NAHRES-71

Vienna, 2002

CO-ORDINATED RESEARCH PROJECT

ON

APPLICATION OF ISOTOPIC NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION-POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF HUMAN SUBJECTS IN DEVELOPING COUNTRY POPULATIONS

Report on the First Research Co-ordination Meeting

IAEA Headquarters, Vienna 6-10 May 2002

INTERNATIONAL ATOMIC ENERGY AGENCY

EDITORIAL NOTE

The views expressed in this report do not necessarily reflect those of the IAEA or of governments of the Member States or organizations under whose auspices the work described herein was carried out. The use in this report of particular designations of countries or territories does not imply any judgment by the IAEA as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries. The mention of specific companies or of their products or brand names does not imply any endorsement or recommendation on the part of the IAEA.

Some of the reports in the NAHRES series are recorded in the IAEA's International Nuclear Information System (INIS).

APPLICATION OF ISOTOPIC NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION-POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF HUMAN SUBJECTS IN DEVELOPING COUNTRY POPULATIONS

Report on the First Research Co-ordination Meeting

IAEA Headquarters, Vienna, 6-10 May 2002

NAHRES-71, IAEA, Vienna (2002)

A report prepared by the

Section of Nutritional and Health-Related Environmental Studies
Division of Human Health
Department of Nuclear Sciences and Applications
International Atomic Energy Agency
P.O. Box 100, A-1400 Vienna, Austria

Single copies of this report are available cost-free on request from the above address

GROUP PHOTO

CONTENTS

PART I:
SUMMARY REPORT

FIRST RCM FOR THE COORDINATED RESEARCH PROJECTS (CRP) ON "THE APPLICATION OF ISOTOPIC AND NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF HUMAN SUBJECTS IN DEVELOPING COUNTRY POPULATIONS"

SUMMARY REPORT

Section of Nutritional and Health-Related Environmental Studies, Division of Human Health, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, P.O. Box 100, A-1400 Vienna, Austria

INTRODUCTION

Ecosystems worldwide are being affected by numerous kinds of anthropogenic activities. Rapid and uncontrolled industrial growth in most part of the developing world has aggravated environmental contamination, some of which are potent pollutants. It is common knowledge that such pollutants significantly affect human health, as is the case with heavy metals and with organic dusts as an emerging concern.

There is a distinction between pollution and contamination, in that a contaminant may be present in a given medium with or without causing harmful effects. Pollutants are regarded as agents causing harm to the environment subsequently leading to impairment of human health. Certain pollutants (e.g. lead absorption by anaemic subjects) cause ill health effects including deterioration of the nutritional status of individuals.

Up until now, nutritional status and environmental pollution have mostly been treated as separate issues and there is very little information available about their relationship. Yet, previous studies have indicated that nutrition and pollution are interconnected with regard to their effects on human health.

In recent years, a great deal of effort has been put into developing sensitive methods of measuring pollutants both in the environment and biological systems. However, the quantitative evaluation of impacts on health is still in its infancy and therefore far from satisfaction. To monitor the interaction between environmental pollution and nutrition, as well as health status, some indicators exist. Breast milk is an example of an indicator used for Real Time Monitoring (RTM). It has been demonstrated that the effect of the presence of pollutants in breast milk has an effect on morbidity and growth faltering in the nursing infant. Similarly, other non-invasive indicators such as urine and saliva and less invasive ones, as whole blood have commonly been used to monitor some pollutants (e.g. toxic metals). Placenta can also be employed as a dual indicator for RTM, reflecting the pollutant status in both mother and fetus. Hair, adipose tissue (to monitor organic pollutants), and hard tissues such as bone and teeth (to monitor selected elements, particularly Pb, Sr and Ur) have been used as Long Term Monitoring (LTM) specimen. In this context, the International Atomic Energy Agency (IAEA) is actively contributing through coordinated research project mechanisms to the development of isotopic techniques applicable for pollution-nutrition interactions.

This CRP is based on the recommendations of a Consultants' Meeting on 'Nuclear analytical and isotope techniques for assessing Nutrition-pollution interactions' held in Vienna, 11-15 December 2000.

This report summarizes the main activities of the first RCM held at the IAEA Headquarters in Vienna from 6 to 10 May 2002.

The countries participating in this CRP are:

Bangladesh, Brazil, Chile, China, India, Kenya, Korea, Morocco, Peru, Sweden and Viet Nam

OVERALL OBJECTIVE

To use nuclear and isotopic techniques to understand and to evaluate how environmental pollution affects the nutritional status of human subjects already exposed to marginal malnutrition. Results of this study will contribute to better knowledge of nutrition-pollution interaction mechanisms and to develop more targeted strategies.

SPECIFIC OBJECTIVE

- Use of non-invasive specimens (e.g. breast milk, blood, hair, urine, placenta; eventually adipose tissue for organic pollutants) as indicators to monitor the interaction between the environmental pollution (both organic and inorganic) and nutritional status.
- Development of protocols to assess the heath impact of environmental conditions on populations living under the risk of malnutrition.
- Validated procedures for chemical analysis in selected environment and nutritional specimens for research in the pollutant–nutrition interaction.

EXPECTED RESEARCH OUTPUTS (RESULTS) (See also Appendix 4)

- Harmonization of the protocols and procedures for sampling and analyses;
- Determination of Pollutant exposure levels based on biological biomonitors;
- Determination of nutritional impacts of pollutant exposures;
- Understand mechanisms and interactions of pollutants and/or nutrients;
- Publications of the study results in an IAEA TECDOC, and in peer-reviewed journals by participants;
- Capacity building in developing countries.

ACTION PLAN (ACTIVITIES) (Table 1, Appendix 5)

- Identification of the study areas and population groups;
- Collection of information for environmental monitoring;
- Harmonization of protocols and use of nuclear analytical methodologies;
- Collection and analysis of biological indicators; and
- Evaluation of possible relationships between human exposures and biological indicators for the pollutants and nutritional status, as judged by body weight and height for age in children (NCHS: National Center for Health Standards) and BMI (Body Mass Index) in adults (WHO Guidelines) and trace metal profile.

Supplementary activities:

- Understand the mechanisms of the interaction between pollutants and nutrients

- Define the functional consequences of environmental pollution
- Assess the efficacy of some nutritional intervention strategies
- Undertake laboratory inter-comparison analysis

COUNTRY PRESENTATIONS

All country presentations were very specific and highlighted the current knowledge in the field. The studies proposed are exploratory in nature (case study) and hence there is a felt need to enlarge the scope of the studies with adequate number of samples to gain more scientific insights into the problems (see country report).

The country presentations stressed on the following:

- To assess the relationship between exposure to environmental pollutants and nutrient/nutritional status.
- To monitor environment pollution, some studies identified air, water/agriculture produced (food) and soil as sources.
- To use biological biomarkers such as blood, placenta, cord blood, breast milk. For long-term exposure hair and nail samples were considered.
- To study the possibility of either nutrient deficiency exaggerating the net effects of pollutants or nutrient supplements reducing the toxicity.
- To define mechanistic explanations in terms of oxidant damage and anti-oxidant enzymes induction of metallothionine as bioresponses were presented.
- To consider Pb, Cd, As and Hg as major pollutants, and trace minerals such as Zn, Fe, Ca, and Se as the major nutrient deficiencies to the be explored.
- To harmonize methodologies starting from sample collection and processing, to methods of estimations, measuring nutritional and socio-economic status and QA/QC. (See appendix 6 and 7)

It was fully realised that there were inherent difficulties for expanding the scope immediately because of a small amount of grant as well as capacity to take on a larger scale studies without some preliminary observations.

TECHNICAL ASPECTS

Most of the participants have selected their sites based on all or any of the following criteria:

- History and/or present status of pollution
- Preliminary studies
- Identification of highly polluted sites
- Medical indications

Criteria for the selection of an appropriate sampling site/number of samples

- Statistical considerations will be taken into account for the design and analysis in each country. Samples selected for testing should be both representative and of sufficient size and frequency so that meaningful results can be obtained.

Questionnaires for socio-economic status, occupational history and personal history will be prepared according to guidelines provided (see Appendix 7).

- To classify subjects into low, middle and high income groups.
- To study occupational exposures.
- To determine the duration of exposure to certain toxic substances such as tobacco, alcohol from personal history.
- To collect simple health and physical examination data.

Types of samples to be collected

- Table 1 provides relevant information.

SAMPLING TECHNIQUES

- A standard operating procedure (SOP) for each type of sample should be developed according to the specific needs of the individual research programmes.
- Processes such as pre-sampling, sampling, packaging, transport, storage, handling, treatment, etc. should be carried out, according to recommended guidelines (see appendix 6).
- Appropriate precautions should be taken to avoid external contamination.
- Sample integrity with respect to chemical components should be preserved.

ANALYSIS

Sample preparation

- Sample preparation guidelines are available (see appendix 6)
- Transport and short-term storage of biological samples should not compromise the integrity of the samples (e.g. temperature, acidity, moisture)
- For long-term storage, drying, deep-freezing, freeze-drying and evaporation may be used, where appropriate
- Non-contaminating devices (e.g. Ti, Teflon, quartz knives, etc.) should be used for sample disintegration and homogenisation
- Laboratory samples should be prepared in sufficient quantities for repetition of analysis
- Samples should be clearly and uniquely marked/labelled.

Recommendations for nuclear analytical techniques

- Nuclear Analytical Technique (NAT) such as INAA (Instrumental Neutron Activation Analysis), PIXE (Particle Induced X-ray Emission), XRF (X-Ray Fluorescence) should be the primary technique of analysis.
- Neutron Activation Analysis (NAA) can be used where it is available.
- Although each NAT should be optimized for the elements of interest, a slight adjustment of irradiation, decay and counting time may provide data for additional elements (e.g. co-contaminants) that could be useful for the purpose of interpretation of data.
- Energy Dispersive-XRF (ED-XRF), Care should be taken in matching the matrices of the RMs to be used with those of the sample to be analysed.
- Total Reflection-XRF (TR –XRF) Ensure that the sample holder is cleaned for every sample.

Recommendations for isotope related and other analytical techniques

Other techniques such as AAS (Atomic Absorption Spectrometry), AFS (Atomic Fluorescence Spectrometry), ICP-AES (Inductive Coupled Plasma Atomic Emission Spectrometry), ICP-MS (Mass Spectrometry), ASV (Anode Stripping Voltmetry), HPLC (High Performance Liquid Chromatography), GC (Gas Chromatography), and RIA (Radio-Immuno Assay) may be used as necessary.

- *ICP-MS:* Care must be taken to avoid, or correct for mass overlaps and matrix effects.
- *AAS*: The choice of graphite furnace, flame, hydride generation and cold vapour, should be based on the concentration of the analyte and interferences encountered. The instrument should have a functioning background correction system. Calibration should be based on calibration curve or, in the case of interference, on the method of standard addition.
- *ASV*: Standard methods are available for a number of elements. A prerequisite for interference free analyses is that the sample solution is completely free from organic residues

REFERENCE ANALYTICAL LABORATORIES

IAEA's Seibersdorf laboratories and/or participating laboratories may be requested to provide guidance regarding the specific needs of the participants:

- To advise for a specific request
- To assist other participants regarding specific samples and/or analytes
- For cross-checking purposes

DATA MANAGEMENT AND STATISTICAL EVALUATION

General procedure for statistical treatment is guided as follows:

- Collect sample data: A sample data sheet to collect the data from each participant is attached as an excel sheet
- Design experiments specifically for statistical treatment.
- Decide how many variables you need for the meaningful result through statistical treatments
- Perform experiment
- Record the data on master data in an excel file (see Appendix 8)
- Go through the general statistical test
- Data Randomness Test
- Outlier Test
- Data Transformation if (SD/Avg)X100 > 33 %
- t-test for Accuracy between Laboratories or Methods (optional)
- Mann-Whitney test or more for the non-parametric test (optional)
- More statistical treatment if needed (optional)
- ANOVA (Analysis of Variance for precision test)
- Factor Analysis
- Pattern Recognition
- Result & Report

- Graphically present the result
- Box plot is generally recommended

QUALITY ASSURANCE AND CONTROL

Existing written protocols for sampling, sample preparation, analysis

The wide diversity of research interests amongst all participants will most likely require a large variety of protocols. Participants may request protocols from other laboratories and make their own available through the list-server. Reference is also made to paragraph in this regard.

In-house QA/QC standards

The wide diversity of research interests amongst all participants will most likely require a large variety of in-house standards. Participants should provide information on their relevant standards and where possible make them available for distribution to interested participants through the IAEA.

Recommended QA/QC procedures for the analysis

- Certified Reference Materials (CRMs), having the same or similar matrix composition and analyte levels, should be analysed in parallel to the samples and/or for linking them to in-house reference materials.
- Analytical quality control measures should be pursued. This can be done through in-house reference materials linked to CRMs, or intercomparison of two or more independent analytical methods, self-verification principle by e.g. NAA as a referee method.
- Possible external Central Reference Laboratory (CRL) will be considered (e.g. Korea), CDC (Centre for Disease Control, USA), EPA (Environmental Protection Agency, USA).

ORGANISATIONAL ASPECTS

Co-operation within the group

- Participants are requested to communicate with one another by e-mail.
- Participants are encouraged to co-operate on a bilateral and/or multilateral basis, especially if their research interests are closely related. The co-operation may take any form including exchange of sampling and analytical methodologies, expertise, data, and samples for comparative and/or complementary analyses (e.g. to increase the number of analytes).
- Participants who may experience difficulties in any project-related area should feel free to contact other participants who may have more experience.
- Participants are requested to share relevant publications and reports from their own countries which may not otherwise be available in the open literature.
- Participants are requested to acknowledge the contribution of the IAEA quoting the CRP in their publications and circulate these amongst the participants.

CO-OPERATION WITH OTHER INSTITUTIONS

- Participants are strongly encouraged to interact with local, regional, national and/or international institutes/organizations/ministries, for example of environment, health and nutrition, with a view to obtain reports, information, guidelines and regulations from them. The data collected in the participant's own country and/or in the framework of this CRP could be communicated to the appropriate authorities.

NEXT RCM

- The next RCM will be planned in the second half of 2003. Location will be decided later.

PART II:
COUNTRY REPORTS

BIOMONITORING OF ARSENIC AND LEAD IN HEALTH INDICES (HAIR, BLOOD, ETC.) AND THEIR INTERACTIONS AND IMPACTS ON THE NUTRITIONAL STATUS OF BANGLADESH POPULATION.

MUHAMMAD ALI

Chemistry Division, Atomic Energy Centre, 4, Kazi Nazrul Islam Avenue, P.O. Box No. 164, Ramna, Dhakla 1000, Bangladesh

Abstract

Atomic Energy Centre, Dhaka under the Bangladesh Atomic Energy Commission was recently awarded a research contract from the International Atomic Energy Agency to investigate the levels of micronutrients (K, Ca, Mn, Fe, Ni, Cu, Zn) and pollutants (As, Pb) in health indices (hair, blood, etc.) to study their interactions and impacts on the nutritional status of Bangladeshi population.

The project was scheduled to start in December 2001 and to be completed by November 2002. To date, sampling and sample preparation techniques for heavy metal analysis in hair and blood using XRF/PIXE have been investigated, and some preliminary work on sample analysis has been performed. It indicates that both PIXE and XRF methods can be used for the determination of nutritionally important trace metals in health indices after a simple sample treatment for volume reduction either by oven or freeze drying.

Results of Biochemical assessment of nutritional status of Bangladeshi pre-school children under normal and malnutrition conditions from previous study has been given in RESULTS section of this paper.

There has been found a positive correlation of malnutrition with some nutritional parameters such as fasting blood glucose, serum total protein, serum total albumin, and serum Cu and Zn levels.

Hair Zn level had no significant correlation ($p>0.05$) with serum Zn level but hair Cu level had a positive correlation with serum Cu level.

The trace element concentrations in hair of both normal and malnourished children in the age group of 1-5 years, as studied do not show any regular dependence on nutritional status of the subjects. Only the low copper content in the hair of malnourished group can possibly be linked with nutritional disorders.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Bangladesh is one of the most densely populated countries in the world with about 124 million people in 147,570 sq. km. area. Pollution from an increasing population and industrialization with a small land mass threatens the over all environment in Bangladesh. Growing population, intensification of agriculture, industrial and other economic activities are affecting the quality of life in many different ways. As a result of these activities, deterioration of the environment and its consequent affect on human health has been emerged as a major concern in Bangladesh as elsewhere in the world. Depletion of the soil nutrients and its effect on productivity, industrial atmospheric emission of particles and gases, industrial and municipal discharge of liquids and solids, applications of pesticides and fertilizers to soils, natural waters contaminated with agriculture run-off, mine waste, geogenic arsenic contamination of Bangladesh ground water and its carried over effects on foodstuffs, etc. have created high national demand for measurement of contaminants in food-chain and human specimens (hair, blood, placenta, breast milk etc.) for the study of trace metal nutrition and pollution and their possible interactions in Bangladesh populations. The present CRP proposal and its fruitful utilization may provide a scientific basis for better understanding of the transfer of elements from pollutant environment to the food chain, and to identify and elucidate the health effects associated with the consumption of contaminated foods and drinking water.

The above forecast and IAEA-CRPs on trace elements in human nutrition generated a spate of activities all over the world to observe, monitor and assess nutrition-pollution from in situ, natural; and anthropogenic sources and their possible impact on the nutritional status of the human life, whilst not restricting the pace of essential economic development activities.

The impact of human activities on nutrition pollution has been increasing and therefore, research on contamination monitoring and its effect on human health is carrying out in many countries of the world.

The control of nutritional pollution is now one of the most serious problems in better health management. Bio-monitoring of nutrition pollution through micronutrient analysis using bio-indicators such as hair, blood, placenta, breast milk etc may be efficient, and economic.

The specific specimen for the nutrition pollutant accumulator has not yet been selected but suggestions for the nutrition pollutant accumulator include hair, nail, urine, venous blood, skin, breast milk, placenta, cord blood etc. Several studies have shown that a survey of the metal concentration in bio-monitors (hair, blood etc.) is a valuable mean of identifying sources of health contamination. In recent years there has been a considerable growth of interest in using bio-monitors for human nutrition pollution studies.

We are continuing to provide our existing analytical facilities to the students (medical/non medical) for their higher degrees such as M. Sc., M. Phil., M.D., Ph.D. etc. within the country. We have also running national CRP with other institutes and organizations apart from this CRP. We are also engaged in IAEA/ RCA CRP and TCP`s.

2. METHODS

For large scale biomonitoring **PIXE** is the method of choice. But for overall study, the proposed methods are: (a) Proton-induced X-ray Emission (**PIXE)**, X-ray Fluorescence (**XRF**), Atomic Absorption Spectrophotomer (**AAS**) and inductively coupled plasma atomic emission spectrometry (**ICP-AES**).

3. RESULTS

Results on “Biochemical assessment of nutritional status of Bangladeshi pre-school children under normal and malnutrition conditions” from earlier study has been presented.

3.1. Introduction

The global indiscriminate pollution due to ever increasing release of foreign chemicals into the environment from various human activities and the subsequent pollution of the living environment leading to contamination of the human food resources with trace toxic elements is very likely to affect human health. These environmental pollutants find their way to the human body through the food chain, water and the ambient atmosphere and may ultimately accumulate in the critical organs.

Of the biological tissues and fluids, human hair is unique in that it reflects the biological and environmental history of an individual. Consequently, there has been a considerable interest in using human hair as an indicator of trace element nutritional status [1-13] as well as a biological indicator of environmental pollution [4-6]. The low level of essential bioelements such as Ca, Fe, Cu, Zn, etc. in human hair was found to be typical of deficiency diseases [14-16], metabolic disturbances and physiological disorders [17].

The prevalence of varying degrees of energy-protein malnutrition among the preschool children in Bangladesh [18] is widespread. Since early age protein-energy malnutrition has marked effects on the nutritional status of the trace elements which have significant clinical roles in pediatric nutrition, this study was undertaken as the first step towards understanding the effects of protein-energy malnutrition on the nutritional status of some essential trace elements in preschool aged rural children. How much of the level of malnutrition is due to the trace elements deficiency state has not yet been properly evaluated. The present study was, therefore, undertaken to determine the concentrations of biological trace elements in scalp hair (Ca, Cr, Mn, Fe, Ni, Cu and Zn) and blood serum (Cu, Zn) of malnourished and normal children with an object to find out if there is any correlation between the contents of these elements in scalp hair/blood serum and/or nutritional status among the children of 1 to 5 years age groups in Bangladesh. The multielement analytical information obtained from such studies may help to develop a strategy for trace element supplementation programs in order to eliminate precursors of biochemical changes leading to different degenerative diseases.

3.2. Materials and Methods

3.2.1. Selection of subjects

Three clinical types of 55 malnourished children, aged between 1 and 5 years, were selected for this study. Forty-nine apparently healthy children were selected as control, matching age and socio-economic status with the study population. Screening was done by histories, clinical examination, anthropometric measurements and biochemical tests. Growth chart introduced by the National Centre for Health Statistics (NCHS) was used to assess the nutritional status of these children [19], and Welcome classification [20] was used to classify different clinical types of malnourished children.

3.2.2. Sample collection and preparation for analysis

Sample collection: Approximately, 5 g of hair was cut from the closest distance of the scalp and from different sites around the head with stainless steel scissors. Six milliliter (ml) of venous blood was drawn from each children. In the case of malnourished children, both the hair and the blood samples were collected immediately after their admission into the hospital before any treatment was initiated and from the control children, on the day of their first visit to the hospital. For fasting glucose levels, about 0.5 ml of venous blood was collected from each children in the morning after 6-7 hours fasting and was kept immediately in a test tube containing a mixture of sodium fluoride and potassium oxalate which act as preservative by inhibiting glycolytic enzymes.

Blood samples were collected by means of a stainless steel needle and sterile plastic disposable syringe into a contaminant free pyrex test tube.

For the preparation of serum, blood samples were allowed to clot at room temperature for about 1 hour and then centrifuged at 1000 rpm for about 10 minutes. The clear supernatant

was considered as serum and then transferred by a pasteur pipette into another clean dry test tube.

Hair samples were kept in clean paper envelopes in a desiccator and all blood and serum samples were kept in test tubes in a refrigerator until preparation for analysis.

Sample preparation: The hair samples were washed according to the procedure described by the International Atomic Energy Agency (IAEA) [21] to remove external contaminants and the pellets were made from the homogeneously mixed charred (180^{o}C for 2 to 3 hours) samples after finely powdering them in an aluminum carbide mortar according to the prescribed procedure reported earlier [22].

The serum samples stored in deep freeze were at first thawed at 56^{o}C for 1 hour in a water bath. Then 2 ml of serum from each sample was taken into a clean dry contaminant free test tube and then freeze dried for 72 hours to constant weight of the samples. The dried mass of serum thus obtained was ground to fine powder with an aluminum carbide mortar and pestle.

From the powdered material of serum, pellets were prepared with a graduated stainless steel hand-press pellet maker (Perkin-Elmer). Similarly, pellets were made from the NIST orchard leaf powder as standard for concentration calibration.

The pellets prepared from each sample and standard were then mounted on a 35-mm slide frame with adhesive tape and preserved in a desiccator until analysis.

3.3. Analytical methods

In the present analysis, the external beam PIXE method **(Fig. 1)** was applied to determine the trace element composition of hair, and blood serum. In this methodology, similar experimental conditions were maintained as reported earlier [23]. For analyte concentrations, a NIST orchard leaf standard (SRM 1571) was used for concentration calibration. The calibration curve was constructed as shown in **Fig. 2.**

Fasting blood glucose levels were determined by glucose oxidase method [24]. Serum total protein and serum total albumin were measured by Biuret method [25].

3.4. Results and Discussion

3.4.1. Biochemical assessment

The analytical results of some biochemical nutritional parameters such as fasting blood glucose, serum total protein and albumin, serum copper and zinc, and hair trace metals, obtained in this work for biochemical assessment are summarized in **Tables I-VI.**

3.4.2. Fasting blood glucose levels

The mean levels of fasting blood glucose of control (96.6 mg/100 ml), kwashiorkor (60.2 mg/100 ml), marasmic-kwashiorkor (58.5 mg/100 ml), and marasmic children (50.8 mg/100 ml) are presented in **Table I**. There are significant differences between the fasting blood glucose levels of control children and those of all three clinical types of malnourished children ($p<0.001$). The fasting blood glucose level **(Table I)** was found correlated with the degree of malnutrition. The blood glucose level was found to decrease with the increase of disease.

TABLE I: COMPARISON OF FASTING BLOOD GLUCOSE LEVELS BETWEEN NORMAL AND MALNOURISHED CHILDREN WITH VARYING DEGREES OF MALNUTRITION.

Nutritional status	No. of children	Fasting blood glucose (mg/100 mL)	t-value	Level of significance*
Normal	49	96.6 ± 20.4	-	-
Kwashiorkor	10	60.2 ± 19.3	5.19	p<0.001
Marasmic-Kwashiorkor	18	58.5 ± 13.6	7.33	p<0.001
Marasmus	27	50.8 ± 10.7	10.85	p<0.001

Uncertainties are standard deviations.
* Two sample student t-test.

3.4.3. Serum total protein and albumin levels

The mean serum total protein of control, kwashiorkor, marasmic-kwashiorkor, and marasmic children are 6.6, 3.6, 3.2 and 4.2 (g/100 ml) respectively and mean serum total albumin of control, kwashiorkor, marasmic-kwashiorkor, and marasmic children are 3.8, 1.4, 1.8 and 2.1 (g/100 ml) respectively. These values are compared between normal and three clinical types of malnourished children and summarized in **Tables II and III**. It is found from these results **(Tables II** and III) that both the serum total protein and albumin levels do not seem to be related with the degree of malnutrition but in the patient groups they are significantly lower (p<0.001) than those observed in the control group.

TABLE II: COMPARISON OF SERUM TOTAL PROTEIN LEVELS BETWEEN NORMAL AND MALNOURISHED CHILDREN WITH VARYING DEGREES OF MALNUTRITION.

Nutritional status	No. of children	Serum total protein (g/100 mL)	t-value	Level of significance*
Normal	49	6.6 ± 0.7	-	-
Kwashiorkor	10	3.6 ± 0.4	13.06	p<0.001
Marasmic-Kwashiorkor	18	3.2 ± 0.3	19.87	p<0.001
Marasmus	27	4.2 ± 0.1	17.66	p<0.001

Uncertainties are standard deviations.
* Two sample student t-test.

TABLE III: COMPARISON OF SERUM TOTAL ALBUMIN LEVELS BETWEEN NORMAL AND MALNOURISHED CHILDREN WITH VARYING DEGREES OF MALNUTRITION.

Nutritional status	No. of children	Serum total albumin (g/100 mL)	t-value	Level of significance*
Normal	49	3.8 ± 0.6	-	-
Kwashiorkor	10	1.4 ± 0.4	12.07	p<0.001
Marasmic-Kwashiorkor	18	1.8 ± 0.7	11.56	p<0.001
Marasmus	27	2.1 ± 0.8	10.48	p<0.001

Uncertainties are standard deviations.
* Two sample student t-test.

3.4.4. Serum copper and zinc levels

The mean serum copper levels in control, kwashiorkor, marasmic-kwashiorkor and marasmic children between 1 to 5 years of age are 120.5±12.6, 62.6±20.4, 54.7±31.5 and 84.6±28.4 μg/100 ml respectively. The mean serum zinc in control children is 85.6±16.7 μg/100 ml, in kwashiorkor 40.8±10.2 μg/100 ml, in marasmic-kwashiorkor, 52.2±11.6 μg/100 ml, and in marasmic children, it is 54.4±14.7 μg/100 ml. These values are compared between normal and three clinical types of malnourished children and summarized in **Table IV.** It is observed in these results that there is a significant difference (P<0.05) in the serum copper and zinc levels between different clinical types of malnourished and healthy children. This observation leads to the conclusion for further investigation that serum levels of Cu and Zn may well reflect upon the clinical state of malnutrition in children of 1-5 years age groups. The status of Fe should also be examined along with these two elements of vital interest in health status evaluation.

TABLE IV: CONCENTRATIONS OF SERUM COPPER AND ZINC IN CONTROL AND MALNOURISHED CHILDREN (IN μG/100 ML).

Nutritional status	Number of children	Cu	Zn	Comparison with control group*
Control	49	120.5±12.6	85.6±16.7	-
Kwashiorkor	10	62.6±20.4	40.8±10.2	p<0.05
Marasmic-Kwashiorkor	18	54.7±31.5	52.2±11.6	p<0.05
Marasmus	27	84.6±28.4	54.4±14.7	p<0.05

Uncertainties are standard deviations.
* Two sample student t-test.

3.4.5. Hair trace element levels

The level of trace elements in scalp hair of malnourished and normal preschool children in the age group of 1-5 years were calculated only for those elements which were observed above the detection limit of the PIXE method. The concentrations of these elements (Ca, Cr, Mn, Fe, Ni, Cu, Zn and Pb) are given in **Table V**. In the hair of normal children, the distribution pattern of the elements was found to follow the order: Ca > Fe > Zn > Cu > Pb > Ni > Cr > Mn. However, in the patients, the concentration of these elements did not follow the same order indicating possible disorders in the trace metals balance barring any disturbance from external factors. The zinc level is higher than the iron level in almost all the hair samples examined and the order of distribution of the elements is Ca > Zn > Fe > Pb > Cu> Ni > Mn > Cr. Table VI lists the results of student t-test of each of two mean values for each element at 5% level of significance. In **Table VI**, the characteristics of the hair results for diseased children in general are the significantly low ($p<0.05$) contents of Cr, Fe, and Cu compared to those in normal children. No significant correlation is observed in case of Ca, Mn, Ni, and Zn and Pb levels between the two groups.

TABLE VI: COMPARISON OF MEANS HAIR TRACE ELEMENT CONTENTS (μG/G) BETWEEN NORMAL AND MALNOURISHED CHILDREN.

Element	**Normal**	**Malnourished**	**t-value**	**Level of significance**
	Mean±SD	Mean±SD		
Ca	487±175(49)	498±194(55)	0.30	P>0.05
	464*			
Cr	6.3±3.7(49)	3.9±1.2(55)	4.53	P<0.05
Mn	6.2±3.2(42)	7.5±3.0(50)	2.00	P>0.05
	6.2**			
Fe	312±206(49)	63±33(55)	8.84	P<0.05
Ni	14.4±9.8(47)	11.5±7.4(52)	1.67	P>0.05
Cu	21.7±11.3(49)	12.5±6.9(55)	5.08	P<0.05
	21.7+			
Zn	154±59(49)	135±47(55)	1.83	P>0.05
	163*			
Pb	17.4±8.7(40)	15.6±9.2(28)	0.82	P>0.05

Values in parentheses indicate the numbers of children on which estimation was made
* Ref. 26.
** Ref. 27 (sexes combined).
+ Ref. 28 (sexes combined).

4. PLANS FOR FUTURE WORK

The detailed work plan within the framework of the present Coordinated Research Programme is as follows:

- Selection of target population.
- Collection of hair from clinically diagnosed patients suffering from chronic arsenic poisoning.
- Collection of blood from Dhaka city general population and target group populations residing in Dhaka.
- Preparation of collected hair and blood samples according to standard procedures.
- Analysis of prepared samples for micronutrients (Ca, Fe, Cu, Zn, etc) and pollutants (As and Pb) using PIXE, XRF, ICP-AES and other techniques.
- Study of possible interactions between Pb and/or As with other micronutrients, under conditions of deficient dietary intake of the essential elements such as Ca, Se, Fe and Zn to mitigate ill effects in target populations at risk.
- Finally, assessment of nutritional status of Bangladesh population using present results and comparing with available national dietary data.
- Reporting and publications.

REFERENCES

[1] MAUGH JR, T.H., Hair: A diagnostic tool to complement blood serum and urine, Science, **202** (1978) 1271.

[2] GENTILE, P.S., TRENTALANGE, M.J. and COLEMAN, M., The relationship of hair zinc concentrations to height, weight, age, and sex in the normal population, Pediatr. Res., **15** (1981) 123.

[3] SANER, G., The effect of parity on maternal hair chromium concentrations and the changes during pregnancy, Am. J. Clin. Nutr., **34** (1981) 853.

[4] BHAT, K.R., ARUNACHALAM, J., and YEGNASUBRAMANIAN, S., Trace elements in hair and environmental exposure, Sci. Total Environ., **22** (1982) 169.

[5] PHELPS, R.W., CLARKSON, T.W., KERSHAW, T.G. and WHEATLEY, B., Interrelationships of blood and hair mercury concentrations in a North American population exposed to methylmercury, Arch. Environ. Health, **35** (1980) 161.

[6] BAKER JR, E.L., HAYES, C.G., LANDRINGAN, P.J., HANDKE, J.L., LEGER, R.T., HOUSWORTH, W.J. and HARRINGTON, J.M., A nationwide survey of heavy metal absorption in children living near primary copper, lead and zinc smelters, Am. J. Epidemiol., **106** (1977) 261.

[7] HILDERBRAND, D.C. and WHITE, D.H., Trace element analysis in hair: An evaluation, Clin. Chem., **20** (1974) 148.

[8] CHITTLEBOROUGH, G., A chemist's view of the analysis of human hair for trace elements, Sci. Total Environ., **14** (1980) 53.

[9] KOZUKA, H., Activation analysis of human head hair. VIII. Factors having influence on the trace elements contents in hair, J. Hyg. Chem., **18** (1972) 7 (in Japanese).

[10] GIBSON, R.S., Hair as a biopsy material for the assessment of trace element status in infancy, A review, J. Human Nutr., **34** (1980) 405.

[11] LANGHAM, W.H., Some considerations of present biospheric contamination by radioactive fallout, Agric. Food Chem., **9** (1961) 91.

[12] YURACHEK, J.P., CLEMENA, G.G and HARRISON, W.W., Analysis of human hair by spark source mass spectrometry, Anal. Chem., **41** (1969) 1666.

[13] HADA, T., Metals in normal human skin and hair: Report II. Metals in hair, Hiroskaki Med. J., **34** (1982) 363 (in Japanese).

[14] STRAIN, W.H., STEADMAN, L.T., LANKAU, C.A., BERLINER, W.P. and PORIES, W.J., Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man, J. Lab. Clin. Med., **68** (1966) 244.

[15] HAMBIDGE, K.M., HAMBIDGE, C., JACOBS, M. and BAUM, J. D., Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children, Pediatr. Res., **6** (1972a) 868.

[16] HAMMER, D.I., FINKLEA, J.F., HENDRICKS, R.H., SHY, C.M., and HORTON, R., Hair trace metal levels and environmental exposure, Am. J. Epidemiol., **93** (1971) 84.

[17] BACSO, J., KOVACS, P. and HORVATH, S., Investigation of some inorganic compounds in human hair, Radiochem. Radioanal. Lett., **33** (1978) 273.

[18] AHMAD, K. and HASSAN, N., Nutrition Survey of Rural Bangladesh, 1981-1982, Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh, 1983.

[19] National Centre for Health Statistics (NCHS). NCHS Growth Curve for Children Birth-18 Years. United States, Washington, D.C., US Department of Health, Education, and Welfare, 1977 (Vital and Health Statistics Series 11, Publication No. (PHS) 78-1650).

[20] FAO/WHO Expert Committee on Nutrition., 8th Report, WHO Tech. Rep. Ser. No. 477, Geneva, 1971, p. 38.
[21] RYABUKHIN, Y.S., Activation Analysis of Hair as an Indicator of Contamination of Man by Environmental Trace Element Pollutants, Report, IAEA/RL/50, 1978, pp. 1-134.
[22] HUSSAIN, M., KHALIQUZZAMAN, M., ABDULLAH, AHMED, M., I., and KHAN, A.H., Trace element content in hair of adult population in Bangladesh, Int. J. Appl. Radiat. Isot., **31** (1980) 527.
[23] ALI, M., X-ray Analytical Studies of Trace Elements in Food and Drink: A Nutritional Assessment in Bangladesh, Ph. D. Thesis, JU, Dhaka (1955).
[24] WOOTTON, I.D.P., Micro-analysis in Medical Biochemistry. 4th Edn., J. and A. Churchill Ltd., London (1964) 97 pp.
[25] OSER, B.L., Hawk's Physiological Chemistry, 14th Edn., Mc-Graw Hill Book Company, New York (1965).
[26] MOON, J., et al., Trace metals in scalp hair of children and adults in three Alberta Indian villages, Sci. Total Environ., **54** (1986) 107.
[27] TERAI, M., et al., An application of neutron activation analysis to biological materials: The comparison of trace element contents in normal and diseased infant hairs, J. Radioanal. Chem., **52** (1979) 143.
[28] IMAHORI, A., FUKUSHIMA, I., SHIOBARA, S., YANAGIDA, Y., and TOMURA, K., Multielement neutron activation analysis of human scalp hair: A local population survey in the Tokyo metropolitan area, J. Radioanal, Chem, **52** (1979) 167.

TABLE V: TRACE ELEMENT COMPOSITION OF HAIR OF NORMAL, KWASHIORKOR, MARASMIC KWASHIORKOR AND MARASMUS CHILDREN OF BANGLADESH.

Nutritional status		**Concentration in μg g^{-1}, dry hair**							
		Ca	**Cr**	**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Pb**
Normal	*Range*	218-905	2.1-15.7	1.7-33.2	31-803	3.7-40.3	6.2-56.5	63-377	4.6-43.6
		(49)	(49)	(43)	(49)	(47)	(49)	(49)	(40)
	*Mean**	487±36%	6.3±59%	6.2±51%	312±66%	14.4±68%	21.7±52%	154±38%	17.4±50%
Kwashiorkor	*Range*	158-852	3.2-5.6	4.3-14.3	7-93	4.2-31.6	4.8-21.9	76-194	14.3-17.9
		(10)	(10)	(9)	(10)	(10)	(10)	(10)	(2)
	*Mean**	507±48%	4.2±29%	7.6±42%	54±63%	12.8±75%	9.7±55%	137±24%	16.1±41%
Marasmic-Kwashiorkor	*Range*	277-771	2.8-4.8	3.2-19.3	43-110	1.9-34.4	2.4-40.3	22-239	2.4-28.1
		(18)	(18)	(16)	(18)	(18)	(18)	(18)	(10)
	*Mean**	503±28%	3.8±18%	8.0±25%	71±54%	12.4±78%	13.7±59%	135±40%	13.6±64%
Marasmus	*Range*	47-897	2.0-5.4	2.0-27.7	25-166	2.6-39.0	6.1-45.4	45-286	4.0-40.1
		(27)	(27)	(25)	(27)	(24)	(27)	(27)	(16)
	*Mean**	485±42%	3.6±42%	6.9±53%	64±40%	9.4±40%	14.2±52%	134±41%	17.1±71%

The figures in parentheses indicate number of subjects in each group. *: Uncertainties are standard deviations.

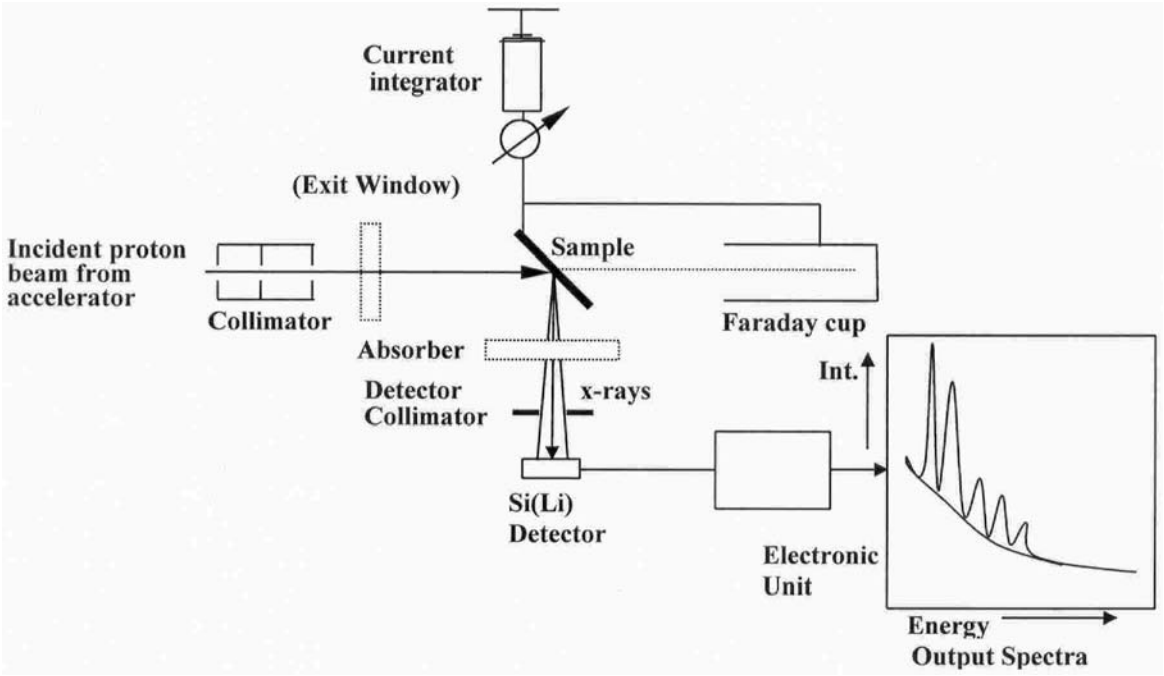


FIG 1. Schematic of PIXE setup with basic physical parameters.

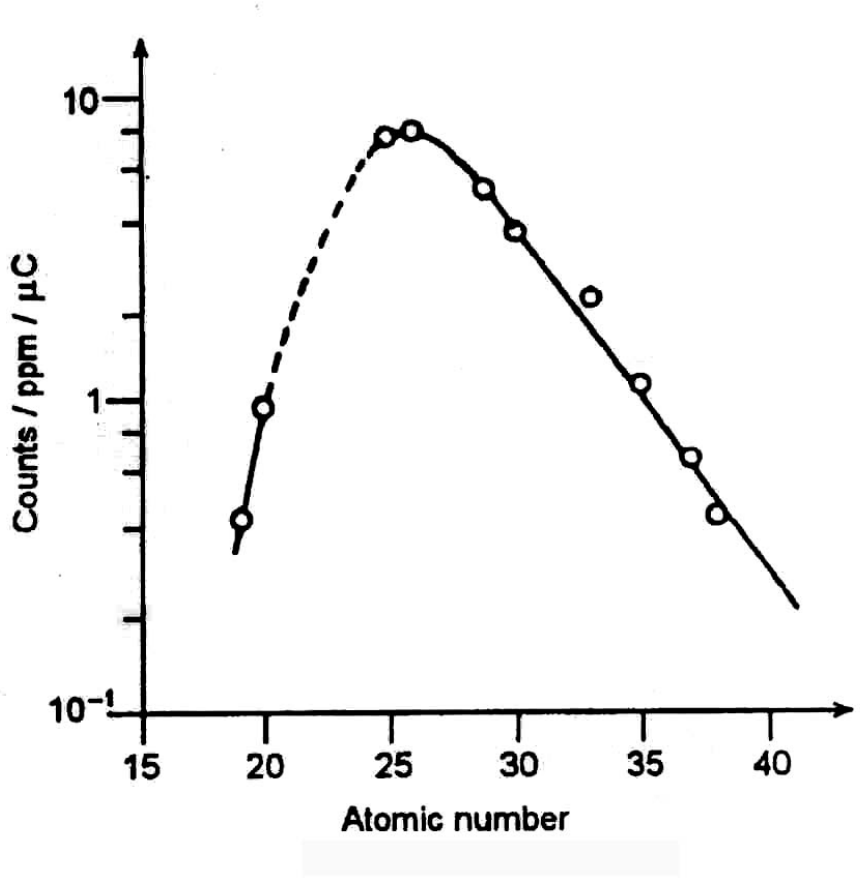


FIG. 2. The x-ray yield curve for concentration calibration constructed from the NIST orchard leaves standard SRM 1571; Ep = 2.0 MeV, absorber ~ 44 mg/cm^2 plastic, detector geometry = 0.018 sr.

MICRONUTRIENT COMPOSITION AND THE PRESENCE OF NUTRIENTS: POLLUTANTS IN MOTHER'S MILK FROM RURAL AND URBAN ENVIRONMENT

JOSÉ EDUARDO DUTRA DE OLIVEIRA, MD
J. SERGIO MARCHINI, MD

Medical School of Ribeirão Preto, University of São Paulo, Brazil. Department of Medicine, Laboratory of Mass Spectrometry, Rua Lafaiete, 1222 – Ap. 71, 14.015-080 - Ribeirão Preto, SP, Brazil

Abstract

Mother's milk is the universal and the best complete food for the first 4 to 6 months of newborns' life. Its mineral content is shown to be relatively stable. Several studies have been and are carried out concerning breast milk composition in different parts of the world. The amount of chemical pollutants in breast milk is shown to be rising, becoming a matter of concern. Pesticides are found to be excreted in appreciable amounts in human milk. This could be toxic to the baby, result in nutrient imbalance and could produce undesirable interaction with some of its components. In developing countries as in Brazil, one of the world largest agricultural countries, sugar cane, soya, cotton, corn are produced in large scale and the use pesticides is going on for several years. We are proposing to analyzed the mineral composition and the presence of pesticides in breast milks of mothers exposed to rural and urban environments. The objective of this project is to get data on breast milk concerning mineral composition, presence of pesticides and possible relationship among them using nuclear analytical technology and non-nuclear methods.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

A large number of studies on macro and micronutrient composition of breast milk in the world have shown some but not always large differences when socioeconomic factors, nutritional status, mothers age and other factors are considered.

In São Paulo, Brazil the situation is similar, macro and micronutrients breast milk composition in a large series of lactating mothers were studied and published by Nobrega et al. [1]. They support the world literature. Calcium, magnesium and phosphorus, iron, zinc and other minerals, as well as a few vitamins, showed small variation in several breast milk samples analyzed. It is also noteworthy publications of Dorea et al. [2], from the University of Brasilia concerning not only breast milk mineral composition of several other areas in Brazil, but also the presence of mercury in the milk from nursing mothers of the Amazon region.

Contamination of the environment, crops and animals and persons through the use of large amounts of pesticides and herbicides to increase agriculture production is being responsible for the presence of increased amounts of chemical pollutants in mother's milk in Brazil. Pesticides presently banned by WHO and no more allowed in developed countries were previously used in Brazil and some are still applied in a few areas of the country. New pesticides products have been introduced, but their presence in human milk have not been checked.

Matuo demonstrated in 1978 in Brazil for the first time in Ribeirão Preto area, the presence of DDT and DDE metabolites in human milk [3]. More recently, in 1992, the same group reported again the presence of DDT in human milk and of other organochlorine pesticides used in the region. Lindane was present in 32% of the milk samples, heptachlor in 65% of the

samples and DDT and DDE were found in all samples (lower levels than in the previous study). Breast milk from a small sample of these farm workers showed higher values than non exposed urban lactanting women [4].

Considering the possible interaction of pollutants, heavy metals and pesticides, with mother's milk nutrients the objective of this project is closely review all the literature data on the subject and at the same time to analyze and compare on the same milk samples both the micro mineral content and the presence of pesticides (mercury as far as we know is a pollutant not present in the area). In the farmland where the work will be carried out the main crop is of sugar cane. Other crops such as corn, soya, oranges and vegetables are grown. They use different pesticides, Milk from mothers exposed to pesticides will be localized, their milk collected and analyzed. A control urban group of the same socioeconomic level, not exposed to pesticides will be included in the study. The analyzes will be carried out with the use of nuclear and non-nuclear technology allowing simultaneous measurement of pesticides and micronutrients in human milk.

It is to be expected that this study could bring out data on mineral composition, and presence of pesticides in the breast milk of mothers working in rural and urban areas. We will also be looking to any nutrient-pollutant relationship in the analyzed samples.

2. PLAN OF WORK

- Literature review of the past and recent publications on human milk composition and the presence of pollutants in the breast milk.
- Contact with a small rural community where a great number of its population work in large sugar cane plantations.
- Select paired groups of post partum women, one working in urban area and the other in rural areas.
- Clinical and nutritional examination and collecting samples for routine studies.
- Collecting breast milk samples after delivery (colostrum).
- Breast milk will be obtained with the help of a trained nurse.
- Assessment of nutritional status of mothers and babies.
- Laboratory analysis.

3. METHODS

Neutron Activation Analyzes will be used to measure the mineral composition of the breast milk samples. This is planned to be carried out at the Radiochemistry Division of the Brazilian Council of Atomic Energy in S Paulo, Brazil. There is also the alternative of these determination be carried on an available atomic absorption spectrometer in our lab, to be equipped with gas furnace.

Gas Cromatography Shimadzu is already available in our lab and is being adapted for the pesticides analysis. Specific columns and other small parts (ECD-Electron Capture Detector (63Ni)), to be looked for as needed.
(See annex for details)

4. RESULTS

The project is expected to start May 2002.

5. PLANS FOR FUTURE WORK

If the presence of pesticides is found in human breast milk from rural or urban areas and this could be in some way linked to the milk composition, analysis of crops, soil, water and farm animals could be considered as a future follow up of this project. Studies of other crops, such as oranges, cotton and vegetables could also be included, they use different pesticides.

6. COLLABORATION WITH OTHER GROUPS IN BRAZIL

Two groups in Brazil have been already contacted concerning these studies:

- Research Institute of Nuclear Energy, National Institute of Atomic Energy, Radiochemistry Division, S. Paulo, Brazil. They work with Neutron Activation Analysis and pilot samples of breast milk and urine were sent to them for preliminary analysis.
- Dr. José G. Dórea, Nutrition Lab., University of Brasília, Brazil. He is Brazilian investigator with more published papers on human milk. Several of his publications are found in the literature. He works mostly with atomic absorption analysis.

REFERENCES

[1] NOBREGA, F.J., Human milk composition. Revinter, S. Paulo, Brazil (1996) 235 pp.

[2] BARBOSA, A.C., DÓREA, J.G., Indices of mercury contamination during breast feeding in the Amazon Basin. Environ. Toxicol. Pharmacology **6**(1998) 71-79.

[3] MATUO, Y.K., DDT levels in maternal milk in the Ribeirão Preto Region. Master's Thesis Nursing School of Ribeirão Preto, USP, Brazil (1978).

[4] MATUO, Y.K., LOPES, J.N.C., CASANOVA, I., MATUO, T., LOPES, J.L.C., Organchlorine pesticides in human milk in the region of Ribeirão Preto, State of S. Paulo, Brazil. Arch. Environ. Toxicol. **22**(1992) 167-175.

ANNEX

ANALYSIS OF PESTICIDES IN MOTHER'S MILK:

The analysis will be performed according Campoy *et al.* (2001).

1. Sample preparation and analysis:

Four to eight tubes of milk samples from each woman will be mix in a glass jar. To this mixture 4–8 ml of milk, half of the same volume of methanol will be added and the solution shake for 5 min; 0.1 g of sodium oxalate will be add and shaked. The extraction will be performed using 10 ml ethyl ether/hexane (1:1 v/v). The extract will be centrifuged for 15 min at 3000 rpm. The organic phase will be obtained and the extraction procedure will be repeated twice more. The three organic residues will be collected. These residues will be concentrated at low pressure in a vacuum concentrator to a volume of 1 ml. To this residue, 0.5 ml of concentrated sulfuric acid will be added and centrifuged for 10 min at 3000 rpm. The acid residue will be extracted twice more with the addition of 1 ml hexane. The three organic phases will be collected and dried in a nitrogen flow. The dry residue will be redissolved in 1 ml of hexane and cleaned up.

Clean-up: the organic extract obtained from any of the extractions were purified with the use of silica Sep-Pak after the prior treatment of the cartridge with 2 ml hexane. The extract will be eluted with 10 ml hexane and then with 10 ml hexane: methanol: isopropanol (45:40:15; v/v/v). Both eluates will be collected and dried in a nitrogen stream.

The dry residue will be dissolved in 1 ml hexane, labeled with the p-p0 dichlorobenzophenone internal standard and analyzed with GC–ECD.

2. Gas chromatography–Electron Capture Detector (GC–ECD):

A 1-ml aliquot of the extract will be injected in a Shimadzu GC 17A with ECD-Electron Capture Detector (63Ni) The injector and detector temperatures were 250 and 300 ºC, respectively. The column will be a DB5-MS (J&W Scientific, Folsom, CA, USA) chromatographic column (30 m x 0.25 mm id. 0.25 mm film thickness). The temperature column will be programmed from 130 to 150ºC at 20ºC/min; 150 to 200ºC at 10ºC/min; 200 to 260ºC at 20 C/min. The carrier gas will be at a flow rate of 30 mL/min at 190ºC oven temperature. The carrier and auxiliary gas will be nitrogen.

3. Analysis of micronutrients in mother's milk:

3.1.1. Sample preparation and analysis:

- The dried milk samples (150 mg) will be sealed in a clean quartz ampoules for irradiation during 8 hours under a thermal neutron flux of 10^{13} n cm^{-2} s^{-1} in the IEA-R1 nuclear research reactor.
- Analytical Procedure: After a cooling time of 2 days, the milk samples will be transferred to Teflon Digestion Bombs (Parr Bombs) and dissolved in HNO_3/HF (5:1) at 150ºC for 10 hours. An aliquot of 0.5 ml of the non irradiated-multielemental standard solution will be

used as carrier. After dissolution, the resulting solutions are brought to dryness by evaporation at 60-70ºC to avoid possible losses by volatilization and the residues will be redissolved in 10 ml buffer solution (0.1M ammoniun acetate at pH 4.8). Thereafter the solutions will be percolated through the Chelex and TOD columns. The Chelex 100 resin will be previously conditioned with 0.1 M ammonium acetate buffer and the TDO inorganic exchanger will be conditioned with 6.0 M HCl. During the percolation through the Chelex columns the TOD will be maintained in strong acidity by adding dropwise 6.0 M HCl directly onto the TDO columns. The Chelex columns will be then washed with 100 mL of buffer solution using the same operating procedure. The gamma-ray spectra will be obtained in a ORTEC EG&G high resolution solid state Ge detector, type POP TOP, Model 20190 with resolution of 1.9 keV for the 1332 keV γ-ray peak of ^{60}Co. This detector will be coupled to an EG&G ORTEC ACE8K card and associated electronics. The spectra will be analysed using the VISPECT2 software.

OXIDATIVE STRESS AND ANTIOXIDANTS IN PLACENTAS OF WOMEN WITH LOW WEIGHT BIRTH NEONATES. CORRELATION WITH TOXIC AND ESSENTIAL TRACE ELEMENTS

MIGUEL LLANOS, ANA MARÍA RONCO, ARGELIA GARRIDO

Instituto de Nutrición y Tecnología de los Alimentos. Universidad de Chile, Casilla 138-11. Santiago. Chile

Abstract

Low weight at birth (LWB) is a serious problem in developing countries. Although there are multiple factors contributing to neonates with low LWB, we are interested in those related to placental dysfunction. It is suggested here that mothers having children with LWB could have increased levels of reactive oxygen species, due to an impairment of placental protection mechanisms. This placental protection to oxygen radicals includes an enzymatic system integrated by glutathione peroxidase and superoxide dismutase, both of which are dependent on essential trace elements for optimal activity. Then, if toxic trace elements increase in placenta (as a consequence of environmental exposure), or if essential trace elements decrease (as a consequence of nutritional status with dietary deficiency of essential elements), the protective enzymatic system would be insufficient to eliminate reactive oxygen species. This situation would lead to oxidative damage in placentas with detrimental effect on their functionality. The magnitude of the proposed damage would contribute to LWB, very likely due to foetal growth restriction.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

1.1. General Antecedents

Low weight birth (LBW) constitutes a major concern in different countries, especially in those developing countries because of its potential risks in several public health problems. The percentage of infants born with LWB in developing countries is within the range of 15 to 50 %. In Latin America the mean figure is about 15%, but with wide variability between and within countries. For example in Bolivia, the figure is about 30 %, with percentages up to 50% in some rural areas. In Chile, the mean percentage has dropped from 15 % to about 5 % in the last two decades, although some variance still prevails in different areas [1].

At this point, it is worth to mention that LWB should be considered a limited concept and raises another significant problem when trying to identify newborns having also associated an abnormality of intrauterine growth. Most of LWB neonates born in developing countries, in contrast to industrialised societies, are the result of intrauterine growth retardation (IUGR), rather than prematurity per se, characterised by an appropriate weight for gestational age. Low weight birth as a result of IUGR is not only a significant cause of organ development, infant mortality and morbidity, but it also may affect quality of life throughout the whole life cycle. Some of the effects that may become apparent in adult life include increased prevalence of diabetes, insulin resistance, high levels of lipids in blood, hypertension and obesity.

1.2. Foetal Growth and Gestational Age: Terms and Definitions

During a long time, literature has been dealing with appropriate terms and definitions to characterise foetal and neonate development. At present, two terms are commonly accepted and used: small for gestational age (SGA) and intrauterine growth retardation (IUGR). An infant with a birth weight lower than a reference limit according to his/her gestational age is

considered SGA, and IUGR supposes that foetus was retarded in its growth due to a pathological process during foetal life. Recent attempts to proportionate accurate and appropriate definitions to classify growth in foetuses and infants have raised the new term "foetal growth restriction" (FGR). Thus, restriction indicates an abnormal process during pregnancy leading to a detrimental and irreversible effect on foetal growing. This concept is better than retardation because this last term suggests a reversible condition. It is possible then to distinguish two different concepts: SGA referring to infants who failed to reach a standard weight or length threshold for their gestational age, and, FGR referring to infants not able to achieve their genetic growth potential in utero. This supposes to determine the genetic growth potential of an infant [2]. Since this may be a difficult task, it has been recently introduced the term "constitutional individual growth potential" [3]. In this definition, apart from the role of sex, which is clearly considered as a genetic determinant, other factors such as maternal age, parity, maternal height and pre-pregnancy maternal weight are considered as constitutional determinants playing important roles in foetal growth.

Nevertheless, terminology is still confusing and IUGR or FGR are still employed in reference to the same definition as SGA. In addition, the same terms SGA, IUGR and now FGR are used for diagnosis at birth or for screening during foetal life by means of sonography tools [4]

More accurate definitions, such as those recently proposed by a French group [3], seem to provide a more complete picture to recognise and infant with SGA and its possible relationship with FGR. At this point is important to reinforce the idea that maternal constitutional determinants provide a physiological influence on foetal growth. In addition, environmental or pathological factors, which may generate clear or subtle abnormal processes, may lead to impaired foetal growth. In this way, smoking, alcohol intake, non-adequate nutrition, infection, toxic consumption and vascular pathologies constitute risk factors for normal foetal development and growing.

We can venture that some maternal habits and/or environmental conditions may lead to a detrimental imbalance of nutrition-pollution factors and thus generating FGR and infants with SGA, all of them with clear low weight birth at term. At this point, we would like to highlight the role of placental tissues during foetal growth and developmental

1.3. Placenta and Foetal Growth

The role of the placenta as a component of the response to a restrictive uterine environment is reinforced by the observed compounding effect of a small placenta and low weight birth in increasing the risk of insulin resistance. Placenta plays an important role not only as a transport system able to deliver oxygen and nutrients to the foetus, but also as an endocrine organ that produces and metabolises hormones and growth factors. Essential trace elements delivered from placenta to the foetus are also important factors associated to foetal growth and development. There are some other trace elements, like heavy metals, that may cause damage to the mechanisms involved in foetal growth. Although placenta acts as a barrier for some toxic elements and thus avoiding transference of them to the foetus (e.g. Cd), it does not have a restrictive barrier to protect against other heavy metals (e.g. Pb) transference [5,6]

Heavy metals are known reproductive toxic agents. It has been recently reported that Pb contamination in Swedish women is a negative predictor of birth weight, length and head circumference, indicating that lead may restrict foetal growth in utero even at very low exposure levels [7]. Other studies conducted in our Institute demonstrated that smoking mothers had infant with reduced birth weight [8]. Smoking increases blood and tissue Cd

levels, and maternal exposure to Cd during pregnancy has been linked with low weight birth, reflecting FGR, attributed to placental damage and/or dysfunction in nutrient transport [9]. A recent study from an industrialised city found that in placental samples, Cd was present in almost all of them, Pb in 22% and Hg in 28 % [10]. At molecular level, some of the important effect of heavy metals may be related to impairment of defence molecules against negative effects of oxidative damage, due to an abnormal accumulation of reactive species of oxygen [11]. Thus, possible placental oxidative damage may have negative consequences for foetal growth and development.

1.4. Reactive Oxygen Species, Oxidative Damage and Defence Mechanisms

Normally foetus-placental system is exposed to different metabolites, some of them that may be toxic for cell function are destroyed by different defence mechanism systems that ensure normal intrauterine growth and development of the embryo. Within the unavoidable intermediates produced during metabolism are the reactive oxygen species (ROS), and although they may have beneficial effects to cell function, their level and balance between production and degradation must be controlled, to avoid risk of oxidative damage. Increased levels of ROS like superoxide anion, hydrogen peroxide, peroxynitrite and hydroxyl radical, are involved in pathogenesis of many diseases and ageing [11,12]. ROS are able to block activity or modify several macromolecules such as proteins, lipids and DNA, thereby destroying integrity of cells [13]. Human placenta and foetus may be also a target for this toxicity. Placental oxidant-antioxidants imbalance may release different oxidation products to the circulation, causing damage to endothelial cell membranes related to several pathological conditions during pregnancy e.g. pre-eclampsia, hypertension and pregnancy induced diabetes [14-16].

Efficiency of various ROS scavengers, enzimatic or non-enzimatic present in the placenta and the foetus ensure normal physiology of this system and as a consequence, normal intra-uterine growth.

The role of antioxidants enzimes superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and glutathione reductase (GR) in inhibiting lipid peroxidation in placenta and neonatal blood has been already reported [17-18]. Two of these enzymes are dependent of essential trace elements for its functional activity. Thus, GPx is dependent on selenium (Se-GPx) and SOD dependent of Copper/Zinc (Zn/Cu-SOD).

Then, it is conceivable that any decrease in these essential trace elements or the replacement of them with some other toxic heavy metal in the placental enzymes will result in decreased activity of them. This may result in augmented oxidative damage in the foetus-placental system with a potential risk for normal foetal growth.

Balance between essential and toxic metals may reflex balance between nutrition-pollution factors. Highly polluted environments such as those produced by metal smelter areas, and smoking habit may decrease activity of enzymatic defence systems.

1.5. Antioxidants Status in Placental Tissues at Parturition and During Pregnancy.

Studies by Qanungo et al. in 1999 [19] have shown activity of the four antioxidants enzymes in unfractionated placenta, placental syncytiotrophoblast brush border membrane (BBM) and umbilical cord blood (UC). Among all of them, CAT in UC blood showed the highest activity, compared to the other enzymes in both placental and foetal systems. In addition, lipid peroxidation expressed as levels of malondialdehyde (MDA) and conjugated dienes showed a consistent decrease in both oxidation products from early gestation to term. This was clearly observed in BBM a most susceptible site to peroxidation of its membrane lipids as it is directly exposed to the high oxygen tension of maternal vascular system [20]. Therefore, it could be concluded that strong protective mechanisms against ROS are present in the foetus-placental unit at the time of parturition.

More recent studies [21] have demonstrated a characteristic profile of antioxidants enzymes and lipid peroxidation during pregnancy in human placenta. Results demonstrated that activities of SOD, GR and CAT in placental homogenate and BBM show a significant increase with gestational progress. Nevertheless, GPx remains unchanged with gestational progress. Additionally, ontogenic profile of lipid peroxidation as a measure of thiobarbituric acid reactive substances (TBARS) clearly shows a dramatic decrease from early gestational age to term. This result is in clear accordance with those previous results indicating an increased activity of antioxidants enzymes through gestational age (early, mid and term). All these facts indicate that in order to meet the increasing demands of the growing foetus, more oxygen must enter the foetus-placental circulation during ontogeny and thus exposing it to a greater risk of oxidative damage due to ROS. Increase in the activity of SOD and CAT indicates an increased protection of placenta and foetus against damage actions of superoxide anion ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2). Although GPx does not change during gestation, occurrence of substantial activity in placental tissues reveals an efficient defence against H_2O_2 toxicity and/or some other peroxides. At this point, it is worth to mention that CAT and GPx are considered molecules able to destroy H_2O_2 and its associated detrimental effects in several biological systems.

Progressive increase of SOD, CAT and GR with increase in gestational age clearly reveals a positive correlation and crucial importance of these antioxidants enzymes with growth and development of the foetus-placental unit. Thus, any constant constitutional or environmental factor able to disrupt the activity of these enzymes could have detrimental effects on the foetus during the whole pregnancy period.

1.6. Toxic Elements Exposure and Oxidative Damage in Placenta

It has been shown that increased placental burden of arsenic is found in mothers living in copper smelters areas compared with non-smelters areas. In addition, it has been also shown that smoking (a habit known to increase body burden of both arsenic and cadmium) contributed to an additional increase in placental content of arsenic and cadmium [22]. In the same study, the combination of smoking and smelter area exposure was also associated with lower glutathione antioxidant protection in both maternal and cord blood. This is important because it has been reported a higher efficiency of glutathione recycling in the foetus at birth as compared with the adult [23]. Thus, antioxidant protection of normal foetuses at term is better than that of the mothers. It has been also found that lipids peroxides tend to be high in maternal blood, cord blood and placenta, in those smoking women exposed to the smelter

area. These results, though not statistically significant, suggest that a lowered glutathione concentration do not provide enough protection to avoid the occurrence of oxidative damage.

1.7. Proposal

The antecedents lead us to propose the study of some aspects of Nutrition-Pollution interaction imbalance and its impact first in placental function with subsequent consequences on the newborn birth weight (appropriate or not for gestational age). Specifically, we would like to know if an imbalance between essential and toxic trace elements could impair mechanisms of defence against oxidative damage in placental tissues. In addition, we would like to establish a relationship between increased placental oxidative damage and neonates with low weight birth (infants with SGA). The importance of this study resides in the fact that the effects of the imbalance of nutrition-pollution factors on placental function, due to oxidative damage, may be recognised at molecular level. Since some non-voluntary environmental conditions are difficult to avoid, therapeutical intervention with other known potent antioxidants (e.g. some vitamins) could constitute an appropriate tool to avoid undesirable foetal risks during pregnancy.

2. METHODS

2.1 Selection of Placentas

Placentas will collected from the Sótero del Río Hospital (South-East Santiago) immediately after parturition. Two groups will be established:

Group A: Placentas from mothers delivering neonates with birth weights over 2.500 g and over 36 weeks gestation age at birth (term)

Group B: Placentas from mothers delivering neonates with birth weights between 1.000 and 2.500 g and over 36 weeks gestation age (term).

Exclusion criteria will include: birth weight under 1.000; major congenital malformation, chromosomal disorders or other genetic causes; pathologies such as rubella, chagas disease, HIV/AIDS, syphilis; neurological impairments (Apgar < 6) and history of drug use during pregnancy other than cigarette smoking

2.2 Sample Preparation

The entire placenta will be collected immediately after parturition. They will be placed in a clean polyethylene bag and frozen at –20ºC. Further preparation will be carried out in the laboratory of the Chilean Nuclear Energy Commission (La Reina, Santiago, Chile) and managed with teflon and titanium material of high purity. Placentas will be ground, lyophilised, homogenised and passed through an appropriate sieve. Ions determinations will be performed with dry powdered placentas, utilising appropriate standards for each ion.

2.3. Zinc, Copper and Iron Determinations

Zinc, copper and iron levels in dry powdered placentas will be determined in the laboratory of Trace Elements at INTA, University of Chile by acetylene flame atomic absorption spectrometry (AAS) Perkin-Elmer Mod 2280 using Varian Techtron Model AA-6. Final results will be expressed in μg/g of dry weight. Known amounts of Zn, Cu and Fe in 0.5 M nitric acid will be used as references.

To evaluate the quality and reproducibility of results obtained at INTA, 10 samples for Cu determination will be confirmed in the Chilean Nuclear Energy Commission. Samples will be irradiated for 5 min at thermal neutron flux of 10^{13} n $cm^{-2}seg^{-1}$ and analysed by instrumental neutron activation analysis (INAA), with radiochemical separation. After cooling during 1 h, samples will be immediately digested in a mixture of sulphuric acid and perhydrol and passed through an analytical grade chelating resin Chelex-100, 100-200-mesh, sodium form column, in order to retain Cu. The resin will then be measured and the radionuclide ^{64}Cu will be immediately determined using the 511 KeV gamma-line. The yield of these radiochemical separations will be controlled using CRM.

2.4. Cadmium Determination

For Cd determination, a preliminary homogeneity study with the solid sample will be performed. Samples will be read in an Atomic Absorption Spectrometer (AAS 5 E-A) with ion exchange resin and graphite furnace for solid samples (Carl Zeiss Technology) [24].

The samples or standards (0.5 g) will be irradiated for 24 hrs at a thermal neutron flux of 10^{13} n $cm^{-2}seg^{-1}$ and then analysed by neutron activation (INAA), using the neutron facilities of the Chilean Nuclear Energy Commission. After a suitable cooling time, samples will be immediately digested in a mixture of sulphuric acid and perhydrol, and passed through an analytical grade anion exchange resin Bio-Rad AG 2x8, 100-200 mesh chloride form resin column which retained Cd. The resin was removed and counted after 24 hrs on a high-resolution gamma spectrometry system using the 336 KeV gamma-line of ^{115}Cd.

2.5. Arsenic Determination

Arsenic will be determined in samples of dried powdered placentas with Instrumental Neutron Activation Analysis (INAA) at the Chilean Nuclear Energy Commission.

2.6. Lead Determination

Pb will be determined by the Atomic Absorption method for solid samples. Samples will be read in an Atomic Absorption Spectrophotometer (AAS 5 E-A) with ion exchange resin and graphite furnace for solid samples (Carl Zeiss Technology) [24].

2.7. Selenium determination

Selenium will be determined in the dried powdered placenta samples with the Instrumental Neutron Activation Analysis (INAA) at the Chilean Nuclear Energy Commission. Samples will be irradiated for 24 hrs at a thermal neutron flux of 1013ncm-2 s-1. After 15 days decay, the radiation gamma from the long-lived radionuclide ^{25}Se will be measured in a high-resolution gamma spectrometer [25].

2.7.1. Determination of the Enzymes Superoxide Dismutase and Glutathione Peroxidase.

Superoxide dismutase (SOD) activity will be evaluated by the method of Sun and Zigman [26]. This is a spectrophotometric method based on the inhibition of the spontaneous degradation of adrenaline to adrenochrom at pH 10.2 by SOD. The increase in absorbance at 525 nm from zero during 10 min, is compared with controls in which SOD sample is replaced by 0,9 % NaCl. The activity of SOD is expressed as the percentage of inhibition of spontaneous degradation of adrenaline.

Glutathione Peroxidase (GPx) will be assayed by the method of Paglia and Valentine [27]. Cellular GPx catalyses the reduction of an organic peroxide while forming oxidised glutathione (GSSG). In the test, the rate of GSSG formation is measured by the following decrease in absorbance of the reaction mixture at 340 nm as NADPH is converted to $NADP^+$, between the 2^{nd} and 4^{th} min after the initiation of the reaction.

2.8. Determination of Glutathione and Lipid Peroxidation

The method used to evaluate total glutathione content (reduced GSH and oxidised GSSG) will be that reported by Tietze [28]. This method is based on the DTNB reduction by the TPNH-glutathione reductase system using total glutathione present in the samples (placental homogenate) as the substrate. Rate of colour development in the reaction mixture will be followed spectrophotometrically at 412 nm at 25 or 37ºC.

Lipid peroxidation is a process associated to oxidative stress in the cell. The assay of lipid peroxidation implies a secondary product of this oxidative reaction, malondialdehyde (MDA) able to react with thiobarbituric acid generating thiobarituric acid reactive substances (TBARS) [29]. The reaction products that can be measured by spectrophotometry at 530 nm.

3. RESULTS

Since this project is in its early beginning, a few preliminary results can be shown, these are mainly related to: (a)- the characteristics of mothers selected for this study, and (b)- levels of essential trace elements and Cd in placentas of mothers delivering normal weight neonates (non-SGA, group A) in comparison to those levels found in placentas of mothers delivering low weight birth newborns (group B) (Tables I and II). Low weight birth (LWB) neonates are considered to weigh 2.500 g or less at term (SGA). The small sample of those LWB does not permit at this time to establish statistically significant differences with the control group.

TABLE I: DESCRIPTION OF SUBJECTS

SUBJECT	**LWB Average (range)(n=2)**	**Controls Mean ± SD (n=10)**
MOTHER		
Age (y)	27,5 (18-37)	22,9 ± 6,1
Height (cm)	159 (167-151)	160 ± 0,04
Parity	(1-4)	1 ± 1
Urinary Cotinine (ng/ml)	(12,2-2.400)	41,4 ± 31,6
INFANT		
Birth Weight (g)	2.315 (2.160-2470)	3.372 ± 381
Height (cm)	44,5 (44-45)	49,9 ± 0,74
Gestational Age (weeks) at delivery	37,5 (36-39)	39,9 ± 1,24

TABLE II. TRACE ESSENTIAL AND TOXIC ELEMENTS IN TOTAL PLACENTAL TISSUE

Condition	**Fe (μg/g dry wt)**	**Cu (μg/g dry wt)**	**Zn (μg/g dry wt)**	**Cd (ng/g dry wt)**
CONTROL MEAN ± SD (N=10)	**424 ± 154**	**12 ± 3**	**90 ± 15**	**23.6 ± 3.1**
LWB Aver. (n=2) (range)	**520** (355-685)	**12,5** (15-10)	**82.5** (85-80)	**41.2** (24.3-58.1)

4. FUTURE WORK

This will include the following determinations in placentas of both, group A and B:

- Determinations of toxic elements such as Pb, As and Cd.
- Determination of essential trace elements: Cu, Zn and Se
- Determination of TBARS concentration, as one of the typical biomarker of lipid peroxidation
- Evaluation of the catalytic activity of the antioxidants enzymes SOD and GPx, both of them dependent of appropriate levels of Cu/Zn and Se respectively.
- Determinations of the antioxidant reduced glutathione (GSH).

REFERENCES

[1] ACUÑA, S., MAURO, J., URRUTIA, J.C. ABARCA, G., Indicadores de atención de salud. Unidad de Estadística y Computación. Secretaría Regional Ministerial. Ministerio de Salud, Chile. Anuario. Vol. **11** (1996).

[2] GOLDENBERG, R.L., CLIVER, S.P., Small for gestational age and intrauterine growth restriction: definitions and standards. Clin. Obstet. Gynecol. **40** (1997) 704-714.

[3] MAMELLE, N., COCHET, V., CLARIS, O., Definition of foetal growth restriction according to constitutional growth potential. Biol. Neonate **80** (2001) 277-285.

[4] MONGELLI, M., EK, S., TAMBYRAJIA R., Screening for foetal growth restriction: A mathematical model of the effect of time interval and ultrasound error. Obstet. Gynecol. **92** (1998) 908-912.

[5] LI, P.J. et al., Transfer of lead via placenta and breast milk in human. Biomed. Environ. Sci. **13** (2000) 85-89.

[6] ROMERO, R.A., et al., J. Placental transfer of lead in mother/newborn pairs of Maracaibo city (venezuela) Trace Elem. Electrolytes Health Dis. **4** (1990) 241-243.

[7] OSMAN, K., et al., Toxic and essentials elements in placentas of Swedish women. Clin. Biochem. **33** (2000) 131-138.

[8] SALAZAR, G., et al., Efecto del tabaquismo materno en el crecimiento de sus hijos.Rev. Med. Chile **126** (1998) 1059-1064.

[9] REICHTOVA, E., URSINYOVA, M., PALCOVIKOVA, L., WSLOVA L., Contents and localisation of of heavy metals in human placentas. Fres. J. Anal. Chem. **361** (1998) 362-364.

[10] ZADOROZHNAJA, T.D., et al., Concentration of arsenic, cadmium, copper, lead mercury and zinc in human placentas from two cities in Ukraine. J. Toxicol. Environ. Health **61** (2000) 255-263.

[11] HALLIWELL, B., GUTTERIDGE J.M.C., Oxygen toxicity, oxygen radicals, transition metal ions and disease. Biochem. J. **219** (1984) 1-14.

[12] AUTOR, A.P. (ed), Pathology of oxygen. New York. Academic Press. (1982)

[13] SIES, H., DEGROOT, H., Role of reactive oxygen species in cell toxicity. ToxicolLett. **64** (1992) 547-551.

[14] PONAREN, A.K., EKBLAD, U., UOTILA, P.,AHOTUPA, M., Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta **17** (1996) 401-405.

[15] ZNAMENSKAIA, T.K., Role of antioxidant enzymes of the foetus-placental complex in neonatal adaptation to maternal diabetes mellitus. Ukr. Biokhim. Zh. **66** (1994) 93-97.

[16] CESTER, N., STAFFOLANI, R., RABINI, R.A., MAGNANELLI, R., ROMANINI, C., Pregnancy induced hypertension: A role for peroxidation in microvillous plasmamembranes. Mol. Cell. Biochem. **131** (1994) 151-155.

[17] NOVAK, Z., et al., Comparative study: The antioxidant enzymes and lipid peroxidation in cord and maternal red blood cell hemolysates. Clin. Chim. Acta **180** (1989) 103-106.

[18] WATSON, A.L., PALMER, M.E., JAUNIAUX, E., BURTON, G.J., Variation in expression of copper/zinc superoxide dismutase in villous trophoblast of the human placenta with gestational age. Placenta **18** (1997) 295-299.

[19] QANUNGO, S., SEN, A., MUKHERJEA, M., Antioxidant status and lipid peroxidation in human foetus-placental unit. Clin. Chim. Acta **285** (1999) 1-12.

[20] WILLIAMS, J.W., CUNNINGHAM, F.G., MACDONALD, P.C., GANT, N.F., The

Placenta and foetal membranes. In: J.W. William (ed). Williams Obstetrics. Appleton and Lange. Norwalk. Connecticut, USA. (1989) pp 39.

[21] QANUNGO, S., MUKHERJEA, M., Ontogenic profile of some antioxidants and lipidperoxidation in human placental and foetal tissues. Mol. Cell. Biochem. **215** (2000) 11-19.

[22] TABACOVA; S¸BAIRD, D.D., BALABAEVA L., LOLOVA, D., PETROV, I., Placental arsenic and cadmium in relation to lipid peroxides and glutathione levels in Maternal-Infant pairs from a copper smelter area. Placenta **15** (1994) 873-881.

[23] CLAHSEN, P.C., MOISON, M.W. HOLTZER, C.A.J., BERGER, H.M., Recycling of glutathione during oxidative stress in erythrocytes of the newborn. Pediatr. Res. **32** (1992) 399-402.

[24] DE GREGORY, I., PINOCHET, H., DELGADO:D., GRAS, N., MUÑOZ, L., Heavy metals in bivalve mussels and their habitats from different sites along the Chilean coast. Bull. Environ. Contam. Toxicol. **52** (1994) 261-268

[25] RUZ, M., CODOCEO, J., HURTADO, S., MUÑOZ, L., GRAS, N., Characterisation of the regional distribution of selenium in Chile using selenium in hens' eggs as a monitor. J. Trace Elements Med. Biol. **9** (1995) 156-159.

[26] SUN, M., ZIGMAN, S., Determination of superoxide dismutase in erythrocytes using the method of adrenaline autooxidation. Anal. Biochem. **90** (1978) 81-89.

[27] PAGLIA, D.E., VALENTINE, W.N. (1967) Studies on the quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. **70** (1967) 158-169.

[28] TIETZE, F., Enzymic method for quantitative determination of nanogram amounts ofoxidised glutathione: Applications to mammalian blood and other tissues. Anal. Biochem. **27** (1969) 502-522.

[29] OHKAWA, H., OHISHI, N., YAGI, K., Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. **95** (1979) 351-358.

APPLICATION OF NUCLEAR TECHNIQUES TO DETERMINE THE COMPOSITION OF HUMAN PLACENTA AND CORD BLOOD IN THE STUDY OF INTERACTION BETWEEN ESSENTIAL AND TOXIC TRACE ELEMENTS

JI-XIAN WANG

Chinese Academy of Medical Science, Institute of Radiaton Medicine-Cams, Tianjin, 300192, China

Abstract

For a better understanding of interactions between frequently encountered toxic trace elements in the environment and nutritional elements and development of strategies to reduce ill effects in pregnant woman and fetus. Application nuclear technique to determine the composition of placenta and cord blood as dual biological indicators is proposed to study the interactions between nutritional and pollution elements and identify the ill effects caused by the deleterious interactions. A part of results obtained from related study are introduced. The methods to be used to the present study and plans for future work are presented in detail.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Rapid and uncontrolled industrial growth in most part of the developing world has aggravated environmental contamination, some of which are potent pollutants. The area of nutrition-pollution interactions is an emerging research discipline. The deleterious health effects resulting from these interactions are considered to be critical issues by the WHO.

Up until now, nutritional status and environmental pollution have mostly been treated as separate issues and there is very little information available about their relationship. It is therefore of great importance to investigate and understand the interaction between pollution and nutrition to reduce ill effects of pollutants and improve the health status of populations, especially in developing countries.

Biomonitoring has now been accepted as a powerful tool to assess the impact of pollutants on human health. During pregnancy pollutants are not only a health risk to the mother but also to the fetus and infant. Placenta can also be employed as a dual indicator for real-time monitoring, reflecting the pollutant status in both mother and fetus.

The scope of the project is application of nuclear technique to determine the composition of human placenta and cord blood in the study of interaction between essential elements, (calcium, selenium, zinc) and toxic trace elements (cadmium, lead). The objectives of the project are as follows:

To establish a non-invasive method that is application nuclear techniques determining the composition of placenta and cord blood as dual biological indicators of pollutant level in the mother and fetus.
To validate procedures for determining the composition of human placenta and cord blood for research in the area of nutrition-pollution interaction.
To identify deleterious interactions between toxic trace elements (Cd, Pb) and essential elements (Ca, Zn, Se).

It is expected that the work will lead to a better understanding of the interactions between frequently encountered toxic trace elements in the environment and human nutrition and to develop strategies to reduce ill effects in pregnant women and fetus.

A stable coordination work group was formed through the IAEA-RCA Reference Asian Man phase 2. It included the main institutes working in the field, such as China Institute of Atomic Energy, China Institute of Radiological Protection, Institute of Nutrition and Food Hygiene-CAPM and so on. We possess all of the facilities to be used in the project, for example equipment for NAA, Miniature Neutron Sourer Reactor, made in China Institute of Atomic Energy, and equipment for ICP-MS, Perkin-Elmer sciex Elan 5000, made in USA.

Also some nuclear techniques and methods for analysis trace elements in biological samples were established and validated with NIST-SRMs during the study on ingestion and organ content of trace elements of importance in radiation protection [1-7].

2. METHODS

2.1. SITE SURVEY

- Determination of environmental Cadmium and/or Lead levels in air, water, soid and food, and identification of the sources of the pollution.
- Estimation intakes of Cadmium and/or Lead.
- Estimation of the nutritional status, especially for Ca, Se, Zn intakes.

The ideal condition of the study site is that the intakes of Cd and/or Pb are high when the intakes of Ca, Se and Zn are deficient among the local residents.

2.2. SAMPLING STRATEGY

Two-stage sampling should be taken. First stage is cluster sampling for getting the primary sampling units. The second stage is random sampling from the primary sampling units to get the subjects to be studied.

2.3. EPIDEMIOLOGICAL SURVEY

Each sampled subject will be interviewed face to face according to the questionnaire, to get the following information: inhabitant history in the area, dietary composition and consumption of various food, reproductive history, family history of multifactoria disease, history of disease, general status, including education, economic condition, smoking and drinking habit, height and weight of total body.

2.4. EPIDEMIOLOGICAL INTERFERENCE EXPERIMENT

The subjects will be random divided into 2 groups, one group is called interfering group in which Ca, Se, Zn will be supplemented, another group do not supplement Ca, Se, Zn as the control group to study the interaction between nutritional and pollution elements and to observe the impact on the health status.

2.5. SAMPLE COLLECTION, STORAGE, AND PREPARATION

Placenta and placenta blood, cord and cord blood to be collected from each pregnant woman when childbirth by using clean polyletre-fluoethylene (PTFE) vacutainer or vessel with a lid (made in USA, Becton Dickinson), stored in freezer at temperature less than -20^{o}C, and dried samples by freezing.

2.6. ANALYTICAL METHODS

NAA and ICP-MS, or AAS will be used for Ca, Se, Zn, Cd and Pb analysis in urine, blood and tissues samples. See reference [1-7].

2.7. BIOLOGICAL INDICATORS FOR DETERMINING THE HEALTH EFFECTS

- X-ray photograph to see the changes of femoral upper end trabeculae grade. It can be used as an early diagnostic index of bone damage induced by Cadmium [8-9].
- Urinary β_2-MG (β_2-miroglobulin), low molecular albumin, total protein, aminonitrogen, creatinine, NAG isoezzymes (N-acetylglucosamindase isoezzymes) will be determined, to observe the renal tubular dysfunction [10-18].

2.8. DATA ANALYSIS

SAS software will be used in calculation, and logistic regression model analysis of the data.

2.9. QUALITY ASSURANCE AND QUALITY CONTROL (QA/QC)

- Development of standard operation procedures covering all aspects of the study. Particular point of emphasis included: (1) Representation of sampling (2) avoid to cause external contamination in sample collection, sample preparation and chemical analysis, including checking of the purity of standards and reagents.
- Validation of the analytical methods using the NIST-SRMs supported by the IAEA.
- During the element analysis phase internal quality control measures will be adopted by using some suitable RMs, and duplicates and retention of a sample aliquot for reanalysis if necessary.
- Attend the external quality control measures, if it is available.

3. RESULTS

Part of results obtained from the IAEA-RCA "study on ingestion and organ content of trace elements of importance in radiation protection, Reference Asian Man Project Phase II" are introduced here [1-7], as these are concerned with the present project.

The concerned elements in the present project are Cd and/or Pb as the polluted trace elements and Ca, Se, Zn as the nutritional elements.

The average concentrations of above 5 elements in various food categories for the whole country are listed in table 1.

TABLE 1: AVERAGE CONCENTRATION OF ELEMENTS IN VARIOUS FOOD OF CHINA

Foods	Ca (mg/kg)		Se (μg/kg)		Zn (mg/kg)		Cd (μg/kg)		Pb (μg/kg)	
	X	S	X	S	X	S	X	S	X	S
Grains	466	210	58	27	14.2	4.8	86.6	29.6	436*	43
Beans	1456	950	22	7	16.5	5.5	17.6	8.9	136	82
Yam	217	66	9	4	3.2	1.0	26.7	19.1	359	113
Meat	148	71	79	17	20.9	7.5	61.0	67.3	498	301
Egg	671	376	187	18	12.4	1.8	94.3	39.9	164	72
Aquatic product	2271	3285	351	123	18.4	13.5	47.7	68.3	91	46
Milk	1210	316	18	4	3.7	0.5	29.3	--	326*	363
Vegetable	724	244	11	7	4.5	1.2	42.4	30.0	106	52
Fruit	123	54	1.5	0.6	0.6	0.3	35.6*	--	369*	399
Sugar	294	176	3.5	3.0	0.8	--	8.0	--	59	96
Drink & water	296	13.6	0.6	--	0.1	0.2	1.1	--	10	8
Alcoholic drink	31.5	13.5	2.0	1.4	0.4	0.3	8.6	--	50	18

* Over the national limit of concentration of the element in the food category

Daily intakes of each element were calculated by multiplying the element concentration in food by dietary composition of Chinese adult man. The average intakes of each element and regional difference are shown in table 2.

TABLE 2: DAILY DIETARY INTAKES OF ELEMENTS FOR CHINESE ADULT MAN (WITH REGIONAL DIFFERENCES)

Element	Unit	Region				x	S
		Hebei	Shanxi	Shanghai	Hubei		
Ca	mg	913	620	676	683	723	130
Se	μg	63.7	43.1	46.2	57.8	52.7	9.7
Zn	mg	11.6	11.8	14.2	12.3	12.5	1.2
Cd	μg	90.1	106.2	68.0	37.9	75.6	29.6
Pb	μg	428	563	291	286	392	132

The comparison of obtained intakes with authorized recommended value and data in 1982 list in table 3.

TABLE 3: DAILY INTAKES OF ELEMENTS AND HYGIENE EVALUATION

Elements (unit)	Daily intake (a)	Recommended value (b)	Ratio a/b	Daily intake in1982 (c)	Ratio c/b
Ca (mg)	723	800 (RDA)	0.90	582	0.73
Se (μg)	52.7	50 (RDA)	1.05	42.3	0.85
Zn (mg)	12.5	15 (RDA)	0.83	9.8	0.65
Cd (μg)	75.6	60 (ADI)	1.26	13.8	0.23
Pb (μg)	392	214.3 (ADI)	1.83	86.3	0.40

RDA: Recommended Daily Dietary Allowance
ADI: Acceptable Daily Intake

Daily intakes of nutritional elements such as Ca, Se, Zn were increased in 1992 than that in 1982. The ratios with the RDA rose from 0.73 to 0.90 for Ca, 0.85 to 1.05 for Se and 0.65 to 0.83 for Zn. It means the dietary composition of Chinese much improved recently, the intakes of essential element are increased than them before. But the intakes are still insufficient. The intakes of potential toxic elements Cd and Pb increased even more, from 0.23 to 1.26 for Cd, 0.40 to 1.83 for Pb. An excess of ADI 26% for Cd and 85% for Pb was found. More attention should be paid to this finding. This is why Cd, Pb, Ca, Se, Zn were chosen as the target elements in the current study.

Through the analysis of the individual food materials among the regions the main food ingredients which made significant contribution to the daily element intake were identified, such as for Pb the significant contribution came from grains, fruit, milk and meat, for Cd mainly from fruit, grains and egg.

Table 4 and 5 show the 5 elements concentrations and contents in muscle, skeleton, liver, kidney and lung. Some elements are concentrated in certain organs and tissues. If assuming the concentrations of elements in muscle are 1, the relative concentration of element in skeleton is 1837 for Ca, 11 for Pb; in liver is 5 for Cd, 4 for Pb; in kidney is 5 for Cd, 4 for Se.

TABLE 4: ELEMENT CONCENTRATIONS IN MAIN TISSUES AND ORGANS OF CHINESE ADULT MAN (G^{-1} FRESH), N=31

Elements	Unit	Muscle		Skeleton		Liver		Kidney		Lung	
		X	S	X	S	X	S	X	S	X	S
Ca	mg	0.07	0.03	120	26	0.07	0.04	0.10	0.04	0.12	0.13
Se	ng	151	30	110	32	347	64	671	146	140	34
Zn	μg	55.0	9.9	60.5	12.6	57.1	13.6	34.5	11.5	12.9	3.6
Cd	ng	173	214	128	110	782	730	844	1117	240	270
Pb	ng	113	67	1197	524	441	254	188	81	226	124

TABLE 5: AVERAGE CONTENTS OF ELEMENTS IN MAIN ORGANS AND TISSUES OF CHINESE ADULT MAN

Elements	Unit	Muscle	Skeleton	Liver	Kidney	Lung
Ca	g	1.73	960.0	0.10	0.03	0.15
Se	mg	3.78	0.88	0.49	0.19	0.18
Zn	mg	1380	484	80.5	10.0	16.0
Cd	mg	4.32	1.02	1.10	0.25	0.30
Pb	mg	2.82	9.58	0.62	0.06	0.28

4. PLANS FOR FUTURE WORK

- Definition of the environmental conditions of the site where to be chosen to undertake this project, including pollutant levels in air, water, soil, food and the sources of such pollutions.
- Development of protocols concerning selection of pregnancy subjects and procedures to specify the nutrition and health status of chosen subjects.
- Development of procedures of investigation the deleterious interaction between nutrients-pollutants by conducting interference study among the chosen pregnancy subjects.
- Development and validation of health indices to be used to assess health effects.
- Specification of the types of samples to be collected and justification to choose them.
- Validation of analytical methods and development of procedures of QA and QC.
- Development of procedures for processing, storage and analysis of samples.
- Setting of strategies for evaluating analytical data and data interpretation.

REFERENCE

[1] WANG, J.X., CHEN, R.S., ZHU, H.D., Study in China on ingestion and organ content of trace elements of importance in radiological protection. Food and Nutrition Bulletin, in Press.

[2] LIU, G.D., et al., Study on content and distribution of trace element in tissues of normal adult. World Elemental Medicine, 7 (2000) 27-30.

[3] ZHU, H.D., et al., Concentrations and contents of 42 elements in main organs and tissues for 31 cases of Chinese man. Chin. J. Radiol. Med. Prot, 21 (2001) 1-8.

[4] ZHU, H.D., et al., Food concentrations and dietary intakes of elements for Chinese Man. Chin. J. Radiol. Med. Prot, 20 (2001) 378-384.

[5] WANG, J.X., et al., Data of anatomical physiological and metabolic characteristics for Chinese Reference Man. Atomic Energy Press, Beijing (1999).

[6] WANG, N.F., et al., Detection of trace elements in human tissues by ICP-MS technique. Symposium of Nature Radiation Exposure and Control, Beijing (2000), China 574-576.

[7] ZHU, H.D., et al., Chinese dietary composition and intakes of elements and radionuclides by ingestion. Chin. Med. Sci. J,12 (1997) 195-201.

[8] SINGH, M., et al., Changes in tubercular pattern of upper end of the femur as an index of osteoporoses. J. Bone Joint Rush, 52 (1970) 457-461.

[9] XU, S.Q., et al., Study on early diagnostic index of bone damage induced by environmental Cadmium. Chin Act Public Hygiene, 14 (1995) 331-333.

[10] YAN, C.H., et al., Lead exposure level in umbilical cord blood and its related factors. Chin. J. Prev. Med, 31 (1997) 9-12.

[11] QIN, X.Y., et al., Relation between Free Erythrocyte Protoporphrins and blood lead among pregnant women and their infants in shijingshan district, Beijing. J. Environ. Health, 16 (1999) 261-263.

[12] LU, YI, et al., Study on the environmental lead pollution causes by sewage irrigation and health effects. J. Environ. Health, 14 (1997) 10-12.

[13] GAO, Y.G., et al., A study on the relationship between Cadmium body burden and environmental Cadmium pollution. J. Environ. Health, 12 (1995) 244-246.

[14] HUANG, W.G., et al., Investigation of Urinary Cadmium –micro globulin in Cadmium polluted village. J. Environ. Health, 9 (1992) 166-168.

[15] YUAN, Y.A., et al., Study on the effect of Cadmium pollution on health of residents in Zhangshi irrigation area. Chin. Environ. Sci., 12 (1992) 173-177.

[16] WANG, C.H., et al., A study on the effect of Cadmium burden upon Calcium balance in population. Chin. Environ. Sci., 11 (1991) 109-112.

[17] WU, X.W., et al., Urinary secreation of NAG isoenzymes of a populated in Cadmiumpolluted area. J. Environ. Health, 14 (1997) 202-205.

[18] SATIN, K.P., et al., Umbilical cord blood lead levels in California. Arch Environ Health, 46(1991) 167.

ROLE OF NUTRIENTS IN ENVIRONMENTAL TOXICITY

B. DINESH KUMAR, PH.D

Sr. Research Officer, Food and Drug Toxicology Research Centre, National Institute of Nutrition, Jamai Osmania, P.O, Hyderabad – 500 007 INDIA Tel. No. +91 (40) 701 8909, Fax No. +91 (40) 701 9074 E-mail: dineshkalpa@eth.net

1. INTRODUCTION

Metals have been used since time immemorial for medicinal and other purposes. Among those oldest known metals, Lead, Mercury, Cadmium, Aluminum and Arsenic are still considered indispensable because of their wide spread use in medicinal, automobile industry and in defence hardware. However the outbreak of the mysterious Minamata disease in a Japanese village 1953, drew global attention to the need to monitor the concentration of toxic metals in the environment.

Some metals have important physiological functions. Iron is essential for synthesis of oxygen carrier protein hemoglobin and is a part of cytochrome which participates in xenobiotic metabolism. Copper is closely linked with iron metabolism and monoamine oxidase activity while zinc is an important constituent of metalloenzymes viz d-Amino levulinate, Pyruvate carboxylase etc. However, certain metals such as Pb, Cd, Hg As and Al are toxic in nature and exposure to them leads to adverse health consequences.

Among the various metals, the heavy metal lead is a major environmental pollutant and its toxicity continues to be a major public health problem in many segments of the population. There is a growing consensus that lead causes toxic injury to humans at a level of exposure that only a decade ago was considered to be safe. The classical symptoms and signs of lead poisoning are uncommon at prescribed safe limits of blood lead levels of 40-60 µg/dl. Chronic exposure of lead even at low levels can result in a slow progress and most of the time, irreversible damage to the nervous, haemopoietic and renal systems. In addition the lead burden experienced by children having blood lead level around 10 µg/dl is reported to be neurotoxic and damaging to neuropsychological functions.

The diagnosis based on the lead levels does not always give an accurate estimate of the total body burden of lead, duration of exposure and extent of sub-clinical toxicity, which is more common than acute toxicity. In developing countries like ours with rapid industrialization and prevalence of malnutrition the problem of lead toxicity gets further aggravated.

Traditionally, treatment of chronic lead toxicity is carried out with metal chelators like EDTA, BAL, DMSO etc. However, in view of their adverse side effects they have limited therapeutic value particularly when lead toxicity is sub-clinical. Of late, it has been suggested that the toxicity of heavy metals like lead / cadmium can be modulated by various nutritional factors. Literature evidence indicates that deficiencies of certain essential elements like iron, calcium, zinc, copper, magnesium and vitamins etc. may lead to increased body burden of certain non-essential toxic elements and the supplementation of the former may mitigate the toxic effects of the latter. Though there is enough literature in this respect from the West, similar information from the third world countries, where problems of poor nutrition and probable metal toxicities are likely to be prevalent, is scanty.

The research efforts are therefore, directed towards quantification of the impact of lead exposure on human health particularly from the environment. In addition there is an urgent need to develop safer compounds, which can be used in prevention/treatment of chronic lead toxicity.

2. STUDIES CONDUCTED AT NATIONAL INSTITUTE OF NUTRITION

The data from developing countries has established the extent of lead poisoning and identified its sources and how to prevent the same. However, limited information from India has been generated to study the impact of lead toxicity in various groups of population.

Lead toxicity is likely to become a major environmental health problem in India in the near future. Our earlier investigation in lead mining areas of Andhra Pradesh (South India) indicated the prevalence of neurological disease in cattle due to lead poisoning. The study also cautioned the impending danger which may arise due to consumption of milk of affected cattle or through consumption of crops raised in the affected areas by humans.

Most of the recent studies used biochemical makers such as decreased erythrocyte Delta Amino Luritine Acid Dehydratase (ALAD)s and increased urinary excretion of N-Acetyl β-D-Glucosaminidase (NAG) and $Beta_2$ Microglobulin (B2 M) along with the monitoring of lead levels in blood to assess the extent of lead toxicity in various group of subjects exposed to lead.

2.1. Occupational groups

The blood lead levels of employees working in various automobile garages were found to be double with a 4-7 fold elevated enzymuria as compared to the normal subjects with no history of anaemia, renal damage, hypertension, diabetes and occupational exposure. There was no significant difference in routine parameters such as hemoglobin (Hb) serum albumin and creatinine as compared to normal. Similarly in another occupational group (monocastor) who are constantly exposed to lead fumes during the preparation of lead press metals, the blood lead levels were again found to be twice the normal, coupled with a 40-60% inhibition in basal ALAD activity and a 4-8 fold elevated excretion of urinary enzyme NAG compared to normals.

2.2. Children

Three categories of children who were exposed to lead toxicity owing to their nature of occupation were screened for their blood lead levels. The mean blood lead levels were found to be higher in all these groups. It is alarming to note that the children working in “petrol bunks” (Gas stations) have very high blood levels (35 mg/dl). Seventeen percent of the 55 children engaged in bangle making industry had mean blood lead levels more than 30 µg/dl. About of children with vicarious eating habits (pica) 47% and 23% of those working in petrol bunks were suffering from retarded intellectual development as assessed by Raven’s Progressive Matrices.

The studies conducted in children and pregnant women belonging to different traffic congested areas have also indicated high blood lead levels. The studies in various groups highlight the fact that even mild chronic lead exposures, which are not significantly reflected in blood lead levels can lead to sub-clinical toxicity. A battery of simple biochemical tests carried out routinely in high risk groups would indicate the impending harmful effects.

Preliminary studies in children employed in the bangle making industry in Hyderabad indicated blood lead levels much above the safe limit of 10 µg/dl. Elevated blood lead levels in children coupled with under nutrition have the potential to further aggravate the adverse effect on their cognitive and neuro-psychological functions in children involved in bangle making in poor urban slums.

A pilot study in monocasters suggests that the administration of thiamin (50-100 mg) not only restored 30-50% of the basal ALAD activity and reversed the urinary NAG activity, but also reduced 25-30% blood lead levels in a span of 10 months. In vitro studies using NMR spectroscopy also indicate that thiamin can chelate lead. Experimental studies also showed that thiamin administration helps in preventing the accumulation of lead in tissues and in leaching out of stored lead gradually.

3. PROJECT PROPOSAL

3.1. Aims and Objectives

Since past two decades, global concern about the toxic effects of metals like Pb, Cd etc., particularly through environmental exposure, is steadily increasing due to its long term consequences on public health. Strict implementation of legislation on environmental aspects becomes difficult in a developing county like India, owing to its rapid industrialization. Prevalence of malnutrition further compounds the problem and complicates the evaluation of risks with heavy metals like lead on human health. Therefore, the present proposal is being undertaken with the following aims and objectives:

3.2. General

- To assess the blood lead levels and identify the extent of lead toxicity among the various groups of children and pregnant women.
- To assess nutrients intake and correlate it with the lead toxicity and its impact on function.
- To develop intervention strategies viz. nutrient supplementation and education for prevention of lead toxicity.

3.3. Specific

- To evaluate the use of non-invasive techniques to assess the sub-clinical lead toxicity of renal, haemopoietic and nervous systems.
- To assess and correlate the cognitive and neuropsychological changes in the children with poor nutritional status and having risk of lead toxicity.
- To determine the potential therapeutic effect of thiamine in subjects having risk of lead toxicity specially at sub-clinical level.
- To study the impact of change of leaded gasolene to unleaded gasolene on blood levels in children from various traffic, industrial zones, including urban, rural and remote areas.
- To study the impact of nutritional component in the population having risk of lead toxicity.

4. METHODOLOGY

The two major groups of population viz. Children (between 5-18 years), pregnant women from the high, moderate, mild risk areas of lead exposure will be selected randomly using appropriate statistical procedures. The clinical history of subjects, nutritional profile, psychological profile in addition to socioeconomic status will be assessed. The blood samples will be analyzed for the presence of heavy metals viz. Lead, cadmium, zinc, mercury, iron etc. The blood smears will be collected for morphological examination of Red Blood Cells (RBC).

5. STUDY DESIGN

5.1. Subjects

5.1.1. Children

The following group of children from various areas will be enrolled in the study with 50% between age group of 5-12 years and remaining 50% between 13-18 years. These children will be selected randomly from the available total number of children in the respective areas.

S.No.	Exposure risk*	Areas of operation	No. of Subjects
1	Heavy Traffic zone	Urban areas	100
2	Moderate zone	Urban	100
		Semi Urban	100
3	Mild traffic zone	Urban	100
		Semi urban	100
		Rural	100
4	Industrial/ Occupation zone	Battery	100
		Lead Manufacturing Unit	100
		Petrol bunks	100
		Any other potential	100
5	No risk zone	Remote rural	100
		Tribal	100
TOTAL CHILDREN TO BE SCREENED			1200

*The exposure levels will be assessed based on the secondary information on automobile traffic level, at peak time (recorded emission level), industrial belt etc. available from the Regional Transport Authority and Pollution Control Boards of respective areas.

5.1.2. Pregnant women

The following groups of pregnant women belonging to various traffic/ industrial areas coming for antenatal checkup/ delivery to different heath center's in urban and rural areas will be registered for the study. To ensure the appropriate representation from various areas of traffic/ industrial zone a selection criteria will be developed for enrollment in the study. In addition to blood lead levels cord blood samples will also be analysed.

S.No.	Hospital details	Exposure levels *	No. of Subjects
1	Govt. Hospital Urban areas	Heavy traffic	50
		Moderate traffic	50
		Mild traffic	50
2	E S I Hospitals	Battery industry	50
		Petrol bunks	50
		Any other potential	50
3	Dist. Hospital	Moderate traffic	50
		Mild traffic	50
4	Community Health Centres	Mild traffic	50
		No traffic	50
5	Primary Health Centres	Mandal	50
		Villages	50
6	Private Nursing Homes	Urban	50
		District	50
		Any other	50
TOTAL PREGNANT WOMEN SAMPLES			750

5.1.3. Clinical Examination / Nutritional and socioeconomic profile

- The information on socio-economic and demographic profiles of the house holds will be recorded in the standardized schedule developed by a National Nutrition Monitoring Bureau.
- Assessment of nutritional status will be recorded using weight and height for age (percentage of NCHS standards) indices by the trained investigator.
- Apart from routine clinical examinations of the study population, the specific signs and symptoms of heavy metal poisoning will be recorded.

5.1.4. Psychological examination

At least ten percent of the children in the age group of 6-18 years willing to participate in the study will be selected from various zones described above. They will be assessed for their psychological profile using the following tests:

- Malin's Performance Intelligence Scale for Indian Children
- PGI Memory test
- Bender-Gestalt test
- Tests of attention and concentration

Psychological tests will be administered on a blind basis i.e., without the tester having information about the children's family occupation to avoid testing and scoring bias. After completion of psychological testing the height and weight measurements and blood samples will be collected.

5.1.5. Haematological Procedures

Estimation of hemoglobin (Hb%) and peripheral smear examination are to be undertaken in all subjects. Presence of anemia as well as morphological changes in RBC if any, shall be looked into using Romauowsly stained smears (Leishman stain):

Hemoglobin levels will be estimated by routine laboratory procedures.

5.1.6. Determination of heavy metal concentration

Quantification of heavy metals in whole blood/plasma viz. lead, arsenic, cadmium, mercury, selenium, iron, fluoride, zinc will be done using (1) Graphite furnace atomic absorption spectroscopy (GFASS) (2) Anode stippling voltammeter (ASV) (for field monitoring of lead) (3) Inductive coupling plasma atomic emission spectroscopy (ICPIAES), Inductive coupling mass spectroscopy (ICP / MS) and other equipments.

The BARC laboratories, Hyderabad have agreed to be the collaborators in the study and will quantify heavy metal estimation using ICPIAES and ICP / MS.

5.1.7. Intervention strategies

- The therapeutic efficacy of thiamin on blood lead levels will be determined after supplementation @ of 50 mg per day per child having lead levels above 10 µg/dl for 6 months.
- Development of education materials. Suitable education materials viz. Brochures, pamphlets, audio/video progrmmes etc. will be developed to create awareness about the problem of lead toxicity. Popular lectures will be also be conducted to create awareness on the poisoning effects of heavy metals.

CHRONOGRAM

(WORK PLAN)

S.No	Details	MONTHS					
		0-6	7-12	12-18	18-24	24-30	30-36
1	Staff recruitment & procurement of equipment/ chemicals/ other related material	X					
2	Selection of areas/ subjects	X					
3	Data collection		X	X			
4	Clinical examination & Blood samples collection			X	X	X	
5	Analysis Laboratory/ standardisation			X	X	X	
6	Intervention programme			X	X		
7	Impact of intervention programme					X	
8	Report Writing					X	X

6. EXPECTED OUTCOME

The information on blood lead level pattern among children and pregnant women will be established and the risk of toxicity will be assessed. The impact of the association of other metal ions viz. iron, selenium, zinc, flouride etc. with lead toxicity will be determined. The therapeutic efficiency of nutrient /vitamin (thiamin) will also be obtained. Further, the results of the study will help the government, NGOs, and policy makers to develop appropriate strategies to combat the problem of lead toxicity in the country.

APPLICATION OF ISOTOPIC AND NUCLEAR TECHNIQUES IN THE STUDY OF IRON-LEAD INTERACTIONS IN CORD AND PLACENTA BLOOD IN SUBJECTS LIVING IN HEAVILY POLLUTED AREAS OF KENYA

ANSELIMO O. MAKOKHA[1], RUTH K. ONIANG'O[2], JOSEPH M. MUTUKU[1], DAVID MAINA[3] AND CHARLES MAINA[3]

[1]Department of Food Science, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya.
[2]Rural Outreach Programme, Nairobi.
[3]Institute of Nuclear Sciences, University of Nairobi, Nairobi, Kenya.

Abstract

The level of malnutrition is relatively high. Iron deficiency anaemia affects about half of the children and women. The same vulnerable population is also exposed to high levels of industrial and traffic pollution, particularly in the industrialized urban centres of Nairobi and Thika. Perhaps the severity of the malnutrition situation is worsened by the pollution situation, as the iron deficiency increases lead poisoning levels. However, there have been no studies to obtain data about the situation, particularly reflecting the interaction of iron deficiency and lead poisoning in the vulnerable population. In recent years nuclear and isotopic methods have been successfully applied to determine micro-nutrient stautus, and the level of pollutants such as lead. The main objective of this study is therefore to determine using nuclear techniques, the lead intakes resulting from industrial and traffic pollution, and the iron deficiency status among populations living near industries and highways in Nairobi and Thika in Kenya. It is anticipated that that the project will avail data about lead levels in the air, and in fresh foods around Nairobi and Thika. It will also avail data about the levels of lead in the placenta and cord blood of mothers living around Nairobi and Thika, and hence about the transmission of lead from mother to foetus. Similarly, the project will generate information about the status of iron deficiency of the target population, obtained from the iron determination in the placenta and cord blood of mothers living around Nairobi and Thika. The project also train and assist in capacity capacity building in the use of nuclear/isotopic methods in nutrient and pollutant analysis, in Kenya.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

Kenya is a country of about 30 million people, located in East Africa. As in many developing countries, the level of malnutrition in the country is relatively high. Stunting in children under five years ranges between 20 and 40% [1]. A recent survey reported that the level of moderate to severe iron deficiency anaemia was 19.2% and 54.2% respectively [2]. The prevalence of this deficiency was much higher in children below 30 months, where the incidence of the prevalence was 76%. Half of the mothers were anaemic, and pregnancy increased the risk of being anaemic.

Though about 80% of the population live in the rural areas, there has been a sustained rural to urban migration in search of employment, as most of the manufacturing industries are based in the urban centres. The majority of these migrants do not secure jobs which can guarantee a decent living, and they end up living in slum areas, where they are exposed to unsanitary living conditions.

Most of the industries in the country are located in two urban centers, Nairobi and Thika. It is in Nairobi, which is also the capital city of the country that most of the slum dwellers are to be found. There is heavy motor vehicle traffic between the two urban centers, which are about 50 kilometers apart. Regulatory measures on emissions from these vehicles are not strictly enforced. Further, lead levels in motor vehicle fuel has not been of concern. It is therefore

probable that there is considerable lead pollution around these urban centers. This pollution is likely to be worse along the highway connecting the two urban centers.

The severity of the malnutrition situation among the urban poor in Kenya is therefore likely to be exacerbated by environmental pollution. It has been reported that in such conditions, environmental pollutants such as lead have a debilitating effect on already nutritionally compromised individuals [3]. Individuals, particularly children, with iron deficiency anaemia may absorb more lead than those who are not iron deficient [4]. A combination of iron deficiency such as that observed in Kenya, and lead toxicity can therefore have a devastating effect on them. The inter-element interactions between lead and iron are therefore among the nutrient-toxicant interactions that have become of great concern. Of similar concern is placental transmission of nutrients and toxicants from the mother to the foetus [5].

Nuclear and isotopic techniques have proved to have an important role in identification and monitoring of nutrition and health issues. For instance, stable isotopes provide the only direct way to measure iron uptake and bio-availability [3]. Analysis of foods by neutron activation analysis is very effective because of the exceptional sensitivity, and the possibility of simultaneous determination of several trace elements. Nuclear and related analytical techniques are applied in measuring pollutants in air [6]. These isotopic and nuclear techniques have been extensively used in industrialized countries in nutrition research. However, they have only begun to be applied in developing countries. In some countries such as Kenya, where there is high prevalency of micronutrient (iron) deficiency and air pollution, such methods will be very useful in determining the micro-nutrient status and pollution levels.

The overall objective of this study is therefore to determine, using isotopic and/or nuclear techniques, the lead intakes resulting from industrial and traffic pollution, and the iron deficiency status among populations living near industries and highways in Nairobi and Thika in Kenya.

The specific objectives are to:

- To determine the levels of lead in the air and some fresh foods sold around Nairobi and Thika.
- To compare the use of chemical analysis with that of isotopic and/or nuclear techniques in measuring iron and lead in human beings.
- To determine the iron deficiency status of the populations exposed to higher than normal levels of lead around Nairobi and Thika in Kenya.
- To evaluate the use of placenta and cord blood as indicators of the status of lead poisoning and iron status, in populations living around Nairobi and Thika.
- To evaluate the rate of mother to child transmission of lead poisoning.
- To train and build capacity in the use of isotopic and/or nuclear techniques in the measurement of lead and iron in human beings in Kenya.

The major activities which will be undertaken to achieve the objectives are:

- Desk review, particularly on industrial and traffic pollutants, including government policy issues related to pollution.
- Determination of lead levels in fresh foods (fruits and vegetables), particularly those sold near the road.
- Determination of lead and iron in placenta blood.
- Analysis of the relationship between serum lead and iron status from subjects living in the high pollution environment of Thika and Nairobi.

1.1. Institution and collaborations

The work will be based in the Department of Food Science and Post-harvest Technology, Jomo Kenyatta University of Agriculture and Technology. The Department has some relevant facilities such as the atomic absorption spectrophotometer for the analysis of lead in food samples. No prior work in nuclear and isotopic techniques has been done in the Department.

The project will work in collaboration with the Institute of Nuclear Science at the University of Nairobi. Among the relevant equipment at the institute are:

- X-ray tube based X-ray fluorescence (XRF)
- Total reflection X-ray fluorescence (TXRF)
- Radioisotope based XRF

2. METHODS

Materials for analysis: Food samples, particularly fresh fruits and vegetables grown or sold near the Thika Nairobi highway, will be purchased. Placenta and cord blood will be obtained from Thika District Hospital in Thika Town, and from Pumwani Maternity Hospital in Nairobi. This will be transported and kept at 4°C before analysis.

Determination of lead and iron: Determination of lead and iron in the food samples will be done by both Atomic Absorption Spectrophotometre (7) and by XRF (8). Determination of lead and iron in the cord and placenta blood will be determined by XRF (8).

3. EXPECTED OUTPUT (RESULTS)

- Availability of information on lead levels in the air, and in fresh foods around Nairobi and Thika.
- Availability of data on the levels of lead in the placenta and cord blood of mothers living around Nairobi and Thika.
- Availability of the status of iron deficiency obtained from the placenta and cord blood of mothers living around Nairobi and Thika.
- Training and capacity building in the use of nuclear/isotopic methods in nutrient and pollutant analysis.

4. PLANS FOR FUTURE WORK

Future work will involve:

- Determination of the effect of pollution on other micro-nutrients in Kenya, particularly zinc.
- Determination of the pollution levels in other parts of the country.
- Training of more people in the use of nuclear/isotopic methods in nutrition work.

REFERENCES

[1] Central Bureau of Statistics (CBS). Kenya demographic and health surveys. Office of the Vice President and Ministry of Planning and National Development, CBS, Nairobi, Kenya (1996).

[2] MWANIKI, D.L. et al. Anaemia and status of iron, vitamin A and Zinc in Kenya, The 1999 National survey. Executive summary, overview of findings, conclusions and recommendations, Nairobi, Kenya. (2001)

[3] IYENGAR, V. nuclear and isotopic techniques for addressing nutritional problems, with special reference to current applications in developing countries. Food and Nutrition Bulletin **23** 1 (2002) 3-10.

[4] GOYER, R.A. Toxic and essential metal interactions. Annual Review of Nutrition **17** (1997) 37-50

[5] GOYER, R.A. Transplancental transport of lead. Environment and Health Perspective **89** (1990) 101 – 105

[6] IAEA. Harmonization of health related environmental measurements using nuclear and isotopic techniques. Vienna, International Atomic energy Agency, 1996.

[7] IAEA. Sampling, storage and sample preparation procedures for X-ray fluorescence analysis of environmental materials. International Atomic Energy Agency, Vienna (1997).

APPLICATION OF ISOTOPIC AND NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION-POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF KOREAN PEDIATRIC POPULATIONS

SEUNG YEON CHO, PH.D

Department of Environmental Engineering, College of Health Science, Yonsei University, Korea

Abstract

Isotopic and nuclear techniques such as Inductively Coupled Plasma-Mass Spectrometry(ICP-MS), Neutron Activation Analysis(NAA) and Biomedical Accelerator Mass Spectometry(BAMS) will be used to understand and to evaluate how environmental pollution affects the nutritional status of pediatric populations already exposed to marginal malnutrition. Results of this study will contribute to better knowledge of the deleterious effects of nutrition-pollution interactions and to develop remedial strategies to alleviate such ill effects.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

We have used NAA more than 10 years for human health such as environmental monitoring and human nutrition. Air pollution study, reference Korean man study with total diet, food and human tissue analysis have been done in my group. Many researches related with hair analysis were performed already for forensic science and human health. We now want to choose the appropriate pediatric Korean populations, nutritional determinants (Ca, Cu, Fe, K, Mg, Na. P, Se, Zn, etc) for investigation and anticipated pollution (Al, As, Ba, Cd, Cr, Hg, Ni, Pb, etc) influencing the nutritional conditions and the sources of such pollutions. We will use specimens obtainable by non-invasive(hair) methods as indicators of interactions between toxic trace elements and nutritional status in human subjects.

Korea Atomic Energy Research Institute for Neutron Activation Analysis and Seoul National University and NuEYES Inc. for Biomedical Accelerator will cooperate with our research group for the success of the CRP.

- Have Used ICP-MS and NAA for Hair Mineral Analysis for More Than 10 Years
- Toxic: Al, As, Ba, Cd, Cr, Hg, Ni, Pb
- Nutrients: Ca, Cu, Fe, K, Mg, Na, P, Se, Zn
- Target Group: Children under Age of 12
- 100 hair samples from AD/HD children
- 100 hair samples from normal children
- Try to Use Biomedical AMS

2. METHODS

2.1. Neutron Activation Analysis

- Available from Hanaro Research Reactor Center in Korea Atomic Energy Research Institute

2.2. Irradiation Facilities at HANARO Research Reactor

- 30MW Thermal Power
- Automatic Pneumatic Transfer System
- *k*0 method is available
- PGAA facility is also available from 2002
- Neutron Flux at Vertical Irradiation Thimbles

Irradiation Thimble	Neutron Flux, Φ=n/cm2s	
	Fast(>0.82 MeV)	Thermal(<0.025 eV)
NAA 1(CdT)	2.4×1010	3.9×1013
NAA 2(AT)	2.5×1011	9.4×1013
NAA 3(MT)	1.3×1012	1.6×1014

2.3. Activity Counting System

- HP Ge Semiconductor Detector, EG & G ORTEC GMX Series(3keV-10MeV) and GEM Series(50keV-10MeV), 25% Relative Efficiency, 1.9 keV Resolution at 1332 keV of 60Co Peak to Compton Ratio; 45:1
- Low Background Pb Shield for Ge Detector, EG & G ORTEC 4π -10cm thick Low Background Virgin Lead, Graded Cu & Cd Liner 28x41cm Cavity Pb ShieldingBox (35x40cm, 40x45cm Cavity, Cu & Cd Liner)
- Multichannel Analyser, EG & C ORTEC 918A MCB; 8K Channel ADC(10μ s), 8K Data Memory, Counting loss Correction 919A MCB; 16K Channel ADC(7μ s), 64K Data Memory, Digital Stabilizer
- Private and Commercial Application Software for NAA

2.4. ICP-MS

- (Model: PQ Excel by VG, UK): available from HANA Laboratory in EN Technology

2.5. AMS (Accelerator Mass Spectrometry)

- Available from Seoul National University and NuEYES Inc.

3. SHORT HISTORY OF NEUTRON ACTIVATION ANALYSIS IN KOREA

3.1. TRIGA MK-II Research Reactor (1962)

- 100 KW thermal power at first, upgraded to 250 KW in (1969)
- Quantitative analysis of Au
- Elemental concentrations in Monazite
- Radiochemical analysis of mineral and precious metals
- Quantitative analysis of Pd, Pt, Rh in Lead
- Quantitative analysis of toxic elements in biological samples.
- Elemental concentrations in sea water using Chelate resin
- Quantitative analysis of toxic elements in fishes and river water
- Elemental concentrations in Lunar Fines (using SRM from USGS)
- Use NaI(Tl) detector
- Not simultaneous multielemental analysis
- Chemical separation of each element by ion exchange and solvent extraction method before and after the irradiation

3.2. TRIGA MK-III Research Reactor (1972)

- 2 MW thermal power
- Nuclear power plant related material analysis
- U, Th (by Delayed Neutron Counting Method)
- B (by Prompt Gamma Analysis)
- Rare earth elements in UO_2
- Elements in Zircaloy
- Cu and trace elements in high purity Aluminum
- Diverse applications in many fields such as archaeology, rocks, soil, forensic science, medical samples
- Trace elements analysis in airborne particulates, soil, and coal flyash for environmental research
- Basic researches by NAA in Universities
- Cooperative Researches with IAEA
- Improved analytical sensitivity
- HpGe, 8K MCA, PC's since 1980's
- Simultaneous multielemental analysis

3.3. HANARO (Multi-purpose Research Reactor: 1995)

- 30 MW thermal power
- National utilization program mainly for environmental and health related studies is developing

3.4. Accelerator Mass Spectrometry(AMS)

- Developed about 20 years ago
- Appled First for Radiocarbon - Geochemical, Climatological, Archaeological Research
- Few MeV tandem Van de Graaff Accelerator
- Around 40 Accelerators are operating

3.5. Sample Preparation

Pretreatment
(Physically and Chemically)
↓
Combustion to CO_2 gas
↓

Reduction of CO_2
(Graphitization by Catalytic Reduction))

Recently Applied for Biomedicine and Environmental Science

Detect ^{14}C, ^{3}H, ^{26}Al, ^{41}Ca up to 10^{-21} - 10^{-18} mole/mg

4. BAMS

- Great benefit to the pharmaceutical and biotechnology industries
- As well as other life science areas

4.1. Biomedical Applications of AMS

- Pharmaceutical Research and Development
- Cancer Research, Endocrinology, Pharmacology
- Apply for the Fields Using Radiolabel
- Pharmacokinetic Studies
- Mass Balance Metabolism Studies
- Determine Quantitative Metabolic Profiles
- Mechanistic Studies: Elucidate Chemical and Enzymatic Pathways

4.2. ^{14}C

4.2.1. Important for Drug Metabolism Study

- Possible to Study Phase I adsorption, distribution, metabolism, excretion (ADME)
- Use ^{14}C labelled drug below 10 nCi(37 Bq) or 0.9 μ Sv(Normal People Exposure Rate Every Day → 10 μ Sv)
- Below the Limit Value for Radioisotope Usage and Waste Control
- Radioactive study can be converted to a non-radioactive study
- Possible for ‘First into Man’ Study

4.2.2. Applying for Clinical medicine

- Demonstrate metabolic abnormalities including malabsorption, increased turnover and disturbed excretion, e.g. fat reabsorption using ^{14}C-Triolein
- Liver function study
- Demonstrate abnormal activity of gut bacteria activity using ^{14}C-Urea
- Can be measured in expired air or in biofluid samples
- Allows the fate of diagnostic radiopharmaceuticals to be determined from hours to months or years after the administration of the radiolabel

4.2.3. Cancer Research

- Metabolism and macromolecular adduction of food contaminants (heterocyclic aromatic amines PhiP, MelQx and also the hepatocarcinogen Aflatoxin B1)
- Biochemical mechanisms of carcinogens in the body
- Measurement of exposure and associated risk to specific populations
- Treatment of cancer using drug therapies
- Developing new drug therapies and assessment of the efficacy and safety of cancer therapies currently in use

4.2.4. Risk Assessment

Two Extrapolation Methods are Currently Used with Biomarker Study Because of Detection Limit

- From a high dose to a much lower dose (realistic exposure at which populations are thought to be exposed)
- A species extrapolation from the rodent (to whom detectable dosed are administered) to humans (to whom high doses would be carcinogenic)

4.2.5. Many Hypothesis are Established

- Cacinogenic undergoes the same biochemical processes at low and high doses and dispositional factors do not effect the metabolic activation and subsequent adduction of the carcinogen
- Different species will metabolize the chemical in the carcinogen

4.2.6. Use AMS for Risk Assessment

The sensitivity of the technique allows realistic human exposure doses to be applied to both rodents and humans and facilitates detection of adduct levels at these doses

4.3. Dose-dependent binding of trichloroehtylene to hepatic DNA and protein at low doses in mice

4.3.1. Chemico-Biological Interactions, 1997

TCE is a widely used industrial chemicals and a low level contaminant of surface and ground water in industrialized areas. It is weakly mutagenic in several test systems and carcinogenic in rodents. However, the mechanism for its carcinogenicity is not known. We investigated the binding of 14C-TCE to liver DNA and proteins in male B6C3F1 mice at doses more relevant to humans than used previously

5. RESULTS

We have done 50 human hair sampling to find out the relationship of trace elemental distribution between normal and AD/HD(Attention Deficiency/Hyperactivity Disorder) children under age of 12.

6. PLANS FOR FUTURE WORK

- Sampling 100 normal and AD/HD children's hair respectively
- Use NAA and ICP/MS to determine toxic and nutritional trace elements
- Sampling 20 normal pregnant women's hair with their infants' hair and analyze
- Sampling 20 smoking pregnant women's hair with their infants' hair analyze
- Try to Use BAMS for Nutrition-Pollution Study

IMPACT OF WATER POLLUTION BY Hg, Cd AND Pb ON NUTRITIONAL STATUS OF CHILDREN IN THE NORTH WEST OF MOROCCO.

BENAISSA ATTRASSI[1], HASSAN AGUENAOU[1], YAHIA SOBHI[2], MOUSSA BOUNAKHLA[3] AND KHALID LALAOUI[3]

[1]Ibn Tofail University, School of Sciences, Biology Department, Kenitra- Morocco.
[2]Ministry of environment- National laboratory of environment, Rabat- Morocco.
[3]CNESTEN (National Centre of Sciences Energy and Nuclear Techniques), Rabat-Morocco.

Abstract

Heavy metals are natural elements that exist in air, water, soils and sediments but at trace level. However, their level might increase due to various domestic and industrial activities. Man can be exposed directly (e. g. drinking polluted water or eating contaminated vegetables and fruits) or indirectly to these heavy metals (e. g. exposure to cigarette smoke (Pb), tobacco or painting areas (Cd), old dwellings (Pb), dental amalgam (Cd, Hg)).
The north west region of Morocco, represent a zone where meet all sewages charged of heavy metals coming from different industries and human activities as well.
Regarding the nutritional situation of the Ghrab population, many national and regional surveys indicated that stunting (24% children), anaemia (38% children and women), and vitamin A deficiency (40,9 % marginal deficiency in children) are the most known nutritional problem.
The main causes of these nutritional problems are poor nutritional intake as well as a poor socio-economic situation. However, no study exists on the relationship between these nutritional disorders (or other not yet discovered) and heavy metals contaminants. This relationship deserves to be solved especially in this region. Indeed several pollution indicators such as aquatic organisms have always recorded a strong level of contamination by heavy metals going from 1 to 6 µg/g for Pb, 0.4 to 2 µg/g for Cd and 0.15 to 1µg/g for Hg [1].
The health concequence of heavy metals contamination of water in this region are not yet well understood.

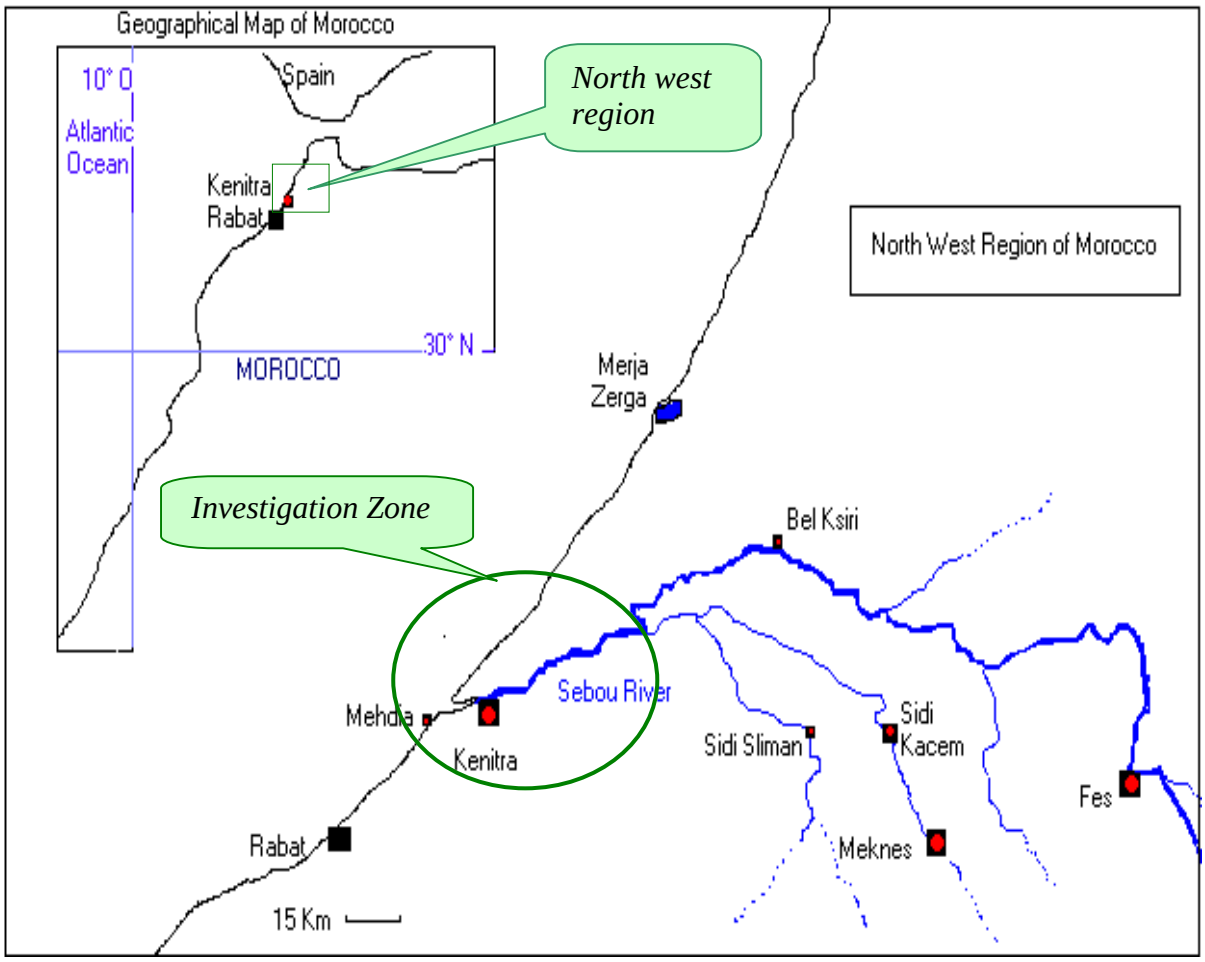
1. SCIENTIFIC BACKGROUND OF THE PROJECT AND SCOPE OF THE PROJECT

Water resources are the principal socio-economic potentialities for the regional development in the north west of Morocco, called the Gharb region.

The basin of sebou covers 40 000 km² and the yearly precipitations are on average 500 mm. In the beginning of the 20th century, the region of the Gharb was populated weakly (130 000 inhabitants in 1900) because of the frequent inundations and several epidemics of aquatic origin [2]. Since this date, the demographic features changed progressively following the reduction of the infantile death rate, development of agricultural sector and reinforcement of socio-economics infrastructures. Thus, in 1994 inhabitant's number reached 1 625 082. It will pass to 2 100 000 in 2005 [3].

The regional economy is based mainly on the agricultural activity that use 53.3% of actives people; this rate is more higher that the national average (40%). The other sectors are especially trade (11.3%) and industry (9.9%) [2].

FIG 1: Geographical localization of the investigation zone



Principal cities polluters of Sebou River are indicated by ■

The Sebou River, the ground water and the lagoon system are the main water supplies in this region. However, during the last 20 years, the population increases in urban and in sub-urban areas, anthropological and industrial activities and weak sanitary conditions have conducted to an extensive water environmental pollution. The waste water in this region is removed through one unique drainage system used for waste water from domestic activity, industrials, hospitals, and rainwater.

This situation leads in general to an increase of bacterial and chemical contaminations of populations which have a direct contact (at risk) with this waste water such as fish consumers, farmers who use this water for vegetables and fruits irrigation, and finally the general population who consume these fruits and vegetables, which are hygienically doubtful.

Moreover, the region of the Gharb receives at the same time waste water from other big Moroccan cities (Fez, Meknes, Sidi Kacem). This water is also carried along the Sebou River (600 km length) (Fig. 1). All these effluents are charged with heavy metals, resulting from many industrial activities such as iron and steel industry, chemicals industries tannery, textile, petrochemistry, paper- making industry. Pb, Cd and Hg are the main pollutants of this water.

The health consequence of heavy metal contamination in this region are not well understood. Therefore, research on health and nutritional profile of the population, living in the polluted environment is needed; there is an urgent need for action since the use of waste water for agriculture irrigation is increasing due of the frequent droughts during the last years.

Regarding the nutritional situation of the Ghrab population, many national and regional surveys indicated that stunting (24% children), anaemia (38% children and women), and vitamin A deficiency (40,9 % marginal deficiency in children) are the most known nutritional problem. The main causes of these nutritional problems are poor nutritional intake as well as a poor socio-economic situation. However, no study exists on the relationship between these nutritional deficiencies (or other not yet discovered) and heavy metals contaminants.

This project is related to health and water quality within a global vision of sustainable development in this region in particular and in Morocco in general. The study aims at the determination of the impact of heavy metal pollution (Cd, Pb, and Hg) on the nutritional status, and on the micronutrients (Fe, Zn) level in children. Finally within this project we also want to understand the relationship between the micronutrients balance and the level of heavy metals.

The proposed work in this project is a multidisciplinary effort leading to address the issues of environment pollution and its impact on human health.

We are delighted currently with the interdepartmental collaboration in the region which facilitates the access to data, suppress the ethical gates and permits to collaborate easily with the population especially when it concerns children.

As a matter of fact, these research works will be done in collaboration with the ministry of health (delegation of health at Kenitra), the ministry of the environment (department of research and the national laboratory of the environment), the ministry of national education and National Centre of Sciences Energy and Nuclear Techniques (CNESTEN – Morocco).

2. METHODS:

2.1. Sampling

2.1.1. Human Population

A sample of school-age children (3-11 years) will be selected randomly from urban and rural areas downstream the Sebou river. The method of sampling chosen is by clusters (4 districts, 2 schools and 2 classes by school (25 children by classes and a total of 400 children). The proportion of 50% of girls will be respected.

A questionnaire will be made for collecting data on socio-economic status of each children as well as nutritional habits.

Samplings of blood, urine will be collected in the dry tubes or with anticoagulant. And stored at –85 C for needed analysis

2.1.2. Environment

The samples of vegetables and fruits (lettuces, potatoes, oranges, bananas, tomatoes) from the areas irrigated by the water of Sebou River or by the non treated wastewaters will be stocked in the appropriated conditions in order to measure out their contents in heavy metals.

Samples of drinkable water from the Sebou River and from the rural community of Mnasra near of Kenitra city will also be taken, in order to determine their content of heavy metals.

2.2. Methods of analysis

2.2.1. Heavy metal

The heavy metals (Pb, Cd, Hg) and essentials metals (Fe and Zn) will be measured out by the classic methods atomic absorption spectrometry (AAS) [4-9] after mineralization according to the method of Hoenig and Vanderstappen [10]. Atomization were conduced using graphite oven with an automatic system and auto sampler injection to measure trace heavy metals and hydride System (cold atomizer) to measure Hg. We will also proceed to dosage of trace heavy metals by neutron activation analysis (NAA) [11] using neutrons generator (14 MeV) and by X-ray fluorescence [12-13] using total reflection method. These analyses will be done in collaboration with Ministry of Environment and National Centre of Sciences Energy and Nuclear Techniques (CNESTEN – Morocco).

2.2.2. Analyses of blood

Iron status of children will be assessed by measuring haemoglobin, serum iron, hematocrite and serum ferritin. Heavy metals as well as Zn in blood will be determined by AAS.

2.2.3. Nutritional statute of children

In addition to the questionnaire up mentioned, anthropometrique measures of children will be done to assess stunting level as well as growth problems.

The interrogation of children parents presents a big importance. It permits to collect precious information on the history of the children health. It also informs on signs clinics being able to be in relation with problems of the environment in the region.

Interaction nutrition pollution study go through the table of the food frequency that permits to orient the investigation on the food habits basis.

2.3. Data collection and analysis

Results of heavy metals concentration in the population investigated and in environment will be analyzed by comparison of variance and covariance. The significance of effects attributable to transfer of metals to the population will be determined using Student test. Analysis of results using statistical software AFC and ACP [14-15] will be permitted to illustrate nutrition pollution interaction.

3. RESULTS

To our knowledge it is for the first time that this kind of work will be achieved in Morocco. However, previous works have already showed that the level of aquatic contamination by the heavy metals in the region have increased considerably to reach concentrations currently in the aquatic organisms (mussels) in the order of 1 to 6 µg/g for Pb, 0.4 to 2 µg/g for Cd and 0.15 to 1µg/g for Hg [1].

The main sources of pollutions are rivers that are transformed currently in a non treated sewages [16]. The main results on the state of the environment in the Gharb region are brought back in the table I.

TABLE I: CONCENTRATION OF METALS TO THE MOUTH OF THE SEBOU RIVER AND IN THE MUSSELS OF THE ATLANTIC COAST AT THE DOWNSTREAM OF THE RIVER. RESULTS REPRESENT A YEARLY AVERAGE [17].

Metallic element	**Waters of river In mg/l**	**Mussels of the coastline of Kenitra in µg/g**
Copper	0.01	10
Zinc	1.30	215
Iron	12.00	800
Cadmium	0.001	1.2
Lead	0.015	4.0
Chromium	0.012	8.6
Mercury	-	0.87

This project will complete these data and in addition will find out the relationship between the pollutant and the level of nutrient

4. PLANS FOR FUTURE WORK

- Literature review to identify all pollution sources of water mainly of sebou river and lagoon system.
- Setting up the sampling protocol of children, water and foods.
- Setting up the analytical methods of heavy metals by AAS, AAN and X ray fluorescence techniques.
- Preparation of ethical clearance and identification of other potential national collaborators (Ministry of Health and Ministry of national education).
- Starting the field work:
- Identification of the population to be studied.
- Compilation of data: socio-economic, environmental, nutritional and of health relating to the population target
- Collection of water and food samples in the nearest environment.
- Laboratory analyses: water, food, blood and urine analyses by isotopic methods and other ordinary laboratory techniques.
- Analysis of results using statistical capabilities of our university (Mathematics departments)

REFERENCES

[1] SOBHI, Y., Etude des tendances des polluants chimiques métalliques: Hg, Cd, Pb, Cr, Ni, Ti, Zn, Cu dans les organismes marins des côtes marocaines. Aspect environnementaux et expérimentaux. Ph D. Biologie. Université Sidi Mohamed Ben Abdellah. Fes Maroc (1997) 200pp.

[2] Monographie régionale. Caractéristiques démographiques et socioéconomiques de la région du Gharb. Maroc (2000) 123 pp

[3] Direction des statistiques. Annuaire statistique du Maroc (1994). 500 pp

[4] MERANGER. J.C., SUBRAMANIAN, K.S., LANGFORD, C.H. et UMBRASAS, R. Use of an one site integrated pump sampler for estimation of total daily intake of some metals from tap water. Int. J. Environ. Anal. Chem., **17** (1984)

[5] UNEP/AIEA. Sampling of selected marine organisms and sample preparation for trace metal analysis. Reference Methods for Marine Pollution Studies. N° 7, Rev. 2 UNEP (1984)

[6] UNEP/AIEA. Determination of total mercury in selected marine organisms by cold vapor atomic absorption spectrometry. Reference Methods for Marine Pollution Studies. N° 8, Rev. 1 UNEP (1984)

[7] RYU, J.E., ZIEGLER, E., NELSON, S. et FORMON, S. Dietary intake of lead and blood lead concentration in early infancy. Am. J. Dis. Child., **137** (1983)

[8] UNEP/FAO, Biogeochemical studies of selected pollutants in open water of the Mediterranean (MED POL VIII). Addendum, Greek Oceanographic Cruise (1980).

[9] US/NAS, Geochemistry and the environment. The relation of selected trace elements to health and disease. Washington DC, US National academy of Sciences. (1974) 113pp.

[10] Hoenig M., Vanderstappen R. Dosage de Cd, Cu, Pb, Zn et Mn dans les végétaux par spectrométrie d'absorption atomique en flamme. Effets de la minéralisation. Analusis, **6** 7 (1978) 312-316.

[11] MINAI Y., TSUNOGAI U., TAKAHASHI H., ISHIBASHI J., MATSUMOTO R. AND TOMINAGO T. Neutron activation analysis of deep sea sediments from the regions near Japan, J. Rad. Analy. And Nucl. Chem., **261** (197) 265-277.

[12] GARG M.L., MEHTA D., KUMAR S., MANGAL P.C.AND TREHAN P.N. X ray spectrometry **14** (1985) 165.

[13] BAHLOULI M., SABIR M.A., ELMAGHRAOUI M. ET BOUNAKHLA M. Analyses multi élémentaires des sédiments marins marocains par activation neutronique et par fluorescence X. Rencontre Franco-Marocaine de Physique Nucléaire, El Jadida Maroc 10-13 mars (1999) 139-142.

[14] SAKAL, RR. AND ROHLF, F.J. Biometry, end ed. W.H. Freeman & Co. San Fransisco. (1998) 776 p.

[15] CALVELO, S. AND BACCALA N. Analytical and statistical analysis of elemental composition of lichen. Journal of Radioanalytical and Nuclear Chemistry. 222 (1997) 99-104.

[16] CHRAIBI, N. Etude écotoxicologique de deux espèces de moules: Perna perna et Mytilus galloprovincialis dans leurs sites naturels Atlantique et Méditerranéen (Maroc). Ph D. Biologie. Université Mohamed V. rabat Maroc (2001) 190 pp.

[17] SOBHI, Y., Transferts des polluants des villes intérieurs vers l'estuaire de Kenitra via oued sebou. Communication personnelle, 2002.

DETERMINING OF THE LEVEL OF LEAD AND CADMIUM CONTAMINATION IN PREGNANT WOMAN LIVING IN AN EXPOSED MINING AREA OF PERÚ

GUILLERMO LÓPEZ DE ROMAÑA[1], KLAUS SHÜMANN[2], PATRICIA BEDREGAL[3], ROSE MARY BENDEZÚ[4], MARÍA INÉS NÚÑEZ[5], RAINER GROSS[6]

[1]Universidad Nacional Agraria La Molina, Av. La Universidad s/n La Molina, Lima, Perú, PO Box 12-108 Lima E-mail: gromana@lamolina.edu.pe
[2]LM-University in Munich, Germany
[3]Instituto Peruano de Energía Nuclear, Lima Perú
[4]Universidad Nacional Agraria La Molina. Av. La Universidad s/n La Molina, Lima, Perú
[5]Universidad Nacional Agraria La Molina, Av. La Universidad s/n La Molina, Lima, Perú
[6]Universidad Nacional Agraria La Molina, Av. La Universidad s/n La Molina, Lima, Perú

Abstract

It is the objective of this study to determine in the first year the level of acute pollution and their nutritional status in pregnant women living in a lead and cadmium exposed mining area of Peru. The complete project will focus on delivering women and their infants who are one of the most vulnerable groups of the life cycle. It is of particular interest to investigate the impact of pollution not only in the mother but also the possible transfer to the infant.

1. SCIENTIFIC BACKGROUND

Peru is a mining country and consequently, there is concern about a high risk of heavy metal contamination of food and the environment on human beings. Therefore, the Ministry of Health (DIGESA) has created a new program to investigate possible sources of heavy metal contamination in different regions of the country. Initial results showed that a high percentage of children in Lima, where a large part of the mineral production of the Andean region is openly storage for export, have elevated concentration of lead in blood, which is known to correlate with impaired cognitive and social development.

The study is being conducted in La Oroya a 33,000 inhabitants city located at 4,000 meters above sea level and the largest mining center of the area. Previous evaluations showed high levels of pollution; air lead concentration 27.5 ug/m^3 (accepted 1.5 ug/m^3), river water lead concentration 2.1 mg/L (accepted 0.03 mg/L). A resent evaluation carried out in school age children showed high levels of lead concentration in blood. Ninety nine percent (99%) of children had lead concentration levels above 10 ug/dl, 86% above 20 ug/ml and 19% above 44 ug/ml

Until now nutritional status and environmental pollution were mostly treated as separate issues. However, there is a tight interaction between both areas, which can be defined as one system consisting of the elements: Agent (air, water, soil), pollutant, host (compartment, age, physiological stage), and environment (geographical, socio-cultural). The interaction between the elements of the system pollution-nutrition is regulated by a complex mechanism, which is only understood in fractions. This project will help to understand the wider impact of pollution on human and economic development and to develop measures to reduce and alleviate the health risks.

Several studies have showed a high association between the lead status in the human organism and the environmental exposure. Especially children are at risk due to their high lead intake via dirt (1) in contaminated environments. There is an increased absorption for lead (**2**) and cadmium during growth (3,**4**). Besides, children are more sensitive to toxic lead effects (**5**). Part of the lead load in infants is transferred from exposed mothers in utero (**6**) or via breast milk (**7**).

High lead intake from food and environment has been shown in mining and smelter areas, leading to elevated blood lead concentrations (**8, 9, 10**). Such exposure may result in impaired neuronal conductance, impaired Vit. D metabolism and reduced haemoglobin synthesis (**5**). Even low lead levels in whole blood impair children´s mental development (**11, 12**). Reduction of lead exposure from industrial emission and leaded petrol (**13**) reduced lead in milk teeth from early school age children (**14**). Cadmium, in contrast, is taken up mainly via crops grown on contaminated soil or via meat or milk products from animals fed on contaminated crops (**15**). Cadmium intake is highest during growth (**3, 4**), while cadmium-related damage to bone strength is mostly found in postmenopausal women with increased urinary cadmium excretion (**16**). Thus, to collect data on lead and cadmium exposure throughout the life cycle in mining country like Peru, to increase our understanding of the level of contamination in pregnant women living in a lead and cadmium exposed mining area seems highly desirable.

2. METHODS

The work plan for the first year of study is as follows:

- A general hospital in La Oroya was selected as the study facility.
- Four OB-GYN teams (doctors, nurses) were selected and are being trained.
- Forty delivering women will be enrolled after obtaining of a signed consent. A pre-coded form will be completed to assess socioeconomic, nutrition, health and environmental characteristics of families.
- Forty blood and placenta tissue samples from mothers, and umbilical cord blood of babies, will be obtained using the appropriate materials and techniques for lead and cadmium concentration analysis
- Forty samples of mothers´ finger nails, hair, urine, colostrum and breast milk will be obtained for further analysis during the second year.
- All samples will be weighed and frozen immediately after collection and send, in appropriate containers with dry ice, to the laboratories of the Nuclear Energy Institute of Perú in Lima for analysis.
- Lead levels will be determined by anodic stripping voltammetry (SV), total reflection X Ray fluorescence and by radiochemical neutron activation analysis.
- Cadmiun concentrations will be determine by SV and radiochemical neutron activation analysis
- Results will evaluated statistically

In the second year, nails, hair, urine, colostrum and breast milk samples will be analyzed to evaluate the possibility of chronic exposure.

REFERENCES

[1] KAISER U, ROSSKAMP E (1992) Gesamtbelastung das kindlichen Organismus mit Schwermetallen. Bundesgasundhbl. 35: 197 – 203

[2] ZIEGLER EE; EDWARDS BB, JENSEN RL, MAHAFFEY KR, FORMON SJ, (1978) Absorption and determination of lead by infants. Ped. Res. 12: 29 – 34

[3] HENKE G, SACHS AW, BOHN G (1970) Cadmiumbestimmung in Leber und Nieren von Kindern und Jugendlichen durch Neutronenaktivierungsanalyse. Arch. Toxikol. 26: 8 – 16

[4] SCHÜMANN K, FRIEBEL P, SCHMOLKE G, ELSENHANS B (1996) State of iron repletion and cadmium tissue accumulation as a function of growth in young rats after oral cadmium exposure. Arch. Environ. Contam. Toxicol. 31: 483 – 487

[5] CHAO J, KIKANO GE (1993) Lead poisoning in children. Am. Fam. Phys. 47: 113 – 120

[6] BELLINGER D, LEVITON A, WATERNAUX C, NEEDLEMAN H, RABINOWITZ M (1987) Longitudinal analysis of prenatal and postnatal lead exposure and early cognitive development. N. Engl. J. Med. 316: 1037 – 1043

[7] SCHRAMEL P, HASSE S, OVCAR-PAVLU J (1988) Selenium, cadmium, lead, and mercury concentrations in human breast milk, placenta, maternal blood, and the blood from the newborn. Biol. Trace Element Res. 15: 111 – 124

[8] LANDRIGAN PJ, GEHLBACH SH, ROSENBLUM BF, SHOUTS JM, CANDELARIA RM, BARTHEL WF, LIDDLE JA, SMREK AL, STAELING NW, SANDERS JF (1975) Epidemic lead absorption near an ore smelter. N. Engl. J. Med. 292: 123 – 129

[9] ROELS H, BUCHERT JP, LAUVERY R (1976) Impact of air pollution by lead in the biosynthetic pathway in school-age children. Arch. Environ. Health 31: 310 – 316

[10] POPOVAC D, GRAZIANO J, SEAMAN C (1982) Elevated blood lead in a population near a lead smelter in Kossovo, Yugoslavia. Arch. Environ. Health 47: 19 – 23

[11] MCMICHAEL AJ, BAGHURST PA, WIGG NR, VIMPANI GV, ROBERTSON I, ROBERTS R, J (1988) Port Pirie cohort study: Environmental exposure to lead and children´s ability at the age of four years. N. Engl. J. Med. 319: 468 – 475

[12] DIETRICH KN, KREAFFT KM, BIER M, SUCCOP PA, BERGER O, BORNSCHEIN RL (1986) Early effects of fetal lead exposure: Neurobehavioral findings at 6 months. Int. J. Biosci. Res. 8: 151 – 168

[13] FERGUSSON JE (1986) Lead: Petrol lead in the environment and its contribution to human blood lead levels. Sci. Tot. Environ 50: 1 – 54

[14] BEACH JR, HENNING SJ (1988) The distribution of lead in milk and the fate of milk lead in the gastrointestinal tract of suckling rats. Pediatr. Res. 23: 58 – 62

[15] COPIUS-PEEREBOOM STEGEMAN JHJ, COPIUS-PEEREBOOM JW (1989) The intake of cadmium in the Kempen, an area in the south of The Netherlands. Ecotox. Environm. Safety 18: 93 – 108

[16] STRASSEN JA, ROELS HA, EMILIANOV D, KUZNETSOVA T, THIJS L, VANGRONSVELD J, FAGARD R (1999) Environmental exposure to cadmium, forearm bone density, and risk of fractures: a prospective study. Lancet 353: 1140 - 1144

HUMAN EXPOSURE TO MERCURY FROM FISH CONSUMPTION IN LATIN AMERICA AND AFRICA: EFFECTS OF MERCURY-SELENIUM INTERACTIONS ON MERCURY METHYLATION RATES IN TROPICAL WATERS.

MARKUS MEILI[1)] AND LARS D. HYLANDER[2)]

[1)] Stockholm University, Inst. of Applied Environmental Research (ITM),
S-106 91 Stockholm, Sweden
E-mail: Markus.Meili@itm.su.se
[2)] Dept. of Limnology, Evolutionary Biology Centre, Uppsala University,
Norbyvägen 20, S-752 36 Uppsala, Sweden
E-mail: Lars.Hylander@ebc.uu.se

Abstract

The dominating human exposure to mercury is generally via fish as food. The Hg levels in fish depend crucially on parameters influencing net methylation and on interactions with selenium. Other variables known to be important are organic acids, metals such as Fe and Mn, geochemical factors such as pH, and microbial activity. However, parameters affecting methylation and other transformations of Hg in tropical environments are not well understood, why a profound study is needed. Methylation potential will be determined in sediments and in the root zone of floating macrophytes by incubating samples with 203Hg. We will also determine total Hg and Se concentrations in fish species important to human consumption. The bacterial production, determined by incorporation of 14C leucine, will be related to Hg methylation potential, among othesr to evaluate the importance of different groups of bacteria for methylation. Similar experiments will be conducted in the presence of variable Se concentrations. Pilot studies and screening surveys at selected sites will be performed during the first year. The study is expected to increase the knowledge about Hg-transformations in the tropics and to point out proper measures to reduce health hazards due to Hg-exposure.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

1.1. Background and information on relevant national programmes

Human health impacts of mercury (Hg) due to its toxicity are well documented. The present, large anthropogenic Hg emissions in many tropical countries from gold mining, chlor-alkali factories, coal combustion etc. is a serious health risk for present as well as future generations. A major Hg exposure pathway is the consumption of fish. With an increasing global population and a corresponding food demand, restriction of fish consumption due to elevated methylmercury content is not a feasible option since fish are a key protein source in many tropical regions. In many of these regions, Hg levels are further elevated due to natural processes influencing the transformations between metallic, oxidized, and organic forms of Hg (in particular methylmercury). While these transformations have been studied to some extent in temperate and boreal areas, little is known about processes such as methylation/demethylation, deposition/volatilization, and reduction/oxidation at tropical conditions. An even less explored issue is the question how these processes are influenced by varying background levels of selenium (Se), an essential nutrient that is known to interact with the Hg cycle. Hence it is necessary to increase the knowledge about factors influencing the transformations of Hg, in particular in in tropical environments. Such factors may be related to the organic environment, geochemical constituents, and bacterial processes. In the proposed project we aim at determining Hg methylation potential, bacterial production, and geochemical parameters, with a focus on the Se content in the water, sediment, and soil of tropical regions.

Mercury levels in Sweden have been extensively studied in several projects [e.g. 1, 2]. The Swedish Food Administration has for decades issued recommendations for the consumtion of fish and other food, to avoid health impacts from Hg and other contaminants. Also Brazil has an extensive research program on Hg in the environment [e.g. 3] and the coupling to human Hg exposure [4]. Corresponding studies are initiated in Suriname [5].

1.2. Studies on mercury and related topics earlier performed by participating researchers

Hg levels in tropical soil, sediment and biota has been surveyed by Hylander et al. in the Pantanal, Brazil, in 1992 - 1994 [3] and 1998 - 2000 [6, 7], and by Guimarães, Malm, and Meili in 1995 [8]. With respect to transformations of Hg, Guimarães et al. showed a large Hg methylation potential in floating macrophyte roots [7, 8]. Fluxes and turnover of environmental Hg and the coupling to organic/biotic matter cycling in tropical and boreal ecosystems have been studied among other by Meili [2, 10, 11, 20, 21].

1.3. Collaboration regarding mercury or nuclear and isotopic techniques apart from this CRP

Markus Meili has a long experience with mercury studies in freshwater ecosystems in Sweden, Canada, and Brazil, supported mainly by SEPA (Swedish Environmental Protection Agency) and SIDA (Swedish Agency for Research in Developing Countries). He is presently involved in European efforts under UN convention (UN/ECE-CLRTAP) to determine critical limits of atmospheric mercury pollution. At the same time he is monitoring and evaluating the remediation of a highly Hg-polluted lake using a novel capping method. Over the years has also conducted a large number of laboratory experiments using radio-labelled tracers to assess the bioavailability and biological turnover of Hg, methyl-Hg, Se, and Cs in microcosms containing dissolved organic matter, microalgae, zooplankton, macroinvertebrates, and fish. His field studies often include the use of ^{13}C and ^{15}N to identify energy sources and actual trophic position of aquatic organisms in various environments, initially a collaboration with the Ecosystems Center in Woods Hole, USA. He has also carried out detailed studies on the distribution of ^{137}Cs in sediments and biota from the Baltic Sea and many adjacent freshwater systems, partly in order to assess the fate of ^{137}Cs deposited after the Chernobyl nuclear accident in 1986, partly to use this nuclide as a tracer of sediment turnover and food web dynamics. This research includes projects supported by SSI (Swedish Radiation Protection Institute) and SKB (Swedish Nuclear Fuel and Waste Management Co). Recently he has started to study the regional and global distribution of ^{129}I in seawater and freshwaters, in particular the fate of the discharges from western Europe in Baltic, Atlantic, and Arctic areas.

Isotopic methods are regularly used by our institutes for estimating bacterial activity by using ^{3}H thymidine and 14C-leucine incorporation, and have among other been a central part of projects in developing countries (Nicaragua, Brazil). We also have applied fallout 137Cs in the Pantanal to study sediment mobility. Isotope methods (ICP-MS, 32P) are also used for studies of heavy metal and phosphorus content in waste water and its uptake by plants. The studies are performed by Lars Hylander in co-operation with researchers from Royal Institute of Technology, Stockholm, Sweden and Kanagawa Environmental Research Centre, Yokohama, Japan.

2. METHODS

All data collection will be systematically organized, among other by determining coordinates of all sampling sites using GPS (Global Positioning System). Water parameters such as pH, redox potential, conductivity, turbidity, temperature, etc. will be measured in the field when samples are collected or incubations performed. Air temperature, light intensity, wind speed, etc. will also be documented.

Determinations of Hg, Se, C, N, S, and 137Cs in waters, sediments, and biota (fish) are performed at Uppsala University (Dept. of Earth Sciences and Dept. of Limnology), equipped with beta- and gamma-counters, and C/N/S-analysers, and at Stockholm University (Dept. of Applied Environmental Research, ITM), equipped with ICP-MS, atomic fluorescence and absorption spectrophotometry for Hg and Se analyses (CV-AFS, CV-AAS). Other institutes in Uppsala can provide ICP-AES, AMS, and other techniques. The quality of obtained data is assured by certified reference materials (ITM is also responsible for national quality control of analyses of water and biological materials).

Different microbial methods will be used in order to determine: (i) bacterial production; (ii) bacterial abundance and biomass; (iii) active/inactive bacteria and respiring bacteria.

Isotope methods for quantifying **bacterial production** include: a) the incorporation of ^{3}H-thymidine into bacterial DNA [12, 13] and b) incorporation of ^{3}H leucine into proteins [14, 15] (or ^{14}C-leucine when combined with ^{3}H thymidine incorporation as a dual label technique). Thymidine incorporation into DNA is specific for bacteria, while leucine incorporation into protein is a more general method for measuring microbial production. The methods can either be used separately or combined in order to obtain an increased amount of ecological information.
Bacterial abundance and biomass will be determined on formaldehyde (5%) preserved samples by standard epifluorescence microscopy after appropriate staining. The most suitable stain will be tested and used. Possible stains include DAPI, acridine orange and SYTO 13.
If possible, the proportion of **active and inactive** bacterial cells will be determined on live samples using epifluorescence microscopy and Live/Dead staining (Molecular probes). The number and proportion of **respiring** bacteria will be determined on live samples using epifluorescence microscopy and the tetrazolium salt CTC (5-cyano-2,3-ditolyl tetrazolium chloride).

The comparability of bacteriological data is more difficult to assure than for data from chemical and isotope analyses. One way to proceed is to divide a homogeneous sample into subsamples for preparation and counting of bacteria cells at the participating laboratories. A library of prepared reference slides is another option.

The **potential net Hg methylation** potential in sediments and selected biota will be determined with in-situ and laboratory incubations using ^{203}Hg [16, 17]. Fresh samples corresponding to about one gram dry weight are incubated in volumes of 30-50 mL (2 -3 samples and one acidified control) with $^{203}Hg^{2+}$ at concentrations of 30-1000 ppb dry weight. Methylation is stopped by addition of HCl and the samples are frozen until MeHg extraction into toluene containing scintillation salts and measurement by liquid scintillation. Samples incubated in Brazil are analyzed at the Federal University of Rio de Janeiro, laboratory of radioisotopes, which is equipped with liquid scintillation counter, centrifuges, and chromatography. Samples incubated in Suriname are analysed at Environmental Research

Center, University of Suriname, which is equipped for commonly performed limnological analyses, or brought to Sweden for analysis. Samples incubated in Tanzania are analyzed at Tanzania University of Dar es Salam, Dept of Geology, or brought to Sweden for analysis. The comparability of methylation analyses can be tested by dividing a homogeneous sample into subsamples for analysis at the participating laboratories.

Parameters potentially influencing MeHg-formation during incubation will be studied by incubating parallel samples at different temperatures, different periods (hours to days), in different atmospheres (air versus nitrogen), and at different pH, conductivity, and light conditions. The influence of micro-organisms and their carbon source on Hg methylation will also be studied.

Selenium interactions with Hg may explain Hg levels and transformations (methylation) diverging from expected patterns [18]. Consequently, survey studies will be performed on Se concentrations in fish, water, sediment, and soil, in combination with incubation experiments, to assess how total and methyl Hg levels are influenced by different Se concentrations.

3. PREVIOUS RESULTS

In the Pantanal fish Hg content was generally below the limit for human consumption in the most intensive gold mining area at the border to Pantanal, as well as in the central Pantanal, while fish Hg content at times was above 0.5 mg Hg g-1 fresh weight at intermediate distances [3, 7]. This shows that the vicinity of Hg emissions is not the only factor of importance for Hg levels found in biota (food). It is likely that environmental factors are at least as important in controlling these levels [4, 11], for example by influencing methylation or volatilization of Hg or Se [22, 23].

This could be a result of climatic conditions such as high temperature and seasonal flooding, and is particularly relevant in tropical flood plains and other wetlands [9], where also natural Hg levels in fish can be very high, suggesting a very high susceptibility to Hg pollution [4, 20]. Eutrophic conditions may contribute to a fast growth and subsequently a reduced bioaccumulation of Hg in fish. It is also possible that the large soil content of oxy-hydroxides of Al, Fe and Mn in soils and sediments may sorb Hg and prevent it from reaching the water bodies as long as soil erosion is prevented [6]. This motivates more detailed studies on the influence of dissolved organic matter and oxy-hydroxides of Al, Fe and Mn, as well as on the influence of microbial activity on the methylation and volatilization of Hg and Se.

4. PLANS FOR FUTURE WORK

4.1. Planned experiments

We will apply experiences from a recent co-operative project between Sweden, Uppsala University and Brazil, the Federal Universities in Rio de Janeiro and Cuiabá [6 - 9]. In these projects, we have studied the methylation potential in floodplain lake sediments and in the root zone of floating macrophytes by incubating samples with 203Hg [9]. Some of the studies were performed in the Pantanal wetland near areas where Hg is used in artisanal gold mining. The results can be complemented with bacteriological studies and further studies of methylation as influenced by parameters such as selenium, organic acids, and geochemical factors. The influence of water pH, conductivity, salinity, organic matter, plant growth stage, plant species, root section, bacterial population etc. on methylation and demethylation rates can be determined under controlled conditions in the laboratory and in the field.

Methylation studies will be performed in rivers and flood plain lakes, where fish are a major source of protein in human nutrition. Some of these systems are exposed to Hg emissions from artisanal gold mining. Since Hg in sediment is transferred to biota, ^{137}Cs will be determined in sediment profiles collected in floodplain lakes to study sediment turnover and its influence on the mobility of Hg, Se, and other elements. A survey of present Hg and Se content in fish and biota can be combined with methylation experiments as described above.

Mercury levels in environmental samples can be compared with Hg levels determined in human hair in the actual study areas. Risk assessment of Hg impact on human health will include an assessment of Hg, MeHg and Se mass balances for tropical regions such as the Rio Negro basin in the Amazon, among other for comparisons with existing mass balances for temperate and boreal regions.

Obtained results will be compared with earlier performed and planned laboratory experiments in Sweden, including water, sediment and biota from temperate and boreal environments. Climatic conditions can be also manipulated in laboratory. Such studies are expected to contribute with valuable information about the importance of methylation and demethylation at different water quality (in particular Se levels and microbiology), which might diverge between temperate and tropical areas.

4.2. Time schedule

Two field campaigns in Tanzania and Suriname are planned for the second half of 2003 and for the first half of 2004. Parallel to the field campaigns laboratory experiments will be performed in Tanzania, Suriname, and Sweden starting in 2001.

REFERENCES

[1] LINDQVIST, O., JOHANSSON, K., AASTRUP, M., ANDERSSON, A., BRINGMARK, L., HOVSENIUS, G., HÅKANSON, L., IVERFELDT, A., MEILI, M. & TIMM, B. Mercury in the Swedish Environment - Recent Research On Causes, Consequences and Corrective Methods. Water, Air, & Soil Pollution **55** (1991) 1-281.

[2] MEILI, M. The coupling of mercury and organic matter in the biogeochemical cycle — towards a mechanistic model for the boreal forest zone. Water, Air, & Soil Pollution **56** (1991) 333-347.

[3] HYLANDER, L.D., SILVA, E.C., OLIVEIRA, L.J., SILVA, S.A., KUNTZE, E.K. & SILVA, D.X. Mercury levels in Alto Pantanal - a screening study. Ambio **23** (1994) 478-484.

[4] SILVA-FORSBERG, M.C., FORSBERG, B.R. & ZEIDEMANN, V.K. Mercury contamination in humans linked to river chemistry in the Amazon basin. Ambio **28** (1999) 519-521.

[5] MOL, J.H., RAMLAL, J.S., LIETAR, C. & VERLOO, M. Mercury contamination in freshwater, estuarine, and marine fishes in relation to small-scale gold mining in Suriname, South America. Environmental Research **86** (2001) 183-197.

[6] HYLANDER, L. D., MEILI, M., OLIVEIRA, L.J., SILVA, E.D.E., GUIMARAES, J.R.D., ARAUJO, D.M., NEVES, R.P., STACHIW, R., BARROS, A.J.P. & SILVA, G.D. (2000): Relationship of mercury with aluminum, iron and manganese oxy-hydroxides in sediments from the Alto Pantanal, Brazil. Science of the Total Environment **260** (2000) 97-107.

[7] HYLANDER, L.D., PINTO, F.N., GUIMARAES, J.R.D., MEILI, M., OLIVEIRA, L.J. & SILVA, E.D.E. Fish mercury concentration in the Alto Pantanal, Brazil: influence of season and water parameters. Science of the Total Environment **260** (2000) 9-20.

[8] GUIMARÃES, J.R.D., MALM, O., MEILI, M. Mercury in Soils, Sediments and Fish Around the Poconé Gold Mining Area, Pantanal, Brazil: Some Mobilisation but No Health Risks. In: Barbosa, J., Melamed, R., and Villas Bóas, R. (eds.) Mercury as a Global Pollutant 5th International Conference, May 23-27, 1999, Rio de Janeiro, Brazil. CETEMCenter for Mineral Technology. Rio de Janeiro, Brazil (1999) 154.

[9] GUIMARÃES, J.R.D., MEILI, M., HYLANDER, L.D., SILVA, E.D.E., ROULET, M., MAURO, J.B.N. & DE LEMOS, R.A. Mercury net methylation in five tropical flood plain regions of Brazil: high in the root zone of floating macrophyte mats but low in surface sediments and flooded soils. Science of the Total Environment **261** (2000) 99-107.

[10] MEILI, M. Sources, concentrations and characteristics of organic matter in softwater lakes and of the Swedish forest region. - Hydrobiologia **229** (1992) 23-41.

[11] MEILI, M. Mercury in lakes and rivers. In Sigel, A., Sigel, H. (eds): Mercury and Its Effects on Environment and Biology. Metal Ions in Biological Systems, Vol. 34, Marcel Dekker Inc., New York, (1997) 21-51.

[12] FUHRMAN JA, AZAM F, Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica and California. Appl. Environ. Microbiol. **39** (1980) 1085-1095.

[13] BELL RT, AHLGREN I, Thymidine incorporation and microbial respiration in the surface sediment of a hypereutrophic lake. Limnol. Oceanogr. **32** (1987) 476-482.

[14] KIRCHMAN D, Particulate detritus and bacteria in marine environments. In: Ford TE (ed.) Aquatic microbiology. An ecological approach. Blackwell Scientific Publishers, Boston, (1993) 321-341.

[15] TÖRNBLOM, E., SØNDERGAARD, M., Seasonal dynamics of bacterial biomass and production on eelgrass Zostera marina leaves. Mar. Ecol. Prog. Ser. **179** (1999) 231-240.
[16] FURUTANI, A., RUDD, J.W.M., Measurement of mercury methylation in lake water and sediment samples. Appl. Environ. Microbiol. **40** (1980) 770-776.
[17] GUIMARÃES, J.R.D., MALM, O., PFEIFFER, W.C., A simplified radiochemical technique for measurements of net mercury methylation rates in aquatic systems near goldmining areas, Amazon, Brazil. Science of the Total Environment **175** (1995) 151-162.
[18] FATOKI, O.S. Biomethylation in the natural environment: A review. South African Journal of Science **93** (1997) 366-370.
[19] ROULET, M., GUIMARAES, J.R.D. & LUCOTTE, M. Methylmercury production and accumulation in sediments and soils of an amazonian floodplain - Effect of seasonal inundation.Water Air and Soil Pollution **128** (2001) 41-60.
[20] MEILI, M., MALM, O., GUIMARÃES, J.R.D., FORSBERG, B.R. & PADOVANI, C.R. Mercury concentrations in tropical (Amazon) and boreal freshwater fish: natural spatial variability and pollution effects. 4th International Conference on Mercury as a Global Pollutant, Hamburg, Germany, 4-8 Aug. 1996, Book of Abstracts, GKSS (1996), p. 403.
[21] MEILI, M., MALM, O., GUIMARÃES, J.R.D., PADOVANI, C. & FORSBERG, B.R. Bioaccumulation of mercury in tropical river food webs (Amazon): Similar patterns as in boreal lakes. 4th International Conference on Mercury as a Global Pollutant, Hamburg, Germany, 4-8 Aug. 1996, Book of Abstracts, GKSS (1996), p. 404.
[22] ZHANG, Y.Q. & MOORE, J.N. Selenium fractionation and speciation in a wetland system. Environ. Sci. Technol. **30** (1996) 2613-2619.
[23] ZHANG, Y.Q. & MOORE, J.N. Environmental conditions controlling selenium volatilization from a wetland system. Environ. Sci. Technol. **31** (1997) 511-517.
[24] DE KOM, J.F.M., VAN DER VOET, G.B. & DE WOLFF, F.A. Mercury exposure of maroon workers in the small scale gold mining in Surinam. Environmental Research **77** (1998) 91-97.

APPLICATION OF ISOTOPIC AND NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION - POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF CHILDREN SUBJECT IN VIETNAMESE POPULATION

TRAN BANG DIEP[A], TRINH THANH HA[B], NGUYEN DUY LAM[A], DINH THI QUI[C] AND TRAN DAI NGHIEP[A]

[a] *Institute for Nuclear Science and Technique, VAEC, P.O. Box 5T-160, Hoang Quoc Viet Road, Cau giay, Hanoi, Vietnam*
[b] *Food Industries Research Institute, 301- Nguyen Trai Road, Thanh Xuan District, Hanoi, Vietnam*
[c] *Hanoi Children Hospital, La Thanh Road, Hanoi, Vietnam*

Abstract

A review on malnutrition of Vietnamese children and its features is presented. In spite of the recent economic development, the proportion of malnutrition for children in the country is still rather high at about 33.8%. The rapid and uncontrolled industrial growth has aggravated environmental pollution, which is one of the reasons for malnutrition of children. The nuclear techniques such as neutron activation analysis (NAA), atomic absorption spectrometry (AAS) and X-ray fluorescence (XRF) are proposed to analyze the nutritional elements and pollutants (Zn, Se, Ca, Cd, Hg, Pb,) in the study on nutrition - pollution interactions. Three groups of malnourished children with adequate real-time monitoring (RTM) (placenta, breast milk, urine), long-term monitoring (LTM) (hair, nails) and food specimens are chosen for the study. The protocol for sampling and sample preparation of placenta is proposed.

INTRODUCTION

In recent years, the malnutritional situation in Vietnamese children has partly ameliorated but the proportion of malnourished children is still rather high, about 33.8%. Beside problems on quality, sanitation and safety of food, the increase in environmental pollution created by anthropogenic socio-economic activities have caused harmful effects on public health, especially on children. In the scope of Co-ordinated Research Project (CRP) of IAEA, the application of isotopic and nuclear techniques is expected to understand and to evaluate the effects of environmental pollution on the nutritional status of children already exposed to marginal malnutrition as well as to develop remedial strategies to alleviate such ill effect.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

1.1 Malnutritional situation in Vietnamese children

Adequate nutrition is fundamental to the maintenance of good health and optimum human performance. Health authorities of Vietnam are concerned about the nutrition of population and have proposed measures to eliminate poverty and malnutrition among women and children. The percentage of malnourished children was significantly reduced from 1985 (51.5%) to 1995 (44.9%); the mean reduction was about 0.66% per year. Since 1995, the mean reduction was about 2% per year, which was recognized by international agencies as quick reduction. Thus, every year about 200,000 Vietnamese children under the age of five are spared from malnutrition [1]. The successes have been achieved through the national nutritional monitoring programs such as “Maternal and child health care program” and

"School lunch support program". These programs are principally designed to address to the micronutrient deficiency of iron, iodine, zinc... and vitamins (e.g. vitamin A, D, E, B-1, B-2, B-6, B-12) [2]. According to data surveyed by the Health Statistics and Informational Department (Ministry of Health) the percentage of malnourished children in Vietnam was about 33.8% in 2000, still higher than the World Health Organization (WHO) standards [1].

The malnourished children suffer symptoms of low weight for height, low height for weight and chronic under-weight. The malnutritional situation of children under 5 years of age in whole country from 1996 to 2000 is shown in Table 1.

TABLE 1: MALNUTRITIONAL CASES IN VIETNAMESE CHILDREN UNDER 5 YEARS OF AGE FROM 1996 TO 2000

Year	**No of children < 5 surveyed**	**Low weight for age (%) (underweight[a])**	**Low height for age (%) (Stunted[b])**	**Low weight for height (%) (Wasted[c])**
1996	...	43.9	44.2	14.8
1997	18690	40.7	44.1	14.2
1998	12900	39.0	34.4	10.6
1999	93469	36.7	38.7	9.8
2000	94469	33.8	36.5	8.6

Source: National Institute of Nutrition [1].

[a]Underweight: weight-for-age of reference population.
[b]Stunting: below – 2 S. D. height-for-age of reference population.
[c]Wasting: below – 2 S. D. weight-for-age of reference population.

1.2. Features of malnutrition

Malnutrition is usually divided into three levels: moderate, severe and very severe. Table 2 shows prevalence of severe undernourishment for children under 5 years of age in 2000. We can see that the very severe prevalence of malnutrition drastically reduced (0.6%) and now malnutrition cases in Vietnam are essentially moderate and severe prevalence. Malnutritional patterns show distinction between different ecosystems and provinces. There is no clear-cut distinction in malnutritional rate based on sex in Vietnam. Children at 6-24 months of age belong to the group, which was seriously affected by malnutrition. This group is starting to transfer from nursing to complementary feeding and inadequate regimes in nutrition could seriously affect the nutritional status of this group.

TABLE 2: MALNUTRION CASES IN VIETNAMESE CHILDREN UNDER 5 YEARS OF AGE BY SEVERITY IN YEAR 2000

Province/ City	No of children < 5 surveyed	Underweight (%)				Stunting (%)			Wasting (%)
		Total	Moderate	Severe	Very severe	Total	Moderate	Severe	
1. Whole country	94 469	33.8	27.8	5.4	0.6	36.5	23.8	12.7	8.6
2. Northern Highland	24 192	37.6	29.9	6.9	0.8	42.3	25.4	16.6	10.6
3. Red River Delta	13 726	31.6	27.5	3.9	0.2	32.7	24.4	8.3	7.7
4. North Central Coast	8 996	40.1	33.0	6.7	0.4	43.1	26.9	16.4	9.9
5. Central Coast	12 138	35.1	28.5	6.1	0.5	36.0	24.3	11.7	8.6
6. Central Highland	6 161	40.9	31.8	7.8	1.2	45.5	26.5	19.0	10.3
7. South-Eastland	10 953	26.7	22.6	3.7	0.4	27.0	16.6	10.3	7.3
8. Mekong River Delta	18 373	30.0	25.2	4.5	0.3	33.9	21.5	12.5	7.9

Source: National Institute of Nutrition [1].

Malnurition is compounded by many factors (regimes of feeding, quality of nutrition, infections, nursing etc.) in which poverty is primary reason. The sanitation and safety of food is an important factor in North Highland, South Central Coast and Central Highland, while the regime of feeding is the major contributory factor of the malnutrition in the Delta areas. In megacities, hunger and under-nutrition were not prevalent and quality of child feeding was improved so the disabilities infected at birth and the effects of environmental pollution are additional risks encountered by malnourish individual.

Report of the International Food Policy Research Institute (IFPRI) showed that knowledge of women has been contributed 43% to the reduction of malnutrition while the contribution of security of food was only 21%. Hence, feeding and care of children (expressed through knowledge of women) is playing an important role in the prevention and reduction of malnutrition.

Long-term protein-energy deficiency of women (Body Mass Index of less than 18.5kg/m^2) in Vietnam was 38% in 1997 and was recently reduced to 32% in 2000 [3]. Malnourished women have reflected the restriction in women carrying and involved in intrauterine growth retardation (IGR), fetal malnutrition and disorder related to short gestation and low weight at birth which are among leading causes of mortality in Vietnam (Table 3).

TABLE 3: RATE OF INTRAUTERINE GROWTH RETARDATION (IGR), FETAL MALNUTRITION AND DISORDER RELATED TO SHORT GESTATION AND LOW WEIGHT AT BIRTH IN VARIOUS AREAS OF VIETNAM IN YEAR 2000

Unit: Per 100 000 inhabitant

Provinces/ City	Cases %	Deaths %
1. Whole country	15.35	0.96
2. North Highland	11.21	0.85
3. Red River Delta	9.21	1.28
4. North Central Coast	-	-
5. Central Coast	11.63	1.44
6 Central Highland	-	-
7. South-East Region	42.37	1.76
8. Mekong River Delta	-	-

Source: Ministry of Health [1]

1.3. Environmental pollution and nutrition-related diseases

The human environment is one of major contributory factor to the overall picture of nutrition-related diseases in Vietnam.

Pollution in a large context encompasses all those determinants, both anthropogenic and non-anthropogenic. Rapid and uncontrolled industrial growth in Vietnam has induced environmental pollutants as a significant factor affecting health. Lead chromium, cadmium, mercury, arsenic and other heavy metals form a major group of pollutants generated by human activity. They have a profound influence on growth and development in infants and children and on the utilization of essential nutrients (e.g. iron, vitamin A) [4]. Similarly, biological agents are no less detrimental to human well-being. Parasitic infection and communicable diseases form major segment of environmental component of nutritional diseases in children and their impact is further enhanced by poor nutrition. However, studies on nutrition - pollution interactions are limited in Vietnam. We hope the effect of environmental pollution on the nutrition status of children will be evaluated in the framework of the present CRP.

Our project is carried out in cooperation with Hanoi Children Hospital, Hatay Hospital, Food Industries Research Institute, Center for Environmental Analysis and Dalat Nuclear Research Institute.

2. BIOINDICATORS AND ANALYTICAL METHODS FOR NUTRITION - POLLUTION INTERACTIONS STUDY

2.1. Bioindicators for monitoring pollutants

Biomonitoring has now been accepted as a powerful tool to assess the impact of pollutants on human health. Real-time monitoring (RTM) and long-term monitoring (LTM) have provided analytical techniques to study the interaction between environmental pollution and nutrition, as well as health status [5].

Specimens such as breast milk, is an example of an indicator used for RTM. It has been demonstrated that the effect of presence of pollutants in breast milk has an effect on mobility and growth faltering in the nursing infant [5]. Similarly, other non-invasive indicators such as urine, saliva and less invasive ones, as whole blood have commonly been used to monitor some pollutants (e.g. toxic metal). There is enough evidence on record to show that the developing fetus and the newborn are particularly vulnerable when exposed to toxic situation [6]. Although the principal role of placenta is to nurture the fetus, the same processes that aid the transport of nutrients can also act as pathways of toxic constituents due to chemical similarities with some of the nutrient metabolites, or simply as a result of passive diffusion [6]. These features make the placenta as dual indicator for RTM, reflecting the pollutant status in both mother and fetus [6]. Hair, adipose tissue (to monitor organic pollutants), and hard tissues such as bone, teeth and nail have been used as LTM specimens to monitor selected elements [5].

2.2. Analytical techniques

The analytical methods applied for the elements of interest in the biomonitors and specimens are showed in Table 4.

In the neutron activation analysis (NAA) the samples are irradiated in Dalat Nuclear Reactor at thermal neutron flux of $3 \div 4*10^{12} ncm^{-2}sec^{-1}$. The combination with high-resolution gamma ray spectrometry and radiochemical separate (RNAA) increases the sensitivity of the method [7].

TABLE 4: ANALYTICAL METHODS FOR SOME ELEMENTS OF INTEREST IN BIOMONITORS AND SPECIMENS

Elements	Methods
Ca	AAS
Se	RNAA
Zn	AAS, INAA/ XRF
Cd	RNAA
Hg	RNAA/ INAA, AAS
Pb	AAS/ XRF
As	NAA

3. ACTIVITIES OF THE PROJECT

3.1. Selection of children subjects for the study

The investigation is planned to be carried out in Hanoi. In such a large city like Hanoi, where hunger and under-nutrition are not prevalent and quality of child feeding has improved, the effects of environmental pollutants are considered to be the major contributory factors causing malnutrition in children. Statistical data in the last three months of 2001 showed that total malnourished children under 5 years of age in Hanoi was 28,720 (about 17%) and the count of low birth weight baby was 2,393 (4.15%) of total children surveyed [8].

In our study the main subjects are malnourished children. The study was conducted in Hanoi Children Hospital and Hatay Hospital. These children were divided into 3 groups based on their nutritional status and age.

Group 1: Low weight newborn.
Group 2: Nursing malnourished babies.
Group 3: Malnourished faltering babies.

The children who have normal nutritional status at the same age with above-mentioned groups were chosen as the 3 reference groups.

Bio-indicators used in this study are placenta, breast milk, urine (indicators used for RTM), hair and nail (indicator used for LTM). Bio-indicators were chosen based on the characteristics of each group (Table 5).

TABLE 5: CHILDREN GROUPS AND BIOINDICATORS FOR STUDY

Group	**Subject**	**Choice criteria**	**Adequate bioindicators/ Specimens**	**Stages for studies**
1	Low weight newborns	The weight less than 2500g.	Placenta (RTM)	1st year
2	Nursing malnourished babies	Under 5 months of age; Breastfeeding fully; Slow growth, reduction or not increase in body weight; Having clinic symptoms of malnourished children at this month of age.	Breast milk, (RTM), hair, nail (LTM)	2nd year
3	Malnourished faltering babies	Malnutrition at 1-3 years of age.	Urine (RTM), hair, nail (LTM)/ food	3rd year

The main objective in the first year will be to analyze the first group with placenta as dual bioindicators. However, bioindicators for other groups (breast milk, urine, hair, nail...) will be considered to investigate primarily the sensitivity of the used methods.

3.2. Placenta as bioindicators for the malnourished newborns

The placenta has a dual function: 1) to provide nutrients and oxygen to the fetus and 2) to prevent some other harmful substances [6, 9]. It is proposed that for the malnourished newborns, this function has declined and the toxic agents such as Cd, Hg, Pb, could cross the placental barrier and caused severe health consequences. In our studies we have chosen the placenta as a real-time monitoring for the first malnourished group.

3.3. The method for sampling of placenta for compositional analysis

An abundant review of sampling and sample preparation of placenta was introduced [6]. The method used by various investigators in the review was not harmonized. The following procedures are proposed in our work.

3.3.1. Sampling protocol

The sampling protocol covering the following items

- Delivery time
- Age of the placenta
- Weight of newborn
- Maternal occupation
- Residence (rural/ urban)
- Dietary and smoking habits (living in smoking environment or not)
- Water supply system (central/ local)

3.3.2. Sampling and sample preparation of placenta and hair

The procedures for placenta cover the following steps:

- Collecting entire placenta immediately after delivery (including umbilical cord)
- Washing with deionized water
- Transferring placenta into clean polyethylene bags and storing at -20^0C. Further preparatory work is to be carried out in the clean laboratory
- Thawing, gently squeezing to remove excess blood
- Washing carefully again with ethanol and wiping with fibre - free tissue
- Collecting multiple pieces from three components: cord (detached at point of entry to the placenta), membrane and placenta body
- Drying with filter paper in vacuum oven - dry at 50^0C to constant weight
- Grinding to maximize the uniformity of the sample in a Quartz mortar
- Keeping the samples (100 mg) in sealed polyethylene bags until analyzed

4. BRIEF ON PLANS FOR FUTURE WORK

As shown in Table 5 the first year of the project will concentrate on the activities on the first group of malnourished children and will engage on placenta as dual bioindicators.

The second malnourished group is the subject of the study in the next year. Breast milk (RTM), hair and nail (LTM) are planned to be used for study on nutrition - pollution interactions.

We hope the investigation for the third group could clarify the results of the initial two years of study.

REFERENCES:

[1] MINISTRY OF HEALTH, Health statistics yearbook, Hanoi (2000).

[2] HOP, L.T., BERGER, J., Efficacy of multi-micronutrient supplementation in Vietnamese infants, Report for international research on infant supplementation, Hanoi (2001).

[3] MEDICAL PUBLISING HOUSE, National policy on nutrition for stage of 2001 - 2010, Hanoi (2001).

[4] IYENGAR, G. V., NAIR, P.P., Global outlook on nutrition and the environment: meeting the challenges of the next millennium, The Sci. of the Total Environment 249 (2000) 331 - 346.

[5] IYENGAR, G.V., RAPP, A., Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: Toxic trace elements in placenta and placenta as a biomarker for these elements, The Sci of the Total Environment 280 (2001) 221 - 238.

[6] IYENGAR, G.V., RAPP, A., Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 1: Physiology, function and sampling of placenta for elemental characterization, The Sci. of the Total Environment 280 (2001) 195 - 206.

[7] VERSIECK, J., NAA for the determination of trace element in biological material, Biological Trace Element Research (1994) 407 - 413.

[8] HEALTH STATISTICS AND INFORMATIC DIVISION (MINISTRY OF HELTH), Report for medical statistics at provinces and city, Hanoi (2001).

[9] IYENGAR, G.V., RAPP, A., Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 2: Essential minor, trace and other (non -essential) elements in human placenta, The Sci of the Total Environment 280 (2001) 207 – 219.

PART III:
APPENDICES

APPENDIX 1: AGENDA OF THE MEETING

FIRST RESEARCH CO-ORDINATION MEETING (RCM) FOR THE CO-ORDINATED RESEARCH PROJECT (CRP) ON APPLICATION OF ISOTOPIC NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION-POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF HUMAN SUBJECTS IN DEVELOPING COUNTRY POPULATIONS

MONDAY, 6 MAY 2002

9:00 – 9:30 Registration
9:30 – 10:30 Opening of the meeting

Mr. Steffen Groth, Director, Division of Human Health (NAHU)

Mr. G. Venkatesh Iyengar, Head, Section of Nutritional and Health-Related Environmental Studies Section (NAHRES)

Ms. Najat Mokhtar, Scientific Secretary of the Meeting

Election of the rapporteur
Adoption of the agenda
Administrative arrangements for the meeting

10:30 – 11:00 Coffee break

10:00 – 12:30 SESSION 1: PROJECT REPORTS

Chair: A. Muhammad

Peru (*G. Lopez De Romana*):
Determination of the level of lead and cadmium contamination in pregnant women living in an exposed mining area of Peru

Vietnam (*Bang DiepTran*):
Application of isotopic and nuclear techniques in the study of nutrition-pollution interaction and their impact on the nutritional status of children subject in Vietnamese population

China (*Ji-Xian Wang*):
Application of isotopic and nuclear techniques to determine the composition of human placenta and cord blood in the study of interaction between essential and toxic trace elements

12:15 –13:30 Luncheon

13:30 – 15:00 SESSION 2: PROJECT REPORTS (continuation)

Chair: J.E. Dutra-de-Oliviera

Chile (*Miguel Llanos*):

Oxidative stress and antioxidants in placentas of women with low birth weight neonates; correlation with toxic and trace elements

India (*K. Krishnaswamy*):

Role of nutrient in environmental toxicity

15:00 – 15:30 Coffee break

15:30 – 17:00 SESSION 3: PROJECT REPORTS (continuation)

Chair: Ji-xian Wang

Sweden (*L. Hylander*):

Human exposure to mercury from fish consumption in Brazil and Suriname: Effects of mercury-selenium interactions on mercury methylation in tropical waters

Korea (*Seung Yeon Cho*):

Application of isotopic and nuclear techniques in the study of nutrition-pollution interactions and their impact on the nutritional status of Korean pediatric populations

17:00 RECEPTION

TUESDAY, 7 MAY 2002

9.00 – 11.30 SESSION 4: PROJECT REPORTS (continuation)

Chair: Seung Yeon Cho

Morocco (*B. Attrassi*):

Impact of water pollution by Hg, Cd, and Pb on nutritional status of children in the Northwest of Morocco

Brazil. (*J.E. Dutra-de-Oliveira*):

Micronutrients composition and the presence of pollutants in mother's milk from rural and urban working environment

Kenya. (*A. Makokha*):

Application of isotopic and nuclear techniques in the study of iron-lead interactions in cord and placenta blood on subjects living in heavily polluted areas of Kenya

11:30 –13:30 Luncheon

13:30 – 15:00 SESSION 5: SEMINARS (see separate provisional list of seminars) *Chair: Bang Diep Tran*

15:00 – 15:30 Coffee break

15:30– 17:30 SESSION 6: SEMINARS (continuation) *Chair: L. Hylander*

WEDNESDAY, 8 MAY 2002

9.00 – 11.00 GENERAL DISCUSSION
(See separate list of discussion topics)
Chair: A. Makokha

11.00 – 11:30 Coffee break

11:30 – 12:30 SESSION 7: GENERAL DISCUSSION (continuation)

12:30 –13:30 Luncheon

13:30 – 17:30 SESSION 8: GENERAL DISCUSSION (continuation)
Chair: B. Attrassi

15:30 – 16:00 Coffee break

16:00 – 17:30 SESSION 9: GENERAL DISCUSSION (continuation)

THURSDAY, 9 MAY 2002

8:30 – 12:30 SESSION 10: GENERAL DISCUSSION (continuation)
Chair K. Krishnaswamy

- Drafting of the Meeting Report

12:30 – 13:30 Luncheon

13:30 – 17:30 SESSION 11: GENERAL DISCUSSION (continuation)
Chair: M. Llanos

- Drafting of the Meeting Report (continuation)

FRIDAY, 10 MAY 2002

9:00 – 12:30 SESSION 12: CONCLUDING SESSION
Chair: G. Lopez de Romana

- **Final discussion and adoption of the Meeting Report**

12:30 – Luncheon, open end, personal discussions

APPENDIX 2: LIST OF DISCUSSION TOPICS

FIRST RESEARCH CO-ORDINATION MEETING (RCM) FOR THE CO-ORDINATED RESEARCH PROJECT (CRP) ON THE APPLICATION OF ISOTOPIC AND NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION-POLLUTION INTERACTIONSAND THEIR IMPACT ON THE NUTRITIONAL STATUS OF HUMAN SUBJECTS IN DEVELOPING COUNTRY POPULATIONS

PROVISIONAL LIST OF SEMINARS

1. Breast milk and pollutants interactions = Dr. J.E. Dutra-de-Oliveira (Brazil)

2. Assessment of nutrients and pollutants in placenta; Breast milk samples using nuclear and related techniques = Dr. Ana M. Ronco (Chile)

3. Assessment of pollutants and minerals in hair samples = Dr. Seung Yeon Cho (Korea)

4. History of mercury pollution and preventive measures of mercury exposure – Dr. Lars Hylander (Sweden)

5. Placenta as a dual biomonitor = Dr. G. Venkatesh Iyengar (IAEA)

FIRST RESEARCH CO-ORDINATION MEETING (RCM) FOR THE CO-ORDINATED RESEARCH PROJECT (CRP) ON THE APPLICATION OF ISOTOPIC AND NUCLEAR TECHNIQUES IN THE STUDY OF NUTRITION-POLLUTION INTERACTIONS AND THEIR IMPACT ON THE NUTRITIONAL STATUS OF HUMAN SUBJECTS IN DEVELOPING COUNTRY POPULATIONS

PROVISIONAL LIST OF DISCUSSION TOPICS

PURPOSE AND SCOPE OF THE CRP

1. Review of individual Summaries

2. Identification of research areas following the review

3. Comparison of identified areas with the proposed CRP plan

- 3.1. Review of specific CRP objective
- 3.2. Review of the expected research outputs
- 3.3. Review of the proposed research plan (framework plan/country)
  - 3.3.1. The core programme
    - 3.3.1.1. Topics
    - 3.3.1.2. Priorities
  - 3.3.2. The supplementary programme
    - 3.3.2.1. Topics
    - 3.3.2.2. Priorities

4. Review of the proposed action plan (activities)

5. Review of individual project plans to comply with the adopted overall CRP plan

TECHNICAL ASPECTS

6. Selection of sampling sites and types of samples to be collected

- 6.1. Criteria for the selection of sampling sites
- 6.2. Types of samples to be collected
- 6.3. Number of samples to be collected
- 6.4. Sampling frequency

7. Sampling techniques and equipment

8. Analysis

- 8.1. Sample preparation
- 8.2. Recommendations for nuclear analytical techniques

8.3. Recommendations for other analytical techniques

8.4. Reference analytical laboratories

8.4.1. As sources of specialised advice
8.4.2. To assist less advanced participants for more samples and/or analytes
8.4.3. For cross-checking purpose

9. Data processing and evaluation

9.1. Database management (quality, outliers, software)

9.2. Data evaluation and presentation (techniques, software)

ORGANISATIONAL ASPECTS

1. Co-operation within the group

2. Co-operation with other institution within a country

3. Co-operation among countries and with international organisations

4. Next RCM

LIST OF PARTICIPANTS

Dr. Muhammad ALI
Chemistry Division
Atomic Energy Centre
4 Kazi Nazrul Islam Avenue
P.O. Box No. 164, Ramna
Dhaka 1000
Bangladesh

Tel.: +880 2 8617946
Fax: +880 2 8613051
E-mail: aecd@citechco.net

Dr. Jose DUTRA DE OLIVEIRA
Medical School of Ribeirao Preto
University of Sao Paulo
Department of Internal Medicine
Ribeirao Preto
Brazil

Tel.: +55 16 6104801
Fax: +55 16 6356008
E-mail: jeddoliv@fmrp.usp.br

Dr. Miguel LLANOS
Universidad de Chile
Instituto de Nutricion y Tecnologia de los Alimentos
Casilla 138-11
Santiago
Chile

Tel.: +56 2
Fax: +56 2 2214030
E-mail: mllanos@uec.inta.uchile.cl

Dr. Ji-Xian WANG
Chinese Academy of Medical Sciences
Institute of Radiation Medicine
Department of Radiation Effects
P.O. Box 71
Tianjin 300192
China

Tel.: + 86 22 87891919
Fax: + 86 22 87891317
E-mail: Jxwang@public.tpt.tj.cn

Dr. Dinesh KUMAR
Indian Council of Medical Research
Food and Drug Toxicology Research Centre
National Institute of Nutrition
Division of Food Safety
Hyderabad
India

Tel.: +91 40 7018909
Fax: +91 40 7019074
E-mail: dineshkalpa@eth.net

Prof. Ansilmo MAKOKHA
Jomo Kenyatta University of Agriculture and Technology
Food Science Department
P.O. Box 62000
Nairobi
Kenya

Tel.: +254 2 723775
Fax: +254 2 723775
E-mail: vam@kenyaweb.com

Dr. Seung Yeon CHO
Yonsei University
Industrial Hygiene Department
234 Maeji-Ri Hungob-Myon
Wonju-Kun, Kongwon-Do 222-710
Republic of Korea

Tel.: + 82 371 760 2437
Fax: + 82 371 766 2551
E-mail: sycho@dragon.yonsei.ac.kr

Dr. Benaissa ATTRASSI
Ibn Tofail University
School of Sciences
Biology Department
B.P. 133 Kenitra
Rabat
Morocco

Tel.: +212 61 362476
Fax: +212 37 372770
E-mail: attarassi@hotmail.com

Dr. Guillermo LOPEZ DE ROMANA
Fundacion para el Desarollo Agrario
Camilo Carrillo 325
Jesus Maria
Lima
Peru

Tel.: + 51 1 4335726
Fax: + 51 1 4331130
E-mail: fda@terra.com.pe

Prof. Markus MEILI
Stockholm University
Institute of Applied Environmental Research (ITM)
SE-106 91 Stockholm
Sweden

Tel.: +46 8 6747217
Fax: +46 8 6747636
E-mail: Markus.Meili@itm.su.se

Dr. Bang Diep TRAN
Vietnam National Atomic Energy Commission
Institute for Nuclear Science and Technique
Hoang Quoc Viet Street
Nghia Do tu Liem, PO Box 5T-160
Hanoi
Viet Nam

Tel.: + 84 4 8362597
Fax: + 84 4 8363295
E-mail: tdnghiep@vaec.vista.gov.vn
or
tranbangdiep@vol.vnn.vn

Observers:

Dr. Ana Maria Ronco MACCHIAVELLO
University of Chile
Institute of Nutrition and Food Technology
Casilla 138-11
Santiago
Chile

Fax: + 56 2 2214030
E-mail: amronco@uec.inta.uchile.cl

Dr. Kamala KRISHNASWAMY
Indian Council of Medical Research
Food and Drug Toxicology Research Centre
National Institute of Nutrition
Division of Food Safety
Hyderabad
India

Tel.: +91 40 7018909
Fax: +91 40 7019074
E-mail: nin@ap.nic.in

Scientific Secretary:

Dr. Najat Mokhtar
Section of Nutritional and Health-Related Environmental Studies
Division of Human Health
IAEA
P.O. Box 100
A-1400 Vienna
AUSTRIA

Tel: 0043-1-2600-21652
Fax: 0043-1-26007-21652 or 0043-1-26007
E-mail: N.Mokhtar@iaea.org

Dr. G.V. Iyengar
Head, Section of Nutritional and Health-Related Environmental Studies
Division of Human Health
IAEA
P.O. Box 100
A-1400 Vienna
AUSTRIA

Tel: 0043-1-2600-21652
Fax: 0043-1-26007-21652 or 0043-1-26007
E-mail: V.Iyengar@iaea.org

APPENDIX 4

Framework for CRP on the Use of Nuclear And Related Analytical Techniques in Studying Nutrition and Pollution Interactions.

Narrative Summary	***Objective Verifiable Indicators***	***Means of Verification***	***Important Assumptions***
Overall Objectives: To use nuclear and isotopic techniques to determine how environmental pollution and nutritional status are related.	N/A	N/A	N/A
Specific Objectives: Study the occurrence and toxicity of commonly known pollutants and their effect on specific nutritional indices in malnourished individuals and /or normal subjects.	Research contract and research agreement holders developing compatible approaches to the objectives.	Report of first RCM will demonstrate a coherent and integrated approach to common goals.	National support is provided to institutions. Analytical services are available within each country. Technical officer provide appropriate technical support.
Outputs: 1 Harmonized protocols for sample collection, handling, and storage, and analytical processes. 2 Information on nutrition-pollution interactions and magnitude of the problem under specific conditions. 3 Use of placenta, breast milk, hair and other biological markers for specific interactions. 4 Impact of nutrients, supplements for alleviating effects of pollutants. 5 Publications of results in IAEA TEC-DOC and peer reviewed journals.	1. Number of participants using harmonised protocols. 2. Production of data outcomes in line with the specified objectives. 3. Establishment of bio-monitoring tools. 4. Possibility of intervention strategies. 5. Progress reports and number of publications.	Progress reports and number of publications. Recommendations to national health planning bodies. Awareness generation in relation to nutrition- related toxicological problems.	Protocols are appropriate, workable and validated. Participants follow established protocols. Sufficient resources available in institutions. Quantity and quality of data appropriate for publication.
Activities: 1. Call for CRP applications. 2. Evaluation of contracts and agreements. 3. First RCM organized. 4. Maintain coordination of research. 5. Future RCM should be organized 6. Preparation of final report.	1. Number of applications received. 2. Number of Research Contracts and Agreements awarded. 3. 1st RCM organized 4. Results to be available in 18 months.	Approval of contracts and compatible protocols used. RCM reports will be produced. Progress reports and final reports	Suitable proposals are submitted. Participants have the required expertise and facilities. National support is provided. Participants have access to or ability to obtain relevant health information. Results of quality to attract publication in peer reviewed journals.

APPENDIX 5: COUNTRIES RESEARCH ACTIVITIES

APPENDIX 6: SAMPLE PREPARATION AND ANALYSIS PROTOCOL

Sampling protocols

Sample	References	Sampling
Environmental inorganic samples:		
Water	Hylander et al., 2000.	Coordinates from a map.
	Sci. Total Environ.	Physico-chemical parameters in field.
	261: 9-20.	Selected samples: filter, conserve with acid.
		Polyethylene (PE) bottles, carefully marked with date, sample-ID, site, depth
		Keep cool until analyses.
Sediment	Hylander et al., 2000.	Coordinates from a map or GPS.
	Sci. Total Environ.	75 mm diameter core sampler.
	260: 97-107.	Sample 2-cm different horizons.
		PE-bags, carefully marked with date, sample-ID, site, depth
		Deepfreeze until further treatment.
Soil	Hylander et al., 2000.	Coordinates from a map or GPS.
	Sci. Total Environ.	Dig a pit and not carefully all observations.
	260: 97-107.	Sample different horizons.
		PE-bags, carefully marked with date, sample-ID, site, depth
		Deepfreeze or store dry until further treatment.
Air		Not actual
Food		
Staple food/rice		Representative samples, PE-bags. Keep dry.
Fish	Hylander et al., 2000.	Coordinates of sampling site from a map or GPS
	Sci. Total Environ.	At least 5 individuals of each species. Note length and weight
	261: 9-20.	Deepfreeze.
Human biological		
Real time monitoring		
Blood		Preferable morning before eating.
		5 mL with syringe, add anticoagulent.
		Vaccutainer tubes, store cool 4-5 ^{o}C, max. 6 hours
Urine		One occasion, discard the first 5-10 mL.
		PE-bottles
		Keep cool until analyses.
Breast milk	Parr et al., 1991.	Empty completely one breast at noon, while the kid is suckling the other.
	Biol. Trace Element Res.	Discard first mL. Sample 5-15 mL. Give the rest to the kid.
	29: 51-75.	PE-tubes, deepfreeze, -20^{o}C
Saliva		Not actual
Long time monitoring		
Placenta	Iyengar & Rapp, 2001	Entire placenta
	Sci. Tot. Environ.	PE-bags, deepfreeze -20^{o}C
	280: 195-206.	
Hair	TEC DOC of IAEA, 1993.	Back skull
		0.5 cm from skull
		5 cm long
		PE-bags
Nails		Not actual

Analysis protocols	
Sample	***Analyses***
Environmental inorganic samples:	
Water	Analyze water according to actual IAEA protocols.
	Analyze non-digested water samples. Digest and analyze used filters including blank filters.
Sediment	Dry at maximum 50^{o}C (important for Hg analyses).
	Dry a parallel sample for dry weight correction.
	Digest (aqua regia, lithium borate, HF + HClO4) and analyze filtrate including blanks.
	Analyze not digested samples.
Soil	Dry at maximum 50^{o}C (important for Hg analyses).
	Dry a parallel sample for dry weight correction.
	Digest (aqua regia, lithium borate, HF + HClO4) and analyze filtrate including blanks.
	Analyze not digested samples with nuclear-related techniques.
Food	
Staple food/rice	Wash eventually with double-distilled water and dry at 50^{o}C.
	Dry a parallel sample for dry weight correction.
	Digest (áqua regia or conc. HNO_3) and analyze filtrate including blanks/
	Analyze not digested samples with nuclear-related techniques.
Fish	Digest with acids without preceding drying and analyze
Human biological	
Blood	Digest with acids and analyze.
	In parallel sample, analyze hemoglobin and C-reactive protein (CRP, which indicates infection.
	Positive samples are discarded).
Urine	Digest with acids and analyze.
	In parallel sample, analyze creatinine and standardize element to content of creatinine.
	Filtration may be necessary for certain purposes.
Breast milk	Digest with acids and analyze/ Liophylize if necessary according to analyzing protocol
Placenta	Thawing and wash with bidets, water to remove excess blood.
	If preferable, take out a representative sample with a punch (of e.g. teflon).
	Freeze-drying (or drying at 50^{o}C if not possible to freeze-dry).
	Homogenize and analyze as a powder or analyze after digestion with acids.
Hair	Wash with EDTA (0.5 - 1%) and bide stilled water (ev. ethanol or acetone)
	Digest with acids and analyze / Analyze of solids.

APPENDIX 7 - GUIDELINES TO DATA COLLECTION

Schedule Title should include:

- Project Title:
- Name of the Institute:
- Name of the sponsoring Agency – IAEA:

1. IDENTIFICATION DETAILS

Schedule No. Area Code Location:
Name: Address: Date of Birth: Place of Birth: Duration of stay in present area:Yrs.

** Environmental Condition*
01 Industrial; 02 Non-Industrial; 03 Urban; 04 Rural; 05 Remote Rural; 06Any Other Specify

2. SOCIAL STATUS:

Social Status:
Family Size:

No.	Age	Sex	Literacy	Occupation	Duration stay
1					

Housing Condition:

Source of drinking water: (01) Tap water (02) Lake (03) Open well (04) Tube well (06) River (07) Any other

Sewerage system: (01) Open drainage (02) Under ground (03) Centralized (04) Any other

***** (To be modified according to local requirement of std. guidelines.
Economic status

A) Agriculture	(01) Self farming	(02) Labour	(03) Lease
B) Industrial	(01) Own	(02) Labour	(03) Executive
C) Self Emblaye	(01) Street Vendor	(02) Carpenter	(03) etc.
D) Salary/Wage Employment		(01) Government	(02) Private

3. ECONOMIC CATEGORY

01 Low income group; 02 High income group; 03 Middle income group

Assessment to be according to the local indicators

4. PERSONAL HABITS

Personal Habits (of the subject included in the study); Dietary habits (Food habits; Staple diet)

No.	Habit	Status: yes/no	Duration:yrs	Quantity/day
1	Smoking			
2	Drug abuse			
3	Tobacco chewing			
4	Alcohol			

5. FAMILY DISEASES PROFILE

Family Diseases profile (only (01) chronic / (02) genetic / (03) occupational

1) Type of disease Duration; 2) Any medication (last 6 months)

6. NUTRITIONAL STATUS

Age (yrs); Height (cm); weight (kgs); BMI; Hemoglobin level.

7. PHYSICAL / CLINICAL STATUS

Physical / Clinical Status (Physician to assess – **optional**)

No.	Physical	Status: yes/no	Duration	Any medications	Remark
1	Skin changes				
2	Hair changes				
3	Any other abnormality				

Blood pressure (sitting posture); Pregnant woman; Gravida / Para;
Obst. history (01) abortion no. (02) Still birth

Delivery no's	Normal	Forceps	Caesarian

Weight (first trisemister); Weight before delivery; Weight after 24 hours delivery; Neonatal weight (after 24 hours); Neonatal height (after 24 hours)

8. LABORATORY INVESTIGATIONS:

(01) Hemoglobin; (02) Blood microscopy; (03) Urine qualitative

9. SIGNS AND SYMPTOMS:

No.	**Signs / Symptoms**	**Status yes / no**	**Duration**
1	Headache		
2	Vomiting		
3	Pyrexia		
4	Constipation		
5	Anorexia		
6	Colic pain		
7	Fine tremors		
8	Frequent fractures		
9	Arthritis		
10	Anemia		
11	Others – specify		
	Advance symptoms		
12	Gum line		
13	Ataxic gate		
14	Abnormal coordination		

APPENDIX 8: PROCEDURE FOR STATISTICAL EVALUATION

Data Input Spreadsheet Example

Sample	**Analyst**	**Method**	**Batch**	**Date (YYMM)**	**Analyte**	**Unit**	**Value**	**RSD %**	**ash/wet %**	**Dry Weight (g)**	**Wet Weight (g)**	**Additional Information**
												Location
												Age, Wt, Ht
												Disease
												etc…..