IN SEARCH OF AN ANTIDOTE FOR A POISONED WORLD

AN INAUGURAL LECTURE,
2015/2016

JOHN IBHAGBEMIEN ANETOR

UNIVERSITY OF IBADAN

IN SEARCH OF AN ANTIDOTE FOR A POISONED WORLD

An inaugural lecture delivered at the University of Ibadan

on Thursday, 01 September, 2016

By

JOHN IBHAGBEMIEN ANETOR
Professor of Chemical Pathology and Toxicology
Faculty of Basic Medical Sciences
University of Ibadan
Ibadan, Nigeria

UNIVERSITY OF IBADAN

The Vice-Chancellor, Deputy Vice-Chancellor (Administration), Deputy Vice-Chancellor (Academic), Registrar, Librarian, Provost of the College of Medicine, Dean of the Faculty of Basic Medical Sciences, Dean of the Postgraduate School, Deans of other Faculties and of Students, Directors of Institutes, Distinguished Ladies and Gentlemen.

QUOTES

Does the dose alone determine the poison?
What is there that is not poison?
All things are poison and nothing (is) without poison.
Solely the dose determines that a thing is not poison. Paracelsus (1493-1541)

We are poisoning the air over our cities, we are poisoning the rivers and the seas; we are poisoning the soil itself. Some of this may be inevitable. But if we do not get together in a real and mighty effort to stop these attacks upon Mother Earth, wherever possible, we may find ourselves one day- one day soon, may be- in a world that will be only a desert full of plastic, concrete and electronic robots. In that world there will be no more, nature', in that world man and a few domestic animals will be the only living creatures.

And yet, man cannot live without some measure of contact with nature. It is essential to his happiness.

—Prince Bernard of the Netherlands.

In publishing this book we wish to contribute our share in reviving the personality of an honest man who was a great physician and a staunch fighter for what he considered the truth. It is so easy to be orthodox and to reap honors by repeating what people expect and wish to hear. Progress,

however, is achieved through the clash of ideas, and heretics like Paracelsus are a ferment without which there would be no life"

—Henry E. Sigerist [In the preface to his book *Paracelsus, Four Treatise 1958*].

Preamble

It gives me great pleasure to stand before this distinguished and august audience to give this lecture. It is God who truly takes all the glory as we will come to see and appreciate. It is He who had preordained everything as I have come to realize in my life and that I am just stepping into the sketched pathway. I thank the Vice-Chancellor for this opportunity. By my calculation I would have been on my way out before it gets to my turn. I also thank my Dean, Professor Olusegun George Ademowo for nominating me to give the lecture.

I do not know how it all started but I just knew that I had this love and reverence for scholars and scholarship in whatever field and yearned to be one. I am glad that God made me one. God used my mother and her relations to ensure that my dream is realized. As king James the First observed, 'were I not a king I would be a university man' (James 1606). I am not a king but I thank God that He made me a university man.

The Department of Chemical Pathology, University of Ibadan

Chemical pathology is one of the foundation departments of the then Faculty of Medicine which has grown into the College of Medicine. The department is organized into five main units as follow:

- Endocrine and Metabolism Unit
- Nutrition and Lipid Metabolism Unit
- Immunology Unit (soon to be a full Department of Immunology)
- Toxicology (and Micronutrient Metabolism) Unit (previously Trace Element Metabolism Unit)
- Reproductive Endocrinology Unit

This is the 5th inaugural lecture coming from the Department of Chemical Pathology. The first was given by Professor David Olatunbosun, MD, from the Toxicology and Micronutrient Metabolism Unit, followed by Professor Babatunde Osotimehin, MD from the Reproductive Endocrinology Unit; the third was from Professor Emmanuel O. Agbedana, Ph.D from the Nutrition and Lipid Metabolism Unit. The fourth was given by Professor Francis A.A. Adeniyi, Ph.D from the Toxicology and Micronutrient Metabolism Unit and the fifth is today being delivered by me from the same Toxicology and Micronutrient Metabolism Unit.

Mr. Vice-Chancellor, Sir, the broad lay out of the lecture shall be as follows:

- Brief overview of my journey into medical science
- Today's world as a chemical habitat
- Unearthing the combat between chemicals of life and the chemicals of prosperity
- Research in the Laboratory for Toxicology and Micronutrient Metabolism (describing my contribution; highlighting:
    - overthrow of the chemicals of life by the chemicals of prosperity, disease and death.
    - toxic responses/consequences and lamentation
    - traditional roles of a chemical pathologist
    - a new remit for the contemporary chemical pathologist
    - mechanistic search for an antidote for a poisoned and hungry (susceptible) world
    - the antioxidant hypothesis and the antioxidant controversy
- Concluding remarks (today's toxicity of the academic environment)
- Recommendations
- Acknowledgements

To know what you prefer, instead of humbly saying Amen to what the worold tells you ought to prefer, is to have kept your soul alive

—*Robert L. Stevenson*

The Annunciation: Journey into Medical Science and Chemical Pathology

Just before my school certificate examination, an uncle of mine, indeed, my most senior uncle, Late Mr. Henry Eromosele Eimunjeze who was one of the foundation staff of the Chemistry Department in the then Midwest Institute of Technology (M.I.T.) which metamorphosed to University of Benin, Benin City, mentioned to me of a 4-year course called Medical Laboratory Technology at the time. He said it was a very good and promising discipline that he wanted a member of the family good in science to take advantage of it. He promised to assist me with admission if I accepted to study the course. During the A-Level Science Course at the Faculty of Science, University of Ibadan, I did not forget what my senior uncle told me. Coincidentally, I came across an advertisement for admission into the programme at the University College Hospital, (UCH), and Ibadan leading to the Associate Membership of the Institute of Medical Laboratory Technology (AIMLT). I told my younger uncle, Uncle Vincent what uncle Henry told me but he did not show any interest. All he said was that he was told that one had to know the 'big professors in UCH to get admission'. He promised to contact his friend who said he had no power to help. All the same I went ahead and applied and continued with my A/Level studies in Biology, Chemistry and Physics. I wrote so many letters to my senior uncle, at Uniben, he never replied. One day he visited Ibadan and told me that he had changed his mind, that I should go and read medicine or any of the biological sciences as he did not want 'a bright boy like me to be frustrated'. He told me of how Late Professor Ambrose Alli started with the course and had to change to medicine as well as many of his former class mates at Edo College, Benin. I went ahead and sat for the examination.

About 300 of us must have sat for the examination at Paul Hendricks's Lecture Hall at the then Faculty of Medicine, UCH in 1976. I was not hopeful as I was told that they will take only ten out of the three hundred of us. Surprisingly, I was invited for the interview. I had to look for information about the course and the practitioners. Those days we used to spend almost all day on Saturdays at the British Council Library, Dugbe if we did not have classes at the university in our A/L programme. It was while I was in the library, I think I must have been on my way to the toilet when my eyes caught a book, 'Ask The Lab: Introduction to the Profession of Medical Laboratory Technology'. I cannot remember the author but one thing shocked me, why is it that I was coming across the book now that I am preparing for the interview. I thanked God, read the book which introduced me to the profession, the various branches and their functions—Chemical Pathology, Haematology and Blood Transfusion, Histopathology, Microbiology, Parasitology and Virology, not too sure about the latter. There and then I made up my mind that were I to be given admission, I would specialize in Chemical Pathology; laden with chemistry (my best and beloved subject) and biochemistry. I went for the interview, I knew I did very well, I still recall that one of the people who interviewed me was Emeritus Professor Grace Olaniyan-Taylor, FAS, who later turned out to be my supervisor for my M.Sc. and Co-Supervisor of my Ph.D and has remained a mother ever since. Before I got close to her, I used to admire her elegance and comportment from a distance as an undergraduate student. I am glad I had the opportunity to be mentored by her.

Medical Laboratory Science

Medical laboratory science refers to a defined field of study related to the clinical investigation of disease. It may be formally defined as the application of basic sciences, especially the biological sciences to the study of medicine, in particular, the causes, consequences, diagnosis, and treatment of human diseases. It is called by different names in different

countries, from medical technology to clinical laboratory science in America and Canada, Biomedical Science in the United Kingdom, Clinical Biology in France, Medical Science in Australia and New Zealand. Thus, the practitioners of medical laboratory science are scientifically qualified professionals who conduct investigations on specimens removed from humans and animals largely for the diagnosis, treatment or prevention of disease (Glencross et al. 2011). They are relatively unknown because they are typically active in more laboratory-based work and tend to have relatively limited contact with patients compared with other healthcare professionals. Scientists as we know need serene noiseless environment to do their work.

As will be clearly evident medical laboratory science is very closely related to pathology, in which they have very strong foundation. Pathology may basically be defined as the science of disease. It is a discipline bridging clinical practice and basic science. The scientific focus of pathology is on the cause (aetiology) of disease, the mechanism of its development (pathogenesis) and pathways by which biochemical and morphological changes (alterations) occur. These fundamental events occur at the molecular and cellular levels and it is there that our studies commence. Medical laboratory science and pathology therefore are at least first cousins.

The core of the training in medical laboratory science revolves around biology and all applied forms, chemistry, pathobiology of human disease and analytical methods. This gives rise to the broad specialties as follow:

The broad specialties of medical laboratory science are:

- Chemical pathology or Clinical Biochemistry- involving the study of chemicals, biochemicals and biochemical mechanisms, and their imbalances within the body how this gives rise to disease. Clinical biochemistry is about patients, how they are investigated for their symptoms, diagnosed and how they are treated. Further elaboration follows below.

- Haematology here often combined with blood transfusion science, largely the study of the morphology and physiology of blood related diseases. The blood transfusion component involves studying blood and blood products to ensure they are fit for a needing recipient or patient.
- Medical Microbiology is concerned with the detection, isolation and identification of micro-organisms, including bacteria, fungi that cause disease and treatment of diagnosed disease.
- Histopathology, this involves use of the microscopic changes in the study of tissues to identify disease that may be present. This is almost all, the only discipline the public knows about pathology and medical laboratory science. They say they deal with the dead, which is not completely true; we actually deal more with the living than the dead.
- Immunology – involves study of the functioning of the immune system of the body, or its responses to disease with an overall goal of defending or protecting the the host. It is known as the unifying science among all the laboratory science or medicine discipline; studied in all the branches.
- Virology – involved in the detection, isolation and identification of viruses and associated diseases.
- I will now briefly elaborate on chemical pathology, a discipline I define as my life after my catholic faith and my family.

Chemical Pathology: Traditional Role of the Chemical Pathologist

Chemical pathology also variously known as clinical chemistry, clinical biochemistry, hospital biochemistry, biochemical medicine or medical biochemistry (Canada) was proposed by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) as "the study of the chemical aspects of human life in health and illness and

the application of chemical laboratory methods to the diagnosis, control of treatment and prevention of disease" (Sans and Louis 1971). Chemical pathology is a fundamental science when it seeks to understand the physiological and biochemical processes operative in both health (normal state) and disease state. Chemical pathology becomes an applied science when analyses are conducted on various body constituents such as fluids, (blood, plasma, serum) and tissue specimens in order to provide useful information for clinical decisions in the diagnosis and treatment of various disorders. Sound knowledge of the working of the human body in healthy state or normal condition is essential for understanding of changes that may occur in abnormal or pathologic states.

The major responsibility of the chemical pathology laboratory is the assessment of chemical changes in the body for purposes of diagnosis, therapy and prognosis (forecast) of disease. The key duty of clinical laboratory scientists who do the routine measurements consists in analyses of various chemical constituents in blood, urine and other fluids or tissues. They provide scientific data for sound clinical decision and appropriate management or treatment of disease by the clinician. Scientists and Clinicians work closely together here as in the other disciplines, with the latter focusing on the more clinical responsibilities and as liaison with physicians and surgeons from other clinical departments. A brief background of what is normal to understand abnormalities is probably appropriate.

Chemical Composition in Normal (Healthy) State

Normally, the concentrations of the chemical constituents are relatively constant, but in disease states the levels become altered, the magnitude of changes usually paralleling the degree of disease. In advanced disease states, the alterations or abnormalities are easily detected and present not too much of an analytical challenge to the laboratory specialist. Early detection of organ dysfunction is much more difficult;

however, as the chemical changes are usually minor or slight and have to be differentiated from possible errors in the analytical aspect of the investigations (Kaplan and Szabo 1979). This current view may not be too different from the charter Paracelsus of Hohenheim gave scientific medicine in his acclaimed hypothesis: 'The human body is a conglomeration of "chymical" matters, when these are deranged illness results, and naught but chymical medicines may cure the same' (Gray 2001). This essentially captures the remit of all chemical pathologists or clinical biochemists and particularly this investigator who has set himself the responsibility of advancing the mitigation of toxicity or poisoning through the mediation of the micronutrients to restore altered physiology that leads to disease, in line with Paracelsian doctrine.

Accuracy is very critical to the work in chemical pathology for the proper interpretation of laboratory tests. Scientists in chemical pathology indeed laboratory medicine generally, must always remember that laboratory specimens are taken from humans with a real or potential health problem and that the scientist is an important part of a highly skilled team that contributes to an evaluation of a patient's condition. As a former president of the Institute of Biomedical Science of the United Kingdom, Graham Smart once admonished, "In our pursuit of science and academic excellence, our principal consideration is that the final beneficiary must be the patient. This patient oriented ethos is the touch stone of our profession."

Lectureship Position in the Department of Chemical Pathology

My appointment as a staff of the Department of Chemical Pathology came as a pleasant surprise and like sweet and sour event. As I had always considered my sojourn in IITA, where I spent close to one and a half decades as education and training, for indeed that essentially was what it was for me. After the defence of my Ph.D thesis, I thought it was time to go home. I then applied for a lectureship position at the

Chemical Pathology Department, University of Benin. The then head of department, Professor Israel O. Oforofuo, who is both a product of the University College Hospital (UCH) and the University of London, came to inquire from his colleagues in Ibadan, Professors Taylor, Salimonu and others, if I were a good person that he could employ. I was told they immediately told him that they will not release me to him and immediately put in motion the process of my employment. Then, I was contacted to submit my CV immediately that was how I got to the University of Ibadan as a lecturer. I did not apply to U.I. from the beginning because when I saw the line up, I thought there was no space and from what I heard from others who graduated before me. Though I was later tempted with a Lecturer I position from Uniben from the back door.

I am very grateful to all my teachers who considered me worthy to be employed in the department. I called it sweet and sour because at the time I was offered the appointment I was already a manager in International Institute for Tropical Agriculture (IITA) on a monthly salary of about ₦36,000.00 aside from some bulk payment at the beginning of the year. Now I was offered a position of lecturer II on a salary of about ₦8,000.00 +. A professor's salary at this time was about ₦12,000.00. Though, I had always wanted to be an academic, at the time it came, the circumstances made it difficult to make a decision immediately. That was when I learnt to say a prayer, which I cannot remember how it started, 'God if it is not your will put an obstacle'. I then accepted the position. Here I must say I am very grateful to late Fr. (Professor) Louis Munoz, he was one of the very few who asked me to accept without doubt and leave the rest to God. That turned out to be prophetic. All other, friends, relations etc. thought I was committing a career suicide. Just like all my class mates at Ughelli and many of my relations felt when I was accepting the offer of admission to study Medical Laboratory Science. The only personal safety valve I had in place was that if it became too rough I would emigrate. I thank God that till now there has been no cause for this. God has been very faithful.

For the Love of Chemistry

I must talk about my love for chemistry which has remained unquenchable. Probably if there were nothing chemical or chemistry in medical laboratory science or chemical pathology I would have gone into some other fields.

I do not know how the love of chemistry or anything chemical science grew in me, but I can trace it to the day some booksellers came to introduce some science books to my class in Form two in 1971. Two books were introduced to us by our general science teacher authored by the Oyewoles (Dotun and Philip) I think from Abeokuta Grammar School; 'Introduction to Chemistry (yellow in colour) and 'Introduction to Physics' (red in colour). I cannot remember the full details, they were relatively expensive at the time, I think it was fifteen Shillings each. I got home told my mother we were asked to buy the books, she asked how much, I thought she would postponed or tell me in a week or two, or out rightly no money, instead she went to her purse and handed me the money for both books. I bought the books and found that I never wanted to put them down, particularly the one on chemistry. This is why I think whatever I have become today in the chemical sciences, I owe to my mother and the Oyewoles for the foundation they laid with their books.

Till now I love chemistry and I have only come across a few people with such love, one key one is Linus Pauling, two times Nobel Laureate and Dr. Leon, Goldberg (1915-1987). As Linus Pauling is so well known, I will focus on Dr. Goldberg. I found that his knowledge of chemistry and enthusiasm for its application to human health issues was like mine, the hallmark of his career. He obtained B.Sc. (Honors) chemistry from the University of Witwatersrand, Johannesburg, South Africa, another B.Sc. degree in mathematics, M.Sc. in Physical Chemistry, D.Sc. degree in biochemistry, from the same university ,and D.Phil. from Oxford in organic chemistry, M.A. anatomy and physiology from University of Cambridge, the Medical Bachelor and Bachelor of Medicine (M.B.B.S.) degrees from University College Hospital, (UCH) Medical School, London and an

honorary D.Sc. Degree in Pharmacy and Science from the Philadelphia College of Pharmacy and Science in Philadelphia, Pennsylvania. Goldberg contributed to the award of Nobel Prize in Chemistry to Robinson in 1947; for their study on the synthesis of stilbosterol. Goldberg return to South Africa to lecture in chemistry at his almamater (Wits) and became head of Biochemical Research Laboratory at the South African Institute for Medical Research. His publications focused on medical issues and the linkage between chemicals and health. My research focus as well. Goldberg later accepted a position as senior lecturer in chemical pathology in the Department of Pathology at the University of Manchester in England, and later became the Medical Research Director of Benger Laboratories Ltd, in England. His work here centered on the role of administered iron in health and induced disease, galactosaemia, (IBEM) and lipid and cholesterol metabolism. I adopted him as my academic mentor. He emphasized understanding of the mechanisms of toxicity of chemicals throughout his career.

Later he became the founding Director of the British Industrial Biological Research Association (BIBRA) in London. This new organization, a joint industry government effort was created to investigate mechanisms underlying toxic effect of chemicals, develop new improved toxicity tests and give advice and information on toxicological issues. The organization (BIBRA) was intended to be an impartial forum for improved communication between industry, government and academic personnel in addressing toxicological issues of major public health significance, issues that were emerging with increasing frequency and consciousness as we have it in Nigeria today. Later he focused on how science could address societal issues (McClellan 2011). Leon Goldberg was President of the Society of Toxicology (SOT), 1978-1979. The reason for this elaboration will be clear later. Leon Goldberg's broadly charts my career roughly and remains a reference point to me in toxicology, my sub-specialty just like Linus Pauling who is also interested in micronutrients like me (recall mega-vitaminosis C, vitamin C and the common cold).

The Field of Toxicology and Micronutrient Metabolism

My sub-specialization in this area arose from reading the chapter on analysis of drugs and toxic substances in the *Fundamentals of Clinical Chemistry* by Nobert Tietz (editor), the bible for all those majoring in Chemical Pathology in the medical laboratory science programme. I found that this chapter, and alteration of the haem biosynthetic pathway by lead, made special impact on me. Then I again (hand of God) came across an advertisement in a journal—I think it was *Journal of Clinical Pathology*, later *New Scientist*—on M.Sc. Degree Programme in 'Experimental Pathology (Toxicology) at the Royal Postgraduate Medical School at Hammersmith, University of London. I applied and was given admission three times; I kept deferring, looking for scholarship, which I did not succeed in obtaining. In 1986, I was advised to apply for the Overseas Development Administration Shared Scholarship scheme (ODASS). Was assured once the school sends my name I would be awarded the scholarship. Since a classmate with lower grade got it I was very confident I would get it, having been adjudged the best and got the prize for the' Best Academic Student' in my set. I received all instructions, room allocation reading list everything except the award letter. This has been the most anxious and stressful period in my life. By the end of September 1986, I wrote and a reply came after about three weeks saying we did not send your name. No explanation was given up till date. I then embarked on the Fellowship programme in Chemical Pathology by thesis. I approached then Dr. F.A.A. (now Professor) Adeniyi as supervisor, he kindly consented and knowing my previous attempts to study toxicology advised that I work on cadmium (Cd).

The Field of Toxicology

Toxicology is the scientific study of poisons and poisoning. It has been defined by the Society of Toxicology (SOT) of the United States as "the study of the adverse effects of chemicals, physical or biological agents on living organisms

and the ecosystem, including the prevention and amelioration of such adverse effects" (*www.toxicology.org*). It is applicable to many areas, including the industrial, agricultural, veterinary, environmental, forensic and medical fields. In chemical pathology we employ the term toxicology to imply identification of the concentrations of poisons or biomarkers of their effects in body fluids, tissues and other materials. It is important to understand why toxicological investigations are conducted and the way results are used, how poisons occur, how a diagnosis of poisoning is made and how poisoning is treated. Poisoning itself may be considered as a medical condition caused by a substance (usually a chemical) that is not usually present in the body such as methanol or is present at a much higher concentration than usual e.g. iron (Fe).

Woolley (2008) has observed that we live in a chemical world, that we are composed of chemicals, that an increasing number of chemicals found in our bodies are persistent man-made chemicals such as polychlorobiphenyls (PCBs), bisphenol A or organochlorine pesticides like DDT and that there is increasing need to emphasise safety. He noted that the major concerns of the public that toxicologists must address are cancer, loss of senses embracing neurotoxicity, general debilitation (susceptibility to illness), reproductive defects (teratogenicity) or shortened life span. These have received the attention of this investigator as will be evident during the course of the lecture.

Toxicology today is generally recognized by the lay people as the science of poisons and poisoning. It is tempting not to overlook the recent observation of Lane (2014), that 'history is full of bad intentions, ignorance and folly. Poisoning has been part of each of these for as long as humans have lived. Understanding poisons- to control them or avoid them- has therefore been an integral part of our past. As understanding of poisons progressed, a unique field of knowledge grew into the science of toxicology. As toxicology moved along this path, it evolved from harming people to helping them by protecting them from the adverse effects of

chemical exposure at home, in the environment and in the work place.'

A toxicologist is trained to examine and communicate the nature of the adverse effects of chemical and physical agents on human, animal and environmental health. Toxicological research examines the cellular, biochemical and molecular mechanisms of action as well as functional effects such as neurobehavioural and immunological, and assesses the probability of their occurrence. A fundamental aspect of this is characterizing the relation of exposure (dose) to the response. This leads to risk assessment.

Risk assessment is the quantitative estimate of the potential effects on human health and environmental significance of various types of chemical exposures that may involve pesticide residues on food, contaminants in drinking water as very recently seen in Flint, Michigan in the United States, where public water supply was heavily contaminated with lead. Others have been mercury contamination of fish in a bay in Kyushu, Japan; Minamata Disease and cadmium in the food chain also in Japan, itai itai byo (ouch ouch disease) in the Fuchu Toyama prefecture in Japan.

Risk Estimate

The risk of some agent or event can be estimated as a function of the product of probability (P) of the event and the severity of the harmfulness of the event or agent (H):

$$\mathbf{R = P \times H}$$ (Campbell 1980)

From the simplest view point from Campbell's thesis above, the risk taker may accept harm of greater severity (high value of H) only if the probability of occurrence (P) is very low. Conversely, events that are only modestly harmful (low value of H) may be acceptable at higher levels of frequency or probability. From this argument, safety may be taken as a measure of acceptability of some degree of risk.

Scientific risk estimation should be carried out with the full knowledge of the action of a toxicant e.g. a carcinogenic agent as either a complete carcinogen, or as having a major

action at one or more of the stages of carcinogenesis. Most of the data in environmental toxicology particularly affecting environmental chemicals and poisoning were obtained by bioassay. The extrapolation of bioassay data to human risk estimation is one of the most difficult problems that has faced society and its scientists and will face us for years to come as numerous new chemicals enter the environment. To predict the behaviour of a chemical in the human from data obtained from bioassay is fraught with a number of difficulties. Knowledge of the action of an agent with regard to toxicity and its modulating factors are important in toxicology and are very useful in mitigating toxic states or poisoning.

The variety of potential adverse effects and diversity of chemicals in the environment make toxicology a broad science and very important science for society. Our society's dependence on chemicals and the need to assess potential hazards have made toxicologists an increasingly important part of the decision-making process (Klaassen 2008).

Toxicology has developed as a science from the study of chemicals to induce harm (the art of poisoning) to a study of chemicals in order to prevent harm (the science of preventing poisoning; antidote) and benefit to humankind' my thrust in the science. Starting from the art of poisoning, supplying and using poisons, the toxicologist now studies their mechanisms of action, develop analytical methods to identify and quantify poisons in body fluids and tissues, develops rational antidotes, establishes safe limits of exposure from carefully designed and executed studies, and quantifies and predicts adverse effects' (Lane 2014).

Toxic Effects

Toxic effects develop from perturbation at the molecular level progressing to altered cellular function, eventually giving rise to disturbances of tissue or organ function with resulting influence on homeostasis that may range from mild discomfort to death. Unfortunately, it is only the later that many accept as toxic effects that may delay seeking assistance or remedy.

Cyanide toxicity for instance occurs as follows—cyanide causes tissue hypoxia by binding to Fe in cytochrome c reductase and inhibits electron transfer. Pesticides are chemicals used to kill pests and include organophosphate insecticides and herbicides they inhibit cholinesterase while Paraquat causes poisoning by leading to oxidative stress.

Toxic Responses to Chemical Poisons by the Organism

In discussing toxic responses to a toxic substance it is important to bear in mind the concept of toxicity which involves a damaging, noxious or deleterious effect on the whole or part of a living system, which may or may not be reversible. This is important as many people only understand poisoning to death. They do not recognize adverse effect on part of a living system as a toxic effect.

A number of ways exist for an organism to respond to chemical agents. The response may be dependent on a number of factors. Though many of the toxic effects of toxic agents (xenobiotics) have a biochemical basis, expression of the effect may be very different (Timbrell 2009). Tumour development may be a consequence of attack on nucleic acids, while the other might be the birth of an abnormal offspring as was classically observed from thalidomide toxicity (to be reviewed shortly). The interaction between toxic agents with normal metabolic processes may cause a physiological response such as muscle paralysis or a decrease in haemoglobin concentration, reduced glutathione (GSH) level or a tissue lesion in one organ. A covalent interaction between a xenobiotic and a normal body protein may lead to an immunological response while it may manifest in another organism or population as tissue lesion such as kidney damage (toxic nephropathy as in cadmium or inorganic mercury poisoning).

Though toxic response may have an underlying biochemical basis, they are commonly categorized according to the manifestation of the toxic effect. Although there is recognized overlap between some of the toxic responses; for the purpose of this lecture it is convenient to list them as shown in table 1 below:

Table 1: Types of Toxic Responses to Xenobiotics

1.	Direct toxic action: tissue lesions
2.	Pharmacological, physiological and biochemical effects
3.	Teratogenesis
4.	Immunotoxicity
5.	Mutagenesis
6.	Carcinogenesis

Source: Timbrell (2009)

Detection/Evaluation of Toxic Responses

Toxic responses may be detected or evaluated in a variety of ways in an organism or population. These will be illustrated in some of the studies that will be reported shortly from the investigator's laboratory. Typically a toxic response may be the all –or- none type that manifests as the death of the organism or expressed as graded responses. These are briefly outlined here as described by Timbrell (2009).

Death: This was previously usually determined by the lethal dose 50 (LD_{50}) the concentration of a toxic compound that kills 50% of the population. This has falling into disuse and has been largely replaced by other bioindicators of toxicity or poisoning.

Pathological Change: This may be expressed as tumours or destruction of tissue as will again be evident from some of our studies particularly with Pb.

Biochemical Change: This was widely used in some of the studies reported here and might involve inhibition or induction of an enzyme or alteration in a particular metabolic pathway as classically seen in the haem biosynthetic pathway altered in exposure to lead. It may also involve the appearance of an enzyme or other markers that may indicate leakage from a tissue or an organ due to damage as with the transaminases (ALT and AST) in liver damage as is typically seen in Paracetamol toxicity accompanied by increase in N-acetyl benzoquinone imine (NABQUI) a toxic metabolite of Paracetamol which are indicative of pathological change or

process. We employed this in our study of carbon tetrachloride induced hepatotoxicity and its amelioration by the extract of veronia amydgalina (Babalola, Anetor and Adeniyi 2001).

Physiological Change: This is usually a simple measure in the whole conscious organism or individual and may include change in blood pressure, body temperature or rate of respiration or level of consciousness (Timbrell 2009).

Alteration or Change in Normal Status: A number of markers of toxicity abound which are simple to perform and reflective of toxic response. Changes in body weight or other anthropometric indices, food and water consumption, urine output, changes in organ weight may all be sensitive indicators of general or specific toxicity. Many studies including ours (Anetor, Wanibuchi, Wei, Kakehsshi, Kang and Fukushima 2008) indicate that experimental models consume less food and lose weight after exposure to a toxicant, while increased organ weight may be an indication of a developing tumour, fluid or lipid (triglyceride) accumulation, hypertrophy or enzyme induction that are commonly seen in experimental pathology or toxicology. These changes may have to be confirmed by the toxicologist by chemical, biochemical or histological examinations. Toxicology is a dynamic subject with great importance to the public and places heavy responsibility on practitioners; or those who register or accredit toxicologists. Getting a decision wrong in toxicology can be associated with very serious adverse effects with consequent litigation (as in thalidomide catastrope, no cases were seen in those living in the U.S. because of a sharp scientist) and could lead to loss of vote in politics as has been seen with global warming. Consequently as toxicologists, we should do nothing without thought, without considering the impact of our actions on the community we serve or the public placing thrust in us. Toxic responses may be modulated by nutritional means. This is where the almost dogmatic observation by the father of modern toxicology, Paracelsus that the dose solely determines the poison in my opinion needs revision.

Nutritional Medicine and Chemical Exposure

Requirements for essential nutrients are recognized to vary from individual to individual depending on genetic, physiological, life style and other influences, perhaps chemical exposure (Anetor 1997). What is adequate for one person may not be for another, probably depending on chemical exposure. Illness is inevitably linked with abnormal biochemistry and an alteration in the metabolism of nutrients and their by products. Specific nutrients, particularly micronutrients and macrominerals, dietary manipulation provide a potent means of influencing body chemistry and consequently disease process. By correcting fundamental abnormalities, including altered chemical composition, one can prevent certain diseases including chemical-induced disease or alter the course of the disease for the better (better prognosis) (Tolonen 1990). Toxic oxygen species (free radicals) are common intermediates generated in altered body composition.

Toxic Oxygen Species (TOS)

Toxic oxygen species are also known as free radicals or reactive oxygen species (ROS) or reactive nitrogen species (RNS), they are chemical species with one or more unpaired electrons in their outer orbitals which make them extremely reactive and damaging to biological constituents, DNA, RNA, proteins including enzymes and antibodies, lipids, carbohydrates nucleic acids which together constitute the chemicals of life. The body produces TOS as part of the normal bodily processes (metabolism), they are essential for normal bodily function, signalling and bactericidal activities, but uncontrolled production of free radicals (excess) is damaging to the cells, tissues and organs. Free radical or toxic species are among the means by which many toxic states are elicited or mechanisms of toxicity. Toxic metals such as Pb and as demonstrated by our study in part exhibits this (Anetor and Adeniyi 2001a). When the generation of free radicals exceeds the bioavailability of the antidotes, antioxidants; imbalance between antioxidants and free radicals, a state of

oxidative stress is said to set in. Extracellular and intracellular defence systems that protect cells against free radical toxicity are of increasing importance in public health. Currently, most diseases are linked with free radicals and their antidotes, the antioxidants including metal poisoning.

Oxygen is both essential to life and poisonous: generates ROS which are chemically reactive molecules containing oxygen (Kelly 1999). Free radicals are like inner radiation, to which cellular constituents are exposed for a long time throughout life. When the protective mechanisms by anti-oxidants are lacking (weak) bodily cells become weak or may ultimately die. To again appreciate toxicology it is perhaps appropriate to look at the chemicals of life and their arrangement.

Chemical Composition and Atoms of Life

In the beginning were the chemicals of life and they ensured life. With the quest by man for prosperity, the chemicals of affluence were introduced and they displaced (over threw) the chemicals of life. This is at least in part, directly or indirectly the basis of a disproportionate number of all known pathologic states.

All human cells are composed of the same basic molecules and share the same fundamental machinery of functions such as the synthesis of protein, the means through which cells execute their functions. The cells also share a similar chemical composition, consisting of approximately, 70% water. About 95% of the mass of cells is made up just four types of atoms:

- Carbon
- Hydrogen
- Nitrogen
- Oxygen

These are linked in a variety of combinations to construct almost all of the molecules of the cell. The linking of these atoms in various different ways to form proteins and nucleic acids gives the cell its complexity. The combinations of the

basic units in different ways appear to be a major principle in the life of the cell and that of the organism- what is called life (Wolpert 2009). Inside this is a collection of molecules, what has been described as a society of molecules which carry out all cellular activities, which is achieved by the work of the proteins, the most complex and varied of the molecules. Each of the thousands of the different proteins has a unique sequence of amino acids, which determine the function of the cells and behaviour. Protein function is largely determined by their interaction with other molecules, whose behaviour or structure they alter. This is an over abridged basic chemistry of the cell that can be altered by foreign compounds, chemicals, generally called xenobiotics. In the course of this lecture I shall attempt to show how this orderly arrangement of chemicals is upturned or overthrown by the chemicals of prosperity, disease and death.

'Omnis Cellula e Cellula'

The German physician Rudolph Virchow in his publication (1895) proposed a seminal concept of life: 'Every animal is a sum of vital units, each of which possesses the characteristic of life. It follows that the composition of the major organism, the so-called individual, must be linked to the kind of social arrangement or society, in which a number of separate existences are dependent upon one another, in such way however, that each element possesses its particular activity, and although receiving the stimulus to activity from other elements carries out its task by its own power'. This is the emergence of the society of cells. Rudolph Virchow stressed that cells came from other cells and gave the well-known aphorism, omnis cellula e cellula (every cell from a pre-existing cell), which gave rise to the theory of tissue formation despite gaps in our understanding of the *mechanisms of new cell formation involving the chemicals of life.*

Some Chemicals of Life: The Metals

Chemicals are made up of different type of substances. Metals are among the most common chemicals on earth.

Metals play a significant role in biochemical processes of life. Several fold systems exist in the living organisms ensuring that biometals such as Fe, Cu, Zn, as well as Ca, Mg, and K find their way into cellular and subcellular compartments where they are needed by different transport systems. These transport systems can also be used by chemically closely related metals which elicit only toxic effects. Displacement of the chemicals of life (biologically useful) deserves scientific interest, particularly for the understanding of uptake, distribution, accumulation and disposal (excretion) of either metals (chemicals) of biological (life) or toxicological (malady) significance.

Essential Versus Toxic Chemicals

As the steps promoting the supply of essential metals (chemicals of life) can be investigated in the organism and the exposure of the organism (population) to toxic metals (chemicals of prosperity & malady) we have come to the understanding that these metals (chemicals) compete with each other. The interactions obey physico-chemical and bio-chemical laws broadly as follow:

- There can be impairment of availability (absorption).
- Transport of chemicals to target tissue can be affected (disrupted) where the essential chemical (metal) is needed and finally, the synthesis of metal proteins and metalloenzymes which are necessary for what we call 'Life'.
- Our understanding of life is limited, thus it is difficult to relate interactions between exogenous (toxic) chemicals and chemicals of life down to the molecular level.
- Need to provide more profound (sound) biochemical explanations.

Currently, we have only been able to sketch limited understanding of the complex interactions between essential metals (chemicals of life) like Cu, Fe, Zn (Essenhans et al. 1993). This is elegantly illustrated by the Fenton reaction.

The Fenton Reaction

$$H_2O_2 + Fe^{(II)} \rightarrow OH^{\cdot} + OH^{-} + Fe^{(III)}$$

The above reaction can be inhibited by caeruloplasmin.

Inhibition of the Fenton reaction by the protein, caeruloplasmin and other copper complexes is a common occurrence in toxic states as shall be evident shortly (Gutteridge 1985, 1991). This is practically seen in diseases associated with industrial activities and more of these cases shall be seen in the match towards industrialization, giving rise to diseases of prosperity.

Diseases of Prosperity

Which are the diseases of prosperity? These are the diseases associated with well-being or being prosperous, a fall out or dividend of industrial success and affluence. A few of the most well-known are highlighted below:

- Itai-itai disease (a form of cadmium-induced osteomalacia), exemplified with Cadmium induced bone disease.
- Minamata bay Disease (mercury induced neuropathy).
- Thalidomide (birth defects from consumption of a drug, Contergan® to prevent morning sickness; acts largely by inhibiting angiogenesis).
- All these have led to a cry to protect human health and the environment from the deleterious effects of chemicals.

Protection of Human Health and the Environment

Many scientists, pioneered by Rachel Carson, Osibanjo's group in our chemistry department in this university; recall his inaugural lecture in 2009; 'Giving the Earth a Future: Chemicals, Wastes and Pollution Risk Factor', have continued to call for protection of human life and the environment. Details of the evergreen efforts made by Rachel Carson follow shortly. The Pope has also recently joined this call in his encyclical, Laudato Si' (Our Common Home) (Pope Francis 2015).

Today's World a Chemical Habitat

Rachel Carson was one of the very earliest environemtnalist to recognize what is evident today, that the world is a chemical habitat. She observed the ominous trend at the time and remarked that 'intoxicated with a sense of his own power', mankind appeared to be going farther and farther into more advanced chemical production for the destruction of himself and his world. This situation remains true today if not worse. Rachel tried repeatedly to stimulate the interest of people with influence in society to no avail of the alarming evidence of environmental damage from widespread use of the new synthetic chemicals and other long lasting pesticides needed to improve food yield but consuming the would be consumers. This appears to be the beginning of a chain of reactions in the unending production of chemicals that is thought to increase by about 1500 annually (Trager 2016).

The United Nations Environmental Programme (UNEP) (2012) observed that the exact number of chemicals on the global market is uncertain but under the pre-registration requirement of the European Union's chemical registration, 143, 835 chemical substances have been pre-registered. This is considered a reasonably reliable guide to the approximate number of chemicals in commerce globally. I consider this conservative estimate. Recently, not surprisingly this was upgraded by about 1500 entering the global market annually (Trager 2016). This massive increase in global chemical output had already been predicted and the implications for human health were the focus of Rachel Carson's career.

At the beginning of this year the annual World Economic Forum (WEF) AT Davos shocked the world by the observation that by year 2050 there will be more plastics in the sea than fish and that a lot of money was being wasted on disposable plastics that are neither reused nor recycled. Though they saw the problem only from the economic point of view, it is disconcerting for toxicologists and confirms what we recognize well that the sea is the final sink of all that we dispose of. This observation alludes to the pollution or poisoning of the world and confirms a local observation by Okoye (1994) from the study of fish bought from markets all over the country where they found toxic metals highly

exceeding recommended limits and concluded that Nigeria was heavily polluted. We have confirmed this in some of the studies I will be describing shortly (Anetor and Adeniyi 1999). An unrecognized source of poisoning is the growing mountains of waste, that is unsorted full of a cocktail of toxicants. What I call our waste rising against us.

Fig. 1: Plastics at the bottom of the sea. *Can the Chisso mercury poison be again repeated through degradation of plastics releasing chemical poisons?*

Fig. 2: Unsorted waste: A common site all over the country, laden with toxicants. When we set them ablaze we are unwittingly poisoning ourselves. *Any difference from the drinking of Athenia Hemlock by Socrates?*

Fig. 3: Release of cadmium from tyres.

Fig. 4: Animals in a polluted environment are not spared toxic exposure; grazing on polluted soil.

About 1957, Rachel Carson was convinced that the increasing chemical production was a potential hazard to the whole biota that the indiscriminate use of chemicals emanated from a combination of ignorance and greed. I share Carson's insistence that what science conceived and technology made

possible must be judged for its safety and benefit to the entire spectrum of life. The fruit of Carson's concern for world safety is 'Silent Spring', the product of her concern and an evergreen gift to the world. A book which deliberately challenged the wisdom of government that permitted toxic chemicals to be released into the environment before assessment of their safety. In this immortal book, Rachel Carson, presented faultless evidence of how chlorinated hydrocarbons, of which DDT was the best known at the time and organic phosphorus insecticides, altered the cellular processes of plants, animals and ultimately humans that we just examined above. This astute biologist observed that science and technology, had become the handmaidens of the chemical industry's rush for profits (prosperity) and control of markets. This remains very true even today. Rachel Carson argued that the protection of the public from potential harm of chemicals had been disregarded without any scientific basis. This scientist whose writing and scientific report led to the introduction of the Environmental Protection Act (EPA) that paved the way for the emergence of the Environmental Protection Agency (EPA), the prototype of all environmental protection agencies in the world in 1970, boldly questioned the moral right of government to leave its citizens unprotected from substances they could neither physically avoid nor publicly question. This as we are seeing is callous arrogance that could only destroy the living world. Carson asserted in his days that one of the fundamental human rights must be the "right of the citizen to be secure in his own home against the intrusion of poisons applied by another person". The prediction by Carson that, human health would ultimately reflect the environment's ills has been borne out time and time over within the 54 years since Silent Spring was first published. Scientists including chemical pathologists are among the beneficiaries of the protest by Rachel Carson of the contamination of man's total environment with substances that accumulated in the tissues of plants, animals and humans and have the potential to alter the genetic structure of organisms. She said "poison one corner of the environment and you end up poisoning all."

Many unethical approaches were adopted by multimillion-dollar industrial chemical organizations. About a quarter of a million dollar ($250, 000), a lot of money in the 1950s was voted by the industry to discredit Rachel Carson's research and malign her character. These industrialists as would happen in Nigeria if not worse placed economics above patriotism and the safety of the citizens. Though Carson unknown to her adversaries was battling with a rapidly metastasizing (spreading) breast cancer, she was immune to the chemical industry's effort to malign her; rather; her efforts were concentrated on survival in order to complete the book and bear witness to the truth as she knew it which was acknowledged by the then President of the United States. She died in 1964, two years after the publication of 'Silent Spring'. From our studies we found that exposure to chemicals cause immunosuppression (Anetor and Adeniyi 1998). At the time this was unknown, looking at the life of Rachel Carson, her frequent visits to the woods that were heavily sprayed with insecticides; I am beginning to think that she may actually have succumbed to chemical poisoning and that her breast cancer may indeed be linked to her unintended chemical exposure. Looking back at her health record, she developed duodenal ulcer, while on treatment she came down with a severe case of flu that progressed to pneumonia and finally breast cancer that claimed her life at age 57 years. For immunotoxicologists, these are clinical manifestations at least in part consistent with immunosuppression unconnected at the time. For me she became a victim of a line of research she tried to pursue; tracing the complicated link between pesticide exposure and cancer for which she wrote two chapters in Silent Spring.

After Silent Spring caught the attention of President John F. Kennedy, he ordered federal and state investigations into Rachel Carson's claims. Communities that had been subjected to aerial spraying of pesticides against their wish organized themselves against the continuation of toxic pollution. Rachel Carson was honoured posthumously with the Presidential Medal of Freedom in 1981 (Lear 2009). Today global contamination is a fact of modern life (Lear

2009), and Silent Spring is compelling all of us scientists and lay people alike to reevaluate our relationship to the natural world. The current Pope, Pope Francis has recently joined the league of Environmentalists by authoring Laudato Si (On the Care of Our Common Home) (Pope Francis 2015). Specifically, the Pope lamented:

> Account must also be taken of the pollution produced by residue, including dangerous waste present in different areas. Each year hundreds of millions of tons of waste are generated much of it non-biodegradable, highly toxic and radioactive, from homes, and businesses, from construction and demolition sites, from clinical, electronic and industrial sources. The earth, our home is beginning to look more like an immense pile of filth.....Industrial waste and chemical products utilized in cities and agricultural areas can lead to bioaccumulation in the organisms of the local population, even when levels of toxins in those places are low. Frequently no measures are taken until after people's health has been irreversibly affected.

I am not surprised about this apt and timely lamentation by the Pope as he is a chemist. He has mentioned the price that will be discussed shortly below, a toll on our health that may take a tortuous pathway to evolve and may not be related to exposure to chemical poisons.

The Human Price

With continued manufacture and release of new chemicals from industry a serious public health problem confronts mankind. This was aptly put by Rachel Carson,

> 'only yesterday mankind lived in fear of the scourges of smallpox, cholera, and plague that once swept nations before them. Now our major concern is no longer with disease organisms that

once were omnipresent; sanitation, better living conditions, and new drugs have given us a high degree of control over infectious disease. Today we are concerned with a different kind of hazard that lurks in the environment- a hazard we ourselves have introduced into our world as our modern way of life has evolved (Carson 2002).

Fig. 5: Infected breast feeding mother and child.

One of the greatest dangers of chemicals is that minute causes produce mighty effects; effects often apparently unrelated to the cause, appearing in in a part of the body remote from the area or port of entry of the exposure (Carson 2002).

By the end of the 19th century, about half a dozen sources of industrial carcinogens were known in the 20th century this was increased to countless new carcinogens (cancer causing chemicals) and to bring the general population into intimate contact with these chemicals. In the less than two centuries since the seminal observation of Percival Pott (1775), the environmental situation has changed dramatically' No longer are exposures to dangerous chemicals occupational alone, they have entered the environment of every one- even of children as yet unborn. It is hardly surprising, therefore, that we are now aware of an alarming increase in malignant disease,' 1:4 (one in every four) (Carson 2002). It is painful

that this warrior against chemical poisons and connection to cancer herself became a victim of cancer. Today the situation is much worse there are more cancer cases in the industrializing countries and a larger number of cancer deaths are from the developing countries. Yes, we welcome economic progress but this has to be balanced with implications for health. This was given only very the minimum attention in the classic neuropathy, Minamata bay disease.

Economic Progress and Chemical Exposure: The Story of Minamata Bay

Poisoning by the chemical, mercury, (Hg) in a Chisso Plastic Plant in the Southernly Kyushu sea town of Minamata in Japan, was first manifested by the strange dance of cats on the streets, a strange 'dance', which at times ended in their collapse and death. Minamata was a modest fishing village in the early 1950s. The worrisome story actually began in the 1930s. The town was beginning to shed its heritage as a poor farming and fishing community. The welcome economic up-liftment was facilitated by Chisso Corporation. The corporation had been an integral part of the local economy in the early 1900s, it commenced the manufacture of acetaldehyde for the production of plastics; Hg was a component used in this process releasing mercury as a bi-product. It took many decades to recognize the cause as due to mercury poisoning; part of methyl mercury (CH_3-Hg) that enters the food chain. Residents that were largely fisher men depend heavily on fish and shell fish to meet their protein requirement. At the end of World War II (WW II) the production of acetaldehyde was increased with associated economic boom and attendant great appreciation of the improved socioeconomic status and lifestyle (Michiko 1990). Almost simultaneously, fishes were seen floating in sea in Minamata Bay. Cats also continue to exhibit bizarre behaviour; falling into the sea and dying (cat suicides). About the same time, similar behaviour began to be observed in humans; stumbling while walking with inability to write or button up clothes. Problem with hearing and swallowing and

uncontrolled trembling (Parkinson's syndrome) was also observed (Clarkson 1981, 1987; 1991)

About 1956, a clear epidemic was evident and fear was palpable along with confusion. Two candidate causes were immediately admissible; viral brain inflammation, syphilis. Others were ataxia or alcoholism; they were also called "dancing disease" or "strange disease". Some physiological effects were the loss of motor control (paralyzed and contorted bodies), there was also inability to keep afloat or maintain balance in water on falling from a boat. The patients were also unable to put on sandals by themselves, walk properly or understand others. Men also performed what was called "craze dance", salivated or convulsed, tore their skin with their own finger nails a condition similar to Lesch Nyhan's syndrome. One member of a given family may be the first to be affected probably the husband, then the wife and others.

Scientific Investigation

In nearly all the classic cases of metal poisoning all of which were first reported from Japan; Itai, itai disease, and Minamata disease the power of science was brought to bear. Careful scientific studies revealed that the disease arose from toxic metal poisoning (mercury poisoning) caused by eating fish and shellfish contaminated by mercury from Chisso Plant was responsible. Dr. Hajime Hosokawa who conducted the investigations on cats at the Chisso Company Hospital, provided evidence directly linking acetaldehyde waste water caused the disease. The key experiment showing that cats developed the characteristic disease when administered effluent water from the polluting factory was unfortunately suppressed by the sponsoring company and details were only made available after a delay of about four decades (40 years); extreme case of conflict of interest. About 100 patients were first identified with a mortality of 20. The community's way of life in Minamata itself had been poisoned. Poison from the food from the sea also flowed into the blood of the community (Eto et al. 2001). This remains a challenge to the

present day investigator. It also calls for greater need for government to listen to scientists, especially toxicologists not just wait for fire brigade approach when what could have been averted at the budding stage explodes. We for instance warned about wide spread lead poisoning on the African continent before it blew open in Zamfara State (Anetor and Adeniyi 2000a). I have described the Minamata episode to some detail because of the lessons they hold for us. We shall now report some of the key ones of the research activities in the toxicology unit of our department.

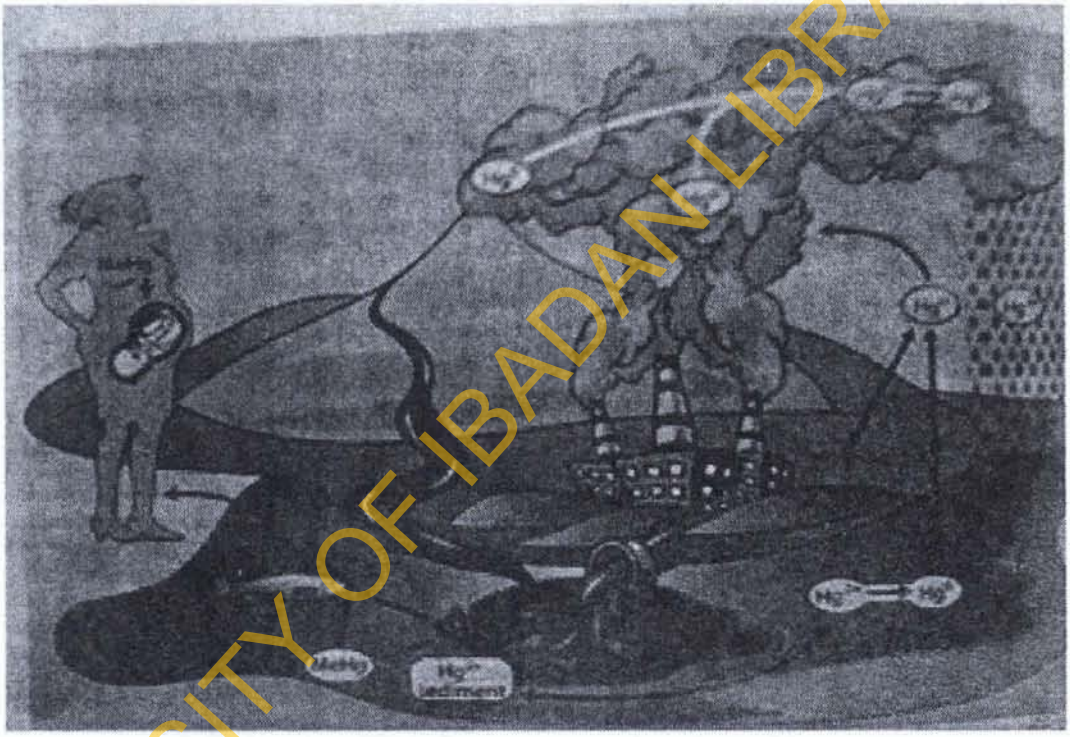


Fig. 6: Mercury cycle in the environment.

Source: Liu et al. (2008)

Research at the Toxicology and Micronutrient Metabolism Unit, Department of Chemical Pathology

One of the very early studies we conducted in our toxicology unit was on cadmium (Cd). The investigation of Cd in smokers along with other toxicants such as lead are also described, suggesting overthrow of the chemicals of life by those of prosperity, disease and death.

Overthrow of the Chemicals of Life by the Chemicals of Prosperity, Disease and Death: Social Toxicants and Cosmetics

Cadmium Toxicity in Cigarette Smokers

Cadmium is a ubiquitous chemical that is only poorly excreted with a long biological half-life ($_{1/2}$) approximately 20-30 years. Cadmium though poorly heralded is especially dangerous; it is cumulative and bioaccumulates in tissues. It has consequently gained recognition in scientific cycles not only in occupational environment but also in environmental pollution. Cadmium is poorly absorbed in the gastrointestinal tract (< 8%) in contrast it is more strongly absorbed through the respiratory tract; inhaled Cd readily penetrates the alveolar epithelial and enters the blood stream. This pathway is particularly an important route for the delivery of cadmium to cigarette smokers; cigarette is rich in cadmium (Lewis et al. 1972). An important biochemical characteristic of Cd is that it is taken up by the epithelial cells where Cd^{2+} is a molecular 'mimic' of Ca^{2+} and enters the cell through calcium channel, a well-known important element. Cadmium is a serious nephrotoxicant (damaging to the kidney) and also impairs bone metabolism. This arises from the tobacco leafy plant which contains substantial amounts of cadmium (Goyer 1997). Consequently, cigarette smokers have many fold Cd intake (Goyer 1997). This can be exercabated by micro-nutrient deficiencies which promote greater intake and toxicity of Cd (Bremmer 1978; Goyer 1995). Cadmium interacts metabolically with three essential metals; calcium, iron and zinc, two of which play key roles in osteogenesis (Goyer 1997). Even in small amounts Cd causes significant organ damage.

Cadmium deposited in osteoid (bone) tissue interferes with calcification, decalcification and bone modelling (Bhattacharya et al. 1978). Itai-Itai disease (translated to it hurts, it hurts for want of name), a bone disease first reported in the Fuchu-Toyama Prefecture of Japan is thought to be due to excessive exposure to Cd in the presence of nutritional deficiencies (Tsuchiya 1969; WHO 1995). Nutritional deficiencies are quite common in this environment. Some controversies still exist as to whether the primary effect of Cd

on Ca metabolism is a consequence of effect on tissue or to its effect on bone. It is remarkable that a population in America with a similar level of exposure but with optimum nutritional status did not come down with the disease.

Concern about the health effects of cadmium has been largely restricted to its possible causal links to hypertension and kidney disease (Lewis et al. 1972; Schroder 1976). The possibility that interaction between cadmium and calcium in bone may result in disorders of bone metabolism is seldom considered. With the growing population of smokers in most developing countries, including Nigeria (Mackay 1994), cadmium may constitute a significant health problem in a significant segment of the population with implications for bone disease.

This investigation was conducted to examine the interaction between Cd status and bone metabolism and the possible risk of cadmium-induced osteopathy (bone disease) in Nigerian cigarette smokers. Our data (Anetor and Adeniyi 2002) (table 1) suggest the existence of both oxidative stress and impaired calcium and bone metabolism which may heighten the risk of bone disease such as osteomalacia and premature on set of osteoporosis in Nigerian cigarette smokers.

Table 1: Serum Total Calcium, Ionised Calcium, Inorganic Phosphate, Total Protein, Albumin and Alkaline Phosphatase Activity in Smokers and Non-smokers

Parameters	Smokers	Non-smokers	"t"	"p"
Total calcium (mmol/l)	2.30 ± 0.02	2.40 ± 0.03	2.81	<0.01
Ionized calcium (mmol/l)	1.00 ± 0.01	0.99 ± 0.02	0.81	>0.05
Inorganic phosphate (mmol/l)	.1.32 ± 0.03	1.29 ± 0.04	1.32	>0.05
Total protein (g/L)	72.00 ± 0.07	78 ± 0.80	2.82	<0.001
Albumin (g/L)	41.00 ± 0.50	44.60 ± 0.40	3.37	<0.001
Alkaline phosphatase (I.U./L)	25.00 ± 0.87	26.00 ± 1.5	1.01	>0.05
Magnesium (mmol/l)	0.81 ± 0.03	0.92 ± 0.02	2.80	<0.01

Values are Mean ± SEM.

Source: Anetor and Adeniyi (2002)

Cadmium Status in Nigerian Cigarette Smokers and Risk of Non-communicable Disease

Next we investigated the effect of rising consumption of cigarette smoke on major diseases such as heart disease and susceptibility to disease generally. Cigarette is a risk factor for at least two very serious diseases, cardiovascular disease and cancer (Pryor 1997) but the mechanisms and modulating factors are incompletely known. In 1985, 10% of all new cancer cases in the developing world were attributable to cigarette smoking and that percentage is projected to quadruple in about three decades (Parkin et al. 1994). Despite this ominous sign, tobacco companies are aggressively targeting markets in Africa and other developing countries whiles sales have stagnated or even declined in the economically advanced nations (Mackay 1994). Thus in dramatic contrast to the declining trends in per person consumption in most developed countries, usage rates across Africa are increasing sharply (Mackay 1994).

A remarkable aspect of Cd, a toxicant and carcinogen is that a small amount of it is needed to increase genetic mutations, and inhibit DNA repair. Cadmium is a significant constituent of cigarette smoke (Lewis et al. 1972), and probably one of the greater than 4000 identified constituents in cigarette smoke. We evaluated the level of Cd in 58 smokers (55 male + 3 females) and 42 non-smokers (Anetor and Adeniyi 2001a) in relation to a number of biochemical mechanisms in an attempt to provide scientific explanations for many of the disorders associated with smoking which had largely escaped previous investigators.

Our major findings were that serum Cd level was significantly higher in smokers ($P<0.001$), at least 3-fold the level in non-smokers, and associated with an elevated serum level of the anti-oxidant component, Cu, (raised caeruloplasmin, marker of acute phase response; constituent of Cu-ZnSOD), while the levels of Zn, Mg and total globulin levels were all significantly lower in smokers than in non-smokers ($P<0.001$, $P<0.01$ & $P<0.01$) respectively (table 2).

Taken together, it appears reasonable to suggest that the combination of antioxidant pro-oxidant –imbalance leading to oxidative stress, depressed immune status as suggested by the reduced total globulin level among others put the Nigerian cigarette smoker at increased risk of CHD and cancer. We on the basis of these data recommend that if smokers cannot quit smoking, supplements of anti-oxidant enzyme components and some related minerals may be used to ameliorate the attendant morbidity and mortality associated with these important and costly diseases.

Table 2: Serum Cd, Cu, Zn, Mg, Total Cholesterol, and Total Globulin Levels in Smokers and Non-smokers

	Smokers (n=58)	Non-smokers (n=42)	t	p
Cd (μg.100ml)	0.015 ± 0.001	0.005 ± 0.00	17.2	<0.001
Zn (μg/100ml)	85.0 ± 2.22	99.0 ± 2.79	3.92	< 0.001
Cu (μg/100ml)	111. 0± 2.59	98 ± 4.93	2.23	<0.05
Mg(mmol/l)	0.81 ± 0.003	0.92 ± 0,03	2.82	< 0. 01
T.Chol.(mg/100ml)	149.0 ± 6.17	161.0 ± 8.05	1.18	> 0.05
T. glob. (g.100ml)	3.0 ± 0.009	3.5 ± 0.11	3.47	< 0.001

Values are mean ± SEM

Source: Anetor and Adeniyi (2001a)

Cadmium and Risk of Prostate Cancer in Cigarette Smokers

After the previous studies associating Cd with bone disease, heart disease and cancer risk, we examined the possible relationship of Cd with cancer that is common in men, prostate cancer. Prostate cancer is a major health problem in both the industrialized and developing countries. It is the most frequently diagnosed male cancer and the second most common cause of cancer death among men accounting for 29% of all new cases of cancer (Jemal et al. 2007). The aetiology of human prostate cancer is complex and poorly understood. Prostate cancer is known to be more aggressive in smokers. The explanation for this is uncertain. Cadmium aside from being a substantial constituent of cigarette smoke

(Satarug and Moore 2004) is regarded as a human carcinogen and the level is rising in the developing countries (Bakshi et al. 2008).

Though Cd has been associated with lung cancer, the relationship with prostate cancer is less clear. Cd is also an antagonist of Zn, a micronutrient abundant in the prostate gland. The relationship or ratio of Zn to Cd and its implications for risk of prostate cancer in cigarette smokers remains unexplored. To clarify the possible role of alteration on DNA repair pathways and prostate carcinogenesis we examined this ratio and related biochemical correlates in active cigarette smokers. In this report, we evaluated the individual and combined effects of Cd-Zn ratio as a possible predictive biomarker of the risk of prostate cancer in cigarette smoker.

We reported (Anetor, Ajose, Anetor et al. 2008) that Zn: Cd ratio was significantly reduced ($p < 0.001$), implying high cadmium: zinc ratio (tables 3 & 4). This ratio was 4.5 fold that of non-smokers. The key plasma proteins and broad sub-fractions, albumin and total globulins were all significantly reduced ($p<0.001$). K^{+}, reflecting potassium channel kinetics, was significantly higher ($p<0.05$), unlike Mg which was significantly reduced in smokers than in non-smokers ($p<0.01$). We observed that altered Zn status, culminating in high Cd: Zn ratio appears to be the central factor in smokers, leading to oxidative stress, DNA damage, mutation, impaired DNA repair mechanisms, p53 expression, impaired signal transduction (altered Ca^{2++}), angiogenic effect of Cu, and impaired vitamin A metabolism. These pathophysiological effects may all converge in the risk of the carcinogenic process, suggesting high Cd: Zn ratio as the critical determinant of the risk of prostate cancer in smokers and possibly a biomarker of susceptibility to the disease.

Table 3: Serum Cd, Zn, Cu, Mg, K^+, and Fe Levels in Smokers and Non-smokers

	Smokers (n=55)	Non-Smokers (n=41)	t	p
Cd (µg/100ml)	0.015 ± 0.01)	0.005 ± 0.00	17.17	<0.001
Zn (µg/100ml)	85.0 ± 2.22	99.0 ± 2.79	3.92	<0.001
Cu (µg/100ml)	111. 0 ± 2.59	98.0 ± 4.93	2.23	< 0.05
Mg (mmol/l)	0.81 ± 0.03	0.92 ± 0.02	2.82	<0.01
K^+ (mmol/l)	4.2 ± 0.05	4.0 ± 07	2.0	< 0.05
Fe (ug/100ml)	139.0 ± 7.0	122.0 ± 12.7	1.98	> 0.05

Values are mean ± SEM

Table 4: Serum Calcium, Total Protein, Albumin, Total Globulins, Alkaline Phosphatase in Cigarette Smokers and Non-smokers

	Smokers (n=55)	Non-Smokers (n=41)	t	p
Ca (mmol/l)	2.30 ± 0.025	2.4 ± 0.275	2.81	< 0.01
T.Chol. (mmol/l)	149 .0 ± 6.2	161 ±8.05	1.18	> 0. 05
T. protein (g/l)	72.0 ± 0.7	78.0 ± 0.80	2.82	< 0.001
Albumin (g/l)	41.0 ± 0.5	44.0 ± 0.4	3.62	< 0.001
T. globulin (g/l)	30.0 ± 0.90	35.0 ± 1.1	3.47	< 0. 001
Alp (I.U/L)	25.0 ± 0.87	26.0 ± 1.5	1.01	> 0.05
Cd : Zn ratio	123.0 ± 8.37	553 ± 66.0	6.51	< 0.001

Chemical-Induced Female Baldness (Alopecia) (Hair Relaxer Induced Alopecia)

Another toxicant we examined in this field is the aspect of cosmetic toxicology, one of the fastest growing aspects of which is the use of hair relaxer among women and the scalp trauma that it elicits by way of female baldness. The discovery in the 19th century of alkaline hair relaxers by Garrett Augustus Morgan paved the way for the use of this agent to alter the basic structure of the hair shaft. A hair relaxer is applied to make hair less curly, and its consistent periodic usage causes the hair to maintain a non-curly appearance. The active ingredient of many relaxers is a strong alkali, although some brands contain ammonium thio-glycolate. Misuse or excessive usage has been reported to

cause hair breakage or alopecia in some groups of women (Borovicka 2009). That this occurs only in certain individuals and not all users suggests that there might be some other underlying causes. Apart from environmental causes such as hair relaxer type or other chemical agents, congenital, infectious, autoimmune, or nutritional causes have also been considered but remain unresolved. We studied women of African-descent who use hair relaxing products for hair strengthening purposes and develop lingering non-resolving alopecia. Three female populations; A, B & C as follow; early users and late users with alopecia and users without alopecia respectively were studied. We attempted to exclude as far as possible confounders in our study population. Figure 7 shows some women with typical non-resolving alopecia.

Altered serum Zn alone was found to be the probable cause of alopecia in early users and long-time users (table 5). We speculated that the women may probably have had a lower Zn level compared to the general population before exposure. We recommend (Iyanda, Anetor and Oparinde 2011) that assessment of Zn level in users of hair relaxer may be an advisable risk assessment strategy and women at risk could be advised to take zinc supplement (even before commencement) as an antidote.

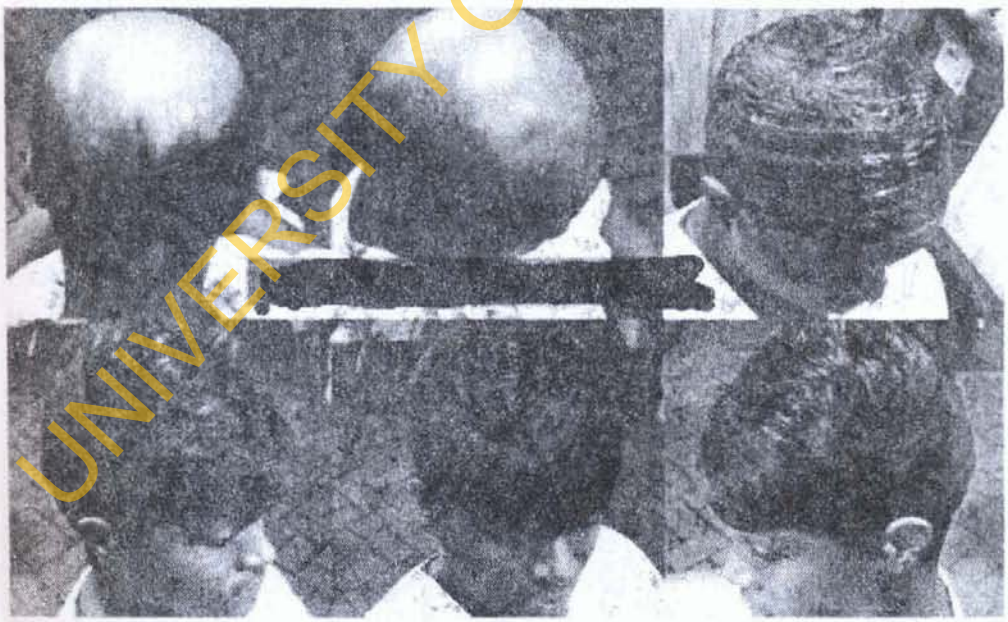


Fig. 7: Women with typical lingering or non-resolving alopecia.

Table 5: Age, Duration of Exposure and Serum Levels of selected Minerals and Vitamins in two Categories of Alopecia Subjects and Controls

	Group A	Group B	Group C	F- value	P- value
Zn (µmol/L)	12.11 ± 0.37	11.96 ± 0.90	14.27 ± 0.34	4.77	0.014*
Cu (µmol/L)	15. 85 ± 0.69	14.55±0.66	15.71±0.82	0.962	0.390
Mg (mmol/L)	0.79±0.05	0.70±0.06	0.70±0.05	1.091	0. 345
Mn (nmol/L)	114.3±7.2	112.86±5.22	108.54±5.23	0.250	0.780
Vit. A (µmol/L)	1.91±0.17	1.96±0.14	2.03±0.11	0.194	0.824
Vit.E (µmol/L)	15.78±1.39	18.1±1.39	16.01±1.16	0.801	0.455
Age (years)	41.5±1.9	42.9±2.2	40.9±1.7	0.259	0.773
Alopecia duration (years)	14.3±1.5	8.1±0.90	-	-	-

Values are Mean ± SEM
*Significantly different

Pregnancy and Developmental Disorders

The Catastrophe of Thalidomide Poisoning

The story of thalidomide (Contergan®) is well known in medical cycles. This was a drug given to pregnant women to prevent morning sickness (neonatarum gravidorum). Thalidomide is one of the typical compounds that can exert teratogenic effects (birth defects; embryotoxicity) in humans. Compounds that belong to this class cause birth defects by interference of the toxicants or their metabolites with morphogenetic differentiation during embryo development (embryogenesis). The drug was introduced in Germany in 1956. Though, it was considered safe as a sedative against morning sickness, with use, an unusual increase in the number of newborns presenting with malformations were first observed in Germany. The predominant symptoms were malformations of the limbs, presenting mainly as shortened arms (phocomelia) or at times out rightly missing arms (amelia). Subsequently the episode was observed in other countries. All over the world about 7000-1000 children with malformations were born (Boesterli 2003). It is remarkable that the catastrophe was not observed in America as the Director of FDA being not convinced with the pre-clinical data declined approval for use in the United States.

It was only about 1961 that an astute physician connected the malformations with the use of Contergan® in pregnant women. The drug was withdrawn from the market a year later, with concomitant observed reduction in the number of malformations. This informed the issuance of the first guidelines for reproductive toxicology by the United States FDA in preclinical toxicity assessment. Classic experiments in the foregoing several decades demonstrated the teratogenic effect of thalidomide in non- human primates (Wilson 1972) (fig. 8). This investigator reported similar types of toxicity to those seen in humans. These malformations could not be reproduced in rats where the oral bioavailability of thalidomide is poor; but can be reproduced when administered intravenously (Stephens and Fillmore 2000; Boesterli 2003).

What is the relevance of this review? It is to show that the thalidomide catastrophe may be unknowingly repeated with

different manifestation that may not be physical; neurological defects, increased disease susceptibility, shortened lifespan etc. Some of our studies point to this possibility as in iron supplementation when not indicated. Iron poisoning may be unwittingly precipitated with manifestations reminiscent of thalidomide toxicity. The role of Cu in angiogenesis is unknown in this catastrophe. What was the Cu level or caerulo-plasmin level like in the mothers of thalidomide babies/ patients? Could it have been a promoter in this disorder?

Fig. 4: Thalidomide embryopathy in non-human primates (Rhesus monkey). After Wilson (1972)

Thalidomide embryopathy in the Rhesus monkey. *Left*, 100-day old monkey after treatment of the mother with a single dose of 30 mg/kg thalidomide on day 26 of gestation; *right*, normal 100-day old fetus.

Source: Wilson, J.G. (1972) Abnormalities of intrauterine development in non-human primates, Acta Endocrinol. Suppl 166; 261-292, with permission of the *Society of the European Journal of Endocrinology*

Fig. 9: Socrates committing suicide by consuming the Athenian state poison, hemlock. After Timbrell (1989)

Unknown to us we may also be committing suicide in little bits. This is practically what we do every day to ourselves in today's chemical world, only that they are in small doses that we consider inconsequential. But for the large functional reserve of most organs and systems in the body and may be endowed efficient xenobiotic metabolic system, which is genetically determined and susceptibility is variable. Notwithstanding, minor metabolic defects or lesions may build up to a climax when the toxic burden perhaps potentiated by hidden hunger (micronutrient deficiency disorders, MDDS) makes a victim succumb. Recently, toxicologists have called for a rethinking of risk assessment, giving synergistic effects of low dose to be a potential permissive factor for disease especially cancer (Colacci and Kleinstreuer 2015).

Occupational Exposure and Poisoning

I shall now present some of the studies in occupational exposure that unearth the combat between the chemicals of life and the chemicals of prosperity to be followed by those

involving our mechanistic search for an antidote for a poisoned and hungry world.

Toxicology of Lead: A Prime Environmental Pollutant and Toxicant

Lead is one of the oldest chemical poisons known. A lot of scientific efforts have gone into its elimination or reduction in the environment. Yet it remains with us today.

A reminder is needed that the weight of Pb adds up and may manifest later in life depending on the metabolic equation.

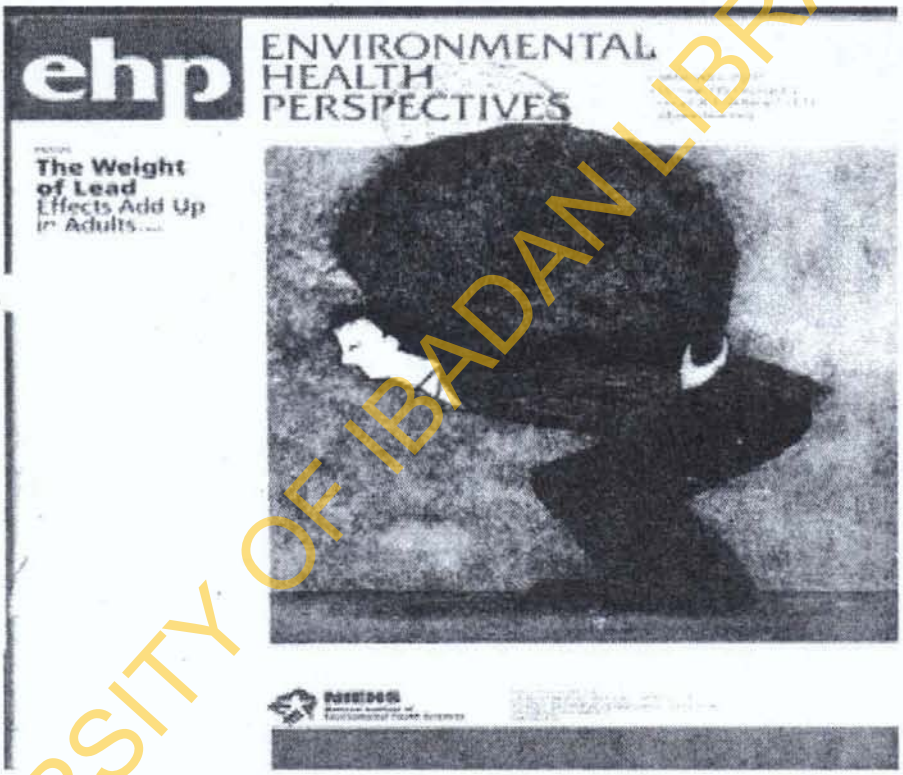


Fig. 10: Burden of lead – prime environmental pollutant. After Spivey (2007)

Immunotoxicity of Lead (Lead-Induced Immuno-supression)

The immune system (fig. 11) in any population is very important for the protection of that population. This is particularly more important understandably in a setting like ours where infectious diseases are endemic and the need for an intact and competent immune system is obvious. There has

been increasing concern about the effects of occupational and environmental pollutants on immune function; the most important of the pollutants is Pb (Faith et al. 1979). Lead and its compounds have been shown to induce immuno-suppression or decreased host resistance to infectious agents in experimental models (Hemphill et al. 1971; DeBruin 1971; Exon et al. 1979). The possible effect of the pervasive effect of lead on the immune system was considered of sufficient importance to receive scientific attention especially that inconsistent data existed in the few and scattered reports in the literature. We were driven by the increasing abundance of lead in the Nigerian environment, a consequence of progressive industrialization which demands that we establish the degree of human risk. It is desirable to know if the risk is insignificant or substantial in order to provide an intelligent occupational and environmental health policy. We examined indices of both cell mediated immunity (CMI) and humoral immunity (HMI) and a key index of micronutrient status, vitamin C. (Anetor and Adeniyi 1998). Total lymphocyte count (TLC), total globulin, IgA, IgG, IgM, CRP and related to lead level (table 6).

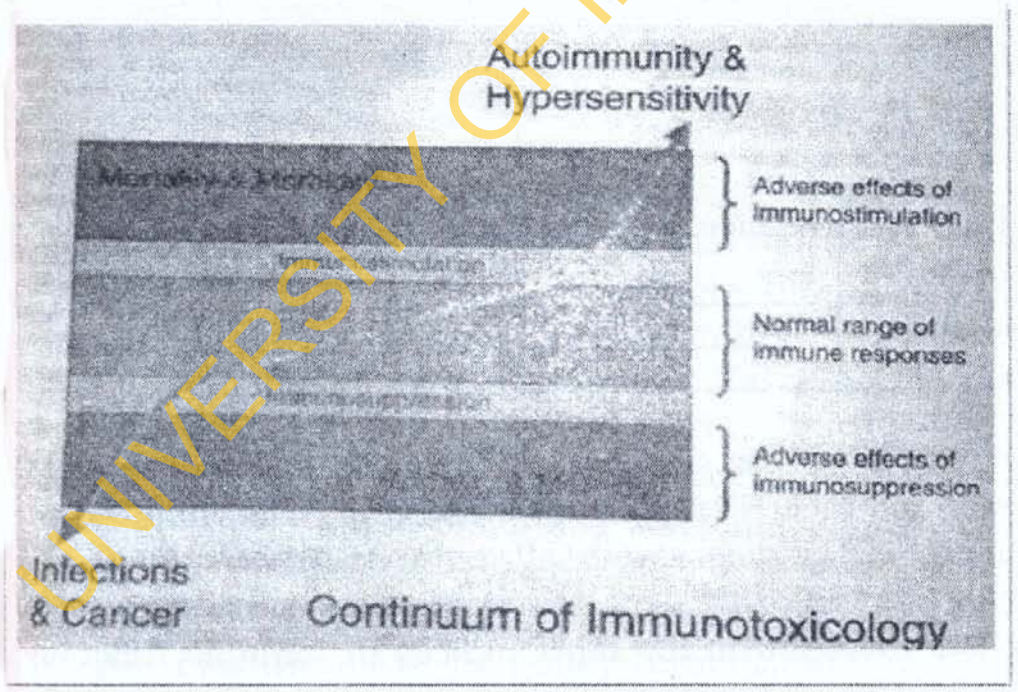


Fig. 11: The immune system in health and disease: A continuum, After Kaminski et al. (2008)

Table 6: Blood Lead Level and Urinary Ascorbate Level in Lead Workers and Control

	Lead Workers	Controls	t	p
Blood lead level (µg/dl)	56.3 ± 0.95	30.4 ± 1.4	18. 91	0.001
Urinary ascorbate (mg/ 100 mg creatinine)	9.0 1.62 ±	14.3 ± 1.23	2.57	< 0.02

Values are mean ± S. E. M.

Table 7: Immunological Indices in Lead Workers and Controls

Immune parameters	Lead workers (n= 80)	Controls (n= 50)	t	p
TLC (mm^3)	2157 ±63	2515 ± 115	2.74	< 0.01
Total globulin (g/dl)	3.73± 0.05	3.20 ± 0.07	6.84	< 0.001
IgA (mg/dl)	143 .79 ± 6. 76	187. 51 ± 14.2	2.62	< 0.01
IgG (mg/dl)	1187.73 ± 65.33	1997. 33 ± 108.33	6.79	< 0.0001
IgM (mg/dl)	190. 87 ± 11.76	215.43 ± 12.66	1.25	> 0.05
CRP (mg/dl)	0.60 ± 0.03	0. 50 ± 0.03	2.56	< 0. 01

Values are means ± S. E.M.
After Anetor and Adeniyi (1998)
n = number of subjects

Data suggesting suppression of both humoral and cell mediated immunity with associated inflammation.

After Anetor and Adeniyi (1998)

Table 8: Correlation between Blood Lead Levels and IgA in Lead Water

Blood lead level Vs IgA	n	r	p
BLL Vs IgA	80	-0.28	< 0. 009

Blood lead level was significantly higher in the lead workers than in controls (P< 0. 001) (table 6). The indices of immunological indices were all reduced; except for total globulin and CRP that were raised suggesting inflammation, while the reduction in the other indices of immune function are suggestive of immunosuppression (depressed immune function) (table 7). The inverse correlation of IgA with blood

lead level is particularly noteworthy for its implication for gastrointerstinal tract (GIT) and respiratory disorders (table 8). These data suggest that lead workers may be at greater risk of increased susceptibility to infectious diseases, inflammatory disorders and very importantly cancer. It is on record that we were among the first investigators to link lead with risk of cancer (Anetor and Adeniyi 1998). Inorganic lead is now considered a promoter of cancer (Kwong 2013). This may in part be a contributor to the upsurge in cancer rates in the economically disadvantaged communities. This report appears to provide scientific explanations for the symptoms that patients always presented with; absenteeism from work, un-subsiding coryza, prolonged diarrhoea and increased susceptibility to disease.

Lead is described as the most studied of all toxic substances (Goyer 1993) and remains important and relevant today. Due to its wide spread application in the industry and its massive application as additive to gasoline (leaded gasoline), tetraethyl-lead (TEL) which has been described as the mistake of the 20th century (Shy 1990) and largely phased out now in most developed countries, hopefully also in Nigeria. The great public health significance of exposure of the public to lead; reduced cognition, anaemia, depressed immune function and attendant increased susceptibility to disease, systemic inflammation and implications in reproductive disorders in both male and female. Indeed, lead is strongly believed to be responsible for the fall of the Roman Empire due to reduced fertility (Gilfillan 1965; Watson and Proudfoot 2001; Timbrell 2009). A view strongly corroborated by the report of Carlsen et al. (1992) that fertility has been on the decline (increase in infertility) after a 50-year study. These disturbing scientific reports led us to design comprehensive experimental and non-experimental studies into lead with a number of sub-themes to provide sound scientific basis for policy formulation and regulatory decisions.

One of the first studies was to explore the magnitude of the problem of lead poisoning (plumbism, saturnism) in Nigeria. This first study was divided into two phases. In the phase I which investigated occupationally exposed individuals, 137 subjects comprising of 86 exposed and 51 unexposed (control) participants. The occupationally exposed participants were largely drawn from the occupations represented in table 9 below.

Table 9: Distribution of Lead Workers in Phase 1 Study

	Number of participants
Battery plant A	17
Battery plant B	20
Paint industry	20
Petroleum deport	14
Gasoline distribution centres	10
Insecticide factory	5

Source: Adeniyi and Anetor (1999)

The control, unexposed participants were essentially indoor administrative workers non-occupationally exposed to lead. They were sex and age matched as well as in socio-economic status. Consumers of alcohol and users of cigarettes were carefully noted.

In the second phase of the study, a total of 880 participants who were not occupationally exposed to lead were selected from three sites in Southwest, Nigeria (Iseyin, 115, Shaki, 280 and Ogbomosho, 284). From Sokoto, North-West, Nigeria, 201 participants were recruited. It is remarkable that this latter location bothers with Zamfara state now internationally recognized for its profound and unprecedented episode of lead poisoning (Anetor et al. 2016) in these environmentally exposed individuals. The characteristics of the subjects and blood lead levels, the biomarker of lead exposure and poisoning are shown in table 10.

Table 10: Blood Lead Levels (PbB) in Lead Workers, Controls and Occupationally Unexposed Participants

	Number of subjects	Age (years)	Blood lead level (µg/dl)
Lead-workers	86	24.8 ± 5.8	56.3 ± 0.95 (26- 97)
Control subjects	51	25.3 ± 4.7	30.1 ± 1.47* (10-58)
Other unexposed subjects	880	25.8 ± 6.3	28.8 ± 1.22* (15-63)

All values are mean ± S.D with range in parenthesis

Compared with lead-workers, values were significantly lower ($P < 0.001$) in the general population. The data from these two- phased studies are very instructive; occupationally exposed participants exhibited significantly higher PbB than in controls and environmentally exposed participants ($P < 0.001$) (table 10). Remarkably 95.3% of the exposed subjects had PbB greater than 40µg/dl, the universal upper limit of acceptable PbB in lead workers. About 70% had PbB greater than 55µg/dl, a level considered indicative of excessive exposure. Additionally, about 40% of the lead workers had PbB of 60µg/dl or greater, a level indicative of the need to remove the individuals from further exposure. Only about 5% had PbB below 40µg/dl.

Though the main thrust of this investigation was to examine the levels in lead workers relative to those of controls, the results in the control, (apparently healthy participants) are very disturbing. The data from the lead workers though unsurprisingly demonstrate excessive exposure and toxicity (poisoning), they are consistent with earlier reports from similar populations all over the world. Only about 18% had blood lead levels falling within commonly acceptable PbB, about 7% of controls (occupationally unexposed) had PbB within the range indicative of moderate toxicity; over 8% had PbB above levels acceptable in occupational exposure, while 4% fell within range indicative of severe exposure. Data from the

unexposed (general) population are similar to those of control for the lead workers, with very similar distribution. Taken together the data suggest lead poisoning of high magnitude from both environmental and occupational exposure. These have far reaching implications that will be highlighted in subsequent studies. The data call for measures to reduce exposure. This probably in part informed the intervention of the World Bank in reducing blood lead level in Sub-Saharan Africa by phasing out lead from gasoline, a project in which I was a Member of the National Committee head by Eng. Aminu Jalal. The paper I presented at the national conference has been adopted as one of the Working papers of the World Bank Working Paper Number 6 (Anetor 2001) (fig. 12).

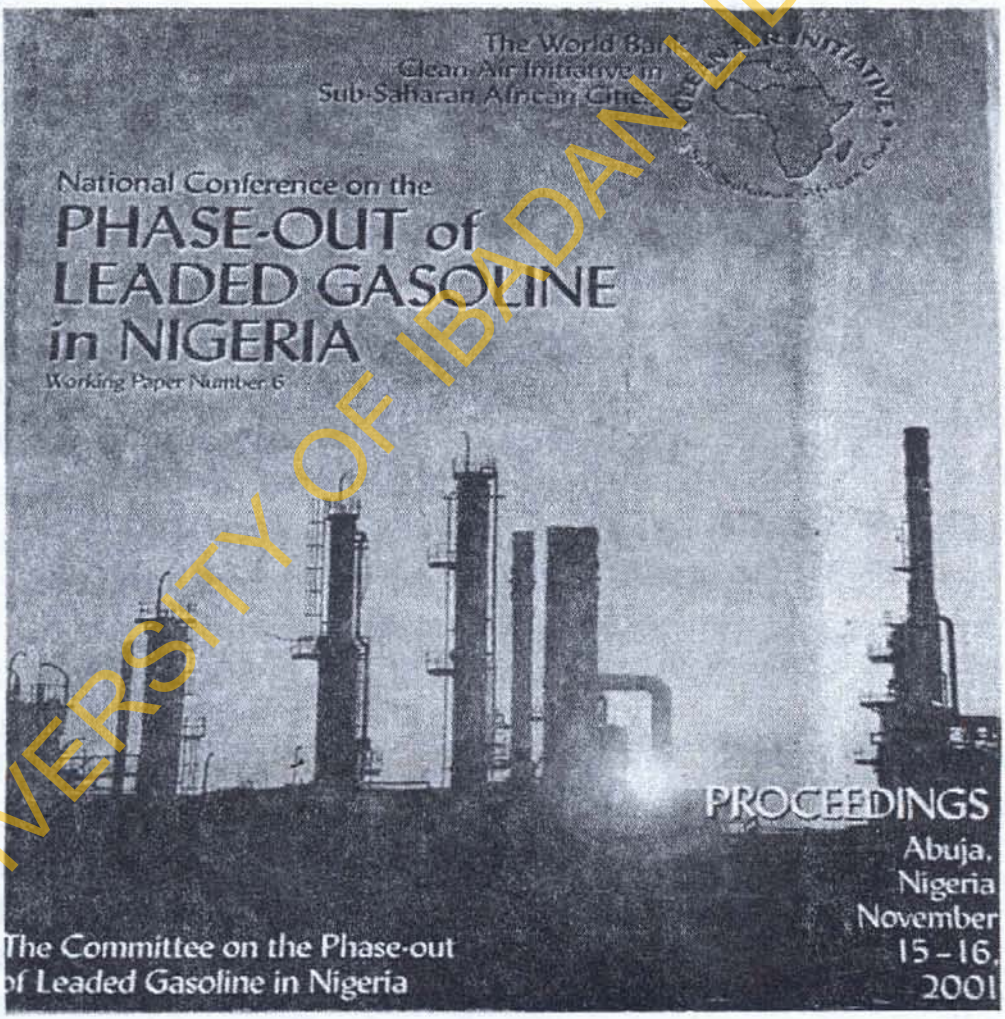


Fig. 12: National committee on the phase-out of leaded gasoline in Nigeria. World Bank Working Paper Number 6.

Table 11: Blood Lead Levels in Lead Workers in Nigeria and in Advanced and Emerging Economies

Country	PbB (µg/dl)	Investigator(s)
United States	37.1	Gartise et. al (1982)
U. K.	39. 4	Ferguson (1986)
Korea	45.7	Kim et. al. (1995)
Jamaica	33.0	Matten et. al. (1989)
Nigeria	56.3 ± 0.95	Adeniyi and Anetor (1999)

The lead level in our occupational cohort falls within levels indicative of severe lead poisoning and may be reflective of a combination of two factors; lack of regulation and two the high lead level in our gasoline at the time of our study (Commencement of phase out was in 2003). Later reports from our laboratory (Anetor et al. 2007b) strongly favour the latter. Lead content of Nigeria' gasoline was one of the highest in the world (Arah 1985) and contributed to the substantial magnitude of lead poisoning in Nigeria (Adeniyi and Anetor 1999).

The periodic studies of the National Health and Nutrition Examination Survey (NHANES) of the United States have provided a useful surveillance programme. It identifies significant trends in exposure to lead. The NHANES II (1976-1980) determined PbB in over 9,000 Americans and demonstrated that 37% population-wide decrease in lead levels paralleled a 55% decrease in the use of leaded gasoline (Annest et al. 1983). The data were useful in further attempts to reduce tetraethyl lead in petrol. The NHANES III (1988 – 1994) identified high-risk populations for excessive exposure to Pb, in the process providing improved strategies for reducing lead toxicity. Table 12 shows the progressive lowering of blood lead level by the Centres for Disease control and Prevention (CDC) leading the way for other nations including Nigeria.

Table 12: CDC Elevated PbB Benchmarks by Year

Year	CDC- elevated benchmark (µg/dl)
Pre- 1970 (adults and children)	60
1971	40
1975	30
1985	25
1991	10
2012	5

Source: CDC (2012) and Richardson (2005)

Indicators of Metabolic Poisoning: Implications for Early Detection

Lead is poisonous without any useful biological function in the organism. It however has excellent physicochemical properties that are very useful in a wide range of industrial activities and occupations that are extremely beneficial to man (Zielhuis 1983). From these occupational activities lead in the form of dust or fumes gains access into the human environment and into the human body culminating in lead poisoning (Landrigan 1990). Acute clinical lead poisoning is easily recognized; in contrast prolonged chronic exposure is insidious. The biochemical manifestations of such an exposure represent what is called metabolic poisoning. Metabolic (sub-clinical) poisoning is considered to exist when it is possible to detect alterations in metabolism secondary to lead absorption. Clinical lead poisoning on the other hand is diagnosed when absorbed lead produces signs and symptoms that are obvious to the patient or physician. Metabolic poisoning can exist without clinical poisoning but not the converse. Owing to the undesirable severe consequences of clinical lead poisoning it is important to investigate the possibility of metabolic poisoning to provide a basis to avert clinical poisoning. In this study we investigated a cohort of 80 lead workers and unexposed individuals. The workers were classified into low, moderate and high exposure categories according to prevailing air lead level in the work environment (NIOSH 1978). Our findings confirm the

intimate metabolic pathways in which lead ions (Pb^{++}) and calcium ions (Ca++) participate. Pb++ have been shown to compete with Ca++ for uptake by Ca++ channels (Simons and Porock 1987) while Pb^{++} also block Ca^{++} efflux from the cells by substituting for Ca- Na ATP pump (Simons 1986). The hypocalcaemia associated with high BLL in our report (Anetor et al. 1999) may be explained by the mechanisms above. Importantly, we think it is reasonable to suggest that cohorts who are exposed to lead and whose intake of calcium rich foods are considered to be borderline or reduced ; levels of total and ionised calcium, reduced calcitriol (1,25 $(OH)_2D$ and raised uric acid level may be some of the biochemical indicators of metabolic poisoning (table 13). We provided evidence that corroborate the renal excretion of uric acid and the biosynthesis of 1, 25 $(OH)_2D$ occur at the same site, the proximal convoluted tubule (PCT) (Ball and Sorensen 1969; Goyer and Rhyne 1973; Brunette et al. 1978). Our findings provide useful information that the combination of hypocalcaemia, hyperuricaemia, decreased calcitriol level are reliable biochemical indicators of metabolic poisoning of lead particularly in nutritionally disadvantaged communities which may serve as basis of early warning signs of impending clinical lead poisoning. This would have been useful in the early detection of the lead poisoning in Zamfara state were we a prepared nation.

Table 13: Biochemical Indices of (parameters) in Lead Workers and Control

	Lead Workers	Controls	t	P-values
Blood lead (μmol/l)	2.72 ± 0.05	1.47 ± 0.07	18.91	< 0.001
Total Ca (mmol/l)	2.22 ± 0.02	2.31 ± 0.02	2.6	< 0.01
Ionised calcium (mmol/l)	0.87 ± 0.001	0.99 ± 0.01	6.67	< 0. 001
Inorg. Phos. (mmol/l)	1.18 ± 0.003	1.21 ± 0.029	1.5	> 0.05
Total Prot. (g/l)	82 ± 5.6	75 ± 1.6	1.05	> 0.05
Albumin (g/l)	44 ± 0.50	44 ± 0.50	0.56	> 0.05
Uric acid (mmol/l)	311 ± 16.6	204 ± 11.3	5. 28	< 0.001
1,25 $(OH)_2D_3$	54.6 ± 21.3	72.8 ± 28.4	8.88	< 0. 001
% with Ca level < 2 (mmol/l)	19	4	-	-

Values are Mean ± SEM
> 0.05 = not significantly different
< 0.01 = significantly different
< 0.001 = very highly significantly different

Nutritionally Essential Metals and Lead Poisoning

The relationship between essential and toxic metals in nutritionally compromised communities and the modulation of toxicity is often ignored. Yet scattered reports suggest this may have beneficial effects (Cerkwleski and Forbes 1976; Papaioannou et al. 1978). Such a study may reveal the level of risk or protection. We evaluated the relationship between Pb and nutritionally essential biometals; Cu, Fe, Zn in Pb lead workers, comprising of, welders/panel beaters, mechanics, painters, battery workers and printers. The Pb workers were classified according to exposure category based on the (NIOSH 1978) standard prevailing air lead levels, PbA; low, moderate & high. Controls were selected from individuals not known to be occupationally exposed to lead or by hobby.

We found significantly higher blood Pb level in lead workers than in controls ($p < 0.001$). The levels of the nutritional metals, Cu and Zn were also significantly higher in Pb workers than in controls. Serum Fe level was not significantly raised (table 14). Among the striking findings in this study were that the blood lead levels in all 3 exposure categories were all significantly higher than that of the controls ($p< 0.001$) in all cases.

Serum Fe level was significantly lower in the low exposure category than in the moderate and severe exposure categories (tables 14-17). Very interestingly, the low exposure category with the lowest Fe level exhibited the highest blood lead level. Also remarkable is that Zn level was significantly higher in the severe exposure category than in the low and moderate categories (tables 14, 15 & 17). The low exposure category with the lowest Zn and Fe levels also exhibited the highest blood lead level; additive effect (Anetor, Adeniyi and Taylor 2001). These results appear to strongly suggest that the dose alone of Paracelsus may not solely determine the poison and that the levels of nutritionally essential biometals may be protective (have antidotal effect) against lead poisoning and that the contrast, very common in many developing (industrializing) countries may put the population at greater risk of lead (metal) poisoning.

Table 14: Blood Lead (PbB) Level, Serum Cu, Fe, Zn and C-reactive Protein (CRP) Levels in Lead Workers and Controls

	Lead workers	Controls	t	p
PbB (µmol/L)	2.72 ± 0.05	1.47 ± 0.07	18.91	< 0.001
Cu (µmol/L)	18.88 ± 0.54	16.17 ± 0.49	3.06	< 0.05
Fe (µmol/L)	10.1 ± 0.59	11. 42 ± 0.84	1.28	> 0.05
Zn (µmol/L)	17.2 ± 0.93	13. 03 ± 0.80	4.06	< 0.001
C- reactive protein (mg/L)	6.0 ± 0.3	5.0 ± 0.3	2.56	< 0.01

Table 15: Serum Levels of Cu, Fe, and Zn according to Exposure Category

	Low	Moderate	High
Cu (µmol/L)	19.36 ± 1.27	16.14 ± 1.38	19. 36 ± 0.64
Fe (µmol/L)	4.73 ± 1. 31	10. 11 ± 0.98	10.63 ± 0.68
Zn (µmol/L)	12.38 ± 1.21	14. 22 ± 0. 74	19.70 ± 1.48
P	< 0.001a	< 0. 002	-

a = Low exposure category versus moderate category
b= Moderate versus high exposure categories

Table 16: Blood Lead (PbB) Levels according to Exposure Categories and in Controls

Expo. Category	n	PbB (µmol/L)	t	p
Low	8	2.92 ± 0.01	1.77a	> 0.05
Moderate	34	2.64 ± 0.02	1. 19b	
High	44	2.75 ± 0.07		
Control	51	1.47 ± 0.07		< 0. 001

a= Low exposure category versus moderate exposure category
b=Moderate versus high exposure categories

Table 17: Serum Zinc Levels in the Major Occupational Groups

	Panel beaters/ welders (n=30)	Mechanics (n= 17)	Battery workers (n=11)	Painters (n= 15)
Zn (µg/dl)	145 ± 2.23	87 ± 1.58	104 ± 3.97	92 ± 1.88
Range of Zn (µg/dl)	70- 300	40- 135	45- 185	55- 130
T	-	4 .19	0.28	2.73
P	-	< 0.001	< 0.05	< 0. 001

Values are mean ± SEM
t and p values are values obtained when level in welders/panel beaters were compared with levels in the respective occupational groups.

Biological Monitoring of Chemical Exposure (Biomarker Mining)

Biological monitoring traditionally refers to the measurement of internal exposure to a toxicant or xenobiotic through the analysis of a biological specimen. Biological monitoring may also be extended to include the measurement of some fairly specific biological tests to measure internal exposure for instance the determination of cholinesterase activity as an indicator of exposure to cholinesterase inhibiting chemicals. The term 'biomarker' may be used in the alternative and three types of biomarkers are described. These are;

- Biomarkers of exposure, these are either exogenous compounds within the system and essentially reflects the interaction between the foreign compounds and endogenous compounds.
- Biomarker of effect, these are indicators of an endogenous component of the biological system or indicators of functional capacity of the system, or an index of an altered state of the system that are recognized as dysfunction or disease; response to the toxicant as is evident from the metabolic poisoning study by Anetor et al. (1999), just described.
- Biomarkers of susceptibility are pointers that the integrity of the system is especially sensitive to the exposure to a toxic substance.

It is debatable if biomarkers have any clear advantage over measurement of the chemical substance itself; advancement in science makes it possible to detect some subtle cellular responses to chemical exposure. Following interaction with a receptor in the target organ, biochemical, physiological or cellular events occur. The agent-receptor interaction such as adducts between DNA and xenobiotics, may be measured directly. At times biochemical, physiological or cellular events due to agent receptor interaction are measured as biomarkers of effect. Owing to genetic, biochemical, and cellular alterations, occupational diseases may occur with

symptoms, signs, and evidence of disordered physiology or pathology, that can be eventually detected by a physician or recorded by an epidemiologist (Blum and Emmet 2013). From the perspective of protection of health, determination of internal biological exposure is very relevant as the biological measurement confirms that absorption of the substance has taken place. One advantage of biological measurement is that it may reflect the effective dose that causes adverse biological consequences. Biological levels from exposure may be utilized in assessing potential toxicity in an individual, in the community or for monitoring an occupational cohort exposed to a toxicant. Biological monitoring of occupationally exposed population is usually conducted to monitor occupational groups to help ensure that working environments are safe. We have used this principle or concept in our safety assessment of occupationally exposed cohorts (Anetor et al. 2000, 2001, 2002). This will be further evident in the study on the effect of Pb in reproduction which follows.

Chemical Poisoning and Infertility

Toxicants, Reproduction and Perpetuations of the Human Race

Toxicants have a number of adverse effects. One very important one is that on the reproductive system and a well-known reproductive toxicant which was actually implicated in the fall of the Roman Empire is lead (Gilfillan 1965; Watson and Proudfoot 2002, Timbrell 2009). With the serious level of pollution of the Nigerian environment we decided to assess the effect of Pb on the reproductive system of males who were more involved in occupations involving lead. The damage to male reproductive system caused by exposure to Pb though thought represent a major public health problem has only received measured attention. Only experimental models indicated adverse effects on fertility and offspring, impairment of spermatogenesis and endocrinological abnormalities. We investigated the effect of Pb on male reproductive function and a biomarker for its detection

(Anetor, Akinpelu and Adeniyi 2001c). Urinary creatine (UCT), a metabolite of the high energy compound creatine phosphate has been shown to be a potential marker of damage to male reproductive system following Proton Magnetic Resonance (NMR) analysis of urinary changes after acute doses of cadmium chloride ($CdCl_2$). The investigators demonstrated a marked rise in urinary creatine which was both dose and severity related to testicular damage as revealed by histopathological studies (Nicholson and Wilson 1989). Timbrell et al. (1994) have shown that the site of the toxic effect of Pb on male reproductive system is the glycogen rich sertoli cells of the testes from where the primitive germ cells obtained nourishment during spermatogenesis. The process is critical to spermatogenesis (Timbrell et al. 1994). It had not previously been linked with infertility and as a possible biomarker of reproductive toxicity. We observed that creatine paralleled elevation in PbB levels, duration of exposure and inversely correlated to most sperm parameters. It may serve as a sensitive, reliable and inexpensive biomarker of depressed spermatogenesis in humans occupationally exposed to Pb (tables 18, 19 & 20). This may be particularly useful in resource poor communities (Anetor et al. 2001).

Table 18: Blood Lead Level, Urinary Creatine, AST, ALT, Levels in Lead Workers and Controls

	Lead Workers	Controls	t	p
Blood lead level (μg/dl)	82.5 ± 18.3	27.7 ± 6.6	17.6	< 0.001
Urinary creatine (mg/100mg creatinine)	303 ± 45	101 ± 20	25.2	< 0.001
AST (I. U/l)	25.5 ± 2.5	25.1 ± 3.1	0.67	> 0. 05
ALT (I. U.)	22. 4 ± 2.6	21.7 ± 2.9	1.1	> 0.05

Values are mean ± S.D.

Table 19: Seminal Fluid Volume, Motility, Morphology and Total Sperm Count in Lead Workers

	Lead Workers	Controls	t	p
Total sperm count ($x10^6$/ cm^3)	61.4 ± 5.0	78. 9 ± 5.3	14.4	< 0.001
Motility (%)	69.7 ± 4.6	77.7 ± 9.5	4.5	< 0.001
Normal morphology (%)	65.9 ± 3.2	76.0 ± 5.7	9.3	<0.001
Seminal fluid volume (cm^3)	2.9 ± 0.40	31 ± 0.50	1.8	> 0.05

Values are means ± S.D.

Table 20: Correlation between Lead and Biochemical Parameters and between Lead and Semen Characteristics

	r	p-value
PbB vs urinary creatine	0.71	< 0.001
PbB vs total semen count	0.03	>0.05
PbB vs duration of exposure	0.47	< 0.05
PbB vs spermatozoa morphology	-0.46	< 0.005
PbB vs sperm motility	-0.46	< 0.05
Urinary creatine vs total sperm count	0.10	>0.05

Values are mean ± S.D.

Source: Anetor et al. (2001)

Lead Poisoning and Bone Disease

Following our study of the effect of Cd on bone we then examined the effect of Pb on bone metabolism. Bone, the largest repository of Pb in the body, is not metabolically inert. Bone thus responds to environmental poisons and pollution. Occupational lead toxicity and exposure to increasingly polluted environments impair bone mineralization which may lead to increased incidence of bone disease such as osteomyelitis and osteoporosis (inflammation in the bone and brittle bones) in human and animal populations. This is already a big health problem in the developed countries. It is notwithstanding frequently ignored in current environmental

health concerns in the developing countries. We examined bone metabolism and its implications for bone disease in lead workers.

We have provided evidence that a clear consequence of occupational lead toxicity or a polluted environment is impaired bone metabolism, specifically defective mineralization arising from decreased calcium and phosphate absorption owing to lead-induced impairment of 1,25-DHCC. This may lead to bone disease in human and animal populations, with serious implications for the livestock industry and the economy of affected nations. This is also important in Zamfara one of the states from which cattle that supply most of the beef consumed in this country comes (tables 21 and 22). The health of children may also be affected, stunted growth and rickets. Impaired bone metabolism in occupational lead toxicity and increased environmental pollution probably contributes to the incidence of osteoporosis already a major concern in the more industrialised economies which may also spread to developing countries with progressive industrialization.

Table 21: Indices of Bone Metabolism in Exposed and Unexposed Subjects

Biochemical Parameters	Exposed	Unexposed	t	p
Total calcium (mmol/l)	2.2 ± 0.02	2.31 ± 0.02	2.6	< 0.01
Ionized calcium (mmol/l)	0.87 ± 0.001	0.99 ± 0.001	6.67	< 0.001
Inorganic phosphate (mmol/l)	1.18 ± 0.003	1.12 ± 0.029	1.5	> 0.05
Total alkaline phosphatase (ALP) (I.U./l)	32 ± 1.48	33 ± 1.71	0.5	> 0.05
Bone isoenzyme of ALP (I.U./l)	27 ± 1.17	24 ± 1.67	0.5	> 0.05
Total protein (g/l)	82 ± 5.6	75 ± 1.60	1.05	> 0.05
Albumin (g/l)	44 ± 0.4	44 ± 0.5	0.5	> 0.05
Percent of subjects with total calcium below 2 mmol/l	19	4	–	–

Values represent the mean ± SEM

Table 22: Blood Lead, Serum Magnesium, Uric Acid and Zinc Levels in Exposed and Unexposed Subjects

Biochemical Parameters	Exposed	Unexposed	t	p
Blood lead (μmol/l)	2.72 ± 0.05	1.47 ± 0.07	18.9	< 0.001
Zinc (μmol/l)	112 ± 6.11	85 ± 2.65	4.06	< 0.001
Magnesium (mmol/l)	0.90 ± 0.05	0.85 ± 0.06	0.66	>0.05
Uric acid (mmol/l)	311 ± 16.6	204 ± 11.3	5.28	< 0.001

Values represent the mean ± SEM

The economic and social implications of inadequate skeletal development and attendant disease have been pointed out by Scott (1995). In the elderly, environmental pollution may exacerbate the abnormal bone metabolism normally associated with ageing. The population of ageing subjects is also increasing in Africa and many other developing countries with improvement in economic indices (Unwin and Alberti 2000). The skeleton also provides important calcium reserve during lactation (breast feeding), if this reserve is impaired owing to environmental pollution, rickets (soft and deformed bones) may result. Rickets, a predominantly nutritional disease, has been reported to be common in developing countries (Thatceth et al. 1999; Bishop 1999). The contribution of environmental pollution to this nutritional and metabolic disease may yet be unknown to the scientific and medical communities. Additionally, lead may be mobilised during normal bone homeostasis which may put the developing brain of children in developing countries at risk of lead-induced brain disorders (neurotoxic effects) such as behavioural abnormalities and reduction in intelligence quotient (I.Q.) levels elegantly enunciated by Needleman (1996) and Rodier (1995).

Observations on the Haemopoietic System in Tropical Lead Poisoning

The haem biosynthetic pathway is one of the most fundamental and remarkable in the chemistry of life. It shows the greatness of nature and of God. The pathway commences with very simple precursors; the simplest amino acid, glycine and an intermediate of the Krebs's or citric acid cycle,

succinic acid, one of the most important systems of life, haem is born. This on addition of the protein, globin gives us haemoglobin the pigment of life that transports oxygen throughout the body. Interestingly, this toxicant has great affinity for and unleash its deleterious and life threatening effects on this system.

The haem biosynthetic pathway was one of the earliest principal targets of lead to be recognized and intensely studied; Sofoluwe et al. (1971) studied this pathway in lead workers in Lagos to infer lead poisoning. Over a century ago Garrod (1892) first identified porphyrinuria in human lead poisoning. In 1895, Stovkis demonstrated its occurrence in both clinical and experimental plumbism. Though now well recognized that lead interferes with haemoglobin synthesis at a number of steps (fig. 13) the mechanisms are incompletely elucidated. These interferences are largely responsible for the haematological toxicity of Pb. Most earlier studies have emanated from the temperate developed world with optimal nutritional status. Our report (Anetor et al. 2002) examined the haematobiochemical effects of lead in tropical lead workers. Lead level, erythrocyte protoporphyrin (EPP, ZPP), porphobilinogen, the degradative product of the haem biosynthetic pathway bilirubin, indices of Fe homeostasis; Fe, transferrin, TIBC, % Fe saturation and the haem co factor biometals, Cu and Zn were investigated in Pb workers. We observed that RNA metabolism was unaltered as indicated by the absence of basophilic stippling (table 25), normally arising from deposition of Fe in the mitochondria due to inhibition of haem synthesis. We also observed complex changes suggesting the interplay of acute phase and antioxidant responses of Cu in caeruplasmin and Cu-ZnSOD. No variation with exposure category was evident; implying absence of dose response relationship. Remarkably, elevated Zn level in Pb workers (table 23) most probably from inhalation (analogous to supplementation) of Zn from welding fumes in the occupational environment was found. The activating effect of Zn appears to have significantly restored the activity of the major haem pathway enzyme d-aminolaevulinic acid dehydratase (d-ALA), a Zn dependent

enzyme exquisitely sensitive to Pb (fig. 8). This modulated the deleterious effects of Pb. Reduction in some haematological indices, Hb, haematocrit (HCT), mean cell haemoglobin concentration (MCHC) etc. were also observed (table 25). These apparent inconsistent changes appear to corroborate the hypothesis that other mechanisms may be involved in Pb associated anaemia. The practical application of the findings here is that this concept of optimum or raised Zn level can be explored as antidote of lead poisoning and may be the explanation for why for instance workers in some workshops where you have welders may have elevated blood Pb level and not have clinical symptoms. This is why over the years I have called for a revision of the 'dogma' in toxicology first enunciated by Paracelsus that the dose alone solely determines the poison.

Table 23: Blood Pb, Serum Cu, Zn, Fe, TIBC, Transferrin, and Percent Fe Saturation in Lead Workers and Controls

	Lead Workers	Controls	t	p
Blood lead (µg/dl)	56.3 ± 0.95	30.47 ± 1.4	18.91	< 0.001
Cu (µg/dl)	118 .0 ± 3.40	104.0 ± 3.07	3.06	< 0.005
Zn (µg/dl)	112.0 ± 6.11	85.0 ± 2.65	4.06	< 0.001
Fe (µg/dl)	77. 0 ± 4.93	87. 0 ± 6.7	1.28	< 0. 05
TIBC (µg/dl)	302.0 ± 37.12	286.0 ± 0.31	0. 42	> 0.05
Transferrin (mg/dl)	183. 0 ± 6.60	193 .97 ± 9.73	0.89	> 0.05
Percent (%) Fe saturation	29.74 ± 1.69	31. 81 ± 2.39	0.71	> 0.05

TIBC = total iron binding capacity, Values are mean ± SEM
After Anetor et al., (2002).

Table 24: Haem-precursors, Erythrocyte Protoporphyrin (EPP) Porphobilinogen (PBG) Haem Degradative Product, Total Bilirubin in Lead Workers and Controls

	Lead Worker	Controls	t	p
Erythrocyte protoporphyrin (EPP)	±	±	-	-
porphobilinogen	-ve	-ve	-	-
Total bilirubin (mg/dl)	0.78 ± 0.03	1.0 ± 0.06	3.35	< 0.001

Values are mean ± SEM; ± = trace

Table 25: Haematocrit (PCV), Haemoglobin Concentration, MCMC, Polychromasia and Basophilic Stippling in Lead Workers and Controls

	Lead Workers	Controls	t	p
Haematocrit (PCV) (%)	41.0 ± 0.33	43.0 ± 0.41	3.73	< 0.001
Hb (g/dl)	13.57± 0.09	14.4 ± 0.13	4.99	< 0.001
MCHC(g/dl)	33.0 ± 0.13	34.0 ± 0.18	4.53	< 0. 001
Polychromasia	Absent	Absent	-	-
Basophilic stipplings (accumulated rRNA)	Absent	Absent	-	-

Values are mean ± SEM
After Anetor et al. (2002)

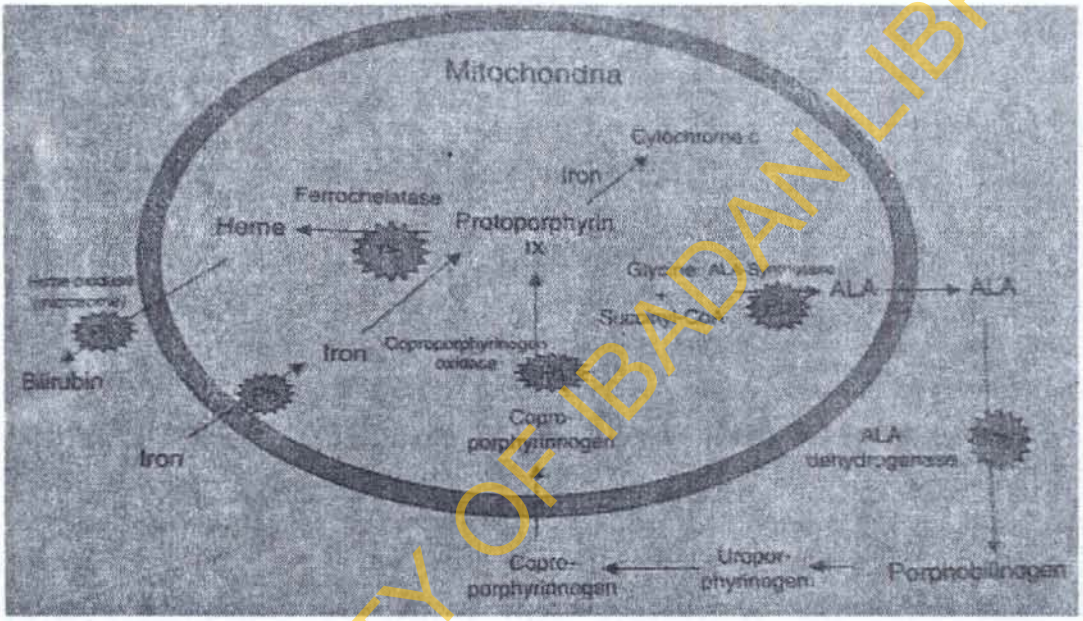


Fig. 13: Haem Biosynthetic Pathway: Points of interference by lead (Pb). After Liu et al. (2008)

Pb = **Point of lead interference with the haem biosynthetic pathway**

The Antioxidant Hypothesis and the Antioxidant Controveresy: Diabetes and Toxic Radicals as a Case in Point

Diabetes mellitus (DM) is one of the metabolic diseases now recognized to be caused or aggravated by free radicals or toxic oxygen species (ROS, TOS). It is widely recognized

currently that the global explosion in the incidence of diabetes mellitus particularly type II has reached an epidemic situation. Recent reports estimate that the total number of individuals with diabetes worldwide will increase from 171 million in 2000 to 366 million by the year 2030 (Wild et al. 2004). It is even more disturbing to learn of the predicted rate of increase in real terms for the next two decades, where it is speculated that one in every four individuals will be diabetic. Not unexpectedly, the picture appears more grue-some for the developing or resource poor countries. The two widely accepted explanations for the current state of affairs have been shift in dietary life style from a less calorie laden, complex carbohydrate-based and active life style to a more calorie dense, simple sugar-based diet and a more sedentary life style permissive of obesity. The possible involvement of TOS is hardly considered.

Reactive oxygen species appear to be involved in both the development and later complications of DM (Oberley 1988; Wolf 1993). Lipid peroxidation is elevated in diabetic patients. Evidence from both animal and human studies suggests that the antioxidant defence system becomes compromised prior to the development of the disease. Magnesium and zinc are important minerals which play important roles in metabolism. Knowledge and implications of the status of minerals though important is often overlooked. Understanding the roles of Mg and Zn may help in lowering the morbidity and mortality associated with diabetes. This may be particularly important in less developed (resource poor) countries like Nigeria with limited financial and health facilities. The growing awareness of the beneficial role that mineral element nutrition plays in amelioration of disease states, restoration of good health and enhanced physiological function was the key motivation of this study.

We investigated 40 type-2 diabetic (21M, 19F) and 20 non-diabetic Nigerians as controls; examining serum Mg, Zn and total cholesterol levels. The concentrations of Mg and Zn were significantly reduced, probably suggesting depressed antioxidant status and increased demand for these nutrients in diabetes mellitus. One implication of this, is greater

susceptibility of low density lipoprotein (LDL) – cholesterol (bad cholesterol) to oxidation which is more atherogenic. The attendant risk of development of premature coronary heart disease (CHD) is enormous. Magnesium and Zn are nutritional minerals that play crucial roles in the regulation of carbohydrate and lipid metabolism as well as the antioxidant system. Zinc is a component of the potent antioxidant, Cu-ZnSOD. Zinc deficiency may also lead to deficiency of other vitamins; E, and A (Goode et al. 1991; Christian and West 1998). Thus Zn deficiency leads to reduced total bio-availability of vitamins A, E supplies and consequently total antioxidant status needed to combat the increased free radical burden in this disorder which is exacerbated by the concomitant low Mg level (Anetor et al. 2002b) (tables 26-27). These data appear to give some credence to the antioxidant hypothesis and weaken the argument of those proponents of the antioxidant controversy.

Table 26: Age Blood Glucose, Total Cholesterol, Serum Magnesium and Zinc Levels in Diabetes Mellitus

	Patients (n=40)	Controls (n= 20)	t	p
Age (years)	56 ± 1.6	51 ± 2.4	1.7	> 0.05
FBG (mmo/l)	8.8 ± 1.1	4. 11 ± 0.2	3.5	< 0.05
T. Chol. (mmo/l)	5.56 ± 0.11	4.5 ± 0.17	5.3	< 0.001
Mg (mmol/l)	0.75 ± 0.05	1.15 ± 0.05	3.1	< 0.001
Zn (µmol/l)	9.23 ± 0. 32	12. 46 ± 0.28	14.4	< 0.001

Values are mean ± SEM; FBG = fasting blood glucose

Table 27: Correlations among Blood Glucose, Total Cholesterol, Magnesium and Zinc Levels in Diabetic Subjects

	r	p
Glucose vs Magnesium	- 0.02	> 0.05
Glucose vs Zinc	- 0.14	> 0.05
Total cholesterol vs Mg.	0.60	< 0.001
T. Cholesterol vs Zinc	0.03	> 0.05

Increasing concern about this well-known metabolic disease that arises from either absolute or relative insulin deficiency that may also involve receptor metabolism led us to conduct further studies. According to the World Health Organization (WHO), about 347 million individuals worldwide suffer from diabetes currently. What is more disturbing is that though this is a global disease, over 80% of those dying from diabetes come from low and middle-income countries (Danaei et al. 2011; Rao et al. 2015). The majority of cases involved in the upsurge are of the type two form (T2-DM). The disturbing number of increase in individuals with diabetes has created enormous problem of immense dimensions, which aside from medical aspects has huge economic challenges to governments, society and individuals. It is clear to both scientists and leaders of government that there is need for paradigm shift in the understanding and management of DM. Though traditionally, lifestyle factors, obesity etc., have been considered to be the major cause of the disease as earlier indicated, emerging reports indicate that toxicants may play a crucial role in this disorder (Edwards and Prolialeck 2009). This is aside from the role of toxic or reactive oxygen species in the aetiology and evolution of the disease. These considerations made it instructive to us in the toxicology unit to conduct a number of studies on this increasingly exploding disease in an attempt to better understand the disease and possibly search for possible mitigating factors.

One of these latter studies (Anetor et al. 2007c) examined the possible contribution of oxidative stress. Oxidative stress is an important component of diabetes as confirmed by our earlier studies (Anetor et al. 2002b) and its complications. Uncoupling protein 2 (UCP-2) has been implicated in the pathway inhibiting glucose stimulated insulin secretion (GSIS). The micronutrient, manganese (Mn) is the key component of the mitochondria antioxidant (MnSOD), and plays a critical role in the superoxide uncoupling of UCP-2 pathway in GSIS. There is a disagreement on this about β-cell function and DM. We studied 50-established type-2 diabetics and 30 non-diabetics (tables 28-30). Our results provided

evidence suggesting absence of significant oxidative stress in the mitochondria, probably excluding a role for UCP-2 – superoxide pathway in the inhibition of glucose-stimulated insulin secretion. We called for caution in the precocious conclusion that interruption of UCP-2 activity may provide a viable strategy to improve β-cell dysfunction in type-2 diabetes. Very importantly our data suggested that the combination of increased Mn, thus increased activity of MnSOD, increased K^+, reflecting potassium channel activity, partial reduction in ascorbate level, increased BMI within reference limits along with hyperglycaemia may suggest absence of superoxide –UCP-2 pathway oxidation. Our finding called for a re-examination or need to explore alternative pathophysiological pathways. Our suggested panel appears a simple approach, readily available to the average clinical laboratory, rather than the purely research-based high technology approach, involving molecular genetics (that are still routinely beyond our reach) in current literature and promises to be of particular value to populations in resource poor countries who have to grapple with better understanding of the current epidemic that diabetes constitutes.

I am glad to report Mr. Vice-Chancellor, Sir, that this paper was quite impressive to the editorial board of Biological and Trace Element Research (BTER) with an invitation to become a member of the International Association for Bioinorganic Scientists (IABS) of the United States.

Table 28: Body Mass Index (BMI), Plasma Glucose, Creatinine, K^+ Levels in Diabetics and Non-diabetics

	Diabetics	Non-diabetics	t	p
BMI (Kg/m^2)	25.27 ± 0.07	23.17 ± 1.65	5.78	< 0.01
Glucose (mmol/l)	11.07 ± 0.08	4.83 ± 0.02	8.91	< 0.001
Creatinine (μmol/l)	170.0 ± 140.0	100.0 ± 0.00	1.32	> 0.05
K^+ (mmol/l)	4.71 ± 0.023	4.17 ± 0.17	3.0	< 0.01

Table 29: Plasma Ascorbic Acid and Manganese Levels and Percent of both in Type-2 Diabetics and Non-diabetics

	Diabetics	Non-diabetics	t	p
Ascorbic acid (µmol/L)	10.0 ± 0.06	20.0 ± 0.3	0.50	> 0 .05
Mn (µmol/L)	3.8 ± 0. 007	1.8 ± 0.005	3.33	< 0.01
Percent (%) of Mn in DM to that of controls	211	-	-	-
Percent (%) of ascorbate in DM to that of controls	50	-	-	-

Values are mean ± SEM

Table 30: Correlation of Serum Mn Level with Serum Levels of Glucose, Ascorbate, K^+ & Creatinine in Diabetics

	r	p-values
Glucose (mmol/L)	- 0.08	> 0.05
Ascorbate (µmol/L)	- 0.05	> 0.05
K+ (mmo/L)	- 0.17	> 0.05
Creatinine (µmol/L)	- 0. 07	> 0. 05

Cadmium and Diabetes

The next in the series of our studies on this disease of growing concern was to examine the possible contribution of cadmium a ubiquitous and rising environmental pollutant (Anetor 2012) which in addition to other harmful effects has emerged as a major concern not only in environmental toxicology but also in metabolic diseases such as diabetes mellitus and its complications. Conflicting data aside, very few studies have examined a clinical population undergoing management as well as the possible modulation by a prominent metabolic antagonist of Cd, zinc. We examined the relationship between cadmium levels, glycaemic control and renal pathology (kidney disease) in established type II diabetic patients in a population exposed to modern environmental health hazards (MEHHs) classified using glycated haemoglobin (HbA1c) to good glycaemic control, fair control and poor control.

Contrary to our expectation Cd level was lower in diabetics than in non-diabetics (Anetor et al. 2016) (table 31). One of the key messages from this study is that the protective or ameliorative nutrient, zinc demonstrated significant inverse correlation with Cd (table 32). The lower Cd level found in diabetics compared to non-diabetics probably reflects the modulating effect of Zn in the treated diabetics (a Zn wasting disease) given nutritional education in addition to their regular regime, including consumption of good sources of Zn. This may be corroborated by the cadmium: zinc ratio which was significantly lower in diabetics than in non-diabetics, suggesting that Zn may have a profound modulating effect on cadmium poisoning or toxicity. It has been consistently demonstrated in our studies and reports from other laboratories that toxic metals mimic essential metals and consequently gain access to and disrupt key cellular functions (Shenkin 2006). This study appears to have elegantly upheld this seminal observation. It is an important concept that could be explored to ameliorate modern environmental health hazard (or poisoning) as illustrated between Cd and Zn in this report. The observed renal insufficiency with increasing Cd level may suggest that the progression of renal impairment may not be responsive to the putative modulating effect of Zn.

Table 31: Fasting Plasma Glucose, Cadmium, Zinc and Cd/Zn Ratio in Diabetics and Non-diabetics (Controls)

	Diabetic (n=45)	Controls (n=20)	t- value	p-value
FPG (mg/dl)	150 .90 ± 79.39	96.62 ± 11.957	4.474	0.000*
Cd (µg/dl)	0.05± 0.02	0.095 ± 0.031	5.763	0.000*
Zn (µg/dl)	102.51± 10.11	98. 1 ± 10.76	1.496	0.144
Cd/Zn ratio	0.002 ± 0.000	0.004 ± 0.001	5.485	0.000*

*Significant at the level indicated
FPG; fasting plasma glucose

Table 32: Correlation of Cadmium with Biochemical Indices

Parameters	r- value	p-value
FPG (mg/dl)	-0.196	0.133
HbA_1c (%)	-007	0.967
Zn (µg/dl)	- 0.317	0.014*
Creatinine (mg/dl)	0.149	0.258
Urinary albumin-creatinine ratio	-0.172	0.196
eGFR (ml/min/1.73m^2)	- 0.127	0.333

Significant at the level indicated
HbA1c = glycated haemoglobin; eGFR = estimated glomerular filtration rate.

Carbon tetrachloride (CCl_4) Poisoning

Carbon tetrachloride is a simple molecule and a common domestic and industrial chemical that is profoundly toxic to the liver. It was previously used extensively in dry cleaning and even as an anaesthetic. It is primarily hepatotoxic (damaging to the liver) where it causes two types of pathological effect. It causes centrilobular hepatic necrosis and fatty liver when administered to different types of species. An important feature of this solvent is that it is very lipid-soluble, thus well distributed throughout the body. Its target toxicity in the liver is because the toxicity of CCl_4 is dependent on metabolic activation of CY2E1; the liver is thus a target as it contains the greatest concentration of CYP450. It is selective for isoezyme CYP2E1 in the rat while CYP1A1 is unaffected. It elicits its toxic effect by oxidative mechanisms, forming hydroxynonenal (HNE), a reactive aldehyde that may lead to cytosolic calcium build up. Though extensively used, possible protective mechanisms have only received measured attention. We investigated the of veronal amygdalina on CCl_4 toxicity (Babalola, Anetor and Adeniyi 2001) and found it to be extremely protective against CCl_4-induced liver damage (hepatotoxicity). The advantage of this observation is that this extract is obtained from a common vegetable found all over the country and can be used as an antidote or prophylaxis to this chemical or related ones in domestic and industrial environment at a very cost effective rate.

Mechanistic Search for Remedy or Antidote of Poisoning

An antidote is a substance which specifically blocks or reduces the action of a toxin or toxicant in general. Nearly all the investigations in the field of metabolism of poisonous compounds called xenobiotic biotransformation arose from the studies of a scientist from Wales, Richard Tecwyn Williams. His early studies on the ring structure of glucuronic acid isolated from the urine of dogs fed borneol which enabled him to crystalize the conjugate that he employed as a base substance for the elucidation of its pyranoid structure. This encouraging outcome stimulated his interest in the biotransformation of exogenous substances, which led to a series of reports on the fate of phenols (major bi-product of the environmental toxicant, benzene), terpenes, and sulphonamides.

The concept of what is today called antidote is traceable to James Gillete, a major figure in biotransformation who along with Bert Ladu, Jr at the Laboratory of Chemical Pharmacology/Heart and Lung Institute at the National Institute of Health (NIH) where B.B. Brodie was the head (Lane 2014). Gillette's studies on cytochrome p450 were very significant and he succeeded Brodie in that Laboratory.

Another remarkable pair of scientists, indeed a couple, James and Elizabeth Miller, made seminal contributions to the field of biotransformation (Xenobiotic Metabolism). They were initially interested in understanding the conversion of synthetic and naturally occurring chemicals to toxic or carcinogenic electrophilic metabolites and the regulation of xenobiotic metabolism. The origin of their studies can be traced back to the late 1940s when the Millers demonstrated that a foreign chemical could be biotransformed to intermediates that covalently bind to macromolecules such as DNA (adducts). The administration of hepatocarcinogenic aminoazo dyes (cancer causing dyes) to rats culminated in the covalent binding of metabolites to protein in the liver. Interestingly, very little binding was observed in non-target tissues which did not exhibit tumor formation

(tumourigenesis). These investigators elegantly demonstrated that factors which influenced in vivo binding of aminoazo dyes to protein also influenced hepatocarcinogenicity (cancer development in the liver), an observation which led to their suggestion that covalent binding of metabolites to liver macromolecules was an essential step in carcinogenicity.

Laying the foundation for possible intervention by exploiting this pathway. They extended this line of thought to carcinogenic polycyclic aromatic hydrocarbons (PAHs) when they observed that metabolites covalently bound only to protein in the skin (Lane 2014). James Miller demonstrated the oxidation of a foreign compound in a cell- free system by enzymes later identified as cytochrome P450. He demonstrated that liver microsomes reduced the azo bond of 4-dimethylaminoazobenzene and that NADPH (a derivative, coenzyme of nicotinic acid, vitamin B_3) was required for the catalytic activity. James Miller also observed that flavin adenine dinucleotide (FAD) a derivative and co-enzyme from riboflavin (vitaminB_2) (a micronutrient), was required for azo dye reductase activity. These results provided a mechanistic explanation for the protective effect of riboflavin on carcinogenicity of the toxicants, aminoazo dyes. This in part gave the impetus for the thrust of my research in employing endogenous modulation of toxicity as an antidote for toxic states or poisoning. These observations implied that dietary substances, largely the micronutrients embracing the vitamins can inhibit the carcinogenicity of chemicals by influencing their biotransformation. This also provided an early foundation of cancer chemoprevention, the use of natural agents to prevent, delay or reverse the process of carcinogenesis. James Miller carried out several other experiments extending this principle; biotransformation of chemicals, though they did not pursue this line of research far enough, it paved the way for the discovery of cytochrome p450.

A very important contribution of the Millers for which many toxicologists and pharmacologists are very grateful is the discovery that foreign chemicals including drugs can

induce the synthesis of liver enzymes that can biotransform the compound administered including foreign (toxic) chemicals. Investigations on microsomal enzyme induction provided a mechanistic understanding of the inhibitory effects of some PAHs on aminoazo dye carcinogenesis. The studies by the Millers formed the basis of the investigations of Allan Conney (Lane 2014), who showed that increase in metabolism could be antagonized by inhibitors of protein synthesis. One contribution of the Millers to biotransformation and the concept of antidote is the important unifying concept that most carcinogenic and mutagenic (mutation causing) chemicals are not carcinogenic or mutagenic by themselves, but that the compounds must undergo biotransformation to reactive electrophilic intermediates that exert their toxic effects by covalently binding to critical sites on DNA or RNA, and protein (Lane 2014). This observation opened up the field of mutagenesis as it relates to chemicals and development of fast mutagenicity tests of potential human carcinogens. The induction of these enzymes is important in toxicology as it leads to an accelerated biotransformation of drugs and environmental chemicals in vivo and so alters their action and toxicity or poisoning for a more favourable outcome or protection.

The other aspect that influenced my line of research in this direction is another seminal observation by Parizek and Zahor (1956); Zahor (1957) on the toxic effect of cadmium on the testes. The potent deleterious effects of Cd on testicular tissue were first observed as early as 1919 by Alsberg and Schwartz (1919). They observed that administration of Cd salts in animals caused "bluish discoloration of the testicles". Their report escaped further examination for about forty years. Parizek (1957) reopened the subject and thoroughly investigated the destructive effects of Cd on testicular tissue. He observed that after the parenteral administration of Cd (2.2mg/kg), the testes became swollen, dark red, or purple. The weight fell rapidly, turning small, hard and yellowish. This sequence of events was followed by haemorrhagic, edematous and ultimately necrotic appearance. Here we may recall the remarks of Timbrell

(2009) on toxic responses. Parizek and Zahor (1956) and Parizek (1957) observed that the effect of Cd on the testes were sterilizing and developed rapidly, making the experimental animals rapidly sterile in as short as 24 hours.

Very importantly, Parizek (1957) demonstrated that large doses of zinc salts could prevent the action of Cd on the testes. Previous studies in the early and late 1960s (Kar et al. 1960, Gun et al. 1961; Mason and Young 1967) made similar observations. Zinc deficiency in animal models affects the gonads; it has been observed that Cd administration can displace significant amounts of Zn (Singhal and Merali 1979). Before then it had been suspected that Cd might exert its initial injurious effects on Zn-dependent spermatogenic elements. This suggested a form of antagonistic metal- metal interaction. Zahor had suggested that Zn might be essential for normal metabolic activity and/ or tissue integrity at some testicular site(s), where Cd could displace Zn. This study reaffirmed the possibility of using the antagonistic relationship of an essential element to a toxic one as a basis of antidote effect, a principle in which I have much interest and faith as one of the most pragmatic approaches to unpoisoning a poisoned world as our studies bore out.

Mason and Young (1967) had also confirmed that both Zn and Se (another micronutrient) are extremely effective in preventing testicular injury produced by Cd. We have consequently examined the effects of micronutrients to assess the metabolic influence of micronutrients in affording protection against the various metabolic and functional alterations caused by toxicants like Pb, Cd, As, Hg and other toxicants like benzene.

It is also of interest that both Se and Zn are capable of significantly ameliorating Cd-induced metabolic disturbances. We demonstrated this in the study in diabetes just discussed above (Anetor et al. 2016). These protective or antidotal effects largely appear to lie in the ability of these biometals to reverse the high affinity of Cd for sulphydryl (-SH) groups. It is postulated (Gunn et al. 1966) that the micronutrient Se complexes with Cd preventing sufficient free cadmium from reacting with bioavailable -SH groups.

Descriptions of some of these studies follow.

Lead Poisoning and Brain Function: A Biochemical Panel for Silent Lead Neurotoxicity

Neurotoxicity from lead exposure is of great concern, especially as lead is ubiquitous in the environment and even at low concentrations can elicit neurological damage; ideal blood lead level is zero. Levels as low as 2µg/dl have been associated with reduced IQ and behavioural deficits (Lanphear et al. 2005; Gilbert and Weiss 2006). Emerging reports suggest that deleterious effects of toxicants may not be clinically manifest for a long time after exposure to toxicants like Pb. This period is referred as the period of silent or latent neurotoxicity (Fretham et al. 2014). It is characterized by persistent biochemical or morphological injury which remains clinically unapparent unless explored by investigations. This situation may be comparable to the process of carcinogenesis in which molecular and cellular damage are evident long before the manifestation of cancer. Mechanisms of lead toxicity are incompletely understood and only few studies have examined these with the multiplicity of contending factors in the developing countries and their implications. We examined metabolic status and common health problems in occupational lead exposure in eighty-six lead workers fifty-one unexposed participants; 137 participants on the whole.

Blood Pb level and serum K^+ were significantly higher in Pb workers than in controls. It should be noted that K^+, like glycine has a potent effect on transmission of nerve impulses and neuron firing. In contrast total and ionised calcium, cholinesterase (ChE) and urinary ascorbic acid levels (nervous system is rich in ascorbate and very beneficial to it's function) were significantly lower. Calcium and ChE appeared in the multiple regression and principal component analyses model, indicating important relationship to PbB level (Anetor, Adeniyi Taylor 2002d) (tables 33 and 34). We suggest that these lead-induced biochemical changes have important neurological implications such as irritability,

insomnia and headache not commonly appreciated. Evaluation of these biochemical changes in combination with blood lead level (PbB) may constitute a panel of biomarker of silent neurotoxicity (braindamage). It may also serve in children as an early marker of functional deficits apparent later in life, congruent with Barker's hypothesis of fetal origin of adult disease. Another important inference from this study is that optimum levels of the nutrients, calcium, ascorbate and K may be protective of neurotoxicity and compromised levels permissive of the disorder. Indeed most of the neurotoxic effects of lead are exerted by interfering with calcium level.

Table 33: Common Health Problems or Complaints of Lead Workers

Health Problem/Complaints	Duration (weeks)	Percent (%)
Headache and pains	43	50
Easily tired/general malaise	14	16
Non-specific	12	14
Abdominal symptoms (Colic)	7	8.1
Insomnia/Sleep disturbance	5	5. 8
Irritability/Loss of memory	5	5.8
No symptoms (Nil)	5	5.8
Impotence/Infertility	3	3.5
Drowsiness	3	3.5
Total with neurological symptoms	71	83

Mainly symptoms and complaints that are neurologically related

Table 34: Blood Lead Levels and Biochemical Constituents Important in Neurological Function in Lead Workers and Control

	Lead workers (n= 86)	Controls (n= 51)	t- value	p - value
Blood lead(µmol/l)	27.72 ± 0.05	1. 47 ± 0.07	18. 91	< 0.001
K^+ (mmol/l)	4.7 ± 0.10	4.2 ± 0.13	2.63	< 0.01
T. Ca (mmo/l)	2.22 ± 0.09	2.30 ± 0.08	2.6	< 0. 01
I .Ca(mmol/l)	0.88 ± 0.03	0. 99 ± 0.04	6. 67	< 0. 001
Serum ChE (I.U/l)	2582 ± 98. 83	3126 ± 82.05	3.07	< 0.005
Urinary ascorbic acid (mg/ 100 mg/ creatinine)	9.0 ± 1.62	14.3 ± 1.23	1.57	< 0.02

Values are mean ± SEM

Lead Poisoning and Neural (Brain) Nutrients

Lead as we have seen above is a prime toxicant, but is particularly important as a neurotoxicant; it is one of the most widely used chemicals both domestically and in industry. The mechanism of the neurotoxic effects of lead are poorly understood (Arena 1979). In recent years there have been numerous indications that more investigations on the mechanism or mode of action of lead are needed (Walker 2000). Magnesium and thiamine (vitamin B_1) play important roles in the adequate functioning of the nervous system. Magnesium deficiency among others is associated with central nervous system symptoms including anorexia, personality changes, weakness, tiredness, vertigo, confusion, nervousness, irritability, tremors, involuntary eye movement, anxiety, insomnia, and the chronic fatigue syndrome (Soldatovic et al. 1998). These are also some of the protean symptoms associated with Pb toxicity (Sepallainen et al. 1983).

Interestingly, these two nutrients play very pivotal roles in energy metabolism. Thiamine (fig. 14), as part of TPP is needed for the generation of acetyl-coA to enter the Krebs's cycle for greater energy yield and Mg as the cofactor needed for ATP activity and as a cofactor for hexokinase. Though the subtle effects of Pb on the developing CNS is well documented, those on the adult CNS are not as well known. This is probably more so in environments with high carbohydrate intake, where magnesium, thiamine and other micronutrients, needed for adequate metabolism and neural health may be required in increased amounts. This study on neural nutrients and lead poisoning was designed to shed more light on this aspect of the subject (i.e. interaction between Pb and neural nutrients) and contribute to improved understanding and possibly as a basis for an antidote for the neurotoxic effects of Pb.

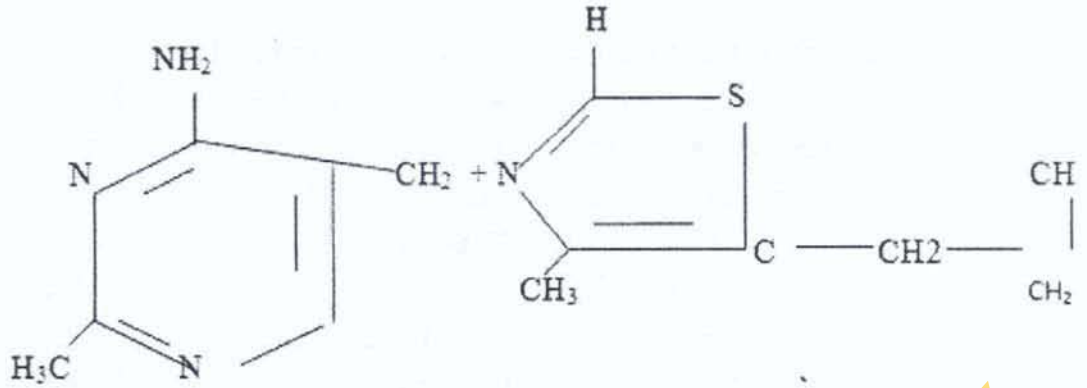


Fig. 14: Structure of thiamine [B_1].

The subjects were classified according to exposure category as in our previous studies. The blood lead level was higher in Pb workers but not significantly different ($P > 0.05$). In contrast magnesium and thiamine levels were significantly decreased ($P<0.05$; $P<0.01$) (table 35) respectively. The calcium level was also lower in Pb workers, than in controls bût was not significant ($P > 0.05$). Additionally, thiamine was significantly inversely correlated with BPb level (r-0.50; $P< 0.01$). Magnesium level was similarly negatively correlated with BPb but was not significantly (r-23, $P>0.05$). Calcium was in addition significantly inversely correlated with BPb level ($r - 0.41$; $P < 0.01$) (table 36). Remarkably, the low exposure category with the lowest thiamine level ($p<0.05$) compared to controls and significantly reduced Mg level ($p<0.05$) showed the highest BPb level, which was significantly higher than in controls ($p<0.05$) unlike when the whole cohort of lead workers was compared with controls (table 37).

Blood lead level in the controls though about half those in earlier reports, is still higher than currently acceptable levels in less polluted environments and may also be harmful in the face of reduced thiamine and magnesium levels. These results demonstrate that relatively low BPb levels, can enhance the absorption and potentiate neurotoxicity in the presence of decreased thiamine and Mg levels, particularly in calorie overdependence; thus modulating dose – response relationship.

Table 35: Age and Blood/Serum Levels of Measured Parameters in all Lead Workers and Controls

	Lead workers (n = 47)	Controls (n =25)	t	p
Age (years)	33.0 ± 1.2	36.0 ± 0.50	2.3	< 0.05
Lead (umol/L)	0.865 ± 0.06	0.754 ± 0.073	1.2	> 0.05
Thiamine (nmol/L	39.91 ± 2.85	58.23 ± 5.46	3.3	< 0.01
Magnesium (mmol/L)	1.05 ± 0.02	1.1 ± 0.05	2.24	< 0.05
Calcium (mmol/L)	2.27 ± 0.1	2.42 ± 0.1	1.14	> 0.05

Table 36: Comparison of Lead, Thiamine, Magnesium and Calcium Levels between Severe Exposure (group 1) and Controls

	Group 1 (n = 23)	Controls (n =25)	t	p
Lead (umol/L)	0.820 ± 0.170	0.754 ± 0.073	0.40	> 0.05
Thiamine (nmol/L	40.68 ± 6.64	58.23 ± 5.46	2.6	< 0.05
Magnesium (mmol/L)	1.0 ± 0.1	1.1 ± 0.05	0.1	> 0.05
Calcium (mmol/L)	2.25 ± 0.25	2.42 ± 0.1	0.4	> 0.05

Table 37: Comparison of Lead, Thiamine, Magnesium and Calcium Levels between Group Three (low exposure category) and Controls

	Group 3 (n = 10)	Controls (n =25)	t	p
Lead (umol/L)	0.995 ± 0.013	0.754 ± 0.073	2.5	< 0.05
Thiamine (nmol/L	37.44 ± 4.42	58.23 ± 5.46	2.3	< 0.05
Magnesium (mmol/L)	1.0 ± 0.01	1.1 ± 0.05	2.5	< 0.05
Calcium (mmol/L)	2.28 ± 0.13	2.43 ± 0.1	1.0	> 0.05

Table 38: Correlation among Lead, Thiamine, Magnesium and Calcium Levels in Lead Workers

	r	p
Lead vs thiamine	- 0.50	< 0.01
Lead vs magnesium	- 0.23	> 0.05
Lead vs calcium	- 0.41	< 0.01
Thiamine vs magnesium	0.20	> 0.05
Thiamine vs calcium	0.20	> 0.05
Magnesium vs calcium	0.07	> 0.05

The non-significantly increased blood lead level in lead workers compared to control, could probably be due to the short duration of exposure; about 1-5 years compared to those studied by Adeniyi and Anetor (1999) who were exposed for over thirty years. Despite this, there was an associated significant reduction in the levels of thiamine and magnesium ($p < 0.00$, $P < 0.05$) respectively in lead workers compared to controls. This suggests that lead has an antagonistic effect on these nutrients. This may be consistent with the observation of Shakman (1974) and Mahaffey and Vanderveen (1979) that acceptable Pb levels in the presence of reduced neural nutrients may be neurotoxic.

The exact mechanisms for this phenomenon have not been completely elucidated: competitive inhibition by toxic elements may be operant. This suggests increased demand for magnesium and thiamine in lead exposure. Magnesium is important for the activation of thiamine to its metabolically functional moiety and involved in its metabolism. Indeed, in the presence of magnesium deficiency, thiamine deficiency cannot be corrected, as it enhances thiamine deficiency (Zieve 1969). The significantly decreased thiamine level suggests antagonistic relationship between lead and thiamine. The exact mechanism may be multifaceted. The side chain hydroxyl group and sulphur in the thiazole nucleus may be chelated by lead (See fig. 14) probably as part of the general affinity of lead and other heavy metals for sulfhydryl (-SH) groups. Thus, more of the vitamin may be required to counter the toxic effects of lead, leading to increased demand in lead toxicity. Thiamine administration has been found to reduce the toxic manifestations of lead (Bratton and Zmudzki 1981; Flora and Tandon 1986). The side chain hydroxyl group and sulphur atom at the thiazole nucleus of thiamine participate in chelation of lead (Flora and Tandon 1986). Tandon et al. (2001) have recently observed that the daily intake of vitamin B1 along with vitamin C may prevent the accumulation of lead and reduce its toxic effects particularly in those regularly exposed to lead).

Thus, it is most probable that lead intoxication causes magnesium deficiency, which in turn interferes with responsiveness to thiamine by probably hindering the

formation of thiamine pyrophosphate, which in addition to its many metabolic roles, is involved in the synthesis of a component of the neurotransmitter, acetylcholine (Berdanier 1998). Very importantly, lead and thiamine levels were significantly inversely correlated (r –0.5, $p < 0.01$). An important implication of this observation for our environment (or the developing countries) where there is a disproportionate consumption of carbohydrate, requiring increased thiamine and magnesium for appropriate metabolism and utilization, is that the increased demand for thiamine implies a need to have a higher recommended daily intake (RDI) or prudent dietary intake (PDI) for this nutrient; to either eliminate or ameliorate the neurotoxic effects of lead exposed population. This is an important public health message for Zamfara and environ.

A striking and important observation in this study is that the lowest exposure category (lowest lead dose, with the least thiamine level) demonstrated higher lead level and was significantly higher than in controls ($P< 0.05$) (table 36). Thus reduced thiamine level has a potentiating effect on the absorption and toxicity of lead especially in the presence of significantly decreased Mg level. Thus, though lead level may be relatively low in a given population, its toxicity, including neurotoxicity can be magnified by relative unavailability of thiamine. Again bringing me to call for a revision of Paracelsus' dictum; 'the dose alone solely determines the poison'.

The findings reported in this study (Anetor et al. 2007c) have largely contributed to the understanding of the subject of lead neurotoxicity. They show that dose-response relationship may not be the major determinant of the absorption and possibly toxicity of lead, particularly its neurotoxicity (table 37). This implies that even relatively low lead levels may be potentiated by decreased thiamine and magnesium levels, particularly in predominantly carbohydrate dependent regions, where there is an increased demand for thiamine and magnesium required for proper metabolism. The observation here appears consistent with the recent views calling for a rethink of risk assessment by toxicologists (Colacci and Kleinstreuer 2015).

Industrial Pollution: Contribution of the Cement Industry

Many substances contribute to environmental pollution. One that is very pervasive and overlooked is the contribution of cement. The cement and steel industries are key to industrialization in any country. Activities related to cement production and utilization are numerous in many developing countries including Nigeria; Dangote group is welcomingly expanding this sector. This has also given rise to an upsurge in the number of factories and paces of production. Following conflicting reports on the composition of cement and possible toxic constituents, we set out to investigate the composition of cement in Nigeria and United States of America, through collaboration with the University of Texas Medical Branch, Houston, United States of America.

Introduction of toxic metals into cement has been related to combustion processes (Bhatty 1995). Owing to sustainability of cement production, many manufacturers have had recourse to alternative sources of energy and secondary fuels derived from industrial by-products that may be toxic (Bhatty 1995; Yan et al. 2010). Examples of such alternative source of fuels include solvents, used tyres, waste oils, paint residues, biomass such as wood-chips and sewage sludge (Bhatty 1995). These varied fuel sources are the probable contributors to the elevated toxic metal levels released from factories reported by different investigators.

Elevated concentrations of toxic metals may result in the following:

- Induction of oxidative stress via production of reactive oxygen species or reduction of antioxidant defences
- Disruption of calcium homeostasis
- Induction DNA damage
- Interaction with sulphydryl (-SH) groups
- Disturbances of haem biosynthetic pathway
- Presence of some known carcinogens (Templeton and Liu 2010)

Some leading investigators, such as Satoh et al. (1994) have reported that chromium induced nasal carcinoma in workers exposed to chromium: the workers were diagnosed of carcinoma of the nasal region after exposure duration of an average of about 8 years. Zhang et al. (2011) in their study reported increased DNA damage in workers exposed to Cr (VI). The findings of these investigators suggest that the health impacts of metals are more of chronic episodes than acute.

The highlights of our findings in the Nigerian- United States studies (Ogunbileje, Sadagoparamanujam, Anetor et al. (2013) conducted using graphite furnace atomic absorption spectrophotometry (GF- AAS) revealed that total Cu, Ni, Cr, Cr (VI) and Mn were significantly higher in USA cement dust. Nigerian cement dust in contrast and disturbingly contained higher concentrations of the key toxic metals, Cd, Pb and Hg. Some several fold than those in the USA samples (table 39 & fig. 15). It is also remarkable that some of the metals found in these dusts are established chemical carcinogens.

Table 39: Statistical Summary of Measured Heavy Metals and Cr (VI) Concentrations in Cement Dust from USA (U) and Nigeria (N) and Clinker from Nigeria (C)

Metals & Cr	U	N	C
*Cu(μg g-^{1})	23.66±7.23	6.92±0.86	6.47 ±0.84
*Ni(μgg^{-1})	47.45±3.21	17.34±0.97	20.82 ±0.81
*Mn(μgg^{-1})	2526.4±223.2	381.2±23.2	359.3 ±27.7
*Cr (μgg^{-1})	597.5±64.9	91.67±19.90	110.3 ±40.7
*Cr (VI) (μgg^{-1})	153.3±8.3	11.47±0.04	5.79 ±0.01
*Cd (μgg^{-1})	0.05±0.01	0.57±0.04	0.49 ±0.03
*Hg (μgg^{-1})	151.5±18.6	297.0±38.1	344.0 ±8.6
*Pb (μgg^{-1})	3.03±0.55	3.86±0.56	4.39 ±0.47
Ca (mgg-1)	369.3±11.7	335.8±4.8	340.6 ± 34.5
Zn (μgg-1)	474.6 ± 28.5	422.4 ± 18.1	466.4 ± 62.2
Fe (μgg-1)	23,587.7± 1268.2	17, 556.5 ±1115.5	21, 133.7±809.6

Source: Ogunbileje, Sadagopanamanujam and Anetor et al. (2013)

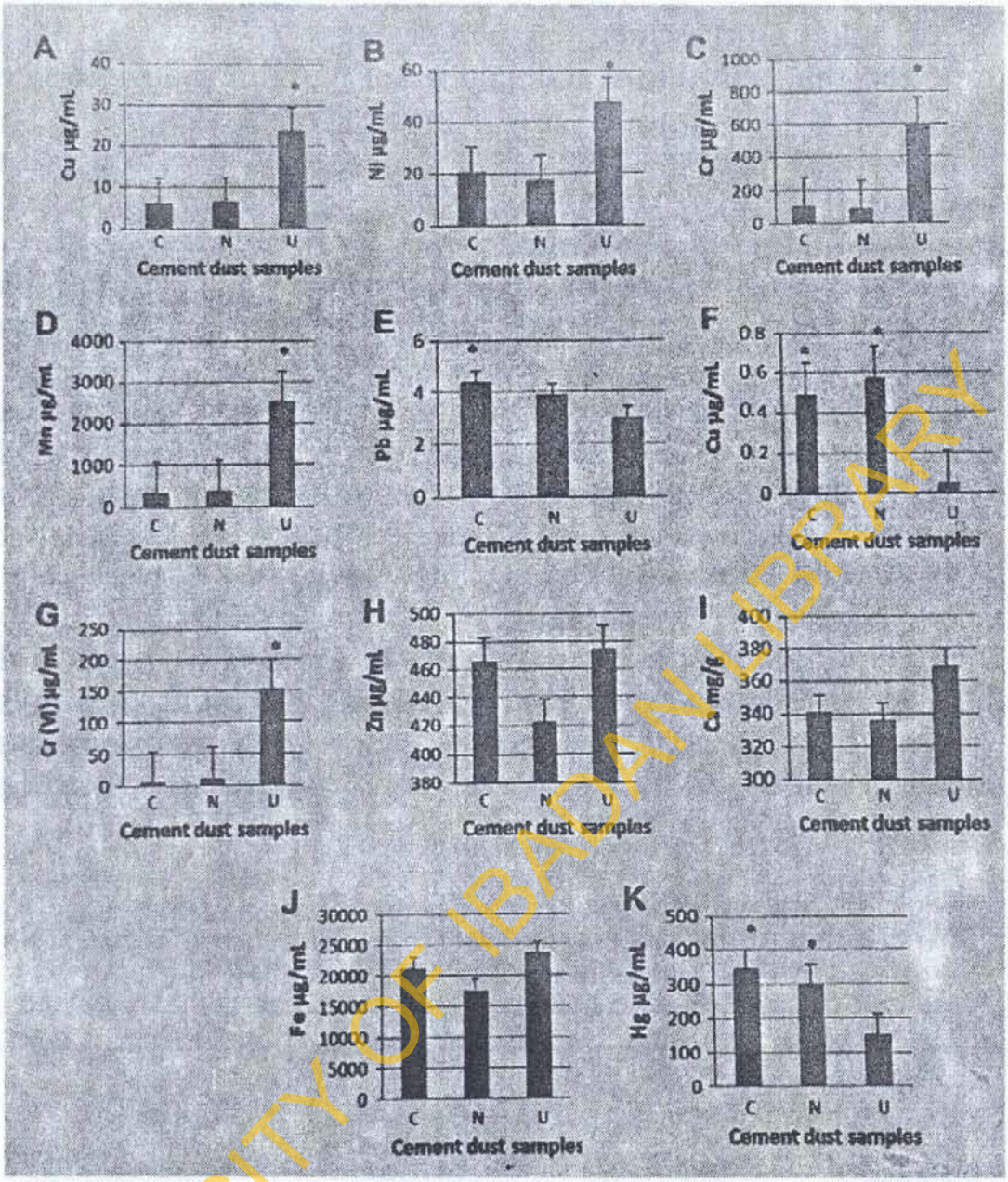


Fig. 15: Metal levels in Cement dust from United States (U), Nigeria (N) and Clinker (C). Cd, Hg and Pb are much higher in the samples from Nigeria (N), Compared to U.S.A. samples (U)

After Ogunbileje et al. (2013)

Data from the study on cement dust support the hypothesis that cement dust contains several toxic metals and Cr (VI) which can increase the risk of metal related diseases, thus acting as an independent risk factor in all those exposed to cement dust. Consequently, it is recommended that long-term consequences of human exposure to cement dust should be

investigated relative to the elements reported in this study. Many studies are in progress in this regard. These data suggest need for additional human studies relating the toxicity of metals and health effects in cement factory workers. Cement factories in Nigeria appear to be major contributors to environmental toxic metal contamination. With the rapid industrialization of Nigeria, it will be imperative that laws should be enacted in order to protect both workers in the industry and residents of the immediate environment. As a prophylactic measure, some of the principles we have enunciated in our studies could be employed.

Modulatory Effect of Zinc on Host Defence (Immune Function) in Welders and Cement Block Moulders

As follow up from the above study, we under took to evaluate the modulatory effect of zinc on a major host defence system of the human body, the humoral immune system. In an attempt to raise the standard of living and improve their economies, developing countries are emphasizing both small and medium scale enterprises (SMEs) on the understanding that SMEs play a substantial role in employment generation and national development (Akingbunola 2011). Welding and cement block moulding are among the most prevalent of these small scale industrial activities but associated with excessive occupational exposure to toxic substances such as Pb, Cd and many others which occur due to lack of proper workplace control measures. The upsurge observed in welding and cement block moulding activities in these countries may be related in part to the relatively low level of education required in these occupational activities. A country like Nigeria is a good case in point to assess the potential human health effects posed by these two occupations where there may be exposure to toxic metals with one also associated with high degree of exposure to an essential metal such as is seen in welding where there is simultaneous exposure to both toxic metals and the essential metal, zinc which has substantial effect on immune function.

Our study was conducted as outlined below. Thirty welders, thirty CBMs, thirty unexposed partici-pants were studied. Cadmium, Pb and Zn were determined using atomic absorption spectrophotometry. Key components of the humoral defence system; immunoglobulins, IgA, IgG and IgM were evaluated using ELISA. Total lymphocyte count (TLC) and monocyte (TMC) were also determined using standard haematological methods. Lead and Cd were expectedly significantly higher in welders and CBMs than in controls. Cadmium was significantly higher in welders than in CBMs unlike Pb. Remarkably; Zn was significantly higher in welders than in CBMs. Interestingly the anti-bodies IgG and IgM in welders were significantly higher than in CBMs; IgG being approximately two fold in welders (table 40). Monocytes were significantly higher in CBMs than in welders. These data suggest antagonism of Pb and Cd to Zn by the lower levels of zinc in the exposed groups and the humoral immunomodulatory effect of zinc, by the higher levels of immunoglobulins in welders with higher zinc levels (Adeniyi et al. 2016).

Table 40: Lead, Cadmium, Zinc Levels and Immunological Parameters in the Study Population and Control

Parameters	Welders Mean ±SD, Mean Rank	CBMs Mean± SD, Mean Rank	Control Mean± SD, Mean Rank	P-value
Monocyte (10^3 /µl)	0.2 ±0.1, 25.1	0.5 ± 0.2, 64.5	0.3 ± 0.2, 47.0	0.001
Lymphocyte (10^3 /µl)	2.5±0.6	2.6± 0.8	2.2 ± 0.5	0.003
IgA(mg/dl)	440.0 ±120, 58.9	380.0 ± 190, 35.6	360.0± 80, 42.0	0.002
IgG (mg/dl)	1530.0±560, 59.6	810.0 ±350, 27.8	1290.0±590, 49.1	0.001
IgM(mg/dl)	160.0 ±30.0	130.0 ±30.0	140.0 ±30.0	0.001
Zinc (µg/dl)	76.6 ± 8.3, 45.0	65.6 ± 10.1, 23.6	93.7 ± 16.3, 67.9	0.001
Cadmium (µg/dl)	0.1 ± 0.0, 68.1	0.01 ± 0.0, 52.2	0.002 ± 0.0, 16.3	0.001
Lead(µg/dl)	5.0 ± 2.1, 60.0	4.4 ± 2.3, 51.1	2.3 ± 1.0, 25.4	0.001

P< 0.003; 0.002 & 0.001 statistically significant
CBMs = Cement block moulders

Source: Adeniyi et al. (2016)

The data from this study suggest that exposure to welding fumes and cement dust perturbed humoral immune system and is modulated by the higher Zn level in the welders. The higher Zn level in the welders and the correspondingly higher lymphocyte and immunoglobulin levels in the same group appear to argue strongly for a modulatory role for Zn in immune response in these workers. This appears supported by the findings in the CBMs with low Zn levels exhibiting significantly decreased IgG compared to the welders: One implication of these data is that the immunosuppressive roles of toxic metals such as Pb and cadmium abundant in our environment may be modulated by raised zinc level. An indirect support for this appears to be demonstrated by the antagonism of Pb and Cd to Zn by the lower levels of zinc in the exposed groups than in controls and associated indices of immune response and may explain why there could be varied susceptibility to illness in occupationally exposed individuals. The data imply that cement block workers may benefit from zinc supplementation. This study appears to corroborate our previous study (Anetor and Adeniyi 1998) and adds to the mitigating or antidotal role of zinc in many pathologic states. (disease conditions)

Iron Poisoning in Pregnancy

Iron (Fe) remains a commonly prescribed supplement in pregnancy owing to the widely held belief that the requirement of Fe during gestation is very high and may be difficult to fulfill particularly in those entry pregnancy with low Fe level. While this may be true in a few instances, the majority of women do not require most of the iron supplements they consume (Whittaker et al. 1991). Ortega et al. (1998) have also contended the usefulness of iron supplementation in pregnancy. Consequently, it has been suggested that Fe status be monitored during pregnancy and Fe given when indicated in addition to improving the consumption of good sources of iron (Ortega 1998).

While this controversy is raging, reports indicate that iron is a free radical generator (pro-oxidant) and that supplements may put an individual at risk of excessive iron intake or poisoning (Halliwell and Gutteridge, 1998). Indeed the chemical properties of Fe render it a potential hazard within the organism in that ferrous ion (Fe^{2+}) in small non protein shielded chelates can catalyze the production of oxygen radicals or reactive oxygen species, which in turn can lead to peroxidation and radical chain reaction with molecular damage. Iron is involved in the Fenton reaction, generating hydroxyl radical ($OH^{\cdot}$) which is particularly toxic. Indeed it is one of the most reactive forms of the free radicals. Thus iron is important in toxic cellular injury (Pietrangelo 1998). The viciously reactive $OH^{\cdot}$ once generated attacks whatever it comes in contact with (Pietrangelo 1998). If the hydroxyl radical is generated adjacent to DNA, it attacks both the deoxyribose sugar and the purine and pyrimidine bases forming multiple products.

Less than a decade ago, Lachili et al. (2001) reported that iron overload could promote the generation of free radicals and these can result in deleterious cellular damage. Additionally these investigators observed that α-tocopherol level was lowered in individuals with iron overload. This suggests a state of oxidative stress. Albumin, urate, vitamins E and C are chain breaking antioxidants within membranes and lipoproteins, these could be easily employed in evaluating antioxidant status in pregnancy. It is important to recall that there is no regulated excretion of Fe in Fe overload. Thus iron overload may exist in pregnant subjects who take iron supplements, and this might cause oxidative stress in them with attendant pathologies. Iron supplementation in pregnancy is currently a routine practice in most pregnancy care centers (ante natal clinics) particularly in developing countries. Indiscriminate iron administration may carry greater risks than hitherto recognized. This possibility and the magnitude of the consequences have not been adequately explored or received the level of attention it

deserves. This study was therefore conducted to examine the possible risk non discretional iron supplementation may constitute in pregnant women with particular attention to oxidative stress and its public health significance.

The pregnant women were divided into two groups pregnant women on Fe supplement, non-supplement pregnant women from non-drug using Christian sect. They were further classified into the three trimesters of pregnancy. Our findings suggest considerable depression of antioxidant status in pregnant women on Fe supplement (tables 41-45) Anetor et al. (2009).

Table 41: Age, Gestation, Anthropometric Indices, Iron, Ascorbate, Copper, α-tocopherol, Bilirubin, Zinc Levels and Fruit and Vegetable Consumption in Supplement and Non-supplement Groups

	Test(n= 58)	Control (n= 55)	t-values	p-values
Age (Years)	26.81 ±3.94	27.71 ± 4.78	1.09	>0.05
Gestation (Months)	6.0 ± 2.29	5.58 ± 2.50	0.929	>0.05
Height (M)	1.58 ± 0.61	1.60 ± 0.61	1.52	>0.05
Weight (Kg)	66.31± 12.43	71.45 ± 12.52	1. 89	>0.05
BMI (Kg/m^2)	26.82 ± 5.75	27.85 ± 4.41	1.07	>0.05
Fe (μmol/l)	20.27 ± 9.29	13.81 ± 5.87	4.39	< 0.001
Ascorbate(mmol/l)	16.85 ± 7.70	20.59 ± 5.00	2.60	<0.05
Bilirubin(μmol/l)	0.84 ± 0.77	2.81 ± 2.0	4.66	<0.001
Cu (μmol/l)	16.12 ± 7.2	19.34 ± 5.9	2.59	<0.05
Vitamin E(μmol/l)	11.0 ± 3.67	11.07 ± 0.77	0.07	>0.05
Uric acid (μmol/l)	178 ± 78.3	205.53 ± 96.3	1.61	>0.05
Zn (μmol/l)	15.53 ± 5.3	18.43 ± 5.0	2.71	<0.01
Fruits &Vegetable Intake/24h (%)	57.17	37.13	-	-

Values are mean ± SD

Source: Anetor et al. (2009)

Table 42: Anthropometric Indices, Serum Iron and Antioxidant Levels in the Three Trimesters of Pregnancy in the Supplement Group

	First Trimester (n=10)	Second Trimester (n=23)	Third Trimester (n=25)	Per cent (%) Changes (between 1st & 3rd Trimesters)
Age (Years)	28.40 ± 4.53	26.00 ± 4.25	26.81 ± 3.94	-
Gestation (Months)	2.10 ± 0.74	5.43 ± 0.73	8.08 ± 0.76	-
Height (m)	1.59 ± 0.26	1.59 ± 0.29	1.57 ± 0.23	-
Weight (Kg)	68.10 ± 6.02	65.57 ± 10.14	66.28 ± 13.2	-2.67
BMI (Kg/m^2)	26.78 ± 5.45	26.07 ± 10.14	27.52 ± 1.51	2.76
Iron (μmol/l)	10.69 ± 4.15	21.30 ± 6.80	23.16 ± 10.14	116.65
Ascorbate (mmol/l)	19.71 ± 4.39	16.82 ± 5.91	14.98 ±2.23	-23.5
Bilirubin (μmol/l)	1.14 ± 0.83	0.70 ± 0. 57	0.60 ± 0.38	-47.36
Copper (μmol/l)	15.00 ± 6.12	15.93 ± 6.26	16.08 ± 10.93	7.2
Vitamin E (mmol/l)	11.96 ± 3.35	10.85 ± 4.24	9.90 ± 2.56	-17.22
Uric acid (μmol/l)	211 .00 ± 80.36	155.42 ± 84. 12	135.59 ± 42.8	-36.02
Zinc (μmol/l)	17. 68 ± 4.69	15. 49 ± 5.23	14.40 ± 4.09	-8.55

Values are mean ± SD; Negative sign (–) before figures indicate decreases

Table 43: Anthropometric Indices, Serum Iron and Antioxidant Levels in the Three Trimesters of Pregnancy in Non-supplement Pregnant Subjects (Controls)

	First Trimester (n=12)	Second Trimester (n=20)	Third Trimester (n=23)	Per cent (%) Changes (between 1st & 3rd Trimesters)
Age (Years)	25.5 ± 4.53	29.20 ± 4.08	25.57 ± 4.88	-
Gestation (Months)	1.92 ± 0.79	5.0 ± 0.86	8.00 ± 0.80	-
Height (Meters)	1.59 ± 0.97	1.61 ± 0.90	1.60 ± 0.90	-
Weight (Kg)	67.33 ± 16.18	70.9 ± 11.63	74.09 ± 14.79	10.04
BMI (Kg/m^2)	26.66 ± 3.24	27.29 ± 3.74	25.95 ± 5.47	-2.66
Fe (mmol/l)	11. 34 ± 3.37	11.89 ± 3.74	17.06 ± 6.98	50.44
Ascorbate (mmol/l)	17.21 ± 9.56	16.96 ± 7.74	16.58 ± 7.15	-3.66
Bilirubin (μmol/l)	2.46 ± 1.78	2.30 ± 1.81	1.23 ± 1.47	-50.00
Cu (μmol/l)	18. 91 ± 5.89	19.46 ± 5.92	19 .47 ± 3.27	2.96
Vitamin E (mmol/l)	11.55 ± 4.42	11.0 ± 5.31	10 .70 ± 3.27	-7.30
Uric acid (μmol/l)	233.87 ± 109.00	205.13 ± 84.59	150 .41 ± 60.47	-35.69
Zn (μmol/l)	18.90 ± 5.89	18.03 ± 4.96	16.09 ± 1.83	-14. 86

Values are mean ± SD; Negative sign (–) before figures indicate decreases

Table 44: Anthropometric Indices, Serum Iron and Antioxidant Levels in the Three Trimesters of Pregnancy in Subjects on Iron Supplements

	1st Trimester (n=10)	2nd Trimester (n=23)	3rd Trimester (n=25)	F-value	p
Age (Years)	28.40 ± 4.53	26.00 ± 4.25	26.81 ± 3.94	4.16	0.073
Gestation(Months)	2.10 ± 0.74	5.43 ± 0.73	8.08 ± 0.76	26.94	0.001
Height (Meters)	1.59 ± 0.26	1.59 ± 0.29	1.57 ± 0.23	5.109	0.051
Weight (Kg)	68.10 ± 6.02	65.57 ± 10.14	66.28 ± 13.2	5.11	0.051
BMI (Kg/m^2)	26.78 ± 5.45	26.07 ± 3.36	27.52 ± 1.51	1.577	0.288
Iron (μmol/L)	10.69 ± 4.15	21.30 ± 6.80	23.16 ± 10.04	135.77	0.0001
Ascorbic acid(mmo/L)	19.71 ± 4.39	16.82 ± 5.91	14.98 ± 2.23	17.055	0.0033
Bilirubin(μmol/L)	1.135± 0.83	0.70 ± 0.97	0.60 ± 0.38	0.466	0.649
Copper (μmol/L)	15.00± 6.12	15.93±6.26	16.08±10.93	0.836	0.478
Vit. E (mmol/L)	11.96 ±3.35	10.85±4.24	9.90±2.56	3.189	0.114
Uric acid (μmol/L)	211±80.36	155.42±84.12	135.59±42.80	4597.6	0.0001
Zinc (μmol/L)	17.68±4.69	15.49± 5.23	14.40±4.09	8.371	0.0184

Table 45: Correlation of Iron with Antioxidant Levels in Supplement Pregnant Women

	r-values	p-values
Ascorbic acid (mmol/l)	-0.299	<0.05
Bilirubin (μmol/l)	-0.278	<0.05
Cu (μmol/l)	-0. 431	<0.01
Vitamin E (mmol/l)	-0. 120	>0.05
Uric acid (μmol/l)	-0. 383	<0.05
Zn (μmol/l)	-0.369	<0.05

Taken together these data add and extend the growing mass of knowledge suggesting iron supplementation is associated with profound oxidative stress with associated pathologic and clinical correlates which may present as teratogenic effects (birth defects), habitual abortions, retarded intrauterine growth, and low birth weight infants, pre-eclampsia and other complications of pregnancy. Thus, there is the need to exercise caution and discretion in the use of Fe supplements in pregnancy with each case considered on its own merit and more importantly calling for the need to evaluate antioxidant status in pregnant women, including determination of Fe level, before and during Fe supplementation when indicated. Owing to the seemingly preferential demand for ascorbic acid in the Fe supplement subjects, efforts should be made to improve the supply of ascorbic acid to combat the intense oxidative stress in these pregnant women which may assist in ameliorating the high maternal and child morbidity and mortality rates which may be unwittingly exacerbated by Fe supplementation culminating in iron poisoning. The data also suggest that an up-regulated supply of antioxidants, particularly vitamin C and Zn may have synergistic antidotal effect in pregnancy.

Oxidative Stress in Pregnancy

Pregnancy is an important state in the life of living organisms, as without this stage there will be no offspring and thus extinction and end of existence. It is associated with a series of small continuous physiologic adjustments that affect metabolism of all nutrients (King 2000) due to raised metabolic rate (hypermetabolic state) in the pregnant state.

Attendant oxidative damage (the imbalance between the rate of generation of free radicals and bioavailability of their antidotes, antioxidants) is an inevitable side effect of cellular metabolism leading to genome instability and associated pathological correlates (Thomson and Orvig 2003). Oxidative stress arising from hypermetabolic state in pregnancy, the concomitant change in nutrient metabolism and their possible contribution to the distressing foetal and maternal morbidity and mortality have only received limited attention particularly in a region of the world where malnutrition is also of serious concern.

Forty pregnant women divided into the three trimesters of pregnancy and 25 non-pregnant individuals of similar demographic and anthropometric characteristics were studied; specifically examining the levels of Cu, Mn, and Zn. The level of uric acid, an end product of nucleoprotein metabolism, and an important endogenous antioxidant was also examined. We observed significant reductions in the levels of Mn and Zn in the pregnant state. Unlike the latter, Cu was increased progressively while the endogenous antioxidant urate was significantly reduced in the pregnant state (Anetor Adelaja and Adekunle 2003) (table 46). Remarkably, Zn levels declined steadily in all 3 trimesters, but only significantly in the last trimester, while Mn and uric acid were significantly more elevated in the third trimester than in the first. Our study revealed that the changes in the antioxidant status are attributable to the second and third trimesters.

Table 46: Serum Levels of Micronutrients (Cu, Mn, Zn) and Uric Acid in Pregnant and Non-pregnant Women

	Pregnant subjects (n=40)	Non-pregnant subjects (n=25)	t - value	P -value
Cu(μg/dl)	204 .7 ± 5.3	137.4 ± 5.8	8.3	< 0.001
Mn(ng/ml)	8.7 ± 0.4	104 ± 0.7	2.4	< 0.02
Zn (μg/dl)	87.3 ± 2.7	97.4 ± 2.6	2.5	< 0.02
Uric acid (Mg/dl)	2.8 ± 0.1	4.9 ± 0.2	10.5	< 0.001

Prevalence of Zn deficiency was 4% in the non-pregnant population while it was 22.5 % in total pregnant state, but increased to 10% in the 1st trimester, to 20% in the 2nd trimester to 33.3 % in the 3rd trimester (table 47). This observation is important and probably alludes to suggestions that Zn, like Fe and folate should be given as supplement in pregnancy. Taken together, these data, suggest that oxidative stress is pronounced in the pregnant state probably from free radical poisoning and that micronutrients supplementation may play an important role as antidote. Considering their role in pregnancy, prevention of deficiency arising from increased demand and attendant oxidative stress may contribute to a reduction in the incidence of foetal and maternal ill- health and complications in pregnancy. We recommend that intervention should be mainly directed at the second and third trimesters.

Table 47: Comparison of Serum Levels of Micronutrients and Uric Acid in Pregnant and Non-pregnant Women in the Three Trimesters

	Non-pregnant	1st Trimester	2nd Trimester	3rd Trimester
Cu(μg/dl)	137.4 ± 5.8	185.6 ± 10.8*	200.4 ± 7.8*	220.4 ± 7.9
Mn (ng/ml)	10.4 ± 0.7	7.4 ± 0.90*	8.4 ± 0.5	9.9 ± 0.5
Zn (μg/dl)	97.4 ± 2.6	95.2 ± 4.5	92.1 ± 4.4	83.3 ± 4.5
Uric acid (mg/dl)	4.9 ± 0.2	1.9 ± 0.2*	2.0 ± 0.1*	3.0 ± 0.2

Values are mean ± SEM
*Significant at $p < 0.05$

An adequate evaluation of micronutrient status may help in assessing risk of maternal morbidity and mortality probably in part attributed to oxidative stress. This has tended to be restricted to iron and folate in the past (Rooney 1992). The practice should be extended to other micronutrients such as Cu, Mn, and especially, Zn which is central in the metabolism and bioavailability of other micronutrients, such as vitamins A and E (also antioxidants) as well as nucleic acid metabolism that are very essential for maternal and

foetal health (table 48). Optimal micronutrient status (thus adequate antioxidant status) may contribute significantly to a reduction in the incidence of ill health and many life-threatening complications of pregnancy that are still common in many developing countries. Indeed, giving priority to antioxidant micronutrients status in pregnancy in Nigeria may be an additional weapon (prophylaxis; antidote) against the distressing maternal morbidity and mortality rates in Nigeria and other affected countries.

Table 48: Prevalence of Zinc Deficiency in the Pregnant and Non-pregnant States

Subjects	Number of Women with low Zn level*	Percentage (%) of women with low Zn level*
Non-pregnant (n= 25)	1	4.0
1st Trimester (n= 10)	1	10.0
2nd Trimester (n =15)	3	20.0
3rd Trimester (n =15)	5	33.3
All pregnant women (n= 40)	9	22.5

* A low level is defined as that below the lower limit of reference range

Pregnancy and Cadmium Poisoning: Ameliorative Potentials of Essential Trace Elements (ETEs)

Cadmium is currently of great concern as it is rising in rapidly industrializing countries, especially India, China and by extension, Nigeria (Yan 2006; Sun 2007). Our study on cement dust (Ogunbileje et al. 2013) and the increasing number of cement factories suggest this may be also be a source of significant Cd contamination among others. Cadmium-products are in increasing demand and consumption with concomitant increased waste laden with Cd that may contaminate the environment (Bakare et al. 2008). It is thus a ubiquitous environmental pollutant to which humans are increasingly exposed. Though scattered reports suggest that Cd may be associated with the delivery of low birth weight (LBW) babies, the mechanisms, possible amelioration

and implications for the already high maternal and child mortality rates had not been addressed. We examined 160 individuals comprising of 125 pregnant and 35 non-pregnant women. We classified the pregnant group into, the traditional three trimesters of pregnancy. The last (third; 55 participants) trimester was followed up till delivery. The following were the key findings (tables 49-52):

- 7% gave birth to high birth weight (HBW) babies.
- 58% of the pregnant women gave birth to normal birth weight (NBW) babies.
- 35% gave birth to LBW babies.
- Remarkably women with LBW babies demonstrated higher Cd and lower, Fe, Zn levels and BMI (It is again important to draw attention to this).
- LBW babies exhibited smaller head circumference and shorter length inversely correlated with Cd level (tables 50 & 51) (Ikeh-Tawari, Anetor and Charles-Davies 2013).

We concluded that environmental pollution be borne in mind in regions with persistent low birth weight babies and that supplements with essential trace elements such as Fe, Se and Zn may play ameliorative roles. These appear to be the new remits for the contemporary Chemical Pathologist (risk assessment in clinical care).

Table 49: Comparison of Demographic Indices, Anthropometric Measurements and Nutritional Parameters of Non-pregnant and Pregnant Subjects in 1st, 2nd, and 3rd Trimesters

Parameter	NP (n=35)	1st TRI (n=35)	2nd TRI (n= 35)	3rd TRI (n= 55)	F- value	p- value
Cd (µmol/l)	0.22 ± 0.1	0.20±0.1	0.21±0.1	0.25±0.1	3.478	0.017*
Zn (µmol/l)	11.7 ± 4.3	11.4±2.9	11.3±2.7	10.4 ± 2.4	1.407	0.043*
Fe(µmol/l)	26.9 ± 6.5	23.7 ± 6.6	25.9 ± 6.2	23.6 ± 5.6	2.742	0.045*
Cu(µmol/l)	14.4±5.7	14.9 ± 3.3	15.4 ± 2.1	16.8 ± 3.6	3.190	0.023*
Se (µmol/l)	3.5 ± 0.8	3. ± 4 0.7	3.5 ± 0.7	3.4 ± 0.8	0. 126	0. 945
T. Prot.(g/l)	60.9 ± 1.0	64.0 ± 0.9	58.0 ± 1.2	63.0 ± 1.2	3. 487	0.017*
Alb. (g/l)	39.0 ± 0.9	35.0 ± 0.7	32.0 ± 0.7	33.0 ± 0.8	8.097	0.000*
Age (years)	28.6 ± 7.1	28.6 ± 5.7	29.4 ± 4.9	29.5 ± 6.5	0.336	0.871
Parity	2.0 ± 1.6	2.1 ± 1.6	1.9 ± 1.7	2.2 ± 1.5	0.236	0. 671
BMI(kg/m^2)	25.3 ± 3.6	26.1± 4.9	27.6 ± 5.4	28.7 ± 1.5	0.997	0.039*

*Significantly different at indicated levels, NP = Non-pregnant, TRI= Trimester, BMI= Body mass index

Source: Ike-Tarawari (2013)

Table 50: Third Trimester Maternal Indices of Women with Low and Normal Birth Weights Babies

Maternal Indices	Low Birth Weight (n=19; 35%)	Normal Birth Weight (n=32; 58%)	t- value	p-value
Cd (µmol/l)	0.03 ± 0.01	0.02 ± 0.01	7.918	0.017*
Zn (µmol/l)	8.9 ± 1.8	11.2 ± 2.3	3.706	0.000*
Fe (µmol/l)	22.6 ± 4.9	25.4 ± 6.5	1.784	0.08*
Cu (µmol/l)	16.5 ± 3.1	16.9 ± 3.7	0.420	0.676
Se (µmol/l)	3.5 ± 0.7	3.6 ± 0.8	1.628	0.109
Total protein (g/l)	69.0 ± 1.54	60.0 ± 0.8	2.756	0.008*
Albumin (g/l)	32.9 ± 0.6	33.1 ± 0.94	0.088	0.930
Age (years)	28.68 ± 7.5	29.97 ± 5.6	0.487	0.707
Parity	2.4 ± 1.6	2.1 ± 1.5	0.598	0.552
BMI (kg/m2)	22.8 ± 2.0	28.8 ± 4.1	4.590	0.000*

*Significant at levels indicated, BMI = Body mass index

Table 51: Neonatal Anthropometric Measurements of Babies with Normal and Low Birth Weight

Parameters	Normal Birth Weight (n=32; 58%)	Low Birth Weight (n= 19; 35%)	t- value	p- value
Birth weight (kg)	3.1 ± 0.4	2.2 ± 0.3	6.952	0.000*
Head circumference (cm)	34.6 ± 3.8	32. 3 ± 2.2	2.675	0.010*
Length (cm)	50.1 ± 3.6	46. 6 ± 3.4	3.818	0.000*

*Significant at the level indicated

Table 52: Correlation of Third Trimester Maternal Indices with Neonatal Parameters

Maternal Indices	Normal Birth Weight (r, p-value) (n =55)	Head Circumference (r, p- value) (n= 55)	Length (r, p-value) (n= 55)
Cd (µmol/l)	-708, 0. 000*	- 0.332, 0.013*	-0.499, 0.001*
Zn (µmol/l)	0.306, 0.023*	0.225, 0.039*	0.242, 0.036*
Fe (µmol/l)	-0.192, 0.160	-0.161, 0.241	0.157, 0.241
Cu (µmol/l)	0.022, 0.872	0.230, 0.901	0.066, 0.634
Se (µmol/l)	-0.305, 0.023*	-0.116, 0.399	-0.357, 0.058
BMI (kg/m^2)	0.781, 0.000*	0.537, 0.000*	0. 488, 0.000*

* Significantly different at indicated levels.

Source: Ike-Tawari (2013)

Cancer as Chemical Poisoning: A Call for Education

Prevention of poisoning requires sufficient knowledge of the hazard of the substances by the users. This is very important in industrializing countries. As we have seen in a number of our studies workers may present chronic poisoning as in metal workers, such as welders or radiator repairers. Central nervous system damage from pesticide exposure may also be evident (Anetor et al. 2001d). While in the advanced countries employers have been mandated to adhere to details of occupational exposure requirement for the use of protective devices these safety requirements are hardly observed in the developing countries, thus higher exposure intensity are expected.

A sizeable proportion of the present incidence of cancer appears be the result of chemical carcinogenesis (cancer from chemical exposure or poisoning). The estimates vary, ranging from as low as 4% to as high as 60% or more (True and Dreisbach 2002). I am not sure if developing countries' specific estimate has been conducted. In the United States, the Occupational Safety and Health Administration (OSHA) has established nil or zero levels of tolerance in work environments for many substances including chemicals suspected to be carcinogens to humans. We need to do the same (educate) in Nigeria as part of the detoxification process.

In addition to this all individuals should be familiar with the concept of risk versus benefit in using chemicals everywhere, at home, in the work place or in the environment. Workers who use toxic substances that generate aerosols must be advised to use well- fitted respirators with organic filters, not simple dust masks that are insufficiently protective (True and Dreisbach 2002). Importantly also, people should know that there are no safe chemicals but only safe ways of using them. Consequently, all dangerous medicines, including aspirin, soluble iron salts, and all house hold chemicals should have poison labels on them. This is particularly important in our country where the literacy level is still low and many drugs can be purchased over the counter

with ease. We investigated the contribution of a common poisonous substance to a common cancer, liver cancer.

Arsenic Poisoning and Hepatocarcinogenesis (Liver Cancer)

Arsenic is one of the highest priority hazardous substances around the world currently and is well known for its toxicity and carcinogenicity in humans (National Research Council, 1999). Methylation of inorganic arsenics is an important step during the process of their elimination in many mammals. Generation of monomethylarsonic acid (MMA (V)), monomethylarsonous acid (MMA (III)), dimethylarsinic acid (DMA (V)), dimethylarsinous acid (DMA (III)) and trimethylarsine oxide (TMAO (III)) is as a result of the action of the enzyme methyltransferase, a reaction requiring S-adenosinemethionine (SAM). Dimethylarsinic acid (DMA) (V) is a major metabolite of organic arsenics in the environment and the main metabolite of ingested inorganic arsenicals in most mammals including humans (Goering et al. 1999; Kitchen 2001; Bramans and Foreback 1973). DMA (V) itself is used as herbicide and pesticide and also naturally exists in some sea food. Studies have revealed that DMA (V) is a genotoxic, multi-site promoter of carcinogens in rodents (Yamanaka et al. 2001). Thus providing a novel clue to investigate the mechanism of arsenicals in carcinogenesis.

Currently, millions of people all over the world are exposed to this ubiquitous toxicant at exposure levels leading to long-term toxicity, particularly cancer (Basu et al. 2001; Gebel et al. 2001a). Epidemiological studies have indicated that populations exposed to high levels of arsenic are prone to develop liver, bladder, skin and lung cancers (Chiou et al. 2001). In addition to its carcinogenic effects, arsenic exposure has been suggested to play a role in black foot disease (a form of peripheral vascular disease) (Tseng et al. 1996), type II diabetes mellitus (Tseng et al. 2000) and cardiovascular disease (Engel et al. 1994). Cancer of the liver is an important member of the various types of cancer associated with chronic arsenic ingestion from epidemiological evidence (IARC 1987). This is of major public health significance as

over 200 million people are exposed to inorganic arsenic in drinking water in many countries of the world. Animal studies have revealed that the liver is a major target organ for toxicity, being the organ most involved with the detoxification of xenobiotics and also the main storage site for glutathione (GSH), the cell protectant antioxidant against oxidative damage and other insults to the liver as well as the vital organ for methylation of inorganic arsenite (Healy et al. 1989).

There is dearth of data on the initiation activity of dimethylarsinic acid (DMA (V)), the major metabolite of this ubiquitous environmental and occupational carcinogen and toxicant. The initiation activity of DMA (V) was investigated on rat hepatocarcinogenesis; the liver being the major target organ for arsenic carcinogenicity and toxicity. A total of 50 rats at 10 weeks old were randomly divided in a 9-week medium-term bioassay into four groups (fig. 16). Groups 1 and 2 received 200 ppm of DMA (V) in drinking water for 4 weeks while groups 3 and 4 drank only tap water until the 6th week when groups 1 and 3 were given 0.01% 2-acetylaminofluorene (2-AAF) in diet as promoter for 2 weeks. All animals were subjected to two-third partial hepatectomy (PH) at the 7th week. Quantitative analysis of glutathione S-transferase placental form (GST-P) positive foci in liver, preoneoplastic makers of rat hepatocarcinogenesis demonstrated higher numbers in group 1(DMA(V) + 2-AAF) than in group 3 (2-AAF alone) at foci consisting of 2-4 cells and 15 or greater number of cells. The number of GST-P positive foci consisting of 5-9 cells was significantly higher in group 1 than in group 3. Figure 16 shows the experimental design. Foci consisting of 10-14 cells were also higher but not significantly. While GST-P positive foci were apparently similar in groups 2 and 4 (DMA (V) alone) and (untreated group; control) respectively. Expression of total GST-P positive foci was significantly higher in group 1 compared to group 2, 3 and 4 respectively. The proliferating cell nuclear antigen (PCNA) test performed to clarify the apparent trend of GST-P data revealed significantly higher PCNA labelling index (LI) in group 1 (fig. 17). These data are consistent with weak or initiation potential of DMA (V) and appear to

provide for the first time evidence for the initiation activity in DMA (V)-induced hepatocarcinogenesis in rats and call for caution in holding on rigidly to the existing paradigm that DMA (V) is only a promoter by modulating the signaling pathways responsible for cell growth, until additional data or confirmation of the present study are available (Anetor et al. 2007d).

This study has great implications for this environment in that a lot of food is imported from Asia where arsenic is currently a major problem. Additionally, the mitigation of arsenic poisoning requires adequate supply of GSH derivable from protein, which may be grossly inadequate in populations with compromised nutritional level including MDDs. Thus even low As level may have magnified effect. A number of our studies have repeatedly demonstrated this.

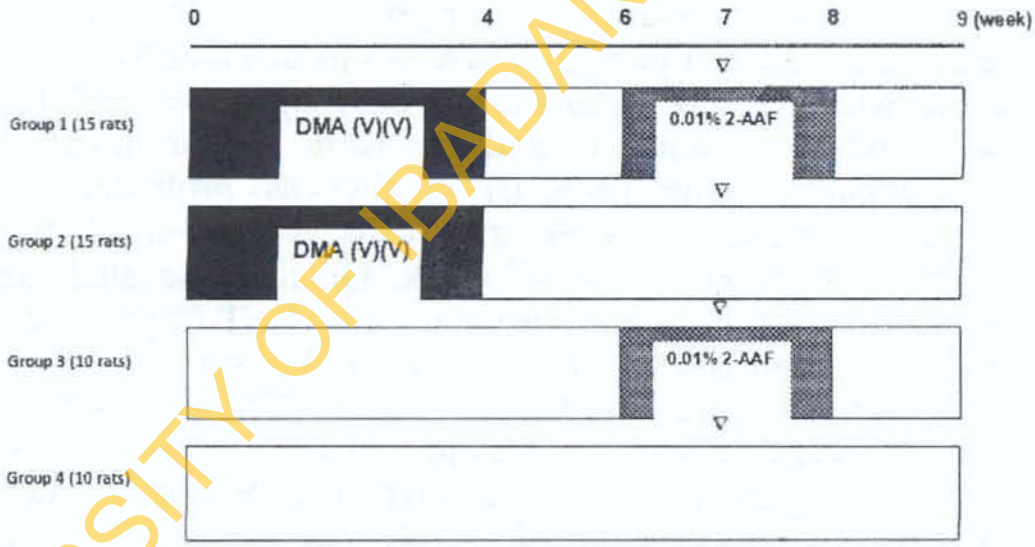


Fig. 16: Experimental design.

Animals: Male F344 rats, 10 weeks old

Test chemicals:
DMA (V): dimethylarsinic acid; 200 ppm in the drinking water.
2-AAF: 2-acetylaminofluorene; 0.01% in the diet
∇, PH: 2/3 partial hepatectomy at experimental week 7

Examination:
GST-P
PCNA

After Anetor et al. (2007d)

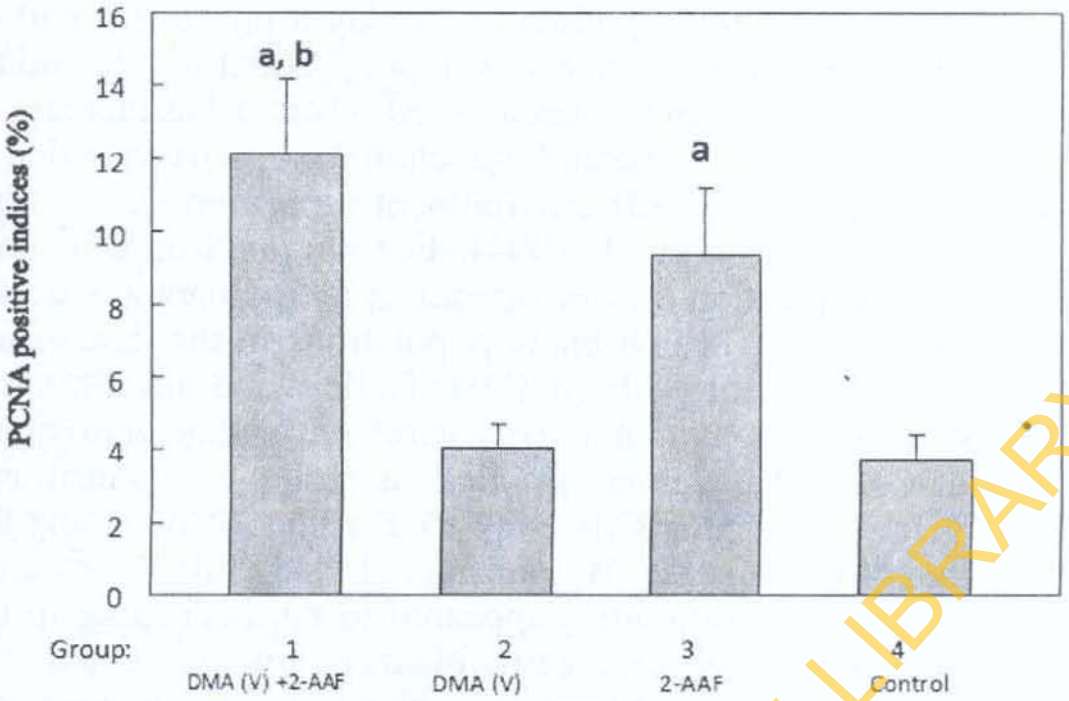


Fig. 17: PCNA indices in rat livers, significantly different from control group (group 4). [b]Significantly different from 2-AAF group (group 3).

Studies on Chronic Obstructive Airway Disease: Asthma

Asthma, one of the commonest chronic obstructive air way diseases was previously considered rare among Africans (Ayers 1995). But it is now known to be common (probably paralleling the degree of pollution in the environment due to progressive industrialization) but poorly managed (Unwin and Alberti 2000). Studies have shown that inflammation in the bronchi plays an important part in the aetiology and development of bronchial asthma (Barnes 1991). Mounting evidence indicate that the allergic disease asthma is caused by a flaw in the free radical-antioxidant balance (Soutar et al. 1997; Kelly et al. 1999). Indeed free radicals (chemical species with unpaired electrons in the outer orbital) are now known to mediate the inflammatory process so characteristic of asthma (Kelly 1999).

Increased environmental pollution or environmental poisons was in the past blamed for the global prevalence of asthma thought to parallel industrialization. Though this has declined in the advanced countries like the United Kingdom

and the United States, pollution is today a prominent part of the environment in many developing countries including Nigeria. The question has been asked whether the increase in the prevalence of asthma and bronchial hyperactivity is due to a more toxic (poisoned) environment or a more susceptible population (Seaton et al. 1994). For our environment both sides of the question may be operative; antioxidant are mostly micronutrients of which many populations in the developing world are deficient (Gibson 1994; Underwood and Smitasiri 1999). We thought that it was good news that antioxidant modulation of asthma implies a role for nutritional intervention and points the way to a means of reversing the upsurge of this disease. We considered that while the medical and scientific communities appeared to be awakening to the potential benefits of using antioxidants to ameliorate (prevent, manage and reverse) asthma its implication for prognosis that had received no attention should be addressed.

Fifty asthma patients, adopting an approximately one in ten random patients of all asthmatics registered at the Allergy Clinic in the Medical Out Patient (MOP) department, University College Hospital, Ibadan were selected. They were classified into two groups; moderate and severe cases, largely based on the level of distress according to the criteria of Eric and Halpren (1994). I am glad to report (Anetor, Ajose, Ige et al. 2003) (tables 53 & 54) that significantly lower levels of magnesium and key antioxidants, vitamin C, albumin and uric acid were found in asthmatics. The very substantial reduction in vitamin C level compared to non-asthmatic (or controls); in this study; over 260-fold is noteworthy (table 53). Surprisingly, the anti-oxidant status did not differ with severity of the disease except for urate level which was up-regulated in the severe group (table 54). Our findings support and extend the emerging concept that antioxidants are consumed in this inflammatory disease which we have demonstrated to have a significant toxic component and that a corresponding increase in antioxidant supply may improve prognosis; also that this may at least in part be the scientific explanation for the varied responses to therapy by patients with this disease.

Table 53: Serum Albumin, Copper, Magnesium Uric Acid and Zinc Levels in Asthmatics and Non-asthmatics

	Asthmatic (n=50)	Non-asthmatics (n=25)	t-value	p-value
Mg (mmol/l)	0.76 ± 0.03	1.09 ± 0.02	4.66	< 0.001
Cu (µmol/l)	22.2 ± 5.0	23.1 ± 6.0	0.62	>0.05
Vit. C (µmol/l)	1.02 ± 0.22	272.54 ± 1.13	28.4	< 0.001
Albumin (g/l)	33.3 ± 0.08	45.0 ± 0.04	12.4	<0.001
Urate (µmol/l)	250 ± 30.0	340 ± 90.0	4.7	< 0.001
Zn (µmol/l)	18.4 ± 4.2	17.5 ± 8.4	0.34	> 0.05

Values are mean ± SEM

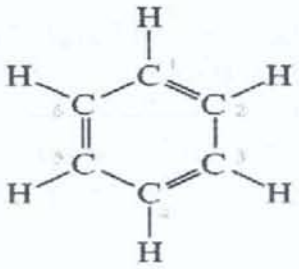
Table 54: Serum Magnesium, in Copper, Zinc, Vitamin C, Uric Acid and Albumin Levels in Groups 1 (Moderate) and 2 (Severe) Patients

	Group 1 (n=36)	Group 2 (n=14)	t-value	p-value
Mg (mmol/l)	1.0 ± 0.04	0.99 ± 0.62	0.54	>0.05
Cu (µmol/l)	22.1 ± 5.93	22.8 ± 8.43	0.31	> 0.05
Albumin (g/l)	33.0 ± 0.13	34 ± 0.13	0.90	> 0.05
Vit. C (µmol//)	0.85 ± 0.06	0.80 ± 0.06	1.06	> 0.05
Urate (µmol/l)	250.0 ± 27.0	470.0 ± 32	3.61	< 0.001
Zn (µmol/l)	18 .0 ± 5.2	16.0 ± 3.2	1.45	> 0.05

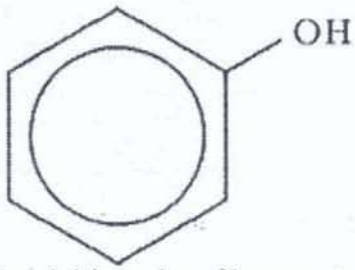
Values are mean ± SEM

Hydrocarbon Poisoning: Benzene

Nigeria for a long time has been a major explorer and exporter of petroleum products from which petrol is obtained and associated hydrocarbons derivatives. One of the commonest of these is benzene which is implicated in the bone marrow disorder, leukaemia. The mechanisms and modulating factors have only received limited attention. We investigated petrol dispensers who are exposed to benzene on a regular basis, using the major metabolite of benzene, phenol (fig. 18). Our results suggest as follow (fig. 19 and table 55):



Benzene Structure



Structure of phenol: Biomarker of benzene toxicity

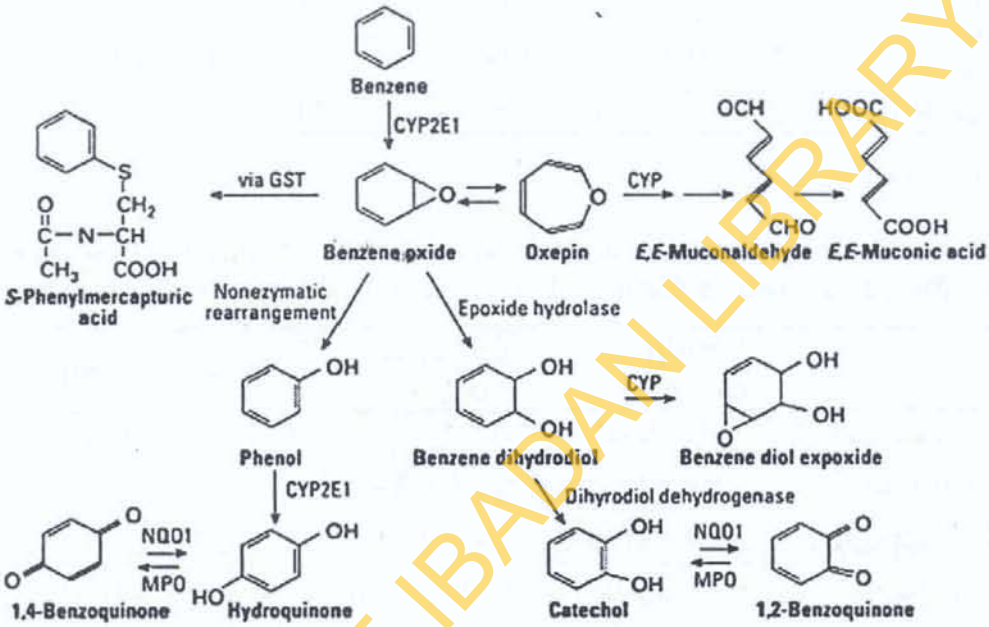


Fig. 18: Pathway of benzene metabolism.

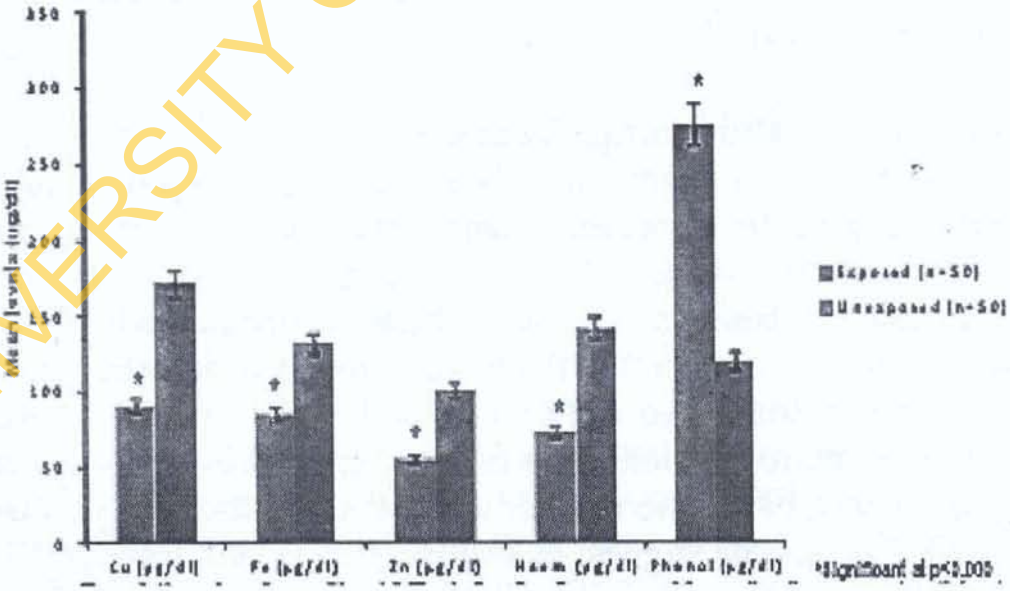


Fig. 19: Level of phenol and micronutrients in chemical workers.

Table 55: Correlation of Phenol with Micronutrients, Haem and Duration of Exposure

Correlating Variables	Gasoline Dispensers		Unexposed participants (Control)	
	r	p	r	p
Age vs Duration of exposure (Yrs)	0.768	0.000*		
Duration of exposure vs Phenol	0.107	0.460		
Age vs Phenol	0.206	0.855	0.187	0.197
Phenol vs Fe	-0.553	0.000*	-0.079	0.583
Phenol vs Haem	-0.375	0.007*	-0.242	0.090
Phenol vs Cu	-0.019	0.898	-0.243	0.089
Phenol vs Zn	-0.368	0.009*	-0.357	0.011*
Duration of exposure vs Haem	-0.107	0.459		
Duration of exposure vs Zn	-0.018	0.902		

Values are mean ± SEM

Marked perturbations of the haemopoietic system resulting from benzene toxicity is suggested in these gasoline dispensers. The major mechanism in part, seems to be a drain on the key micronutrients in this pathway; Cu, Fe and Zn, which play very important roles in the antioxidant defense system, Cu-Zn SOD and catalase and vital molecular activities.

Altered xenobiotic metabolism requiring haem in cytochrome P_{450}; cell cycle dysregulation where Zn plays a key role and p53 suppression also dependent on Zn may all co- exist in these chemical workers. Importantly, depression of the levels of these key micronutrients implies potentiation of myelotoxicity and myeloproliferative disorders arising from alterations in transcription, replication errors, genome instability and derangement in cell signal transduction all of which may raise the risk of carcinogenic potential in this pathway, the commonest of which is leukaemia. Understanding this benzene-induced pathobiology may be important in risk assessment, policy formulations and regulatory safety measures in these chemical workers and the general population (Anetor, Adigun, Bolajoko et al. 2015)

The Electronic Revolution: A Blessing or a Scourge?

There is no doubt that the explosion in the electronic industry has greatly enhanced communication, contributed to welcome

surge in economic activities, improved the quality of life, security but sadly has raised the scope, magnitude and frequency of crime. Where the concern of toxicologists really rests is the afterlife of electrical and electronic devices, they become a huge problem, which though occult to the lay public is worrisome to scientists. The huge electronic waste generated, most of which is shipped to the developing countries as second hand (tokunbo) as evident in figure 20; may be another way of chemical colonization; getting rid of waste in the advanced nations and dumping them on the resource poor recipient nations. Expectedly, Nigeria is one of the leading dumping sites of used electrical and electronic waste. You may ask me why are they of concern? They contain many precious metals that scavengers are searching for as well as abundant toxic ones such as Cd, Pb, As, Hg to mention a few. Again we are lucky in this university to have had one of the leading world experts in this university, Professor Oladele Osibanjo. With his team from the Basel Convention Coordinating Centre (BCCC) they have consistently reported that E-waste is becoming a big problem. But just like when the issue of climate change started their lamentation has fallen on deaf ears.

Fig. 20: E-waste pollution. *Source*: The Conversation, August 19, 2013.

To investigate the possible human health effect of E-waste a Ph.D student, Mr. Godwin Igharo, a lecturer at the School of Basic Medical Sciences, University of Benin is currently working on this. I invited Professor Osibanjo to join in the supervision of this student, he kindly consented. This very hard working and productive doctoral student has generated data that have resulted in four papers so far that are very instructive and disturbing. Expectedly he has confirmed extremely high levels of the key toxic metals in E-waste workers (Igharo et al. 2014). More importantly, the data suggested more serious health problems such as liver damage (Igharo et al. 2015a), increased risk of liver and prostate cancer (Igharo et al. 2015b) and putative mechanism of evolution of these disorders, oxidative stress among others (Igharo et al. 2016). These results demonstrate the magnitude of the health problem that is unfolding and will continue to increase and call for collective action now.

Concluding Remarks and Recommendations

Mr. Vice-Chancellor, Sir, I have attempted to show that we are poisoned from various sources and agents from our polluted environment, from the drugs we use, from endogenous metabolism including the metabolic processes associated with pregnancy and several more. This has led to the overthrow or disruption of the normal levels and relationships of the chemicals of life with dire consequences that set in insidiously. My conclusions will essentially be a summary of some of the reviews of some of our studies highlighting these lesions and suggested approaches to restoring physiological state or their mitigation. Let me start by saying that we have been like prophets; in 2000, it was clear to us that the African environment, using Nigeria as case study was poisoned with lead. In an article entitled 'Lead poisoning in Africa: a silent epidemic' (Anetor and Adeniyi 2000), we warned that there was wide spread lead poisoning and appealed to the African Union (AU) to set up centres of toxicology because of the cost involved in setting up toxicology services that many poor African countries cannot

afford. Shortly an outbreak of Pb poisoning was reported in Dakar, Senegal, then from Nigeria; the now globally recognized Zamfara lead poisoning, then Niger state and several unreported cases that are considered mysterious diseases. For me these are only tips of the iceberg. Nriagu et al. (1996) corroborating our view, had reported that over 90% of the children in Africa are poisoned with lead (BLL greater than acceptable levels; circa 2μg/dl). Based on our studies we made another pronouncement that is very germane to the theme of today's discourse. Industrialization and increasing risk of genome stability in developing countries: nutri-genomics as a promising antidote (Anetor 2008), suggesting genome protective nutrients acting at molecular level were needed to mitigate the situation. Further in another report; education in micronutrients in primary healthcare as prophylaxis for environmental pollution and disease (Anetor 2000a) we recommended that micronutrients were antidotes to environmental disease. We have also suggested that anti-oxidant micronutrients are inter-sectorial link between health and agriculture (Anetor et al. 2005). We argued for a functional partnership between the agricultural and the health sectors, with the agricultural sector mainly producing the sources of the micronutrients, embracing soil analysis to subject polluted farmlands to bioremediation to ensure that the micronutrients from plant and animal sources yield antioxidants that are contaminants free and will ameliorate poisoning, promote damage repair and boost host resistance to poisoning or toxic states. I have maintained that antioxidants if the proper ratios are ensured after scientific investigations are a veritable tool against cancer, other degenerative pathologies and toxic states (poisoning) (Anetor 2009b). The WHO Report 2011 identified some key micro-nutrients (iron, vitamin A and zinc) among the world's most serious health risk factors. Micronutrient malnutrition (Micronutrient Deficiency Disorders, MDDs) contributes to a vicious cycle of poor health and depressed productivity, trapping families in poverty and eroding economic security in many countries worldwide (Anetor 2000). Ensuring adequate intake of these essential nutrients by vulnerable populations

(mainly women and children) will offer enhanced protection against various forms of poisoning, whether endogenous or environmental, children will grow and learn and improve the health and productivity (including reproduction) of women.

This appear probably congruent with the observation of Gey (1986), a former Chief Nutrition Officer of the WHO, that if the key antioxidant levels; vitamins A, C, E, β-carotene, selenium and zinc levels are low, the risk of the population contracting cancer or cardiovascular disease is very high. Similarly, the Japanese during the heart of their industrial development increased intake of nutritional factors like vitamin E about ten-fold the RDA as protection against environmental pollution. My own argument is that the level of a chemical toxicant should not be used solely as the index of poisoning. Even when the toxicant is low or marginal as we have demonstrated, the key protective factors such as, Zn, vitamin C, Se, Mg, vitamin A, vitamin E, β-carotene and others such as GSH, if deficient or low the manifestation of poisoning is certain and that in the converse situation the organism or human will be protected. Figure 21 is an example of good sources of protective nutrients.

On a global level we have presented arguments that poisoning by such toxicants as metals can be mediated by micronutrients. The case of zinc as borne out from our data is particularly striking. Thus we have affirmed that micronutrients are host resistance against toxic states (Anetor, Wanibuchi and Fukushima 2007). It is undoubted that the Nigerian environment is increasingly toxic and that the population is concomitantly susceptible to poisoning owing to the unsung but real problem of hidden hunger or MDDs, thus the advice summarized in one of my reports- 'The increasingly toxic Nigerian environment and an increasingly susceptible population: a challenge to scientists in laboratory medicine and biology' (Anetor 2014) is relevant. The need for assessing of micronutrient levels in at risk populations as a step in risk assessment appears relevant.

Another of our report that may be useful in closing this discourse is the one on the Zamfara lead poisoning entitled, 'The Zamfara lead poisoning episode in Nigeria: An

indication for children's environmental toxicology and micronutrient centre' (Anetor et al. 2016a). Most of the conclusions and recommendations remain very relevant. In the same vein, very recently, we have shown (Adeniyi et al. 2016) that zinc modulated immune response in toxic states in occupational groups. Oxidative stress was amply demonstrated as a major mechanism in our poisoned subjects, suggesting a role for antioxidants as possible antidotes.

Generally, nutritional science is being increasingly recognized as having new and special roles in environmental toxicology and a major determinant of host resistance to susceptibility to chemicals in the environment (Anetor, Wanibuchi and Fukushima 2007). The importance of nutrition and its effect on susceptibility to development of poisoning is becoming better understood, and poor nutritional status apparently can greatly increase chances of poisoning like neurotoxicity as we have demonstrated in a number of our investigations. Nutritional factors influence the toxicity of many environmental pollutants, such as pesticides (common in developing countries) and toxic metals as we have consistently confirmed in our laboratory. We affirm that the concept of nutritional modulation of chemical toxicity is an important one, and one that should be exploited to the fullest by scientists and physicians.

Taken together and recognizing the usual uncertainty associated with science, I unequivocally state that the antidote we have found against current overt and occult poisoning of the world is the use of the micronutrients, which work as receptors, co-factors, co-enzymes, signal transducers, transcription factors, immune response enhancers and antioxidants). They restore the chemicals of life to their proper levels and order including genome stability, a precursor of the carcinogenic state, thus assisting in unpoisoning or serving as antidotes to our contemporary poisoned world. Though several aspects of chemical toxicity or poisoning are still unresolved based on weight of evidence, the precautionary principle is advocated to avoid late lessons from early warnings and should be one of the highest research priorities of developing countries.

Fig. 21: Fruits and Vegetables as antioxidant sources. Courtesy O.O. Ogundipe, M.D.

Before making my recommendations, I will be failing in my responsibility as a member of the global academic community if I do not comment on the toxicity that currently exists in academics in Nigeria. We appear to have forgotten our responsibility to society which Thomas Malone of the Research Triangle, North Carolina, painted as follows: 'The academic community is the principal generator, integrator, disseminator and overall custodian of knowledge' (Malone 1993). This is a great responsibility which we appear to be taking lightly by what I call increasing toxicity to each other and the very unscholarly attitude now very rampant. I have lamented on this elsewhere particularly the shameful situation in the health sector (Anetor and Oyedele 2016) in a book project by the Olusegun Obasanjo Presidential Library; 'Towards a New Dawn For The Health Sector in Nigeria Post 2015'. I was privileged to have been invited by Professor O.O. Akinkugbe, a concerned elder medical man, as one of the reviewers of the report. It is a landmark report I enjoin all to try and read (fig. 22).

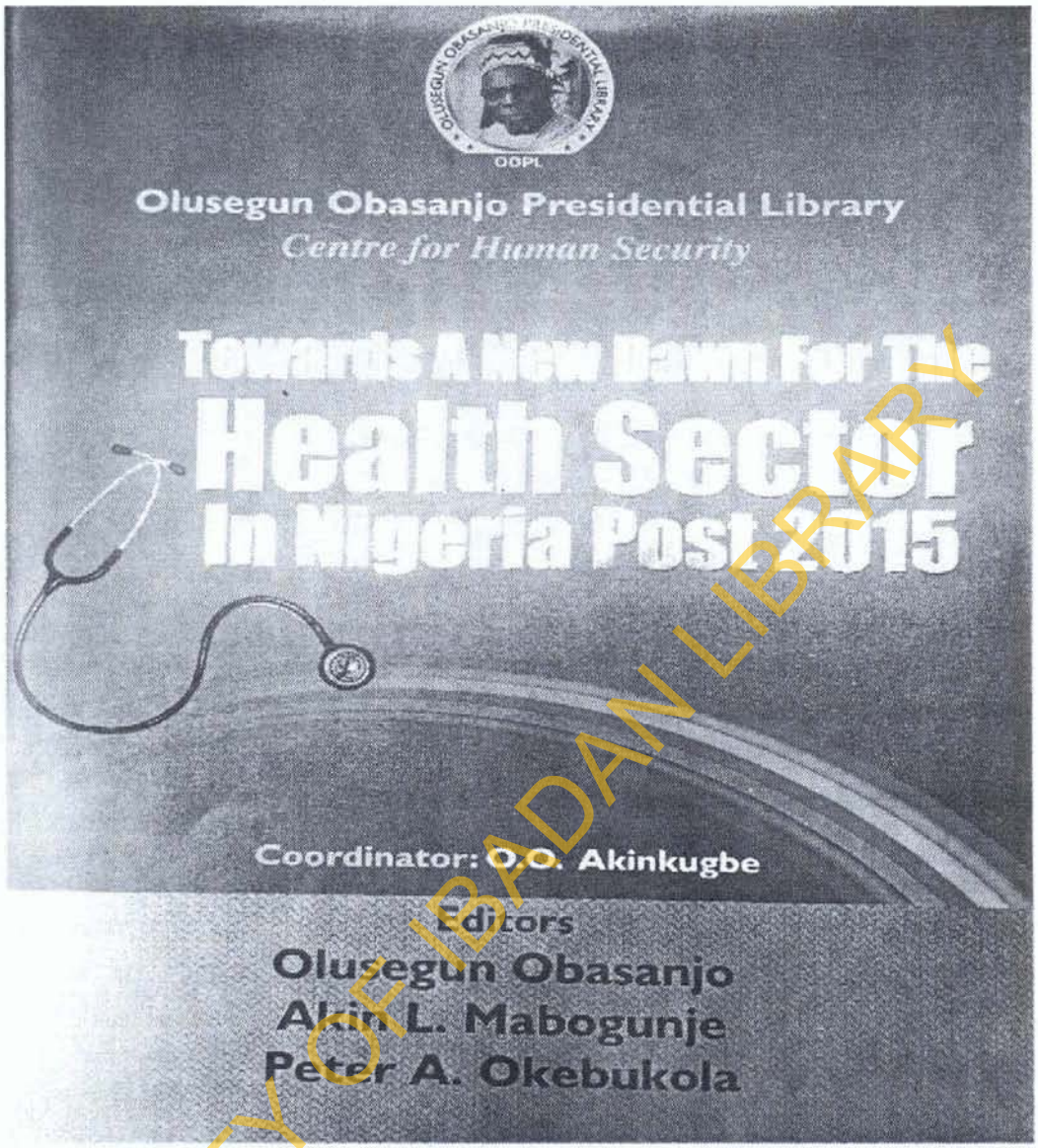


Fig. 22: Dealing with the toxicity among academics and health care professionals.

It is perhaps proper to examine what happens in some progressive centres. From Brown University comes the following, 'The Warren Alpert Medical School of Brown University comprises a community of scholars dedicated to the highest standards of excellent education, research, and healthcare. Our mission is to impart to future physicians and scientists the state of the art of the scientific, ethical and humanistic dimensions of medical science and of public health'. How do we fit in?

'The quality of our research can only be as strong as the foundation upon which it rests' – Robert Berdahl, President, Association of American Universities.

> ***'And if the fundamental operations of our research universities are deteriorating the country's research superstructure will inevitably decline as well.'*** (Ibid)

What are these fundamentals? How do they play out here?

From the University of Leicester I picked what I consider very inspiring and worthy of emulation in U.I.: ***'Elite without Being Elitist'***

We think a university should be about empowering people to explore what they do not know; through passionate, dedicated teaching and innovative, world class-changing research. By embracing the fact that we are all coming at it from a slightly different place, and that every journey is personal, we have managed to achieve some remarkable results in our time. We believe that the best universities are not just the privilege of elites. We're proof that you can stand alongside the best and open up the competition for everyone. Some universities consider their primary purpose to be high quality research, others concentrate on excellent teaching.

At Leicester we think that the two are not only complementary, they are inseparable. We believe that teaching is more inspirational when delivered by passionate scholars engaged in world-changing research- and that research is stronger when delivered in an academic community that includes students. With these ideas at heart, Leicester is reframing the values that govern academia and redefining what a university needs to be in the 21st century. We are constantly finding new ways of being a leading university". Can we say the same of us?

What I see, is that instead of emulating these laudable goals we are increasingly toxic to each other. Aside from my

research I had informally set myself the task of detoxifying our own toxic academic environment by deliberately absorbing whatever happens and enthroning desirable practices in refined societies. This has to be addressed if we are to attain true greatness. Let us embrace true scholarly principles. The situation now, particularly in the college is that academics have been virtually reduced to trade union activities. Even if this happens in other universities in Nigeria, University of Ibadan should resist this tendency of 'crush others by the privileged' group. Let us like Linus Pauling, a double Nobel Laureate pull the best from which ever field to build an enduring and effective system. Let us place the institution and benefits to society first above our personal leaning. Linus Pauling would bring the best from any field and collaborate with them and put California Institute of Technology (CalTech) first. He went from chemistry to nuclear physics, medicine, particularly micronutrients or orthomolecular medicine. His state manly approach to science and global peace was rewarded. Once our intention is right, like Linus Pauling the benefits and fulfillment will be long lasting long after we have gone. Right now what appears operative is 'my Mercedes is bigger than your own'; these thoughts are for little minds. Genuine academics are broad minded and discuss ideas not people or material. Let us make academics what it truly is. Academics is the hope of the world, I am very proud and happy to be a scientist, a scholar, a member of the academic community. It is the highest any one can aspire to be, let us not destroy it in this country. After God the scientific or academic community is the last port of call. We have seen this play out in many situations. Let us be genuine members of the academic community, not a 'Nigerianized' one.

There are some great scientists I have selected as my mentors over the years; Linus Pauling, Peter Medawar, Richard Feynman, Bruce Ames, Leon Goldberg, David Linn Edsall, (the first full time Dean of the Harvard Medical School) who made Harvard medical school what it is today, a home for all. Why cannot we make Ibadan medical school the same?

Recommendations

Mr. Vice-Chancellor at this point what comes to my mind is need for translational research or in this case translational medicine in today's poisoned world. Translational medicine focuses on the conversion of basic biomedical research into practical applications, thus bridging the research-to-application gap (Bench-Bedside). Trans-lational medical research is increasingly recognised as important to the national health service of many nations. There should be focus on translational medicine and both clinicians and scientists should work in collaboration to mitigate the health effects of inevitable chemical poisoning. Translational medicine provides a means for the rapid translation of discoveries in the laboratory into clinical practice. The astonishing advances in understanding the fundamentals of life have not translated into equally astonishing improvement in human health. This observation was indirectly what led to the establishment of the National Academy of Science (NAS) of the United States of America. On March 3 1836, President Abraham Lincoln signed an act that established an independent corporation to "investigate, examine, experiment, and report upon any subject of science or art", when ever asked to do so by the American government. Thus in the middle of the civil war the National Academy of Science was born, an admirable scientific body that has served the American nation, and indeed the whole world creditably. Interestingly more than a century later, precisely, 23 March 2010, another American President, President Barack Obama signed a landmark health care reform bill that, among many other issues created another agency – the Patient Centred Outcomes Research Institute (PCORI) - to carry out a much narrowly defined but focussed scientific mission.

In line with the broad objective of translational medicine, PCORI's purpose is "to assist patients, clients, users, and policy-makers in making informed health decisions by advancing the quality and relevance of evidence" regarding ways in which a broad range of health conditions can be

"prevented, diagnosed, treated, monitored, and managed through research evidence synthesis and considers variations in patient subpopulations". This non-governmental agency is also charged with "dissemination of research findings with respect to the relative health outcomes, clinical effectiveness, and appropriateness of medical treatments, services and items. The ultimate goal of PCORI is to improve patient outcomes by using the best clinical technology, techniques, and medications in existence, as determined by the best available research evidence. This is what we need to ameliorate chemical poisoning that will worsen with diversification of the economy with increasing attention to the mining sector. Scientists must be at the frontier of a similar effort in Nigeria.

The Agency for Health Research and Quality (AHRQ) and the National Institutes of Health (NIH) of the United States have rightly applauded the establishment of PCORI and states that it will build on their agencies' long standing investment in Comparative Effectiveness Research (CER) to provide well validated evidence- based approaches to medical care that can improve patient care. Comparative effectiveness research is designed to inform health care decisions by providing evidence related to the effectiveness, benefits and harms of different treatment options for a given condition, including subgroups within that condition. The evidence is generated through research that compares drugs, medical devices, tests or investigations, surgeries or methods to deliver health care. This principle appears particularly relevant to chemical poisoning considered one of the greatest health challenges that face the global community (WHO 1992). It gives scientific base to the nation's health care system. Scientists are disproportionately charged to use science to improve the nation's health system as enunciated by Laurer & Collins (2010). Are we doing this or are there impediments? If we put impediments in the way of scientists, then we will be unwittingly contributing to the disturbing prevalence of poisoning and its implications, manifesting as cancer, birth defects, increased susceptibility to common

disease (due to immunosuppression) increased crime rates, declining academic performance in our schools, increased allergies and asthma, accelerated aging including baldness and precocious death. Our life span is put at about 55years; chemical poisoning cannot be ruled out as a major contributor through the mechanistic pathways enunciated above.

To be more effective in translational medicine in Nigeria, we need a body similar to the NIH of the United States, which is a recognized leader in supporting CER studies that develop primary evidence of effectiveness, including comparative clinical trials and support for accelerating the path toward personalized medicine. Together with AHRQ, the NIH in America is uniquely positioned to draw on decades of research experience to help PCORI establish its organizational structure, formulate its methodological standards and develop research priorities and processes. In the absence of NIH like bodies in Nigeria, I see no reason why professional regulatory bodies in the country cannot forge this partnership rather than proliferating other resource guzzling ineffective institutions as we have it in Nigeria today and fighting themselves. The statutory regulatory bodies of the Nigerian health system should participate in the fashioning of a robust portfolio of scientific enquiry that builds on current and prior federal research and that remains focused on improving the end result of patients.

A striking feature of the bill establishing NAS is its slim volume, suggesting that legislative length is not always a good measure of a research organization's potential impact on society. Just as it took a dedicated crop of scientists to form what evolved in to a venerable academy to advise the United States on matters of science, Nigeria today stands in need of scientists willing to devote their time and energy to tackle the impact of chemical poisoning in an increasingly susceptible (malnourished) population. This is absolutely vital to the nation's health care system. This should be a joint effort in the name of science for health reform and science in the service of our society or nation. Owing to the threat of chemical poisoning it is more than ever before mandatory to

strengthen the nation's health system, including laboratory services to avoid nationwide repeat of the Zamfara episode of lead poisoning of 2010 which is actually still lingering. This view is truer today in the face of the challenge posed to our health by chemical poisoning.

A cursory observation of our health care system in Nigeria on the state of quality of care and disparities in care shows that, the gap between the best possible care and that which is routinely delivered remains substantial. This if not addressed will further exacerbate the chemical poisoning situation that may worsen with economic reforms focusing on mining and increased geological activities.

The following are therefore specifically recommended:

- The nation's biomedical laboratories must be equipped to ensure easy and early diagnosis of chemical poisoning or chemically-induced diseases.
- All known mining areas should be linked to centres providing toxicological investigations for surveillance-probably under the umbrella of a National Institute of Environmental Health Sciences (NIEHS) (Regional Toxicology Laboratory).
- There is need for a national survey of the major toxic substances preferably combined with nutritional assessment.
- There is the need to establish an agency to study various aspects of the toxic effects of chemicals in Nigeria, comparable to the Agency for Toxic Substances and Disease Registry (ATSDR) in the United States.
- Provision of facilities and strengthening of laboratories in at least some designated Universities/Research Institutes to study the silent epidemic of chemical poisoning is currently a priority.
- Introduction of courses of instruction in toxicology in schools, including undergraduate and postgraduate courses in the universities and establishment of a standing committee to disseminate information through public enlightenment programmes in collaboration

with the Federal Ministry of Information and Culture, emphasizing the adverse effects of toxicants or chemicals are desirable strategies.

- Owing to the special sensitivity of children, Children Survey/Study is needed, possibly under the auspices of Children's National Environmental Toxicology and Micronutrient Programme (CNETMP). This may progress to the establishment of a Children's National Toxicology and Micronutrient Centre.
- Finally, other sources of poisoning to the general population, such as use of pesticides to preserve grains, vegetables and peas, food additives, drugs, chemicals in automobile and home paints, etc should also be addressed.

The Proposed Toxicology Research Laboratory (TRL) in Chemical Pathology

This brings me to the proposed Toxicology Research Laboratory (TRL), a clinically biased toxicology laboratory in the Department of Chemical Pathology. I thank the immediate past Vice-Chancellor, Professor I.F. Adewole, FAS, for being the arrow head of this project. I will seize this opportunity to give a situation report. Before the last administration left office, the proposal was approved by the Senate of this university and awaiting funding, which I am sure our dear Vice-Chancellor will quicken fund release.

I am happy to report that as part of the process of implementing the TRL proposal, I successfully coordinated the first comprehensive training in Basic & Clinical Toxicology (BCT) attracting over 60 participants drawn from many parts of the country (fig. 23). Activities like this and many others are what the TRL plans to be involved in. One of our books (fig. 24) is an example of the type of monographs TRL plans to publish periodically to compressively address the problem of chemical poisoning in Nigeria.

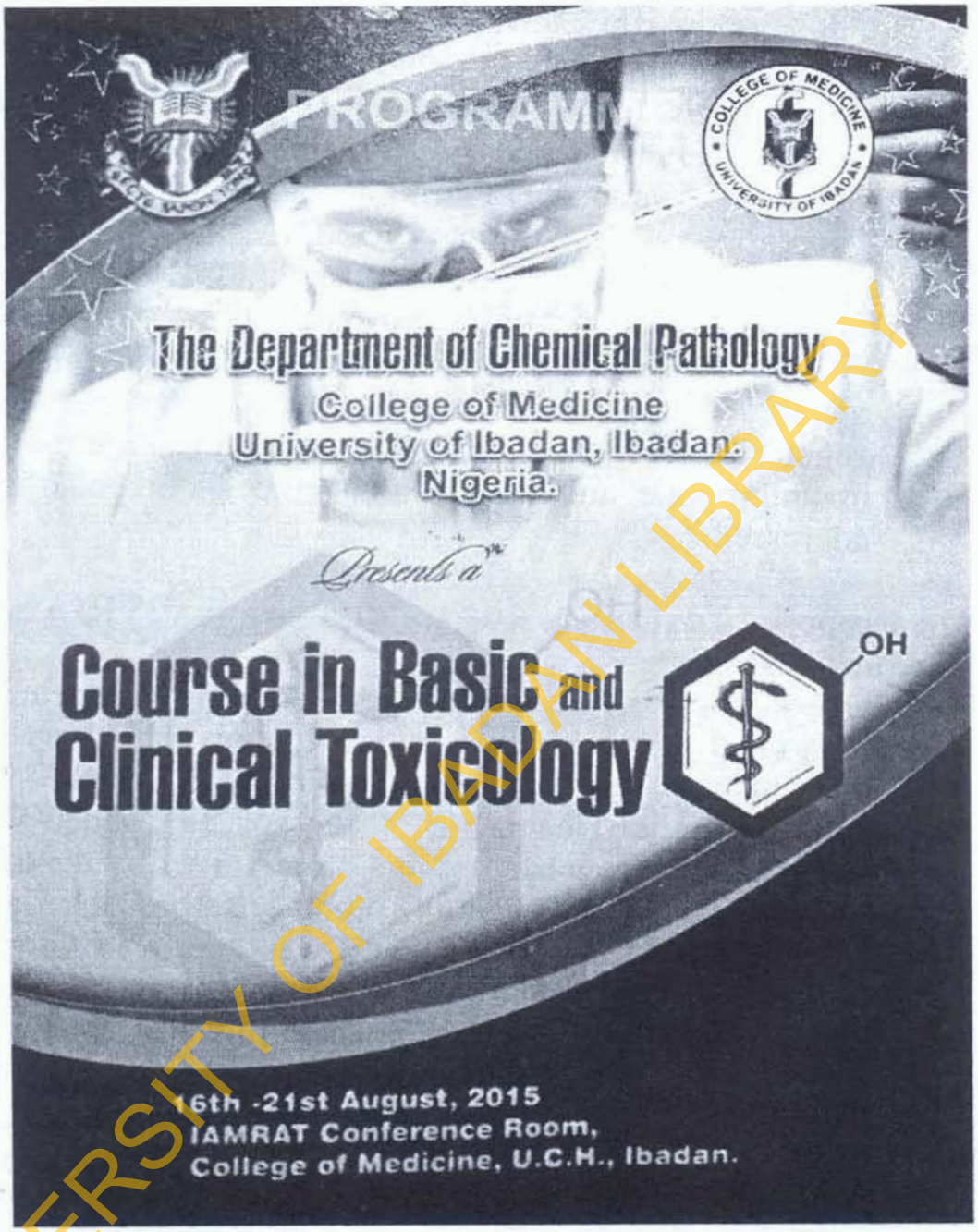


Fig. 23: Programme for the first basic and clinical toxicology course in the university.

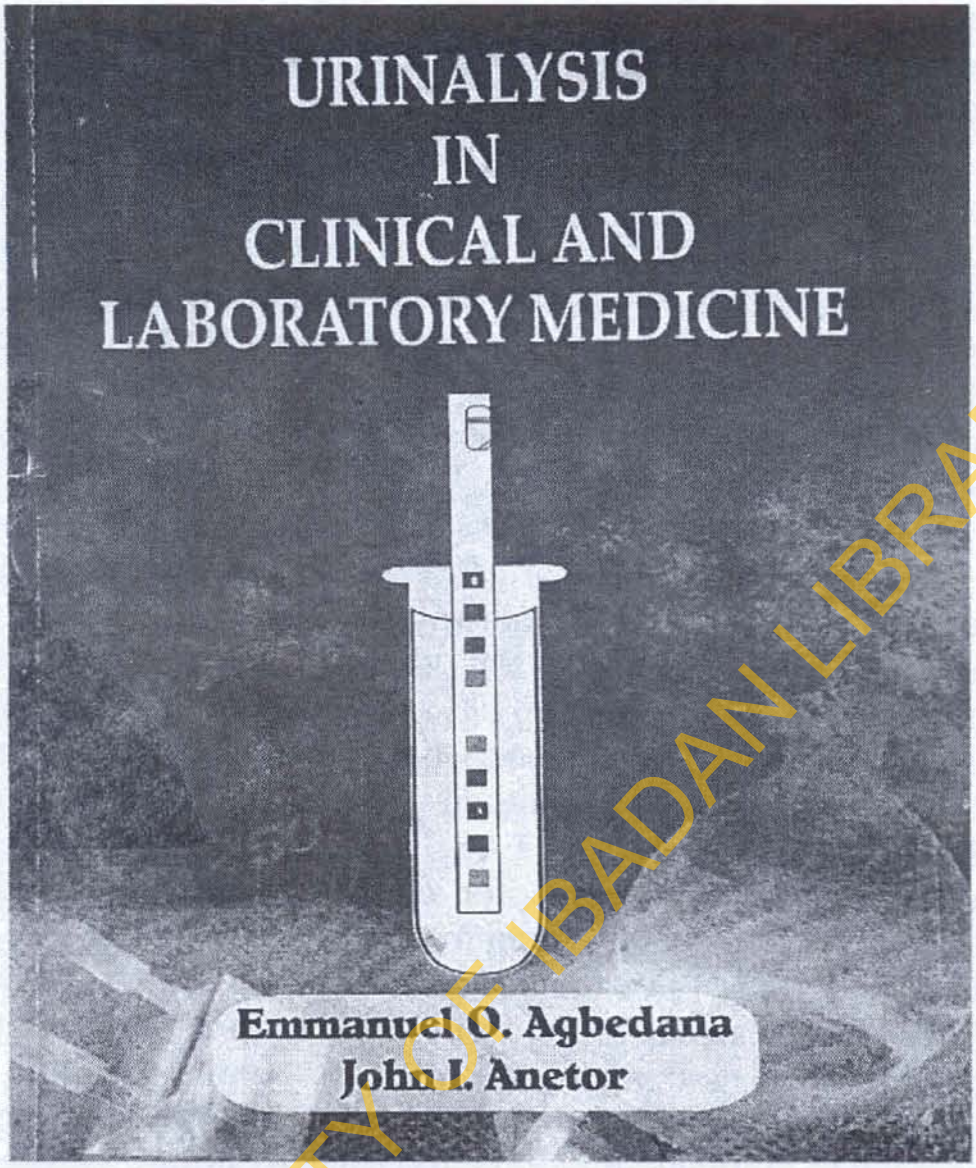


Fig. 24: A book co-authored by the lecturer: the TRL plans to produce monographs in clinical toxicology. A book like this would have been very handy in Zamfara State.

Acknowledgements

First and foremost I give all the thanks, glory, honour, praise and adoration to God, who has made everything possible. Gloria in Excelsis Deo. I thank my parents, particularly my mother for all the sacrifice for my education and especially for buying the first books that opened the doors of science to me, Introduction to Chemistry and Physics by Philip and Dotun Oyewole. I also thank all my maternal uncles (the Eimunjezes) and their wives, particularly, Mr. Henry

Eimunjeze, Mr. Victor Eimunjeze, Mr. Dickson Eimunjeze and Mr. Patrick Eimunjeze for their support to my mother in my upbringing and education.

I thank immensely the Doctors and Rev. Sisters at St. Camillus Catholic Hospital, Uromi, whom I was told were wonderful when I took seriously ill. I would not have been here today. In the words of Nolan Ryan: " I am going to spend my time today, just thanking the people that played a role in my career, because I truly do believe that I was blessed by a lot of people whose path crossed mine as I went down the road of my career".

My deep gratitude goes to my academic mentors beginning with Professor Francis Adefikayo Adebayo Adeniyi, who supervised both my fellowship thesis and was the chief supervisor of my PhD thesis. He was the one who introduced me to research. I thank him for his care, love, inspiration, counsel, guidance, encouragement, and above all prayers. My special gratitude goes to Professor Grace Oladuni Olaniyan-Taylor, FAS, she was a member of the panel that interviewed me for admission into the Medical Laboratory Science Programme in 1976, supervised my M.Sc. dissertation in chemical pathology and a co-supervisor of my PhD thesis (I shifted from lipids to toxicology). She is more like a mother to me and the entire family, her thoroughness, genuine mentorship up till now is greatly appreciated. I thank my PhD External examiner, Professor Umoh, who in addition to examining me counseled me to change from a service career in IITA to the university system and told my department to give me a position. I am indebted to Professor Nurudeen Adedipe for nominating me for the Scientific Committee on Problem of the Environment (SCOPE)/IUPAC International Symposium on Endocrine Active Substances (EACs), Yokohama, Japan. I thank Professor M.K.C. Sridhar my Internal Examiner for the PhD and for recommending me to be a member of the National Committee on the removal of lead from Nigeria's gasoline and his letter of support to the United Nations Scientific Committee on Problems of the Environment (SCOPE)/

IUPAC to attend the symposium on Endocrine Active Substances in Yokohama, Japan.

I owe a lot of gratitude to all my teachers at the School of Medical Laboratory Sciences, University College Hospital, Ibadan, a school that even though unsung has produced well over forty professors and PhDs combined, not counting MSc and Fellowship holders. I cannot but mention, late Mr. F.A. Lukanmbi, M.Phil., FIBMS (UK), a real master of chemical pathology and mentor, Mr. E.A.B. Mould, MSc. FIBMS (UK), a great teacher of chemical pathology, motivator and friend, Professor Theo Olawoye, Professor Ernest Ukaejiofor, Late Professor R.A. Alegbe, a great teacher, Mr. P.F. Idowu, late Mr. J.E.O. Durowoju, Mr. Dennis Opene, Mr. Bayo Lawal and several others. The sound foundation and exposure you laid will forever be cherished. I thank late Mr. Daniels, FRCS (Lond.), formerly of ENT Department, U.C.H., he housed me, cooked for me and guided me in my final year in UCH. May your great soul rest in perfect peace.

I also thank my first employer in my career, Dr. Benjamin Oni Okpako, a surgeon and great administrator, former Medical Director of Benoni Hospital, Benin City, for your love and belief in me and for making me the Secretary to the Clinical Committee of the hospital inspite of clandestine opposition.

I thank my bosses in IITA, Dr. Olufemi Ogundipe for the facilities you put at my disposal and opening your home to me and my family at all times, Dr. Funmi Ajose, for your understanding and support, Mr. Thackway, Head of Human Resources for your interest and your initiation and approval of my training in Scotland. I also appreciate Dr. and Mrs. Joe Mullongoy for their friendship and love throughout our stay in IITA. I thank the management of Igbinedion University, Okada for giving an opportunity for Sabbatical leave between 2008 and 2010. I must thank the following Dr. J.F. Pleysier, Mr. Joe Uponi of the Analytical Service Laboratory (ASL) IITA and Mr. William Ebadan Formerly of the ASL for their considerable technical assistance. Thank you indeed. I will always remember, my former landlord, late Mr. Dapo Aloba,

FCA, a truly detribalized Nigerian who was more like an uncle or father. May your great soul rest in perfect peace.

I bountifully acknowledge all my international teachers, mentors, and collaborators as follow: Dr. Ian Gould, FRCPath, Department of Medical Microbiology, Division of Laboratory medicine, University of Aberdeen, Aberdeen Royal Infirmary (ARI), Scotland, Dr. Ian Ross, former Head, Department of Clinical Biochemistry, University of Aberdeen Medical School, late Dr. Neil Paterson, Ph.D, Chief of Toxicology and Deputy Head, Department of Clinical Biochemistry, University of Aberdeen, Dr. Arthur Dickie, Professor John Broom, Dr. Ian J. Rothnie, Dr. Anthony Seaton, Department of Environmental Health, and Drs John Chesters and John Arthur, Trace Element and Gene Expression Laboratory, Rowett Research Institute (RRI), Bucksburn, Aberdeen.

I thank in a special way professor Shoji Fukushima, MD, Ph.D, Pathology Department, Osaka City University Medical School, Osaka City University, Osaka, Japan. I thank others in this Laboratory, Professor Hideki Wanibuchi, Dr. Anna Kinoshita, Dr. Min Wei, Dr. Kunichiro Doi, Professor Salim, Ms. Tuoma, Ms Mari Doko, Dr Akira Inoue and Dr. Kang.

Late Dr. Junshi Miyamoto, SCOPE LOC Chairman Yokohama, Japan 2002, and former Head, Chemical Evaluation Research Institute (CERI), Tokyo, Japan; Dr. Mark D. Evans, Department of Cancer Studies and Molecular Medicine, University of Leicester; Professor Peter Farmer, Biomarker Laboratory, University of Leicester, U.K., and Professor Marcus Cooke, Department of Environmental and Public Health, Florida International University, Florida. Professor Robert Flanagan, Toxicology Unit, king's College Hospital, London, Dr. Andrew Taylor and Dr. Chris Harrington, Trace Element and Toxicology Laboratory, Sanger Research Park, University of Surrey. Professor Anthony Okorodudu, Clinical Chemistry Division, Department of Pathology, University of Texas Medical Branch, Galveston, Houston, Texas, Professor Jorma Ahonkas, RMIT University, Bundora, West Australia, Anita

Mietttunen, Environment Canada, Dr. Mary J. Brown, Chief Lead Poisoning Prevention Branch, CDC, Atlanta, Georgia.

I wish to acknowledge with gratitude the Funding support of the under listed organizations; Aberdeen Rotary International, Scotland, International Institute of Tropical Agriculture (IITA), Lijima Foundation, Japan, Osaka City Medical School and Medical Centre, Osaka, Japan, C.P. Stewart Memorial Fund, ACB, UK, Royal Society of Chemistry, U.K., IUTOX/SOT, University College Hospital (UCH), the University Ibadan and TETFUND. I thank all my teachers in the Department of Chemical Pathology; I cannot but Mention Professor Bola Osifo for rekindling my interest in Intermediary Metabolism and Professor Abayomi Akanji, FACB, FAS, a true scholar and believer in meritocracy. I am especially grateful to Professor Emmanuel O. Agbedana the only one left among my teachers in the department; I call him 'the last of the Romans,' for helping to hold the department together, for being the link between the past and present and for his myriad advice and counsel. I thank the current Head of Chemical Pathology Department, Professor Ganiyu Olatunbosun Arinola. I thank the following: Professor Babatunde Osotimehin, FAS, one of those who interviewed me for the Job, late Professor Lekan Salimonu, FAS, the Head of Department when I was employed, late Professor Christopher Adesuyi Adejuwon, a great teacher and believer in me, late Professor A.I.O. Williams. I thank all my current colleagues for their comradeship, Dr. K.S. Akinlade, Dr. F.M. Abbiyesuku, Dr. K.S. Adedapo, Dr. O.M. Akinosun, Dr. Mabel A. Charles-Davies, Dr. I.O. Omotosho, Dr. M. Kuti, Dr. A.A. Onifade, Dr. Elizabeth Bolajoko, Dr. Dr. Enitiame Orimadegun, Mrs. Ademola-Aremu. I thank all the non-teaching staff in the department, Mrs. Adeyemo, Mrs. Taiwo, Mrs. Adeoti, Mr. Titiloye, Mr. Taiwo and the indefatigable ever smiling, ever helpful, Mr. Dele Ayede. I thank the administrator and secretary of the Department of Biomedical Laboratory Science for the support in running the department.

I thank all the members of the Faculty of Basic Medical Sciences for miscellaneous assistance at one point or the other. I am very appreciative of the various assistance of the current Faculty Officer, Mr. Akande. I appreciate all staff of the College of Medicine especially, Professor Babatunde Lawal Salako, the immediate past Provost. I wish him well in his new assignment. He will be sorely missed. I thank Professor Oladipo O. Akinkugbe who though did not teach me directly, I have learnt a lot from his words of wisdom and specially for involving me in the Olusegun Obasanjo Presidential Library and Centre for Human Security book project on the Health Sector. I also thank the late professor A. Adetuyibi for his prediction about me and particularly for his book, 'An Introduction to Biomedical Research for Health Workers in the Tropics'. It was very useful in my formative years.

I appreciate the friendship and love of the following Professors—O.O. Olorunsogo, E.O. Farombi, O.G. Ademowo, O.D. Olaleye, E.E.U. Akang, (Late), A.R.A. Alada, S.B. Olaleye, Georgina Odaibo Drs. T.S. Akingbola, F.A. Fashola, Dr. Solomon Adeleye Bakarey, and many others too numerous to mention here. I thank the Deputy Provost Professor Ademola Ajuwon, Director of IAMRAT Professor Catherine Falade and the College Seretary Mrs. Olubunmin Faluyi.

I thank in a special way the following; Professor O.O. Osibanjo, Professor Sunday Ajayi; great teachers of analytical chemistry, Professor Temitope Alonge Chief Medical Director UCH, A/Level course mate and boyhood friend, for the long-term aspiration and dream we shared about science and scholarship, late Professor Theophilus Akinleyele, Professor Mba Ogburuigwe Okoronkwo, OON, National Open University of Nigeria, for his encouragement and brotherly love, Mr. Tunde Anifowoshe, a friend of over forty years, whose contribution to today's event is immeasurable, Professor Anthony Emeribe, Professor, A.A. Alaribe, Dr. Layi Oduwole, Ayo Norman-Williams, Air Commodor (Dr.)

Yomi Akinwale, Dr. Godswill Okara, classmates and friends and many others, Professor Adolphus Udoh, All members of Forum of Ibadan Toxicologists (FIBATOX), especially Drs. Ademola Oyagbemi and Adeolu Adedapo, All members of the management Committee of the Multidisciplinary Central Research Laboratory (MCRL) chaired by Professor Bankole Oke.

I thank all my past and present postgraduate students, I cannot but single out Chukwuemelie Zedech Uche for his 'sonly' attachment and assistance, and for the dream we share. I also thank Mr. Eden for all his technical assistance. I openly confess that I have also learnt from you. I wish to thank Professor Sylvester Osegele Oriaifo, the first Professor in our family to whom I have been attached since my secondary school days for all his care, guidance , admonition and prayers. He was the guardian angel God used to bring me to Government College, Ughelli, the school originally chosen for me by my head master but turned down by my uncle for good reasons. I also thank his wife, Mrs Clara Oriaifo, who is more like a senior sister.

I thank Dr. Adolphus Oriaifo, MD, PhD, FMCS, for all his care. Thank you specially for being there as the doctor of the whole family that gave me peace of mind to face my academic pursuit. It was actually you who first described medical research in my presence when you were describing your Ph.D work on your return, how you broke the legs of chickens; you were applying something and watching the response; the legs healing and straightening. Unknown to me I would decades later find myself in your shoes.

I must thank my late grandmother, Madam Egbakhuangie Eimunjeze for all her sacrifice; it pains me that you did not live to gain anything from me. I also thank all my mother's sisters (my aunts), particularly Madam Monica Ibhahiobheson Eimujeze, who stepped in as the mother of the Eimunjezes when my grandmother passed on. I also thank my mother's younger sister, Madam Beatrice Atuoriakhi Oriaifo, who took special interest in me. I recall your concern that

people were saying they didn't really understand what I was studying. I assured you that it was a good field; wished you were here today. I thank Mrs. Atuegbe Eiwone, the last of my cherished aunts to depart. All your prayers were certainly heard by God. Rest in peace.

My thanks go to all the other Oriaifos; Aunty Stella, Mrs. Ogbebor (especially for accepting her colleagues' suggestion that I repeat primary three), I thank Chief (Mrs.) Victoria Akhere Adedun (our mummy in Ibadan) and the late husband, Chief Ayo Adedun, her twin, Dr. Victor O. Oriaifo all their help when I was studying and now as well. I thank aunty Felicia, Pharm. (Mrs.) F.E. Odaro, FPSN. I thank Professor (Mrs.) Justina Aire and the husband, Professor Tom. A. Aire. The succor you gave to me and my friends, will never be forgotten.

I thank all my siblings and cousins of my age group, Iwobhor Godwin Anetor, Paul Anetor, Mrs. Bennie Anetor, Beatrice Adesua Eimunjeze, Mr. Alexander Oriaifo (Lexy), Barrister Otunba Fidelis Oriaifo (Fido), Rose Eimunjeze, Mrs. Omoso Matankari. I remember in a special way late Barrister Felicia Omonigho Eimunjeze (former Deputy Director of the Law School, Yenagoa, Bayelsa State) who was tragically killed on the 13th of February this year by a careless truck driver. I also appreciate all my younger cousins, too numerous to mention here individually. I am grateful to you all.

I thank specially Dr. and Mrs. Andrew Bella and Professor and Mrs. Peter Aziba as well as late Mr. Shola Bademosi, AIMLS, a classmate and true compatriot, may your soul rest in peace.

I wish to thank all the priests, religious and members of the various pious societies of the Catholic Church, too numerous to list individually I have been associated with all over the world. I cannot but mention The former Metropolitan of the Catholic Arch Diocese of Ibadan, Arch Bishop Alaba Job and his successor Arch Bishop Gabriel Abegurin, members of the Opus Dei Family, Sisters of the Mary Hill

Convent, Ibadan, parishioners of Seat of Wisdom Chaplaincy, U.I., St. Luke Chaplaincy, UCH, Holy Family, Akobo, St. Anthony's Catholic Church, Uromi, The Catholic Cathedral, Bauchi, St. Mary's Cathedral, Huntley Road Aberdeen, St. Joseph Osaka and many more. The Catholic Church has been a home where ever I went. Thank you all. I thank the youngest of my uncles specially, Mr. Vincent E. Eimunjeze and his wife Mrs. Janet (mama Boje) Eimunjeze for he it was who brought me to Ibadan to receive education.

I wish to thank my in-laws for their love care and generosity; Late Mr. Caxston Ainoje' and Mrs. Grace Iyoha Azenabor, Mrs. Clara Azenabor-Awonuga, Mrs. Marie Stella Odanmen, Professor Antony Azenabor, Professor Godwin Azenabor and all others too numerous to list here. I will forever cherish the daughter you gave me.

I thank my jewel of inestimable value, Dr. Gloria Oiyahumen Anetor, a sweet heart and better half indeed, who has toiled with, supported me and sacrificed for me. You have given your all. I will never forget the sacrifice of your shouldering the responsibility of both of us and two children alone when I went on study leave without pay for the M.Sc. programme without our two families knowing it. I do not have the adjective to express the depth of my gratitude to you. All I can say is thank you, thank you and thank you. May God bless you richly. You and the children are the sunshine of my life. I thank the children, Barr. Oseiwe Joan Anetor, Dr. Ehimudiamen Anne Anetor, Dr. Oselumense John Anetor, Mr. Benosehoole Edward Gerald Anetor my nephew, Mr. Eromosele Joshua Anetor, and a close friend of the children who is like a son to me, Eng. Samuel Ailenubhi for their love and understanding. I love you all.

This lecture is dedicated to Our Lady, the Blessed Virgin Mary and late mother, Madam Magdalene Omobhakhamen Anetor (Nee Eimunjeze).

Thank you all for listening.

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BIODATA OF
PROFESSOR JOHN IBHAGBEMIEN ANETOR

John Ibhagbemien Anetor was born on Sunday about midday of May first (May Day) 1955 at St. Camillus Catholic Hospital, Uromi, Edo State to Mr. Aimakhu Ogudo and Mrs. Magdalene Omobhakhamen Anetor (Nee Eimunjeze). The father was a forester, palm produce merchant and a community leader, while the mother was a rice and fish merchant at the famous Ilushi (Ozigono) market, located on the bank of the River Niger. He started his primary education at the Local Authority (L.A.) School at Utako, Uromi in 1963, he could not start earlier as his hand could not cross his head to touch the ear on the opposite side. He transferred to St. Anthony's Catholic Primary School, Okpujie, Uromi in primary three, where he spent two years in primary three, 1965 and 1966. He finished his primary education in December 1969. It is remarkable that many of his school mates rarely called him by his name, instead he was known and called 'Ebo' (White man; due his fair complexion) even up to the first two years in secondary school. John Anetor started his secondary education at Ishan Grammar School (IGS) in January, 1970, and transferred to Government College, Ughelli (GCU) in form three (The school his headmaster chose for him from the beginning but turned down by his uncle) and obtained the West African School Certificate (WASC) in June 1974. Chemistry was his best subject in which he obtained a distinction.

He started his Advanced Level (A/L) course in science (Physics, Chemistry & Biology) at the Faculty of Science, University of Ibadan, under the Extra Mural Programme of the Faculty of Education in the 1975/76 session. He left after one year of the two-year programme to study Medical Laboratory Science at the University college Hospital (U.C.H.), in the 1976/77 (started January 1977) and qualified in June 1981 obtaining the Associate Membership of the Institute of Medical Laboratory Science of Nigeria (AIMLS) with option in chemical pathology. He was adjudged the overall best graduating student winning the then Professor

Kolawole's Prize for the Best Academic Student. He served the mandatory National Youth Service in Bauchi State, with his primary assignment at the General Hospital, Darazo, completing the service in July 1982. There after he took up employment in the Pathology Department as Medical Laboratory Scientist at Benoni Hospital Limited, Benin City on August 2nd 1982 and left to join the Medical Centre of the International Institute of Tropical Agriculture (IITA), November 28 1984, where he rose through the ranks to become a Manager. He got married on August 31st 1985 to Miss Gloria Oiyahumen Azenabor (now Dr. Gloria Anetor) the younger sister of his friend and colleague, Tony Azenabor, now a Professor of Immunochemistry at the University of Wisconsin, Madison, United States of America.

John registered for the fellowship programme (professional postgraduate course) by thesis in chemical pathology in 1987 and successfully defended his thesis in 1989 and was awarded the specialist fellowship of the IMLS (FIMLS) in chemical pathology. He was admitted to study for the academic master's programme (M.Sc.) in the Department of Chemical Pathology, College of Medicine, of the University of Ibadan in the 1990/91 session. John graduated as the best student in his set and the only one who obtained the PhD grade. He registered for the doctoral programme in chemical pathology in January, 1992 and successfully defended his PhD thesis in April, 1997. He had pre-doctoral attachment with the aid of the Aberdeen Rotary International Fellowship at the Laboratory Medicine Division of the University of Aberdeen Medical School/Aberdeen Royal Infirmary (ARI), Scotland in 1995, with visits to the Rowett Research Institute (RRI), Bucksburn, Aberdeen after earlier training in Clinical Laboratory Medicine at the City Hospital, Aberdeen and the Aberdeen Royal Infirmary in 1990.

Dr. John Anetor had his postdoctoral training in the Pathology Department at the Osaka City University Medical School, Osaka City University, Osaka, Japan, under the supervision of Professor Shoji Fukushima, MD, PhD. in 2005. He worked on chemical carcinogenesis and chemo-prevention. He joined the services of the University of Ibadan

from IITA, Ibadan, 15th December, 1998 as Lecture II. He was promoted Lecturer I on October 1, 2001, Senior Lecturer in 2004 and got his Chair in chemical pathology on October 1, 2010. Before this he was appointed a Professor of Chemical Pathology at the School of Clinical Medicine at Igbinedion University, Okada while on Sabbatical leave between 2008 and 2010. They infact argue that they were the first to pronounce him Professor. He was appointed a Consultant Chemical Pathologist to the University College Hospital (UCH) Ibadan, in 2006 and later designated Specialist Adviser in Chemical Pathology in 2011 to date. He has served as a consultant to the Medical Centre, International Institute of Tropical Agriculture on many occasions. He is an Adjunct Professor in the Department of Chemical Pathology and Immunology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State. He has been external examiner at both undergraduate and postgraduate levels in almost all the major universities in Nigeria and a West African country.

Professor John Anetor was a visiting scientist at the Department of Cancer Studies and Molecular Medicine, University of Leicester, U.K. in 2011. He visited the Department of Pathology (Clinical Chemistry Division), University of Texas Medical Branch, Galveston, Texas in 2012. In 2014 he was awarded the C. P. Stewart Memorial Fund of the Association of Clinical Biochemistry and Laboratory Medicine (ACB), U.K. to train in Analytical Toxicology at the Toxicology Unit, Department of Clinical Biochemistry, King's College Hospital, London, with attachments at the Bioanalytics and Toxicology Unit (ASI), Pharmacology Department, St. Georges University, London and the Toxicology and Trace Element Laboratory (Supra-regional Assay Services, SAS) at the University of Surrey, Guildford, Surrey. John Anetor has enjoyed many travel grants/fellowships. He was awarded the UNESCO/IUPAC fellowship in 2002 to attend the International Workshop on Endocrine Disrupting Chemicals (EDCs) in Yokohama, Japan. He won the Gordon Research Conferences (on Genetic Toxicology) Fellowship twice, 2007 and 2008, got the national Universities Commission (NUC) travel grant in

2009, the International Union of Toxicology (IUTOX)/the Society of Toxicology (SOT) travelling fellowship in 2013.

John Anetor is a member of many professional and learned societies locally and internationally. He is a Member, Association of Clinical Chemistry of Nigeria (ACCN), where he was a Treasurer. He is registered with the Medical Laboratory Science Council of Nigeria (MLSCN). He is a Fellow of the Institute of Biomedical Science (FIBMS) of the United Kingdom (UK), Member, Association Clinical Biochemistry and Laboratory Medicine (ACB) of the United Kingdom. He represents Africa on the Committee on Traceability in Laboratory Medicine (C-TLM) of the Scientific Division (SD) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). He is a Member, Society of Toxicology (SOT) of the United States of America. He is a founding member and Adviser to the West African Society of Toxicology (WASOT). He is a Fellow of the Royal Society of Chemistry (FRSC) of the United Kingdom. He is a Fellow of the American College of Nutrition (FACN), he is a European Registered Toxicologist (ERT), a Life member of the Environmental mutagen Society of India (EMSI) and an honorary Fellow, Institute of Industrial Administrators of Nigeria (FIIA). He has published extensively, one hundred and eight papers (108) at the last count, (not counting published abstracts) including several book chapters and a co-authored book, 'Urinalysis in Clinical and Laboratory Medicine'. He has supervised and co-supervised 10 PhD candidates many of whom are occupying senior positions with one in the professorial cadre, 20 MSc candidates and 4 Fellowship candidates.

He is a reviewer for many national and international peer reviewed journals and sits on several editorial boards. He is the pioneer editor of the African Journal of Laboratory Medicine and Biomedical Research, based in the Southern Africa Training Academy (SATA) located in South Africa. Professor Anetor has served the university, the University College Hospital (UCH) and the nation in many capacities. He is the Vice-Chairman of the University College Hospital Research Committee. He was a member of the National

Committee on the removal of lead from Nigeria's gasoline, sponsored by Exxon Mobile and the World Bank. His presentation at the conference is incorporated into the Working Paper No. 6 on the Clean Air Initiative in Sub-Saharan Africa. He was in late 2015, invited to be a member of the team of reviewers of the Olesegun Obasanjo Presidential Library book project, 'Towards a New Dawn for the Health Sector in Nigeria Post 2015' by the coordinator, Professor O.O. Akinkugbe, FAS. John Anetor is an active participant in the Faculty of Basic Medical Sciences, where he is a member of the central committee of the Unibadan Conference of Biomedical Research (UCBR), and was the Chairman of the Scientific Sub-Committee of the 2016, UCBR biennial conference, he is a member of the University of Ibadan Senate Curriculum Committee, a member of the management committee of the Multidisciplinary Central Research Laboratory (MCRL). He is the Chair of the Forum of Ibadan Toxicologists (FIBATOX). He was a former coordinator of postgraduate programme in the department of chemical pathology; and the current chairman of the departmental postgraduate committee of the Department of Chemical Pathology. He is also the Coordinator of the Nutrition and Metabolic module of the departmental postgraduate courses. He is the Head of the Toxicology and Micronutrient Metabolism Unit of the department and coordinated the first certificate course on Basic and Clinical Toxicology (BCT) attracting participants from all over the country organized by the Department of chemical pathology. Professor Anetor is the current head of the Department of Biomedical Laboratory Science, Faculty of basic Medical Sciences, College of Medicine, of this university. John Anetor is a confirmed and committed Catholic. He is married to Dr. Gloria Anetor, a Senior lecturer and Ag. Head of the Department of Public/Environmental Health of the National Open University of Nigeria (NOUN), Lagos and the union is blessed with four children.

NATIONAL ANTHEM

Arise, O compatriots
Nigeria's call obey
To serve our fatherland
With love and strength and faith
The labour of our heroes' past
Shall never be in vain
To serve with heart and might
One nation bound in freedom
Peace and unity

O God of creation
Direct our noble cause
Guide thou our leaders right
Help our youths the truth to know
In love and honesty to grow
And living just and true
Great lofty heights attain
To build a nation where peace
And justice shall reign

UNIVERSITY OF IBADAN ANTHEM

Unibadan, Fountainhead
Of true learning, deep and sound
Soothing spring for all who thirst
Bounds of knowledge to advance
Pledge to serve our cherished goals!
Self-reliance, unity
That our nation may with pride
Help to build a world that is truly free

Unibadan, first and best
Raise true minds for a noble cause
Social justice, equal chance
Greatness won with honest toil
Guide our people this to know
Wisdom's best to service turned
Help enshrine the right to learn
For a mind that knows is a mind that's free