detract from the importance of hair as a useful biopsy material for epidemiological studies of environmental pollutants. The use of hair depends on the scope and objectives of the particular research or monitoring programme, and the expertise and facilities available.

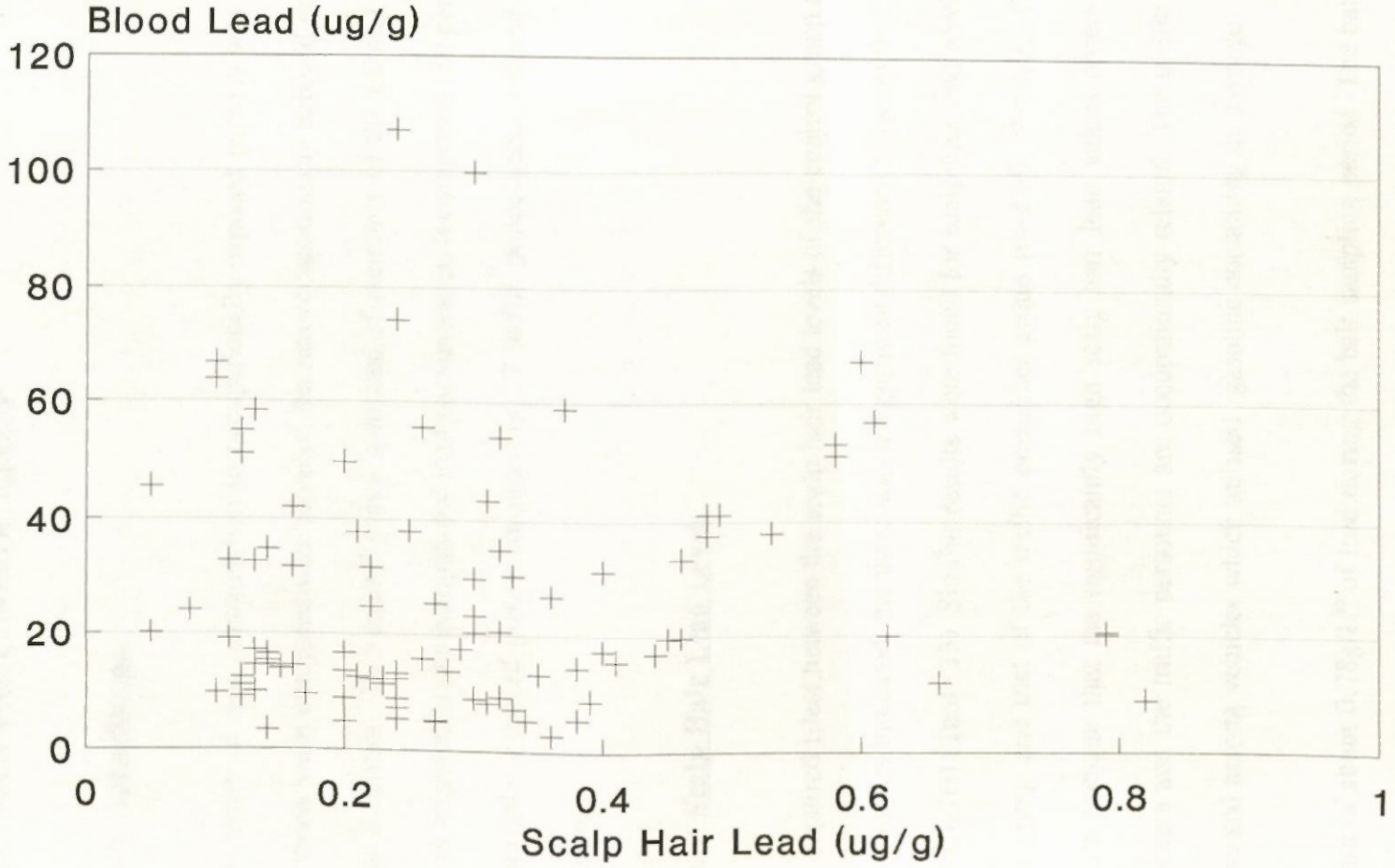


Fig.22 Scatter Diagram Of Blood-Lead Conc. vs Scalp Hair-Lead Conc..Note that no correlation was observed.

## 3:4. COMPARISON OF BATTERY WORKERS AND TRAFFIC POLICE PERSONNEL WITH THE CONTROL GROUP

### 3:4.a Introduction

The extent of lead accumulation in occupationally exposed persons was seen when their lead levels were compared with those of the non-occupationally exposed group which was used as a control. This control group consisted of members of the general population who were not exposed to any occupational lead contaminants. It comprised 189 persons while the occupationally exposed groups consisted of 27 traffic police personnel and 22 battery plant operators.

### 3:4.b Scalp Hair Lead Levels

An unpaired t-test between the scalp hair lead levels of the control group and that of the battery workers indicated that there was a significant difference between the two groups ($t=2.43$ $P<0.05$) (Table 13). Similar results were found for scalp hair lead levels between the control group and that of the traffic personnel group ($t=2.30$ $P<0.05$) (Table 13). These results indicate that the significantly high scalp hair lead values observed by the battery workers and the traffic personnel are occupationally related. The traffic personnel sampled worked among vehicles which utilised gasoline containing an average

concentration of about 0.78g/L[26] of lead during the hair sampling period. The battery plant employees worked in areas that contained high lead particulate loadings. Both categories of

workers were therefore exposed to atmospheric lead for longer periods during their normal working hours compared to the average person sampled from the control group. The scanning electron micrographs (Fig. 3.i and 3.j) of washed hairs (Kleenol® washing procedure) of battery workers and traffic personnel showed a much smaller number of absorbed particles when compared to the unwashed hair samples. It is therefore reasonable to assume that the lead levels obtained from these special groups of workers provided a reasonable estimate of endogenous lead levels. The mean scalp hair lead value for battery workers (418.22ug/g) was much higher than that of the traffic personnel (66.19ug/g). This may be due to the fact that most of the lead contamination of traffic police personnel was from lead particulates from automobile exhausts which has relatively low atmospheric concentrations. On the other hand, in the battery plants, atmospheric lead concentrations are more likely to be much higher.

### 3:4.c Pubic Hair Lead Levels

Significant differences in pubic hair lead levels were found between the control group and the battery workers group ($t=1.92$, $P=0.05$) and between the former and the traffic personnel group ($t=1.74$, $P=0.05$). The mean pubic hair lead level for battery workers (306.17ug/g) was also much higher than that for the traffic police personnel (72.43ug/g, Table 14).

**TABLE 13 SCALP HAIR LEAD LEVELS:**

| CATEGORY | NUMBER SAMPLED | MEAN±SD (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|---|
| TRAFFIC POLICE | 27 | 94.11 ±0.81 | 66.19 | 27.52- 603.17 |
| BATTERY PLANT OPERATORS | 22 | 589.41 ±1.42 | 418.22 | 50.93-1513.00 |
| GENERAL POPULATION | 189 | 29.72 ±0.41 | 20.70 | 1.00- 110.36 |

Explanations given for these results are similar to those given for the distribution of scalp hair lead among the three categories studied. In addition, pubic hair is not normally exposed to the environment and as a result the problem of exogenous lead contamination should be reduced. However the possibility of exogenous contamination still exists. Exogenous lead may be deposited on pubic hair from any lead particles being carried by air currents through the relatively loose clothing used by the traffic police and the battery plant workers.

### 3:4.d Blood Lead Levels

According to the Occupational Safety and Health Administration (OSHA,1991), elevated blood lead levels of 0.4ug/mL or more in persons, is cause for concern of possible lead toxicity. The frequency distribution of lead concentrations in blood lead levels for the three different categories are given in (Figs.10-12). The results show that 65% of the control group had blood lead levels less than 0.4ug/mL, whereas 100% of the battery workers and traffic police had blood lead levels of less than 0.4ug/mL. Unpaired t-tests indicated that no significant differences were observed between the control group and the battery workers ($t=0.95$, $P<0.05$); and the control group and the traffic personnel ($t=0.64$, $P<0.05$). It seems therefore with respect to blood lead levels, the occupationally exposed workers are below the currently acceptable upper limit (0.4ug/mL) (Table 15).

**TABLE 14 PUBIC HAIR LEAD LEVELS:**

| CATEGORY | NUMBER SAMPLED | MEAN (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|---|
| TRAFFIC POLICE | 27 | 72.43 ±1.27 | 319.09 | 13.74-4043.17 |
| BATTERY PLANT OPERATORS | 22 | 306.17 ±10.47 | 419.03 | 98.13-1671.69 |
| GENERAL POPULATION | 189 | 19.77 ±1.36 | 10.43 | 0.14- 167.72 |

**TABLE 15 BLOOD LEAD LEVELS:**

| CATEGORY | NUMBER SAMPLED | MEAN±SD (ug/g) | MEDIAN (ug/g) | RANGE (ug/g) |
|---|---|---|---|---|
| TRAFFIC POLICE | 27 | 0.26 ±0.02 | 0.11 | 0.14-0.48 |
| BATTERY PLANT OPERATORS | 22 | 0.23 ±0.09 | 0.22 | 0.12-0.62 |
| GENERAL POPULATION | 189 | 0.27 ±0.06 | 0.24 | 0.05-0.91 |

The results (Tables 10-12) indicate that in the case of occupational exposure, blood lead reveals little information about the state of lead accumulation in the body. The disadvantages of blood as an indicator relative to hair have already been discussed.

## 3:5 COMPARISON OF THE TWO OCCUPATIONALLY EXPOSED GROUPS

### 3:5.a Introduction

Two occupationally exposed groups were studied in this project. They were workers of a battery manufacturing company and traffic police personnel. The battery plant employees were considered to be occupationally exposed since they constantly worked in an environment with a large particulate loading of lead generated from the lead and various lead compounds used in the manufacture of battery components.

The traffic police personnel were considered to be occupationally exposed since motor vehicle exhaust emissions contained lead particles which were constantly inhaled during their patrols along the busy highways. About six hours a day, five days a week, were spent on the highways and at traffic intersections. These traffic police worked two three-hour sessions (6am-9am and 3pm-6pm) separated by a six hour period per day. On the other hand, persons belonging to the normal population, generally spent about one hour in the morning and one hour in the afternoon on the roads in their journeys to and from their places of work.

### 3:5.b Scalp Hair Lead Levels

For the occupationally exposed groups studied, the mean value of scalp hair lead for Battery Workers was 589.41ug/g (N=22) while that for traffic police was 94.41ug/g (N=27) (Table 13). The much higher mean scalp hair lead value of the battery workers compared to the traffic police personnel is due to reasons already mentioned in section 3:4.b.

In a study done by Fergusson[21] in Christchurch, New Zealand on caucasian battery workers, a mean scalp hair lead of 363ug/g (N=16) was obtained while Nishiyama[81] obtained a mean value of 217ug/g for his Japanese sample population. The median values for the battery workers and the traffic police studied were 418ug/g and 66.19ug/g respectively. The mean and median values in the Trinidad study were much higher than those found by Fergusson[21], and Nichiyama[78]. The median and ranges in this study were also higher than those found by Chattopadhay in Toronto,Canada[15]. The higher lead levels obtained in this project for two occupationally exposed groups were assumed to be at least partly explicable in terms of race. The sample population of workers studied by Fergusson[21] and Nishiyama[81] were basically caucasian and Japanese respectively whereas those studied in this project were of African descent. Possibly the inherent variations in race could result in Africans having a greater susceptibility to lead accumulation in the body than the two other races mentioned. Hair texture is another possible reason for this observation. This is fully explained in section 3:3.a.

### 3:4.c Pubic Hair Lead Levels

Of the two occupationally exposed groups, the average pubic hair lead value for the battery workers (419.03ug/g) was higher than that for traffic police (319.09ug/g). The median value for the battery workers was also higher (306.17ug/g) than that for the traffic police (72.43ug/g). The large disparity between the mean and median values in the two occupationally exposed groups studied indicated that the pubic hair lead values in their sample populations were not normally distributed but skewed (Table 14). Reasons which could have attributed to the higher mean and median pubic hair lead values found for battery workers compared to traffic police personnel are mentioned in section 3:4.b Reasons for the lower values found in pubic hair compared to scalp hair of the occupationally exposed groups are mentiond in section 3:4.c. Some individual values were much higher than the rest of the sample population in both occupationally exposed groups. Two battery workers had individual pubic hair lead levels of 1000.00ug/g and 1094.94ug\g respectively. They were both furnace operators and as such were possibly exposed to larger amounts of lead dust and fumes which was intermittently emitted from the furnace. One traffic police officer studied had a pubic hair lead level of 4043.17ug/g indicating high and prolonged exposure to a source of lead, which is as yet unknown.

### 3:4.d Blood Lead Levels

The mean blood lead levels for battery workers and traffic police found in this study were 0.23ug/mL and 0.26ug/mL respectively (Table 15). The median values for the battery workers and the traffic police were 0.22ug/mL and 0.21ug/mL respectively while the ranges for the battery workers and traffic personnel were 0.12 - 0.62ug/mL and 0.14 - 0.48ug/mL respectively. The blood lead values for both occupationally exposed groups were of the same order of magnitude.

The mean blood lead value for occupationally exposed groups obtained by Dahlgrem[82] was 0.70ug/mL whereas Chattopadhay[15] obtained a range of 0.08 - 0.67ug/mL (no mean value was given). In all three studies the participants were caucasians working in battery plants. A median value of 0.66ug/mL was obtained by Grandjean[20]. The mean, median and range obtained in this study for the two occupationally-exposed groups were lower than those obtained by Dahlgram[82], Grandjean[20] and Chattopadhay[15] respectively. In fact the means, medians and range in this study for the occupationally exposed groups were similar to those found in the general population study (Table 15). Similar results were found by Chattopadh-ay[15]. This is possibly due to the fact that since blood is a circulatory system, it deposits lead in tissues (eg. hair) as it circulates through the body. Thus, blood lead levels would tend to be lower than the concentration found in the hair, brain, kidney or liver of persons, except if the samples are taken immediately after exposure. These observations emphasize the

These observations emphasize the disadvantage of using blood as a bioindicator when the accumulation of lead in the body over extended periods is being studied.

## 3:6 MEDICAL SYMPTOMS

A section of the study dealt with the symptoms associated with lead poisoning. A majority of the exposed workers indicated a combination of these symptoms. The most common symptoms indicated by the exposed workers were leg cramps, metallic taste, headaches, diarrhoea, sluggishness, constipation, vomiting and irritability.

A chi-square statistical test was carried out in order to establish if there was any association between the occupationally exposed groups and the symptoms of lead toxicity. The control group used in this statistical test consisted of persons from the general population. The results (Table 16) of the statistical test indicated that there was a significant difference at the 95% confidence level between the occupationally exposed group and the control in relation to the lead toxicity symptoms (chi-square= 33.39). Occupation may therefore be a factor which affects the health of persons with respect to lead toxicity in this instance.

The results in Table 16 show that for most of the symptoms of lead poisoning both occupationally exposed groups had a higher percentage of persons having these symptoms compared to that of the normal population (Table 16). The large number of persons showing these symptoms in the occupationally exposed groups may be due to exposure to

**TABLE 16 NUMBER OF INDIVIDUALS WITH SYMPTOMS OF LEAD TOXICITY OVER THE PRECEDING MONTH (EXPOSED VS NON-EXPOSED GROUPS)**

| SYMPTOMS | EXPOSED GROUP (N=49) | NON-EXPOSED (N=189) | $X^2$ VALUE | P VALUE | SIG. DIFF. |
|---|---|---|---|---|---|
| abdominal pain | 19 | 15 | 16.11 | <0.05 | YES |
| headaches | 19 | 17 | 15.75 | <0.05 | YES |
| leg cramps | 18 | 13 | 13.35 | <0.05 | YES |
| metallic taste | 27 | 2 | 47.27 | <0.05 | YES |
| irritability | 12 | 8 | 10.20 | <0.05 | YES |
| constipation | 13 | 2 | 20.08 | <0.05 | YES |
| vomiting | 15 | 2 | 25.84 | <0.05 | YES |
| diarrhea | 10 | 11 | 5.01 | >0.05 | NO |
| apathy | 8 | 27 | 0.05 | >0.05 | NO |

enhanced levels of lead since persons belonging to these groups are exposed to lead to a greater extent than those belonging to the normal population. This is supported by the high mean scalp and pubic hair lead levels for the occupationally exposed groups.

It must be noted however that these were the reported symptoms over the month preceeding sampling and that no subject had a combination of these symptoms in such severe forms as to warrant a suspicion of acute lead poisoning.

Furthermore since the mean scalp hair and pubic hair lead values of occupationally exposed workers were significantly higher than persons belonging to the normal population, whereas the mean blood lead levels for both the occupationally exposed groups and the normal population were similar, these results suggest that lead in hair provides a more realistic estimate of long term exposure to lead contamination compared to lead in blood.

## 3:7 SULPHUR AND CARBON:SULPHUR HAIR ANALYSIS

The percentage sulphur and the carbon:sulphur ratio in hair influences hair texture[80]. It has been suggested therefore that the difference in scalp hair texture between the East Indian, African and Chinese racial groups was due to the different sulphur percentages and carbon:sulphur ratios in the scalp hair. Furthermore, the disulphide groups of the hair protein have been proposed as the binding sites for lead[80].

The carbon:sulphur ratios of scalp hair of the general population show a normal distribution (Fig. 23). In order to check for any possible relationship between the percentage sulphur (Table 17) and the carbon:sulphur ratio (Table 18) with that of scalp hair lead, correlation analyses were carried out between:- (1) the sulphur percentages and the scalp hair lead levels and (2) the carbon:sulphur ratios and the scalp hair lead levels. The results indicate no correlation between the scalp hair lead levels with both the sulphur percentages ($r=0.02$) and the carbon:sulphur ratios ($r=0.25$). The lack of correlation may be due to the presence of other binding sites (other than disulphide groups) in hair which bonds to systemic lead or that the hair lead levels are only influenced by environmental lead exposure and not the sulphur percentages or the carbon:sulphur ratios.

The carbon:sulphur ratios of the East Indian and African racial groups were compared since members of these groups generally have different hair textures. Africans have coarse curly scalp hair whereas East Indians have thin straight scalp hair. An unpaired t-test was used to

compare the carbon:sulphur ratios of these two racial groups and the results showed a significant difference in carbon:sulphur ratios ($t=2.12$, $P=0.05$) between them. Since a significant difference was also observed in scalp hair lead levels between the East Indian and African racial groups ($t=2.47$, $P=0.05$) it may be that the carbon:sulphur ratios between these two groups is a factor contributing to the differences in the scalp hair lead levels observed. The curly scalp hair texture of the African racial group compared to the East Indian racial group is due to the higher percent of sulphur in the hair. It is possible that exogenous lead can be chemisorbed into African hair to a greater extent compared to East Indian hair since it spends a longer time trapped on the hair surface of the African hair due to its curly texture.

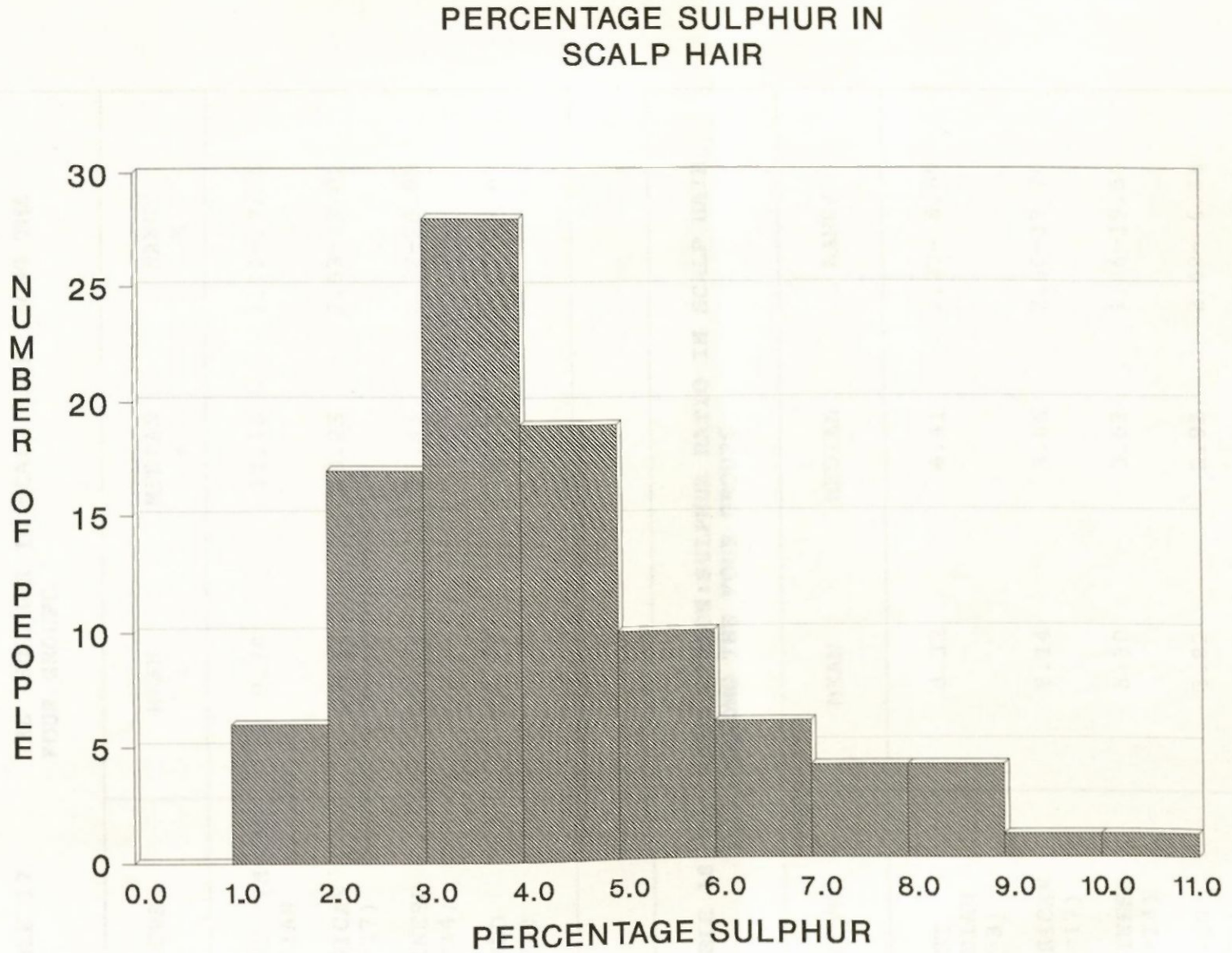
PERCENTAGE SULPHUR IN
SCALP HAIR
NUMBER OF PEOPLE
30
25
20
15
10
5
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
PERCENTAGE SULPHUR

Fig.23

**TABLE 17** **THE %SULPHUR IN SCALP HAIR AMONG THE FOUR GROUPS**

| GROUPS | MEAN % | MEDIAN % | RANGE % |
|---|---|---|---|
| EAST INDIAN (N=35) | 9.78 | 11.15 | 2.11-17.96 |
| AFRICAN (N=17) | 12.17 | 13.23 | 2.53-19.02 |
| CHINESE (N=14) | 12.34 | 12.41 | 2.32-20.85 |
| MIXED (N=8) | 14.09 | 14.73 | 7.29-19.61 |

**TABLE 18** **THE CARBON:SULPHUR RATIO IN SCALP HAIR AMONG THE FOUR GROUPS**

| GROUPS | MEAN | MEDIAN | RANGE |
|---|---|---|---|
| EAST INDIAN (N=3) | 8.32 | 4.41 | 2.32- 6.69 |
| AFRICAN (N=17) | 5.14 | 3.66 | 2.56-17.74 |
| CHINESE (N=14) | 5.30 | 3.62 | 1.66-19.58 |
| MIXED (N=8) | 3.82 | 2.95 | 2.49- 6.88 |

# CHAPTER FOUR

# CONCLUSION

## 4:1 CONCLUSION

As a result of the previously mentioned problems associated with the use of blood as a bioindicator, a project designed to investigate further the possibility of using hair as an alternative was undertaken.

In any study on the use of hair as a biopsy material, finding a suitable washing procedure is of paramount importance. In this project, although the washing procedure used seems to give the best results when compared to others, there are still problems to be addressed. The scanning electron microscope (SEM) photographs taken after hair samples were washed using the Kleenol® method, still indicated the presence of some particulates. Therefore there is the possibility of exogenous lead contamination of the hair samples. In addition, no method was employed to test whether endogenous lead particulates were removed during washing. The problem of identifying a totally acceptable washing procedure therefore remains unresolved based on the results of this study.

A significant difference was found in scalp hair lead levels among the racial groups studied. Since no correlation between scalp hair lead levels and sulphur and carbon:sulphur content was found, this suggested that these factors were not responsible for the significant differences in scalp hair lead levels among racial groups. It is generally assumed that hair texture might be related either to the sulphur content or the carbon:sulphur ratio of scalp hair in some way. The data obtained supported this assumption. A significant difference in the carbon:sulphur

ratio was found between the East Indian and African racial groups. These two groups have different hair texture as mentioned before. It should be noted that the physical structure of scalp hair is different among the racial groups. It is therefore also possible that because of this, lead particles are entrapped to varying extents by the scalp hair of individuals from different races, resulting in the significant differences in lead levels among the races. It may also be that factors not studied in this project are responsible for the differences in scalp hair lead values observed.

In the East Indian and Mixed racial groups, scalp hair, pubic hair and blood lead values between males and females were significantly different. No significant difference was found in the African group. Differences in hormonal activity or hair growth rate between males and females as well as the loss of blood (menstrual discharge) could have influenced the lead values among the East Indian and Mixed groups. It is also possible, that the extent of genetical mixing in the Mixed group (rather than hormonal activity) maybe responsible for the differences observed in this category. Other factors which were more influential to lead accumulation than hormonal activity and which were not influenced by sex may account for the lack of a significant difference in lead levels between sexes in the African group.

A significant difference in lead levels was found between scalp hair and pubic hair of persons within the general population. This difference has been attributed to the difference in growth rate of both types of hair. Pubic hair grows more slowly than scalp hair and therefore lead accumulation in pubic hair would be less than that of scalp hair. In addition, scalp hair is more exposed to exogenous lead contamination than pubic hair.

No significant difference was found in blood lead values between occupationally exposed groups and the general population. This is because blood lead levels reflect intake prior to the time the sample was taken, whereas hair provides a time averaged estimate. The significant difference found in scalp hair lead and pubic hair lead between occupationally exposed groups and the general population suggests that in occupations like that of battery plant employees and traffic police personnel, the increased atmospheric lead concentrations contribute significantly to their scalp and pubic hair lead levels. In spite of the possibility of exogenous lead contamination, it seems that the elevated lead found in occupationally exposed workers is sufficiently different to assume that their occupations are responsible for the difference.

No significant correlation was found between the time spent in "traffic jams" and the lead levels in scalp hair. This was unexpected, but similar to the results of Hall et al[26]. Since the speed at which motor vehicles travel in a "traffic jam" is slow, it may be that the lead particulates emitted from their exhaust systems are deposited close to the ground and are not elevated sufficiently to be inhaled by their occupants.

There was also no significant correlation between the total time spent on the road in motor vehicles and the scalp hair lead levels. It was assumed that at high speeds, automobiles consume larger quantities of gasoline which should result in the emission of larger quantities of lead. However, the force and velocity with which exhaust gases are emitted at high vehicular speeds could result in the lead particulates being elevated to heights beyond the reach of persons travelling.

There was also no significant correlation between the total time spent on the road in motor vehicles and the scalp hair lead levels. It was assumed that at high speeds, automobiles consume larger quantities of gasoline which should result in the emission of larger quantities of lead. However, the force and velocity with which exhaust gases are emitted at high vehicular speeds could result in the lead particulates being elevated to heights beyond the reach of persons travelling.

When the scalp hair lead values and the blood lead values were compared, no significant correlation was found. This reinforced the point that each of the bioindicators mentioned represents different time scales. Blood reflects lead intake in the previous hours or days whereas hair provides a lasting record.

Many problems associated with the use of hair as a bioindicator for detecting exposure to lead still exist. For example, there is the difficulty in obtaining scalp hair from newborn babies and balding adults. Inspite of these limitations, hair analysis is likely to gain more international recognition as an effective bioindicator for determining lead levels.

## 4:2 RECOMMENDATIONS

The following are the recommendations for further work:

(1) A larger sample population should be investigated in order to obtain a sample population which is more representative of the Trinidad population. In addition, the sample population should include more Chinese in the control and occupationally-exposed groups so that suitable statistical analyses can be done.

(2) The sample population studied in this project consisted of only one major age group and therefore no comparison between age groups was possible. Further studies should be done on a population that includes all the major age groups ie. pre-adolescent, adolescent and adult.

(3) Individuals sampled should be instructed to accurately determine the time spent on the road and the time spent in "traffic jams".

(4) Closer medical scrutiny should be carried out on individuals to ascertain whether medical symptoms observed are actually related to lead exposure or are due to other factors.

(5) Air-lead levels at the battery plant and along routes where individuals travel should be monitored. Air currents and rainfall data should also be collected and utilized in the interpretation of the data.

(6) Other occupationally exposed groups such as gas station attendants, taxi drivers, auto-mechanics, persons handling tetraethyllead for gasoline blending etc. should be included in the sample population.

(7) More work should be done to establish a suitable washing procedure which removes all exogenous particulates but avoids removal of endogenous material.

(9) Hair lead determination should also be done by other methods (e.g the X-ray fluorescence (XRF) method) and the results compared with that obtained by atomic absorption spectroscopy (AAS).

(10) An investigation of the growth rate of hair of persons belonging to different racial groups should be conducted so as to determine what impact, if any, it has on lead levels in hair.

## REFERENCES


1. Boeclx, R. L. *Analytical Chemistry*. **1986**, 58, 275-281.

2. Roger, L.R. *Preventing Lead Poisoning in Young Children*; U.S. Department of Health and Human Services. Centers for Disease Control: Baltimore, 1991.

3. Bradley, J. E. *Journal of Pediatrics*. **1956**, 49, 1-6.

4. Billick, I. H.; Curran, A. S.; Sheir, D. R. *Environmental Health Perspectives*. **1979**, 31, 183-190.

5. Annest, J. L.; Pirkle, J. L.; Makuc, D.; Nesse, J. W.; Bayse, D. D.; Kovar, M. J. *New England Journal of Medicine*. **1983**, 308, 1373-1377.

6. Stevenson, R. *Chemistry in Britain*. **1991**, 686, 2214-2221.

7. Hall, L. Presented at the Second Petroleum and Petrochemical Conference, Farrel House Hotel, Trinidad, August 1989; paper 5.

8. Laker, M. *The Lancet*. **1982**, 21, 260-262.

9. Ekanem, E.J.; Bernard, C. L. R.; Ottaway, J. M. *Talanta*. **1986**, 33, 55-59.

10. Chatt, A; Katz, S. A. *Hair Analysis: Applications in the biochemical and environmental sciences*. VCH Publishers: New York, 1988; p17.

11. McKenzie, J.M. *American Journal of Clinical Nutrition*. **1974**, 27, 501-514.

12. Ryabukhin, Y.S. "Activation analysis of hair as an indicator of contamination of man by environmental trace element pollutants"; IAEA Report IAEA/RL 50; International Atomic Energy Agency, Vienna, 1978.

13. Salmela, S.; Vouri, E.; Kilpio, J. O. *Analytical Chemica Acta*. **1981**, 125, 131-137.

14. Hambidge, K. M.; Franklin, M. L.; Jacobs, M. A. *American Journal Clinical Nutrition*. **1972**, 25, 385-89.

15. Chattopadhyay, A.; Roberts, M. T.; Jervis, R. E. *Archives of Environmental Health*. **1977**, 32, 226-236.

16. Hui Min, J.; Gvoan, H. "Study of normal values for zinc in the hair of school children in Jinan, China"; Technical report AA-81; Varian Instruments, China, 1988.

17. Siegfried, M.; Kopito, L.; Schwachman, H. *Journal of American Medical Association*. **1972**, 222, 462-466.

18. Weiss, D.; Whitten, B. *Science*. **1972**, 17, 69-70.

19. Chatt, A.; Katz, S.A. *Hair Analysis: Applications in Biochemical and Environmental Sciences*, 17-26, VCH Publishers, New York, 1988; p29.

20. Grandjean, P. *Archives of Occupational Environmental and Health*. **1978**, 42, 69-81.

21. Fergusson, J. E.; Hibbard, K. A. *International Pollution*. **1981**, 2, 235-248.

22. Renshaw, G. D.; Pounds, C. A.; Pearson, E. F. *Nature*. **1972**, 238, 162-163.

23. Schroeder, H. A.; Nason, A. P. *Journal of Investigative Dermatology*. **1968**, 53, 71-78.

24. Petering, H. G; Yeager, D. W.; Witherup, S. O. *Archives of Environmental Health*. **1973**, 23, 565-566.

25. Hung, P. Q. "PIXE Studies of Ethnic differences of trace and other elements concentration in Hair"; Proceedings of the 2nd International Workshop on the Trace Element Analytical Chemistry in Medicine and Biology, Neuherbery, 1982.

26. Hall, L; Stoute, V.; Maharaj, D. *U.W.I. Biospectrum*. **1990**, 2, 9-13.

27. Tavares, T. M.; Brandao, A. M. *International Journal Environmental Analytical Chemistry*. **1989**, 36, 221-230.

28. Reeves, R. D.; Jolley, K. W.; Buckley, P. D. *Bullitin of Environmental Contamination and Toxicology*. **1973**, 14, 579-587.

29. Klevay, L. M. *Archives of Environmental Health*. **1973**, 26, 169-172.

30. Wedepohl, K. H. *Economic Geology*. **1971**, 66, 240-242.

31. Sheldon, R. P.; Warner, M. A.; Thompson, M. E.; Peirce, H. W. "Stratigraphic sections of the phosphoria formation in Idaho, 1949, Part I"; United States Geological Survey Circular, 304, 1953.

32. Friberg, L. *Handbook on the Toxicology of Metals*. Elsevier/North Holland Biomedical Press: Holland, 1979, p 95.

33. Swaine, D. J. "The trace-element content of soils"; Commonwealth Bureau of soils Technical Communications No. 48; Atlantic City, NJ, 1955.

34. Bernard, G. Master's Thesis, University of the West Indies at St Augustine, 1979.

35. Livingstone, D. A. "Chemical Composition of rivers and lakes"; United States Geological Survey Professional Paper, Paper 440, 1963.

36. Chow, T. J. *Journal of Water Polution Control Federation*. **1906** , 40, 399-411.

37. Chow, T. J.; Bennet, C. F. *Environmental Science and Technology*. **1969**, 3, 737-740.

38. Jernigan, E. L.; Ray, B. J.; Duce, R. A. *Atmospheric Environment*. **1971**, 5, 881-886.

39. Patterson, C. C. *Archives of Environmental Health*. **1965**, 11, 344-363.

40. Chow, T. J.; Earl, J. L.; Snyder, C. B. *Science*. **1972**, 178, 401-402.

41. Engel, R. E.; Hammer, D. J.; Horton, R. J. M.; Lane, N. M.; Plumblee, L. A. *Environmental Lead and Public Health Research Triangle;* North Carolina Environmental Protection Agency. Air Pollution Control Office: 1971, AP 90.

42. Hall, L. The University of The West Indies, Department of chemistry , St. Augustine Campus, personnel communication, 1989.

43. Warren, H. V.; Delavault, R. E. *Journal of Science of Food and Agriculture*. **1962**, 13, 96-98.

44. Ziegfield, R. L. *Archives of Environmental Health*. **1964**, 8, 202-212.

45. Farrell, W. J. Presented at ANZAAS Jubilee Conference, Adelaide, Australia, May 1980; paper 43.

46. Hernberg, S. "Biological Effects of Low Level Lead Doses"; In International Sympoium Encironmental Health Aspects of Lead, 17-629; CEC, Luxembourg, 1973.

47. Newman, P.; & Kenworthy, J. "Lead in Petrol: An Environmental Case study"; Department of Environmental Sciences, Murdoch University, Australia, 1981.

48. Guthrie, R. *Pediatrics*. **1988**, 82, 524-527.

49. Klein, M.; Namer, R.; Harpur, E.; Corbin, R. *New England Journal of Medicine*. **1970**, 283, 669-672.

50. Bose, A.; Vashistha, K.; O'Loughlin, B. J. *Pediatrics*. **1983**, 72, 106-108.

51. Roper, L.A. *Pediatrics*. **1985**, 79, 457-465.

52. Ruther, M. Jones, R.R. *Lead Versus Health*. Wiley: New York, 1982; 35-39.

53. Atkins, R. R. *Journal of Air Pollution Control Association*. **1969**, 19, 591-592.

54. Ter Haar, G.L.; Holtzman, R. B.; Lucas, R. F. *Nature*. **1967**, 216, 353-355.

55. Needleman, H. L.; Gunnoe, C.; Leviton, A.; Reed, R.; Peresie. H.; Maher, C.; Barrett, P. *New England Journal of Medicine*. **1979**, 300, 689-695.

56. Needleman, H.L.; Kenworth, J. R. *New England Journal of Medicine*. **1974**, 290, 245-248.

57. Landrigan, P. J.; Whitworth, R. H.; Baloh, R. W.; Staehling, N. W.; Barthel, W. F. *Lancet*. **1975**, 1, 708-712.

58. Landsdown, R. G.; Shepherd, J.; Clayton, B. E.; Delves, H. T.; Graham, P. J.; Turner, W. C. *Lancet*. **1974**, 1, 538-541.

59. Aldridge, W. N.; Street, B. W.; Skilleter, O. N. *Biochemical Journal*. **1977**, 168, 352-364.

60. Krigman, M. R. *Science*. **1979** , 210, 637-639.

61. Rom, W. N. *Mount Sinai Journal of Medicine*. **1976**, 43, 542-552.

62. Lancranjan, I. *Archives of Environmental Health*. **1975**, 30, 396-401.

63. Sandell, E. B; Onishe, H. *Colorimetric Determination of Traces of Metals*; Interscience: New York, 1978; p604.

64. Skoog, O. A.; West D. M. *Fundamentals of Analytical Chemistry*, 4th ed., Saunders College Publishing: New York, 1982; p459.

65. Skoog, O. A.; West D. M. *Fundamentals of Analytical Chemistry*, 4th ed., Saunders College Publishing: New York, 1982; p573

66. Ma , T. S.; Rittner, R. C. *Modern Organic Elemental Anlaysis*, Marcel Decker: New York, 1979; p95.

67. Shrivastava, A.K.; Tandon, S. G. *International Journal of Analytical Chemistry.* **1984**, 17, 293-298.

68. Clarke, A. N.; Wilson, D. J. *Archives of Environmental Health.* **1974**, 57, 505-514.

69. Creason, J. P.; Hinner, T. A.; Bumgarner, J. E.; Pinkerton, C. *Clinical Chemistry.* **1975**, 21, 603-612.

70. O'Halloran, J.; Myers, A. A. *Environmental Pollution.* **1988**, 52, 19-38.

71. *Statistics at a Glance*; Central Statistical Office: Trinidad, 1986.

72. Klevay, L. M. *Archives of Environmental Health.* **1973**, 26, 169-173.

73. Suzuki, Y. K.; Mishiyama, K.; Matsuka, Y. *Journal of Experimental Medicine.* **1968**, 5, 111-119.

74. Harry, A.; Waldron, M. B. *Archives of Environmental Health.* **1974**, 29, 271-273.

75. Hopps, H. C. *Science Total Environment.* **1977**, 7, 71-89.

76. Singal, G. M.; Gatrad, G. M.; Gatrad, A. R.; Howse, P. M.; Johnson, K. W. *Archives of Disease in Children.* **1988**, 63, 973-975.

77. Hunt, T. J.; Hepner, R.; Seaton, K. W. *American Journal of Disease Children.* **1982**, 136, 538-542.

78. Mahaffey, K. R.; Annest, J. L.; Roberts, J.; Murphy, R. S. *New England Journal of Medicine.* **1982**, 307, 573-579.

79. Ahmed, M.; Kutb, I. I.; Ahmed, P.; Qurashi, M. H. *Environmental Pollution.* **1989**, 41, 103-111.

80. Chatt, A.; Katz, S. A. *Hair Analysis: Applications in Biochemical and Environmental Sciences*, YCH Publishers: New York, 1988, p1.

81. Nishiyama, K,; Nordberg, G. F. *Archives of Environmental Health*. **1973**, 25, 92-96.

82. Dahlgrem, J. *Archives of Environmental Health*. **1978**, 35, 156-159.