STUDIES OF TRACE ELEMENTS IN CANADIAN DUPLICATE DIETS BY NEUTRON ACTIVATION

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For Jesus,
and
for my Mom

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ABSTRACT

Various types of instrumental neutron activation analysis (INAA) methods have been developed for the determination of thirteen major minerals and trace elements in samples of Canadian duplicate diets. The list of elements includes Al, Br, Ca, Cl, Fe, I, K, Mg, Mn, Na, Rb, Se, and Zn. Most of the elements have been analyzed by conventional INAA methods. A number of INAA methods has been evaluated for Se and I. The methods with the best sensitivity and detection limits were found to be pseudo-cyclic INAA (PCINAA) for Se and epithermal INAA (EINAA) with anti-coincidence gamma-ray spectrometry for I. Aluminum has been analyzed using INAA and a combination of INAA and EINAA for the correction of the interfering reaction $^{31}P(n,\alpha)^{28}Al$.

In order to assure the quality of measurement, quality control and quality assessments have been performed. Precision and accuracy have been evaluated through the analysis of reference materials. The measured values have been found to be in good agreement, generally within 10%, with the certified values. The precision of analysis of the diets has been evaluated by triplicate analyses of each of three diet samples. Precision, as assessed by relative standard deviation has generally been found to be <10%. Detection limits have been calculated and found to vary from 30 ppb for Se to 1200 ppm for Mg. Control reference materials have been analyzed with the diet samples in order to prepare external quality assessment (EQA) charts. The traceability has been ensured by developing a laboratory information management system and documentation procedures. The EQA charts have demonstrated the analyses to be in statistical control.

The median daily intakes (mg d^{-1}) of 14 duplicate diets have been determined using INAA are 4.8 for Al, 5.07 for Br, 1080 for Ca, 4750 for Cl, 21 for Fe, 0.280 for I, 3800 for K, 350 for Mg, 3.6 for Mn, 3230 for Na, 3.0 for Rb, and 0.130 for Se. These results are discussed in relation to the recommended Canadian as well as the U.S. intakes and previously reported data in the literature.

Exploratory data analysis has been performed on the elemental data obtained using INAA. The utility of the statistical methods has been demonstrated by revealing associations between various elements and food groups.

A dialysis procedure has been used to determine the fraction of elements in the diets which were insoluble or bound to macromolecules. It has also been found to significantly reduce the levels of interfering elements such as Na and Cl, allowing two elements which have been previously undetected, namely V and Ti, to be analyzed.

An enzymolysis method has been adapted in order to estimate the potentially bioavailable fraction of the elements. This fraction has been calculated by difference, leading to large uncertainties. Results are reported for Al, Ca, Fe, Mn, and Zn. The percent available is generally low for all of these elements, except for Mn, for which it varied from near 0 to 59%.

LIST OF ABBREVIATIONS AND SYMBOLS

α	Alpha-ray
AAS	Flame Atomic Absorption Spectroscopy
ADDI	Average daily dietary intake
AI	Adequate Intake
Carbohydr.	Carbohydrate
DDI	Daily Dietary Intake
DI	Dietary Intake
BMI	Body Mass Index
CINAA	Cyclic Instrumental Neutron Activation Analysis
cm	centimetres
COLR	Colorimetry
CRM	Certified Reference Material
d	day
DDW	Deionized, Distilled Water
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
EINAA	Epithermal Instrumental Neutron Activation Analysis
EQA	External Quality Assessment
FCT	Food Composition Tables
FDA	Food and Drug Administration
FNB	Food and Nutrition Board (of the U.S. National Academy of Sciences)
γ	Gamma-ray
g	gram
Ge(Li)	Germanium-Lithium
GI	Gastrointestinal (as in GI tract)
GSHpx	Glutathione Peroxidase
h	hour
HC	Health Canada
HCA	Hierarchical Cluster Analysis
HGAAS	Hydride Generation Atomic Absorption Spectroscopy
HPGe	Hyperpure Germanium
IAEA	International Atomic Energy Agency
IDD	Iodine Deficiency Disorders
INAA	Instrumental Neutron Activation Analysis
int.	Intensity
kg	Kilogram
L_C	Critical level
L_D	Detection limit
L_Q	Quantitative determination limit
m	metre
MCA	Multi-Channel Analyzer

mg	milligram
min	minutes
mL	millilitres
MS	Mass Spectrometry
n	neutron
NAA	Neutron Activation Analysis
NAS	National Academy of Sciences (of the U.S.)
NIST	National Institute of Standards and Technology (of the U.S.)
PC	Personal Computer
PCA	Principal Components Analysis
PNAA	Preconcentration Neutron Activation Analysis
PGAA	Prompt-gamma Activation Analysis
Prods.	Products
QA	Quality Assurance
RDA	Recommended Dietary Allowance
RM	Reference Material
RNAA	Radiochemical Neutron Activation Analysis
RNI	Recommended Nutrient Intake
rpm	revolutions per minute
RSD	Relative Standard Deviation
s	second
SADDI	Safe and Adequate Daily Dietary Intake
SRM	Standard Reference Material
T_3	Triiodothyronine
T_4	Thyroxine or Tetraiodothyronine
t_c	Counting time
t_d	Decay time
t_i	Irradiation time
TSH	Thyroid Stimulating Hormone
UL	Tolerable Upper Intake Level
UNICEF	United Nations Children's Fund
Vegs.	Vegetables
WHO	The World Health Organization
XRF	X-ray Fluorescence Spectrometry

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1. INTRODUCTION

Food has long been known to play a key role in not only the health status of human beings but also in their social lives. Relationships between food and physical well-being have been cited in ancient Chinese, Greek and Indian literatures. There is renewed interest in various aspects of food. From the nutritional point of view, a human diet should meet the nutrient needs of the body and provide maximum protection against chronic diseases (1). Certain elements are essential nutrients required for life and normal function of the human body, and must be supplied by the diet. Therefore, it is important to assure that the food supply and dietary habits of a population supply the required amounts of the essential elements (2). On the other hand, some elements are not essential for life but may still have adverse physiological activity. Toxicologists are concerned about the levels of these elements in food. Some effort is directed towards the determination of elemental content of food and elemental intake by humans (3). This information along with other parameters form the basis for setting guidelines for minimum intakes of essential elements and maximum permissible levels of toxic elements.

The human body contains varying amounts of almost all naturally-occurring elements. These elements are classified according to various schemes. One such scheme involves grouping the elements as major, minor and trace depending on their levels in the body. An alternative scheme classifies the elements as essential, possibly essential and non-essential (and a subgroup of toxic elements) depending on their biological functions (4). The distinction among the elements in the latter classification is perhaps only a

question of magnitude. Irrespective of how the elements are classified, food is their major source for humans in a natural environment (5), *i.e.* excluding occupational, accidental or similar exposure to massive amounts of one or more elements.

The elemental content of food and the forms in which the elements are found depend not only on the food source but also on the soil and water conditions of the area in which the food is produced, processed, and prepared before consumption (6). Therefore, in a diet study, it is important that the samples be representative of the population under consideration.

The major components of food are the elements C, H, N, O and S which are present in proteins, lipids and carbohydrates, *etc.* They make up the organic structural and functional components of tissues. There are many other elements found in food in varying concentrations. Of these, 21 have been found to be essential for life. These include 6 "major minerals" (namely, Na, K, Mg, Ca, P and Cl) and 15 trace elements (namely, As, Co, Cr, Cu, F, Fe, I, Mn, Mo, Ni, Se, Si, Sn, V and Zn). Monitoring the levels of these elements in foods and determining their daily intake (DI) is important in promoting the health and well-being of population groups, and can only be done through chemical analysis. However, the determination of many elements in foods and diets can be challenging due to the complex matrices and the variable levels in which they are found. Therefore, there is ongoing research into the development of sensitive analytical methods appropriate for this purpose. Neutron activation analysis (NAA) is a sensitive analytical technique for the analysis of several elements in a variety of matrices and is particularly well suited for the determination of elements in diet.

One of the principal objectives of this thesis was to develop, evaluate and apply instrumental NAA (INAA) methods for the analysis of major minerals and trace elements in diet samples. A set of Canadian duplicate diet samples was prepared in this work and analyzed by various forms of INAA. Appropriate quality assurance (QA) procedures were followed. In order to extract the maximum information from the elemental data, another objective was to use multivariate statistical methods to attempt to determine relationships between the elements, food sources and other nutrients.

The total amount of an element consumed is only one factor in assessing the adequacy of a diet in providing the nutrient requirements of the body. For example, some elements may be insoluble or bound to macromolecules, rendering them unavailable for absorption. The bioavailability of an element (7-9) is a measure of its actual utilization to support an organism's structure and physiological process. Bioavailability includes processes involved in absorption as well as transport, excretion, distribution and conversion to a biochemically active form. The availability of an element to be absorbed is an important factor to its bioavailability; if the element is in a form which is not readily absorbed, then it will not be available for use by the body. One of the objectives of this thesis was to use a simple dialysis technique to determine the fraction of the elements which were insoluble or bound to macromolecules compared to those which were soluble. Another objective was to attempt to estimate the potential availability of major minerals and trace elements by the application of an *in vitro* simulated digestion procedure. All of the objectives are described in detail in Section 1.4.

1.1 ESSENTIAL ELEMENTS AND DIETARY RECOMMENDATIONS

An essential element is an element for which dietary deficiency consistently results in a reduction of biological function from an optimal to a suboptimal level (10). This condition is reversible or preventable by ingesting physiological amounts of the element. The determination of essentiality of an element for humans is rather difficult because experiments involving humans are restricted for ethical reasons and also it is difficult to obtain food free of the element under investigation. For these reasons, a number of elements have been demonstrated to be essential through animal studies although no deficiency has been observed in man (11).

The essential elements perform many roles in the body. Elements such as Ca, F, and P perform structural functions as components of skeletal bone. Sodium, K and Cl are involved in electrolyte balance. Many of the other essential elements are part of enzyme systems, such as Co in Vitamin B_{12}.

The normal physiological amount of an element does not relate to its degree of essentiality. However, the actual physiological amount of an element does have a profound effect on the health of the organism. Amounts below minimum requirements may lead to a deficiency syndrome (12). Toxicity may result from many elements, even from some essential ones, if the intake is too high. There is a safe range of intake of essential elements (13-15) which governmental regulatory agencies use to set standards for the intake of minerals and other nutrients (1,13, 16-18).

In Canada, the Bureau of Nutritional Sciences (BNS) of the Food and Drug Directorate (FDD) of the formerly Health and Welfare Canada (1, 13) set Recommended

Nutrient Intakes (RNIs), which are used to formulate dietary recommendations. The RNI is a level of intake believed to be high enough to meet the requirements of almost all individuals in a group having specified characteristics (such as age, gender, body size, physical activity, or type of diet). Since the RNI exceeds the requirement of almost all individuals, a level of intake below the RNI does not mean an individual is malnourished. It indicates only increasing risk of an inadequate intake. The RNI provides a lower limit of safe intake. No upper limit has been defined for the essential elements due to inadequate data (1). For these reasons, the RNI should not be used as an indicator of nutritional status. Nutritional status is a health condition which can only be determined from biochemical, anthropometric and/or clinical examination.

The RNIs for the essential elements in adults, as set in 1990 (1) are given in Table 1.1. No RNI values were given for Na and K, since these elements are already consumed in excessive amounts. There were inadequate data to specify RNIs for Cu, Cr and Mo although the current levels of intake were believed to be adequate. Essentiality of B, Li and Si has been demonstrated in animals but not in humans; therefore, no RNI was given. There is evidence that As, Ni, and V may be essential in animals; again, no RNI was given.

The United States nutrient standards are set by the Food and Nutrition Board (FNB) of the National Academy of Sciences (NAS). These are called Recommended Dietary Allowances (RDAs). The RDA is a level of intake of an essential nutrient sufficient to meet the known nutrient needs of practically all healthy persons. For elements considered to have insufficient data on which to establish a RDA, the FNB sets

Table 1.1. Canadian RNI., and the U.S. RDA and SADDI values of elements for male and female adults (mg d^{-1}).

Element	CAN. R.N.I.		U.S. R.D.A.		U.S. S.A.D.D.I.
	M	F	M	F	
Ca	800	700	800	800	———
Cl	——	——	——	——	750[a]
Cr	——	——	——	——	0.050 - 0.200
Cu	——	——	——	——	1.5 - 3.0
F	1	1	——	——	1.5 - 4.0
Fe	9	13	10	15	———
I	0.160	0.160	0.150	0.150	———
K	——	——	——	——	2 000[a]
Mg	250	200	350	280	———
Mn	3.5	3.5	——	——	2.0 - 5.0
Mo	——	——	——	——	0.075 - 0.250
Na	——	——	——	——	500[a]
P	1000	850	800	800	———
Se	0.050	0.050	0.070	0.055	———
Zn	12	9	15	12	———

[a]: minimum estimated requirement

a range of Safe and Adequate Daily Dietary Intake (SADDI). The RDA and SADDI values for adults (17) are given in Table 1.1.

Health Canada and the U.S. FNB are currently collaborating to revise the RNIs and RDAs, incorporating the latest data on DI in relation to the reduction of risk of chronic diseases (18, 19). There will probably be four reference values (20, 21), namely the Estimated Average Requirement (EAR), RDA, the Adequate Intake (AI) and the Tolerable Upper Intake Level (UL). Collectively, they are referred to as Dietary Reference Intakes (DRI). Establishment of nutrition standards such as the DRI requires the evaluation of data from several fields, including the level of nutrient intakes of populations and individuals.

The present knowledge about the dietary requirements for elements is limited as evident from Table 1.1. The Canadian RNIs have so far been given for only 9 elements (namely, Ca, F, Fe, I, Mg, Mn, P, Se and Zn), and the U.S. RDAs for only 7 elements (namely, Ca, Fe, I, Mg, P, Se and Zn) and SADDI for another 8 elements (namely, Cl, Cr, Cu, F, K, Mn, Mo and Na). Data are also needed for both essential and toxic elements in order to set maximum permissible levels for minimizing health hazards. It is therefore necessary to monitor diets for as many essential, possibly essential and toxic elements as possible, whether they are present in major, minor or trace quantities.

1.2 ESTIMATION OF NUTRIENT INTAKE

There are a number of techniques for assessing the nutrient intake of individuals. Several review articles are available on this subject (22-25). The nutrient

intake is often assessed in two steps: first the determination of food intake and then the calculation of nutrient intake from the food intake data.

1.2.1 Food Intake Determination

The dietary intake of elements can be assessed through various dietary survey methods (26). These surveys can be used to determine the food consumption of individuals or of families or institutions sharing common food supplies. Household food consumption methods include the food balance sheet, food account, food record, food list and weighed household consumption. The food intake of individuals is generally determined by memory methods, food records or diaries, metabolic studies or duplicate portion methods. Memory methods require the participant to remember their food intake or habits and include dietary history, food frequency, and dietary recall. These are advantageous for assessing food intakes of population groups consisting of large numbers of individuals, require little training of participants, and are unlikely to perturb normal dietary habits. However, these methods depend on the ability of the participants to remember accurately and honestly the types and amounts of foods consumed and may depend on the skill of the interviewer to extract this information.

The keeping of food records or diaries reduces the need of participants to remember accurately their dietary patterns. Written entries are made of all foods and beverages consumed over a given period of time. This method still requires volunteers of the study to estimate quantities consumed and does require a higher level of participant training and/or skill.

Metabolic studies for assessing food intake involve the weighing of all foods consumed. This method is perhaps the most accurate of all, but is time-consuming and may perturb normal dietary patterns.

Finally, the duplicate portion technique involves preparation of an exact duplicate sample of all foods and fluids consumed by an individual while eating normally during a period of time, usually 24 h. The duplicate food items are then set aside for later collection by research workers. Usually a food record or diary is also kept at the same time. Duplicate portions are then weighed at the convenience of the researcher and subjected to chemical analysis, if desired. Among the advantages are the preparation of the samples by exactly the same methods as the food items consumed, and the amounts consumed may be estimated with higher accuracy. However, sample collection is costly and labour intensive. It is therefore not suited for sampling large populations. This technique is slightly inferior to the more complicated method of direct weighing which demands much more of the participant's involvement. The duplicate portion technique was used by Chatt and Pegg (27) to collect the food items used in this thesis to estimate the DI of several elements.

1.2.2 Nutrient Intake Determination

Once the food intake has been established, it is necessary to determine the level of nutrient intake. Three methods are generally used for this purpose; these are: calculation from food composition tables, analysis of equivalent portions, and the analysis of duplicate portions.

Food composition tables are formulated by analyzing the nutrient content of "typical" ready-to-eat food items or groups (categories) of food items. The tables are then used to calculate nutrient intake from previously recorded food intake data. Food composition tables are very useful in that an estimate of nutrient intake may be calculated rapidly and therefore are useful for surveying large populations. However, they do not take into account individual food items which are difficult to classify, individual methods of food preparation, or changes in food supply or processing.

In the equivalent composite technique, samples equal to those recorded in the food intake survey are collected by research workers, prepared as necessary, and then analyzed as individual food items, as groups of similar foods, or as a complete composite. This method is relatively simple to perform and less costly than collecting duplicate diet composites. A drawback to this method is that the foods collected may not correspond exactly with those consumed. Unless the collected foods are cooked or prepared exactly as they were done in the food intake survey, the effects of food processing are not taken into account. Food composition tables and the equivalent portion method are the only options available when the memory recall and weighed intake methods of diet survey are used. When the equivalent composite of food is purchased from the merchants in the study area to represent a "typical" diet, it is sometimes referred to as a "market basket". A potential drawback to the elemental analysis of separate food items or portions of food groups is the problem of what to do when the levels are below the detection limit of the method (28). Substitution of the detection limit for the level of an analyte may lead to overestimation of DI, whereas substitution of zero may lead to underestimation of the DI.

Processing and cooking can have significant effects on the content and bioavailability of trace elements in foods (29). For example, the use of Al (30-32) and Fe (33-35) utensils to cook foods can increase their levels in the foods. Therefore, it is important that foods for elemental analysis are prepared in exactly the same way in which they are eaten. The duplicate portion method is advantageous in this respect.

Most of the individual consumption methods do not provide information on the amounts of elements in food as eaten and cannot be used to assess the dietary intake of elements for which values are not currently listed in the food composition tables (*e.g.*, Cr, Cu, Mn, Mo, Se, *etc.*). Direct chemical analysis of the actual food consumed provides the most valid, precise and accurate data, on DIs of various elements (24). Diets prepared for studies on human metabolism, and diets prepared by institutions, such as hospitals and school cafeterias, are often analyzed. Samples obtained from metabolic and institutional diets have the advantage of taking food processing and cooking into account. However, these planned diets may not reflect the foods or amounts that would be chosen by free-living individuals, and sampling often neglects wasted (served but not eaten) food.

The duplicate portions technique is the only one in which the food items collected and analyzed are very close to those consumed. The analysis of duplicate portions can serve as a check on the results obtained by equivalent portions and food composition tables. Therefore, the duplicate portion method has been selected for the work reported in this thesis.

1.2.3 Elemental Analysis of Diets

A variety of techniques has been used for the elemental analysis of foods and diets (28, 36-42). These include: colorimetry (COLR), fluorometry (FLOR), flame emission spectrometry (FES), atomic emission spectrometry (AES), flame atomic absorption spectrometry (AAS), graphite furnace AAS (GFAAS), hydride generation AAS (HGAAS), inductively coupled plasma-mass spectrometry (ICP-MS), ICP-AES, gas chromatography-mass spectrometry (GC-MS), X-ray fluorescence (XRF) spectroscopy, particle induced X-ray emission (PIXE) spectroscopy, anodic stripping voltammetry (ASV), ion selective electrodes and neutron activation analysis (NAA). Daily intakes reported in the literature for several elements determined by a variety of techniques may be found in Chapter 3.

Among the analytical techniques capable of being developed for the reliable measurement of elemental concentrations in food, the AAS, NAA, ICP-AES and ICP-MS techniques are noteworthy. The first of these techniques works best when applied for determining single elements or sequentially for a small number of them, while the latter three have excellent scope for simultaneous multielement analysis at the trace level. The NAA technique is particularly suited to the analysis of food samples because of its excellent sensitivity and detection limits for most elements, the multielement nature of the technique and because little sample preparation is often required. A variety of NAA methods have previously been used for the analysis of foods and diets (42) including INAA (43), cyclic INAA (44, 45), epithermal INAA (46), pre-concentration NAA (47, 48, 49), and radiochemical NAA (47, 206). Other advantages of NAA include analysis

without physical destruction of sample, *e.g.* in INAA; freedom from reagent blanks in INAA and RNAA; virtual freedom from matrix interferences; applicable to solids as well as liquids; qualitative and quantitative analysis; high precision; excellent accuracy; extensive linear range; and fairly rapid. In this thesis, various forms of INAA were evaluated for, and applied to, the analysis of diet samples.

1.2.4 Neutron Activation Analysis

Activation analysis involves the bombardment of a sample with radiation or particles to induce nuclear reactions, forming product radionuclides or excited states of a target nucleus. The decay of the products is generally accompanied by the emission of radiation or particles, which can be detected and used for qualitative and quantitative analysis. The most common type of activation analysis is NAA, in which the neutron is used as the activating particle.

There are a number of texts on the subject of activation analysis, NAA in particular (50-53). Neutron activation analysis was discovered by Hevesy and Levi in 1936 (54). However, it was not until the 1950's and 1960's, with advances in nuclear reactor technology, that it became widely recognized and used. Although other neutron sources are available, nuclear reactors are most common. Neutrons may be classified according to their kinetic energy: thermal, or slow neutrons have energies of about 0.04 eV, epithermal neutrons of 0.1-1 eV, resonance neutrons of 1 eV-1 keV, and fast neutrons of >0.5 MeV. The flux of the Dalhousie University SLOWPOKE-2 reactor (DUSR) is highly thermalized, but does have a significant epithermal component (55, 56).

Epithermal, or more accurately epi-cadmium irradiation of samples can be performed using the Cd-shielded site of the DUSR (57).

The most common neutron reaction in NAA is neutron capture, where the target nucleus absorbs a neutron, increasing its mass number by one, and releasing a prompt gamma-ray. The products may then decay with the release of alpha, beta, or gamma radiation, which can be measured using appropriate detectors. Usually, it is the gamma-rays which are used for analysis via Ge(Li) or HPGe solid-state detectors. The theory behind the operation of these detectors is beyond the scope of this thesis and is discussed elsewhere (51, 52, 58). Briefly, the photoelectric absorption of a gamma-ray by the detector results in the formation of electron-hole pairs, which can migrate in response to an electric field, producing an electrical signal, which is sent to a multi-channel analyzer. However, this is not the only process which can take place. In Compton Scattering, the photon interacts with an atom, but only deposits part of its energy in ejecting an electron and is scattered at an angle from its original direction. If the photon escapes the detector, with only part of its energy deposited, a count is not recorded at the full-energy of the gamma-ray. The result is a continuous Compton background, which can obscure peaks in the gamma-ray spectrum. Anti-coincidence gamma-ray spectrometry involves the use of a guard detector which can detect gamma-rays escaping from the principal detector, allowing such signals to be rejected, and lowering the Compton background. Reviews of anti-coincidence spectrometry are available (58, 59, 60, 61).

The number of counts recorded by the analyzer can be related to the amount of an element in a sample by the activation equation:

$$C = (Nm/M)\ \Phi\sigma\Theta\varepsilon\gamma\ [1-e^{-\lambda t_i}][e^{-\lambda t_d}][1-e^{-\lambda t_c}]$$

where C = total number of counts registered
N = Avogadro's number
m = mass of the element
M = atomic mass of the isotope (g mol^{-1})
σ = cross-section for the neutron capture reaction (cm^2)
Φ = neutron flux (neutrons cm^{-2} s^{-1})
Θ = fractional abundance of the parent isotope
ε = detector efficiency
γ = branching ratio
λ = decay constant of the nuclide (units of reciprocal time)
t_i = irradiation time
t_d = decay time
t_c = counting time

If all variables are accurately known, this equation can be used to calculate the amount of an element in a sample. Since all of these parameters are not always known with high accuracy, the determination is often performed using a comparator method. A comparator standard is prepared with a known amount of the desired element, and subject to identical irradiation, decay and counting conditions as the unknown sample. In this case, the activation equation can be reduced to:

$$C_{unknown} = C_{standard}\ \frac{m_{unknown}}{m_{standard}}$$

where, $C_{unknown}$ = number of counts of unknown

$C_{standard}$ = number of counts of standard

$m_{unknown}$ = mass of the element in the unknown

$m_{standard}$ = mass of the element in the standard

It is important to ensure that the size, sample container, homogeneity and counting geometry of all samples and standards are maintained consistently. Corrections for different timing schemes can be calculated if necessary. The comparator method was used for all analyses in this thesis work.

There are some potential interferences in NAA which require consideration. Interfering reactions produce the radionuclide used for the analysis from an element other than the one being determined. For example, the reaction $^{31}P(n,\alpha)^{28}Al$ can interfere with Al analysis using ^{28}Al. Gamma-ray spectral interferences occur when the gamma-rays of two radionuclides have similar or equal energies. For example, ^{56}Mn and ^{27}Mg have gamma-rays at 846.8 and 843.8 keV, respectively. Overlap of these peaks in a gamma-ray spectrum could prevent the use of either peak in NAA.

1.2.5 Quality Assurance

Assuring the quality of measurement processes is now recognized as a critical concern. Taylor has written a thorough text on quality assurance (62) and defines quality assurance as "a system of activities whose purpose is to provide to the producer or user of a product or a service the assurance that it meets defined standards of quality with a stated level of confidence." It consists of quality control which is intended to control the quality of a product so that it meets users' needs, and quality assessment which

is intended to assure that the quality control job is done effectively. There are many aspects of quality assurance and control to be considered, including good laboratory practices, documentation and traceability. Two important aspects are the assurance that the measurement system is free from bias and in statistical control (63).

Bias refers to a systematic error in the method causing the measured value to vary from the true value. Evaluation of bias requires the measurement of samples having known properties, such as reference materials (63). Two such materials, namely IAEA H-9 Mixed Diet and NIST SRM 1548 Total Diet, are noteworthy since they are mixed diet composites.

Statistical control refers to statistical evidence that the process is in control. A system in statistical control is stable. It varies randomly around a given reference value and the degree of that variation remains constant (64). Control charts are powerful tools for documenting, assessing, and maintaining statistical control. In the most common type called a "property" chart, a control sample is analyzed along with the analytical samples, and the measured value plotted versus time. In addition to the data points, there are two other features in the charts, the central line and control limits. The central line is the best estimate of the variable plotted, usually the mean of all values. If the true, or expected value of the measured property is known, it can be compared with the central line to evaluate bias. The control limits, plus and minus three times the standard deviation (SD), define the boundaries which include almost all values for a system in statistical control. Often control charts have warning limits of twice the SD as well. Points lying outside the warning limits or control limits, or systematic trends may be indicators of loss of

control. When such charts are prepared after the measurement process is complete, they are referred to as quality assessment (QA) charts.

1.2.6 Literature Survey on Dietary Intake of Elements

In this section, Canadian and some noteworthy diet studies from other countries are highlighted. There have been many studies of elements in diets from several countries; these are summarized in various tables in Chapter 3.

The Nutrition Canada Survey carried out during 1970-72 October (65) is the most comprehensive Canadian study on the subject and is recognized world-wide for its organization and breadth of investigation, although actual chemical analysis of total diets was not performed. The dietary portion of the survey involved a recall of all foods and beverages consumed on the previous day and the frequency with which certain foods were consumed over the previous month. The food data of 13 000 participants were used for computing the nutrient intakes based on FCT data. The study concluded that most Canadians' level of intake met or exceeded the RNIs. However, there were certain groups of the Canadian population who were at risk of inadequate nutrition. Unfortunately, this extensive study included only 3 elements, namely Ca, Fe and I and relied on FCT for calculation of DIs.

Most Canadian studies of diet have concentrated on the determination of a few selected elements either in individual food items or total diet samples. For example, Se has been determined in single foods (66-68) and in a prepared composite diet (69); Fe in 24-h duplicate diets (70); Na and K in a prepared composite diet (71); Ca, P

and sometimes Fe in 24-h duplicate diets and using 3- or 7-d food record (72); and Cr and Mn (73) in 24-h duplicate diets. A few multielement studies have been reported (70, 74, 75, 76). Daily intakes of Ag, Cr, Cs, Rb, Sb, Sc and Se have been measured in 24-h duplicate diets (76), and of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in Halifax and Vancouver foods (75). Levels of Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn have been reported in prepared diet composites based either on food disappearance data (74) or on food consumption data (75, 77). Various segments of Canadian population have been studied, including hospital patients, university students, women of various age groups and the residents of some major cities. Not all of these groups (*e.g.* elderly women and hospital patients) may be representative of the general population. Most studies have involved collections using the equivalent composite method, although the duplicate portion technique has been occasionally used (70, 72, 76). Interestingly, there is a lack of data on some elements of nutritional and toxicological significance such as Cl, Mg and Al.

A few large scale studies of nutrient intake and nutritional status have been carried out in the USA including the Nationwide Food Consumption Survey by the Department of Agriculture, the Second National Health and Nutrition Examination Survey (NHANES II) by the Department of Health and Human Services, and the Total Diet Program by the Food and Drug Administration, FDA (12, 78-87). The Total Diet Study uses the equivalent portion method. Two hundred and thirty-four food items were analyzed for 11 essential elements by several techniques. Concentrations of several elements including Al, As, Ca, Cd, Cl, Co, Cu, Fe, Hg, I, K, Mg, Mn, Mo, Na, Ni, P, Pb,

Sb, Se, Sr, V and Zn have been measured in these studies. A variety of analytical techniques have been used, including ICP-AES to determine Na, K, Ca, Mg, Fe, Zn, Cu and Mn levels, COLR for the determination of I, HGAAS to determine Se, and NAA for I and Al (87-89). The FDA's Total Diet Study has been emulated in a number of other countries.

Perhaps the most extensive studies on elemental content of foods and diets have been done in Sweden (2, 90-94), Finland (95-100) and Federal Republic of Germany (101, 102). While in Finland various categories of food including all major food commodities were analyzed, the other two countries used 24-h duplicate diets. The list of elements includes Al, Au, As, B, Ba, Br, Ca, Cd, Cl, Co, Cr, Cu, F, Fe, Hg, I, K, Mg, Mn, Mo, Na, N, Ni, P, Pb, Rb, Sb, Sc, Se, Si, V and Zn.

A review of the Canadian literature indicates that there are relatively few multielement studies of Canadian diets. Most investigations, some of which have been cited above, are based on conventional techniques such as diet history and interview studies in conjunction with the use of standard food composition tables or equivalent composites which suffer from several disadvantages as described earlier. Not all groups studied may be representative of the general Canadian population, elderly women and hospital patients, for example. Furthermore, there are a number of elements for which there is little analytical data, such as Al, Cl, I, K, Mg, and Na. In this thesis project direct multielement analysis of the actual food consumed by individuals was carried out to give a more accurate estimate of the DIs of elements.

1.3 BIOAVAILABILITY

The determination of the total DI of elements through duplicate diets is an important contribution to fill some of the gaps that presently exist in this field. Perhaps equally important is the estimation of the fraction of the total intake that is available for meeting the physiological requirements of the body. The terms availability and bioavailability are often used to describe the proportion of an element that is absorbed and metabolized (7, 8, 9).

1.3.1 Elements, Food and Bioavailability

Bioavailability is "a quantitative measure of the utilization of a nutrient in a food, meal or diet under specified conditions to support normal structural and physiological processes occurring in the body" (9, 103). Bioavailability includes processes involved in absorption as well as transport, excretion, distribution and conversion to biochemically active form. This complex subject is made much more difficult by the numerous variables that can influence the bioavailability of an element.

Trace element bioavailability has been extensively reviewed by O'Dell (8) and McKenzie (9). Estimation of the potentially available fraction of an element in food depends on a number of factors such as the total amount as well as the physico-chemical form of the element, presence of other food constituents in that meal, nutrient status of the person consuming the food, physiological conditions that exist in the digestive tract, *etc*. The availability of an element to be absorbed is an important factor to its bioavailability; if the element is in a form which is not readily absorbed, then it will not

be available for use.

The efficiency of elemental absorption is related to the form in which the elements are present in food, and on other components of the diet (104). There are many forms in which elements may be found. The alkali metals Na and K, and the halogens F, Cl and I, are often found in ionic form or as inorganic salts. The alkaline earth metals Mg and Ca are also found as free ions, but may also form complexes. The elements As, Se and P are found as organic complexes but may also form oxy-anions such as phosphate and selenate. The transition metals tend to form complexes with organic compounds, are frequently bound to protein, and may be active components of enzymes or vitamins. Interaction with food components such as protein, carbohydrate, fibre, phytate and other elements can enhance or inhibit absorption. In the case of Fe, there are large differences between the absorption of Fe bound to haem and non-haem Fe, and between Fe(III) and Fe(II) (105). Iron bound to phytate or insoluble cereal fibre is largely unavailable (106). Many elements such as Na and K are absorbed readily, as they are largely present in ionic form. However, many other elements bound to insoluble molecules are unavailable for absorption unless the binding molecule is broken down. The purpose of digestion is to break food down into components suitable for absorption, storage or biosynthesis, and to separate and eliminate unusable fractions.

1.3.2 The Digestion Process

There are books and review articles on digestion (*e.g.*, 12, 107). The process begins with chewing, grinding and tearing of food by teeth. Digestion of complex

carbohydrates is initiated by salivary amylase, while another enzyme called lingual lipase hydrolyzes triglycerides (fats) to fatty acids and mono- and diglycerides.

After swallowing, the mass of food is delivered through the esophagus to the stomach where it is broken down by HCl and stomach enzymes. The stomach secretes HCl solution of pH 1-2 in its resting state. As food enters the stomach, the pH rises quickly to about 4-5 and then decreases slowly as the stomach continues to secrete acid. Some ions may be reduced, such as Fe (III) to Fe (II). Enzymatic hydrolysis takes place *via* a proteolytic enzyme called pepsin which is secreted via gastric glands. Pepsin cleaves peptides at aromatic amino acid links (*e.g.*, tryptophan, phenylalanine and tyrosine). As the larger components of the food are broken down, metal ions are solubilized as free ions or complexed to other species. Ethanol and some lipid-soluble substances are rapidly absorbed, but most of the contents are passed to the small intestine.

As the mixture enters the small intestine, alkaline fluids from the pancreas, bile from the gall bladder, and enzymes from the intestinal wall are added to the stream. Pancreatic juice contains bicarbonate and digestive enzymes capable of digesting all of the major food nutrients. Bile contains detergent molecules (bile salts), which promote the efficient digestion and absorption of lipids. It also contains endogenous wastes or metabolites (*e.g.*, cholesterol, bilirubin, drugs and environmental pollutants), which are excreted in the bile.

By the time protein material reaches the small intestine, it is a mixture of native protein, proteoses, peptones, polypeptides and some free amino acids. These are further broken down by proteolytic enzymes in the small intestine, including trypsin,

chymotrypsin, elastase and carboxypeptidases A and B. The products of protein digestion by the pancreatic enzymes are small peptides (70%) and free amino acids (30%). Free amino acids are not absorbed as rapidly as dipeptides or tripeptides. There is some evidence for absorption of whole protein although it is not believed to be a major fraction (365).

Ingested carbohydrates consist of starches, glycogen in animal products, sucrose, lactose, glucose and fructose, and indigestible carbohydrates (fibre). Before they can be absorbed, these carbohydrates must be broken into monosaccharides. This is performed by glycoproteins in the brush border membrane. Fibre, including cellulose, hemicelluloses and pectins are indigestible solids which pass through the intestines and are excreted. They can absorb water, Ca, Fe, Mg, Zn and organic materials. Consequently, fibre can reduce the absorption and bioavailability of these substances.

Lipids ingested include triglycerides, phospholipids (such as lecithin), cholesterol esters and fat-soluble vitamins. By the time the lipids reach the small intestine, they have been partially digested and emulsified. They are then solubilized by incorporation into bile salt micelles, and converted to glycerol and fatty acids by the lipolytic enzyme lipase. The fatty acids released are absorbed into intestinal cells by diffusion, from the lumen or from bile salt micelles.

The small intestine is where most elemental absorption takes place. The efficiency of absorption depends on several factors (104). Multivalent ions are absorbed more slowly than divalent ions. Complexation with other species can greatly affect elemental absorption. The presence of insoluble plant materials or fibre may inhibit

absorption of certain elements by binding them. For example, maize, soy-bean or whole-meal products are rich in phytic acids which can bind Ca, Cu, Mn and Zn (108, 109). However, other components such as ascorbic acid, may enhance non-haem Fe bioavailability (110, 111). Elements may also interact with each other to enhance or inhibit absorption. These effects may result from changes in solubility or complexation, or by competition for metabolic or absorptive pathways. It is now recognized that the supply of elements to the body is not determined only by the quantity of a particular element consumed; determination of actual nutritional intake should take into account enhancing and inhibitory factors. Thus, there is a need for studies on the availability of elements from total diets.

1.3.3 *in vitro* Simulated Digestion

Five methods are generally used to assess elemental bioavailability. These include the prevention of gross pathology and measurement of growth rate (or other physiological parameters), chemical balance, tissue concentration and trace element-dependent enzyme activity. Many of these methods may not be used in humans for ethical reasons. The use of laboratory animals can be time-consuming and expensive, and may not be a representative model of the human system. Furthermore, *in vivo* studies may be subject to inherent variability between animals and physiological conditions other than those being studied. They are also generally expensive, time consuming, and complicated. An *in vitro* enzymolysis method simulating gastrointestinal digestion offers an appealing alternative procedure for estimating the absorbable or soluble fraction.

In vitro techniques for measuring potential availability have become more common in recent years. The main principle of the technique is simulation of the gastrointestinal digestion process. Species rendered soluble are assumed to be potentially available for absorption, while insoluble species are assumed to be unavailable. Not all soluble species can be absorbed, but it is generally accepted that a species must be soluble for it to be absorbed (112). Therefore, the technique measures the upper limit of bioavailability or *potential* bioavailability. The advantages of *in vitro* simulation of digestion are that it is relatively inexpensive and rapid, suitable for multielement analysis, and not subject to inherent variability of biological specimens. Good agreement has been reported between the *in vitro* and *in vivo* measurements of Fe absorption (113-115). However, not all important physiological variables can be simulated, and therefore the *in vitro* methods are concluded to be relative rather than absolute measures of absorbability (115, 116).

A brief summary of some *in vitro* digestion studies is given in Table 1.2. One of the first *in vitro* digestion procedures was used by Kaldor in 1957 (117) to study the availability of Fe from human blood. Other similar studies also focused on the availability of haem- and non-haem Fe (118, 119, 113, 120). *In vitro* digestion has since been extended to the study of other elements (notably Ca), protein, peptides, amino acids, phytate and lipid. Samples have included human blood, various food items, meals, protein and phytate (Table 1.2). There have been relatively few studies involving meals, and none of whole diets. Furthermore, only a small number of elements have been analyzed. One of the objectives of the present work was to subject the Canadian total

Table 1.2. Survey of selected *in vitro* enzymolysis procedures

Reference	Sample(s) Studied	Acid Digestion	Alkali Digestion	Separation Method	Species
Kaldor (117)	human blood	0.32-0.64% 0.16-0.32 M HCl 30-60 min, 37°C	———	centrifugn.	Fe
Narasinga Rao & Prabhavathi (113)	edible plant prods.	0.25% pepsin 0.5 M HCl (pH 1.4) 1.5 h, 37°C	NaOH to pH7.5 var. amounts pancreatin 1.5 h., 37°C	centrifugn. filtration	Fe
Hazell *et al.* (120)	rat muscle, beef	0.15% pepsin 0.01 M HCl (pH 2-2.5) 0.03% trypsin 6-24 h, 37°C	$NaHCO_3$ to pH 7-7.5 NH_4SO_4 precip. 0.15% pancreatin 12-48 h, 37°C	centrifugn. dialysis gel filtrn.	Fe
Miller *et al.* (116)	var. meals	0.5% pepsin 0.1 M HCl	$NaHCO_3$ to pH 5	dialysis	Fe, Ca
Schricker *et al.* (362)		2 h, 37°C	0.04% pancreatin 0.25% bile salts 2.5 h, 37°C		
Crews *et al.*. (129)	var. foods groups	0.5% pepsin 0.01 M HCl 0.1 M NaCl 2 h, 37°C	$NaHCO_3$ to pH 7.5 0.2% pancreatin 0.02% bile salts 0.1 M NaCl 2 h	centrifugn.	Cd, Cu, Fe, Pb, Zn
Crews *et al.* (130)	var. foods	0.5% pepsin 0.01 M HCl to pH 1.8 0.1 M NaCl 4 h, 37°C pH maint at 2.0-3.5	$NaHCO_3$ to pH 7.5 0.2% pancreatin 0.02% bile salts 0.1 M NaCl 4 h, 37°C	centrifugn.	Cd, Cu, Fe, Pb Zn

Table 1.2 (continued). Survey of Simulated Digestion Procedures

Reference	Sample(s)	Acid Digestion	Alkali Digestion	Separation Method	Species Studied
Savoie & Gauthier (128)	casein	0.1 M HCl to pH 1.9 0.08% pepsin 50 ppm thimerosal 30 min. 37°C	1 M NaOH to pH 8.0 0.07%pancreatin circulating phosphate buffer 1-24 h, 37°C	continuous dialysis	N, radio-active amino acids
McDowell (66)	bread, eggs	0.01 M HCl to pH 1.8-2.0 0.15 M NaCl 0.7% pepsin 4 h. 37°C pH maint. <2.5	satd. Na_2CO_3 to pH 7.0-7.2 0.75% pancreatin 0.25% amylase 0.04% bile salts 0.15 M NaCl 4 h, 37°C pH maint. 7.0-7.2	centrifugn.	Se
Wolters *et al.* (125)	bread	0.01 M HCl to pH 1.8-2.0 0.15 M NaCl 0.5% pepsin 4 h. 37°C pH maint. <2.5	satd. Na_2CO_3 to pH 7.0-7.2 0.06% pancreatin 0.4% bile salts 0.15 M NaCl 4 h, 37°C pH maint. 7.0-7.2	continuous dialysis	Ca, Cu, Fe, Mg, Zn
Shen *et al.* (131)	baby formula	HCl to pH 1.8-2.0 0.5% pepsin 4 h. 37°C pH maint. <2.5	$NaHCO_3$ to neutral 0.04% pancreatin 0.8% bile salts 0.15 M NaCl 4 h, 37°C pH maint. 7.0-7.2	continuous dialysis	Ca, Zn

duplicate diets to *in vitro* digestion for estimating the potential availability of several elements.

The most simple approach to *in vitro* digestion methods is a sequential pH treatment, simulating the acid environment of the stomach and the alkaline environment of the small intestine (121, 122). Early studies included only an acidic gastric digestion step (117-119). Poor agreement with *in vivo* bioavailability led to the inclusion of a second alkaline intestinal step (113, 114). Most authors have used the two-step process. The first step involves digestion at low pH (1.5-2.5) using pepsin. Then the pH is raised to between 5-8. This is followed by digestion using pancreatin (often with inclusion of bile salts). Some researchers have also added trypsin (120), amylase (66, 123), lipase (124) and erepsin (362) in the alkaline digestion step. This two-stage *in vitro* digestion procedure is commonly referred to as "*in vitro* enzymolysis" (66).

Centrifugation was commonly used to separate the soluble and insoluble fractions of samples subject to enzymolysis. Dialysis has now become much more common. Most studies use equilibrium dialysis. However, removal of dialyzable components through continuous dialysis may be more representative of the situation *in vivo* and give a better estimate of *in vivo* bioavailability (125, 126). Some workers have custom designed dialysis cells for this purpose (125, 127, 128, 131).

The *in vitro* enzymolysis procedure used in this thesis was modified from that of McDowell (66), which in turn derived from Crews *et al.* (129, 130). The modification was the use of dialysis to fractionate the digested samples. In order to remove digestion products during the reaction, the dialysis buffer was replaced periodically.

1.4 OBJECTIVES

The overall objectives of this project were to: (i) develop and apply NAA methods for the determination of nutritionally important and toxic elements in duplicate diet samples, (ii) to carry out a quality assurance program of the methods developed, (iii) to estimate the daily dietary intake (DDI) of the elements determined, (iv) to perform statistical analyses for correlation purposes, (v) to use a dialysis technique to determine the fraction of the elements which are insoluble or bound to macromolecules, and (vi) to determine the potentially available fraction of the elements through *in vitro* enzymolysis.

As stated above, one of the objectives was to develop INAA methods for the determination of several elements in the duplicate diet samples. The elements of interest included Al, Br, Ca, Cl, Co, Cr, Fe, I, K, Mg, Mn, Na, Rb, Se, Ti, V, and Zn. Various INAA methods were to be compared and evaluated for their suitability in the determination of Se. These methods covered conventional INAA, epithermal INAA (EINAA), cyclic INAA (CINAA), pseudo-cyclic INAA (PCINAA) and INAA with anti-coincidence gamma-ray spectrometry, all using the 17.4 s nuclide of ^{77m}Se, and conventional INAA using the 119.8 d nuclide of ^{75}Se. An EINAA method using anti-coincidence gamma-ray spectrometry was to be developed and applied to the determination of I using ^{128}I. The medium-lived nuclides of Ca, Cl, K, Mg, Mn and Na were to be determined by INAA using a few minutes of irradiation, decay and counting periods. A longer irradiation time of 17 to 24 h could be employed for detecting the longer-lived nuclides of Co, Cr, Fe, Rb, Sb, and Zn. The Al content of the diets was to be measured using a combination of INAA and EINAA methods after making correction

for interference from P.

Quality of the NAA measurements was to be evaluated analyzing appropriate RMs, and the development of a laboratory information system (LIMS) to track methods, samples, analyses and results. Exploratory multivariate statistical analysis of the data obtained from NAA was to be performed usin correlation, Hierarchical Cluster Analysis (HCA), and Principal Component Analysis (PCA).

Although INAA methods are capable of determining several elements, analysis for all the elements of interest might not be possible because of either their very low levels in diets or interferences from major elements such as Na and Cl. A simple dialysis technique could be used to reduce the Na and Cl content of the diet samples, allowing previously undetected nuclides to be recorded.

Finally, an *in vitro* enzymatic digestion procedure was to be adapted for the determination of the potentially bioavailable fraction of the elements from the diets. The residues from dialysis of the digested samples could then be subjected to NAA for short-, medium- and long-lived nuclides.

The duplicate diet samples, experimental methods and LIMS are described in Chapter 2. The current status of food chemistry, nutrition and bioavailability pertaining to the individual elements analyzed are briefly reviewed in Chapter 3. Results from NAA, dialysis, and *in vitro* enzymolysis are also presented and discussed in Chapter 3. Exploratory statistical analysis of the NAA data is described and discussed in Chapter 4.

2. EXPERIMENTAL

Total diet samples were collected from 14 volunteers using the duplicate portion technique. Small portions of the duplicate diet composites were subjected to dialysis. Then an *in vitro* enzymolysis procedure was used to simulate gastrointestinal digestion of each composite. The duplicate diet composites, dialysed portions and portions subjected to *in vitro* enzymolysis were analyzed by INAA. The sampling methodology and sample preparation procedures are described in this chapter. Details of the dialysis and *in vitro* enzymolysis treatments are also given. The general experimental conditions for the INAA methods developed in this thesis are also presented.

2.1 SAMPLING METHODOLOGY

2.1.1 Study Group

According to the Nutrition Canada Survey, the nutritional intake of most Canadians is either adequate or in excess of the basic requirements (65). In fact, the most common nutrition-related problems in Canada are those of overweight and obesity, and elevated cholesterol level, which may result in health problems such as cardiovascular disease. There are, however, some Canadians, namely adolescent girls, pregnant women, the elderly and the native peoples (Indians and Eskimos) who are believed to be at risk of inadequate nutrition. It was also reported (65) that there was no detectable effect of season or community size on nutritional status. There was, however, a relationship between income and nutritional status. The volunteers selected for the present study were

intended to represent "typical" Canadian adults.

Diet collection was performed by Chatt and Pegg (27). Fourteen subjects, seven males and seven females, were selected from the cities of Halifax and Dartmouth, and surrounding areas, by way of a questionnaire given in Appendix 1. Only healthy subjects between the ages of 20-39 a were selected (median age in Canada was 30.4 a according to 1985 statistics). Excluded were those on a special diet (*e.g.* vegetarian, diabetic, high protein, *etc.*), who were pregnant or lactating, or who did not normally eat at least one meal per day at home. All participants in this study group were employed full-time. The relevant information on the participants of this study is given in Table 2.1 (132). Included in Table 2.1 are body mass index (BMI) values. The BMI is a measure of obesity or leanness, which may be correlated with risk of a disease. It is defined by:

$$BMI = \frac{\text{median weight in kg}}{(\text{median height in m})^2}$$

A generally accepted safe range of BMI is 20-27. The BMI of all participants were within this safe range.

2.1.2 Diet Collection Procedure

The sampling methodology used was the "Duplicate Diet Technique" described in section 1.2.1 (page 9). One male and one female were selected to collect 24-h duplicate diet composites for each day of the week, including weekends. Each subject was contacted personally and briefed precisely on how to collect a food composite. A duplicate portion of each food item consumed was stored in pre-cleaned

Table 2.1. Information on the Participants of the Total Diet Study.

Sample Code	Gender	Age (a)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Diet Wt. (Wet)(g/d)	Diet Wt. (Dry)(g/d)
(a) Female participants							
HFFF	F	22	165	61.2	22.5	2455	337
HFMC	F	25	168	72.6	25.8	1714	350
HFNA	F	23	163	44.0	16.7	1703	398
HFRR	F	27	165	50.0	18.4	1871	433
HFSR	F	35	198	58.1	14.8	1931	477
HFTC	F	24	152	46.7	20.1	2132	357
HFWH	F	25	165	55.3	20.3	2594	481
Mean		25.9	168	55.4	19.8	2057	405
Median		24	165	50.0	18.4	1931	357
(b) Male participants							
HMFF	M	27	170	69.0	23.9	2768	465
HMMR	M	22	177	68.0	21.8	1277	273
HMNT	M	31	179	70.8	22.1	3244	718
HMRZ	M	27	165	61.3	22.5	2891	597
HMSG	M	37	168	59.0	21.0	2215	458
HMTH	M	32	183	77.1	23.1	2189	527
HMWV	M	30	177	67.1	21.5	3491	807
Mean		29.4	174	67.5	22.3	2582	549
Median		27	170	67.1	21.8	2768	465
(c) Overall							
Mean		27.6	171	61.5	21.0	2320	477
Median		27	168	61.2	21.6	2202	462
Min.-Max.		22-37	152-198	44.0-77.1	14.8-25.8	1277-3491	273-807
HMFS	M	26	168	83.9	29.7	2389	700

polyethylene bags or bottles which were labelled with a marker. A record of each item consumed was also kept on forms provided. Samples were collected between 1987-08-26 and 1987-09-26. All duplicate food items collected from each individual subject were weighed. On the day of collection or the day following, the composites were stored at -30°C. The details of the collection and storage procedures are given elsewhere (133). All diets were stored until homogenized and freeze-dried between 1991-07-01 and 1997-09-01.

A fifteenth duplicate diet composite, coded HMFS, was collected by the author of this thesis for preliminary studies and use as a trial sample. A duplicate portion of each food item consumed, except beverages, was collected on 1991-06-07, using the same protocols established for the 1987 collection.

2.1.3 Composition of Diets

The amounts consumed of all food items in each diet were classified into ten food group categories using the food record forms and the masses of the food items. A complete listing of the contents of the duplicate diet composites is given in Appendix 1. The masses and mass proportions of the total wet mass in each food group are also presented in Appendix 1. These data are summarized in Table 2.2.

2.1.4 Nutrient Analysis using Canadian Nutrient File

A computer analysis of the diet composites was performed by Chatt and Pegg (27) in 1987. The Nutrient Analysis System (NAS) available at the Mount Saint

Table 2.2. Average Food Composition of Duplicate Diet Composites by food category

Food category	Content (g/d)		
	Min.-Max.	Median	Mean±SD
Cereal prods. & nuts	90 - 467	236	246 ± 121
Vegetables	20 - 654	272	282 ± 166
Fruits & berries	0 - 868	346	392 ± 288
Meat & meat products	0 - 251	130	127 ± 86
Fish, seafoods & products	0 - 132	0	25 ± 43
Milk prods. (as liquids)	0 - 1052	388	388 ± 313
Cheese	0 - 52	5	18 ± 22
Food fats	4 - 142	14	24 ± 36
Fluids (excluding fruit & vegetable juices)	0 - 1397	862	801 ± 449
Tea or coffee	0 - 1045	321	360 ± 321
Soft drinks	0 - 708	0	163 ± 244
Mineral water	0 - 862	0	200 ± 313
Wine (beer)	0 - 618	0	82 ± 204
Other foods	0 - 89	0	17 ± 23

Vincent University in Halifax was used for this purpose. The software used the Canadian Nutrient File, a Food Composition Table (FCT) data base prepared by the Nutrition Research Division of Health and Welfare Canada, which contains food composition data on the nutrient content of 3 000 Canadian foods. Daily intakes were calculated for protein, energy, Ca, Fe, K, Mg, Na, P, Zn, vitamin D, vitamin A, vitamin C, thiamin, riboflavin, niacin, vitamin B-6, folacin, and vitamin B-12. The elemental intakes are discussed in individual sections in Chapter 3 while the rest of the data are presented in Appendix 2.

2.2 SAMPLE PREPARATION

2.2.1 Apparatus Cleaning Procedures

All containers, spatulas and scoops were acid-washed according to an International Atomic Energy Agency (IAEA) protocol (364). Sample containers and bottles, caps, beakers, scoops and blender buckets were washed first with liquid soap (Fisher Versa-Clean). They were then rinsed with tap water, followed by several rinses with distilled, deionized water (DDW). Bottles were filled with 4M HCl and all other objects were soaked in a 4M HCl bath. These were allowed to stand for 24 h and were then rinsed thoroughly with DDW. All items were soaked in the same way in 4M HNO_3 and rinsed with DDW. They were then allowed to stand on paper towels or paraffin films until dry. Finally, they were covered with either paper towels or paraffin films until used.

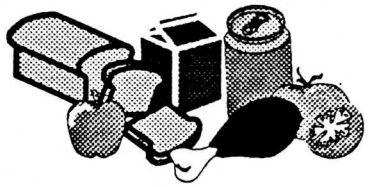
The blender blade assembly was cleaned by washing all components with soap and water, rinsing with tap water followed by DDW. They were then allowed to dry

on paper towels and were covered. The plastic spacer rings supplied with the blender were found not to be acid-resistant. Subsequently, Teflon spacer rings were designed and fabricated, and acid-washed with HCl and HNO_3 as described above. After the first set of blendings, all apparatus was acid-washed again. For subsequent blendings, the apparatus was washed with tap water and DDW.

2.2.2 Homogenization

The preparation including homogenization of the duplicate diets is illustrated in Fig. 2.1. The food blender (Robot-Coupe, Inc.) was assembled with 2 blades, 180° apart, cutting edges up and one spacer between. Only a thin spacer was left under the lower blade, while the remaining 6 spacers were placed over the upper blade.

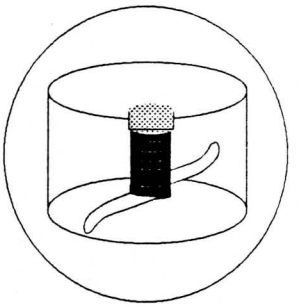
Five duplicate diet samples were removed from the freezer and were allowed to thaw for 2.5 d. Before each diet was added to the blender bucket, the contents were checked against the food record and were supplemented with the recorded amounts of the missing items (*e.g.* tap water and milk) whenever necessary. For the initial set of samples, all food items were added to the blender in one step. The blender was set to 1 500 rpm for 30 s. It was then switched to 3 000 rpm for 1.5 min. Three more blendings were carried out, for 2 min each, with a 2-min interval between blendings. When all food items were added to the blender at once, it was discovered that some items were not completely cut (such as very tough meat) and sometimes leakage from the



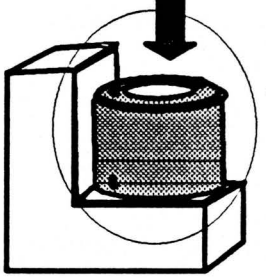
Food items checked against food record

Thawed for 2.5 days

Added to blender



Blender Blade Arrangement

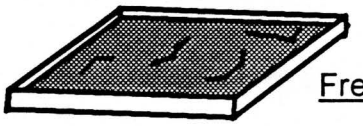


Blending:
Solid items first, then liquids
4 x 2 min with 2 min intervals between

Robot - Coupe Blender

Homogeneity Testing:
5 x 5-15g samples weighed onto watchglasses
oven-dried at 110 C to constant weight

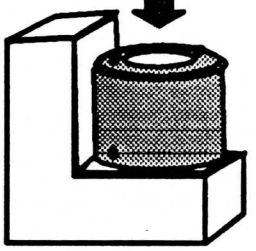
Poured into Teflon-lined trays



Freeze Dried at -35 C

Sub-samples removed for bioavailability studies:
20, 30, 100mL

Solid cake added to blender



Blending:
2 x 2 min with 2 min interval between

Robot - Coupe Blender

0.3 g powdered diet
weighed into irradiation vial

Fig. 2.1. Homogenization of duplicate diet composites

blender bucket occurred. Therefore, only solid food items were added initially to the blender for all but the first three diet samples. Only after three 2-min blendings were the liquid food items added. Two more 2-min blendings were then performed.

Blended slurry was tested for homogeneity as follows (364). Immediately following the blending, 5 subsamples (5-15 g each) of slurry were removed from the blender bucket and placed on clean, oven dried, weighed watch glasses which were then weighed. It was observed that the slurry dried very rapidly. Consequently, the mass continually decreased even after waiting for 1 h. Therefore, the watch glasses and slurry were weighed as soon as possible after subsampling and the first reliable reading on the balance was recorded. After weighing, the watch glasses were placed in an oven at room temperature and allowed to stand for 16 h. The oven was then set to 110°C and switched on for 4 h. The subsamples were allowed to cool for 3 h in the oven, in presence of a desiccant, and were re-weighed. This procedure was repeated until a constant weight was obtained.

In order to facilitate studies on potential bioavailability using blended duplicate diet, the wet slurry was further sub-sampled. One 20 mL, one 30 mL and one 100-mL quantity of each diet was collected, stored in acid-washed containers, and frozen. The remaining slurry in the blender bucket was stored overnight in a covered, acid-washed plastic or glass beaker in a refrigerator. The remaining duplicate diet samples were homogenized following the above procedure.

2.2.3 Freeze-Drying

A tray-type freeze-drier (Virtis model 50SRC6) was used at the laboratories of the Institute of Marine Biosciences, National Research Council of Canada, Halifax. Ultraclean Teflon sheets (Clean Room Products Inc.) were cut into 60 cm x 80 cm sheets using a clean scalpel blade. Handling of the sheets was minimized, and they were stored in acid-washed plastic bags until needed. The Teflon sheets were used to line the freeze-drier trays. After weighing, the blended wet slurry was poured directly onto the Teflon-lined trays. The empty containers and scoops were then re-weighed. The slurries were frozen at -35°C and freeze-dried at constant temperature (a temperature probe was inserted into a separate sample.

The dried slurry cakes were rolled up in the Teflon liner and stored separately in clean Nalgene polyethylene bags. Each cake was weighed and then blended for 2 min at 3 000 rpm. The powder was then scraped off the bottom and sides of the bucket, and mixed. Blending was performed for 2 more min. Finally, the resulting powder was stored in pre-cleaned polyethylene bottles.

Moisture contents, as determined by oven-drying and freeze-drying, of the diets are reported in Table 2.3. The relative standard deviation (RSD) of 5 oven-dried sub-samples taken from each diet was less than 0.3% indicating efficient blending of the samples. The effect of storage on the moisture content was evaluated using the HMFS trial diet; after several months of storage, the moisture content was found to increase to 8.1% by oven-drying.

Table 2.3. Dry weight and moisture content of Duplicate diet composites.

Diet Code	%Moisture (oven)	%Moisture (fr. dryer)	Diet Mass (dry) (g/d)
(a) Females			
HFFF	87.756	86.265	337
HFMC	81.407	79.603	350
HFNA	79.987	76.620	398
HFRR	78.094	76.865	433
HFSR	77.859	75.276	477
HFTC	84.356	83.243	357
HFWH	82.289	81.458	481
Mean	82±4	80±4	400±60
Median	81.407	79.603	398
Min.-Max.	77.859-87.756	75.276-86.265	337-481
(b) Males			
HMFF	84.945	83.198	465
HMMR	82.834	78.660	273
HMNT	80.037	77.869	718
HMRZ	80.201	79.339	597
HMSG	82.026	79.308	458
HMTH	78.000	75.910	527
HMWV	79.093	76.875	807
Mean	81±2	79±2	550±180
Median	80.201	78.660	527
Min.-Max.	78.000-84.945	75.910-83.198	273-807
(c) Overall			
Mean	81±3	79±3	480
Median	80.804	78.984	461.5
Min.-Max.	77.859-87.756	75.276-86.265	273-807
HMFS	71.661	70.7	700

2.3 INAA OF DUPLICATE DIET COMPOSITES

2.3.1 Chemical Reagents

Reagent-grade concentrated HCl, HNO_3, and NH_4Cl were obtained from Fisher Scientific. Ultrex grade HNO_3 was obtained from J.T. Baker Chemical Co. Ultrapure acids and ammonia were also obtained from SeaStar Chemicals. Suprapur grade KNO_3 was obtained from E. Merck Co.

2.3.2 Preparation of Diet Samples for Irradiation

Samples for irradiation were prepared by accurately weighing solid diet materials into precleaned small-size (1.2 mL) polyethylene vials. A balance was calibrated prior to use. An inverted, trimmed polyethylene cap was inserted into the vial to prevent movement of the solid from the bottom of the vial, thus assuring a constant geometry. The small vial was capped, heat-sealed with a soldering iron and placed in a medium-sized (7 mL) polyethylene irradiation vial. An empty small vial was placed as a spacer on top of the sample vial. The medium-size vial was then capped and heat-sealed.

All small-size vials used were acid-washed before placing samples in them. They were immersed in 4M HNO_3 for at least 24 h and rinsed thoroughly with DDW. The vials were then placed in 0.2M Ultrex-grade HNO_3 for at least 24 h, rinsed thoroughly with DDW, and allowed to air-dry on paper towels. A sheet of paraffin film was used to cover the vials until used.

2.3.3 Elemental Comparator Standards

The concentrations of the elements of interest in diet samples were calculated using elemental comparator standards. Multielement comparator standards were prepared in the following manner. If dilution was necessary, multielement stock solutions were prepared by diluting ultrapure (>99.999% purity) plasma emission grade elemental standards (Spex Co) with DDW. Various amounts of these stock solutions or the elemental standards were weighed onto 0.5 g sucrose (Sigma Chemicals Co.) or SiO_2 (99.999%, ICN Pharmaceuticals, Inc.) in precleaned vials. The standards were then allowed to dry in air. Blanks were prepared in the same way as the standards, except that DDW was added in place of the standard or stock solutions. Once dried, the vials were heat-sealed. Occasionally liquid standards were also used. These were prepared by weighing the standard or stock solutions into precleaned vials, and then adding DDW to the volume required for the desired geometry. In many cases, replicate sets of standards were prepared independently and analyzed as a check on their preparation.

2.3.4 Reference Materials

For the purposes of QA, the accuracy and precision of the INAA methods developed were assessed by analyzing reference materials (RMs) and standard reference materials (SRMs). Apple Leaves (SRM 1515), Peach Leaves (SRM 1547), Total Diet (SRM 1548), Non-Fat Milk Powder (SRM 1549), Wheat Flour (SRM 1567a), Rice Flour (SRM 1568), Bovine Liver (SRM 1577b), Whole Egg Powder (RM 8415), Whole Milk Powder (RM 8435), Durum Wheat Flour (RM 8436), and Soft Winter Wheat Flour (RM

8438) were obtained from the U.S. National Institute of Standards and Technology (NIST). Animal Blood (A-13), Mixed Human Diet (H-9), and Animal Muscle (H-4) were obtained from the International Atomic Energy Agency (IAEA). These materials were chosen to represent either edible plant materials and animal products in diets, or diet composites themselves.

In this thesis work, statistical control was assessed by preparing QA charts. Three materials, IAEA H-9, NIST SRM 1548 and SRM 1568, were used at various times during the course of this work as control samples. The control samples were analyzed along with the analytical samples, and the results later plotted on external quality assessment (EQA) charts.

2.3.5 Irradiations and Counting of Diet Samples

All samples, comparator standards, reference materials, and blanks were irradiated at the Dalhousie University SLOWPOKE-2 Reactor (DUSR) facility. Three types of irradiation sites are available at the DUSR, namely five inner sites capable of accepting medium-size (7 mL) vials, two outer sites capable of holding large-size vials (27 mL), and a Cd-shielded outer site for irradiations in epithermal neutrons. When the reactor is operating at a normal power of 8 kW, the total flux at the inner and outer sites are 5×10^{11} and 2.5×10^{11} n cm^{-2} s^{-1}, respectively. The flux in the Cd-shielded site is 1×10^{10} n cm^{-2} s^{-1}. The homogeneity, reproducibility, and stability of the DUSR neutron flux have previous been published (55-57). Measurements have shown that the flux has been highly stable and reproducible since the installation of the reactor. The inner sites

have less than 0.5% cm^{-1} variation in radial and vertical flux. While the flux in the outer sites is very homogenous in the vertical direction (< 0.5% cm^{-1}), the flux varies in the radial direction by about 6% cm^{-1}. In addition to the radial variation characteristic of the outer sites, there is considerable variation in total flux and ratio of thermal-to-epithermal flux in the Cd-shielded site due to a hole in the bottom of the Cd-shield. Inhomogeneity of the flux in the outer and Cd-shielded sites does not create problems as long as the samples and standards have similar geometry.

Concentrations of Ca, Cl, K, Mg, Mn, Na, and Se were determined using conventional (one-shot) INAA. The nuclear data for these elements are given in Table 2.4. The samples were irradiated for 1 min, for all elements except for Se, in an inner site. A longer irradiation time was not selected due to high activities resulting from the high Cl and Na content of the diet samples. A 1-min decay was allowed before counting for 10 min. The dead time was less than 5%. After a decay period of at least 90 min, all irradiated samples were counted again for another 30 min for measuring K and Mn levels.

For Se using conventional INAA, the samples were irradiated for 30 s, allowed to decay for 10 s before counting for 30 s. The dead time was below 6%. Analysis of Se was attempted by epithermal INAA (EINAA) in the cadmium-shielded site. The same irradiation, decay, and counting times were used.

Selenium analysis was also performed using a pseudo-cyclic INAA (PCINAA) method. The irradiation, decay and counting conditions were the same as described above for each cycle. At least one week's decay was allowed between cycles.

Table 2.4. Nuclear Data of Elements Detected.

Element	Target Isotope (% abundance)	Cross section	Nuclide	Half-life	γ-ray energy (keV) (%int.)
Al	^{27}Al (100)	232 mb	^{28}Al	2.24 min	1778.9
Br	^{81}Br (49.3)	2.69 ± 0.06 b	^{82}Br	35.3 h	554.3 (70.8)
Ca	^{48}Ca (0.19)	1.1 ± 0.2 b	^{49}Ca	8.72 min	3084.1 (52)
Cl	^{37}Cl (24.2)	428 ± 5 mb	^{38}Cl	37.2 min	1642.7
Cr	^{50}Cr (4.35)	15.9 ± 0.2 b	^{51}Cr	27.7 d	320.1 (10.1)
Cu	^{65}Cu (30.9)	2.17 ± 0.03 b	^{66}Cu	5.09 min	1039.2
Fe	^{58}Fe (0.31)	1.15 ± 0.02 b	^{59}Fe	44.5 d	1099.3 (56.5)
I	^{127}I (100)	6.2 ± 0.2 b	^{128}I	25.0 min	442.9
K	^{41}K (6.7)	1.46 ± 0.03 b	^{42}K	12.4 h	1524.7 (18.1)
Mg	^{26}Mg (11.0)	38.2 ± 0.8 mb	^{27}Mg	9.46 min	843.8 (71.8)
					1014.4 (28.0)
Mn	^{55}Mn (100)	13.3 ± 0.2 b	^{56}Mn	2.58 h	846.8 (93.9)
					1810.7 (27.2)
Na	^{23}Na (100)	530 ± 30 mb	^{24}Na	15 h	1368.6
Rb	^{85}Rb (72.2)	460 ± 20 mb	^{86}Rb	18.6 d	1076.8 (8.64)
Se	^{74}Se (0.87)	51.8 ± 1.2 b	^{75}Se	119.8 d	264.7 (17.1)
Se	^{76}Se (9.0)	21 ± 1 b	^{77m}Se	17.4 s	161.9 (52.5)
Ti	^{50}Ti (5.3)	179 ± 3 b	^{51}Ti	5.76 min	320.1
V	^{51}V (99.75)	4.88 ± 0.04 b	^{52}V	3.74 min	1434.2
Zn	^{64}Zn (48.9)	780 ± 20 b	^{65}Zn	244.3 d	1115.5 (50.6)

Three cycles were used.

The gamma-ray spectra of the above samples were recorded using the "Analyzer #1" which consisted of a hyperpure Ge (HPGe) detector (Aptec Engineering Ltd.) connected to a Nuclear Data ND66 model 4096 channel pulse-height analyzer. This detector had a resolution of 2.08 keV and a peak-to-Compton ratio of 30:1, both at the 1332.4-keV photopeak of ^{60}Co, and an efficiency of 13% compared to a 7.5 cm x 7.5 cm NaI(Tl) detector.

A Cyclic INAA (CINAA) method was developed for the possible analysis of Se. Irradiation conditions, decay and counting times were the same as described for the PCINAA method above. The samples were cycled every minute for five cycles. Gamma ray spectra were recorded using "Analyzer #2" consisting of a Princeton Gamma Tech Ge(Li) detector with a resolution of 2.02 keV and a peak-to-Compton ratio of 30:1, both at the 1332.4-keV photopeak of ^{60}Co, and an efficiency of 7.1% compared to a standard 7.5 cm x 7.5 cm NaI(Tl) detector.

A conventional INAA method employing anti-coincidence counting was also developed for the analysis of Se using a 10-30 s irradiation, followed by a 15 s decay, and a 30-60 s count. The anti-coincidence spectrometry system ("Analyzer #3") consisted of an ORTEC 25% relative efficiency HPGe principal detector surrounded by a Harshaw 25 cm x 25 cm NaI(Tl) guard detector with a Harshaw 7.5 cm x 7.5 cm NaI(Tl) plug connected to an APTEC multi-channel analyzer. The HPGe detector had a resolution of 1.8 keV. The anti-coincidence system had a peak-to-background plateau ratio of 590 at the 1332.4-keV photopeak of ^{60}Co. The details of this system are

described elsewhere (58, 61).

A method involving reactor flux and epi-cadmium neutron irradiations was developed for the determination of Al. Samples were irradiated in a normal outer irradiation site and in the cadmium-shielded site for 2 min, allowed to decay 1 min, and then counted for 5 min. The detection system used ("Analyzer #4") consisted of a Canberra Ge(Li) detector connected to an APTEC multi-channel analyzer card installed in a PC. Resolution and peak-to-Compton ratio were 1.88 keV and 35:1, respectively, at the 1332.4- keV photopeak of ^{60}Co. Efficiency was 9.5%.

Long-lived nuclides were used in the analysis of Br, Cr, Fe, Rb, Sb, and Zn. The nuclear data are given in Table 2.4. Samples were irradiated in the inner sites of the DUSR for 16-24 h. After about one week's decay, they were counted for 30 min using either the Analyzer #1 or #4. Irradiated samples were recounted for 12 h after about two weeks' decay.

2.3.6 Data Management

As a QC tool, a laboratory information management system (LIMS) was developed to manage experiments and store data acquired during the evolution of this work. The system was developed as a database application in Personal R:Base 1.0 (Microrim Inc., 1990). The database consisted of seven tables, namely "Vials," "Experiments," "Irradiations," "Results," "Final_Results," "Elements," and "Diets." Relational links between tables facilitated look-ups and simplified data entry. Forms and reports were designed for data entry to and output from the various tables. Two

applications were designed, "PkSearch" for quick access to nuclear data on selected elements, and "NAA" for storage of and access to information related to NAA work. The NAA application was divided into separate modules for managing the "Vials," "Experiments," "Irradiations," "Results," "Final_Results", and "Elements" tables.

Since INAA is a non-destructive analytical technique, samples can be analyzed repeatedly and indeed, in this work, samples were often analyzed for various elements using different experimental conditions. The "Vials Database" module was used to manage the information related to all sample vials for irradiation. Each vial was assigned a reference number and code, which were marked on the vial and entered into the laboratory notebook and database. The reference number uniquely identified each vial and the code provided quick reference to the sample material contained by the vial. For example, code "HMNT" referred to the HMNT duplicate diet composite, and H9 referred to the IAEA H-9 Mixed Diet CRM. A sample description, empty vial mass, sample mass, preparation date and extra notes if needed, were also entered into the database.

Having prepared a vial, the next step in its analysis was to select a method. Methods were automatically assigned reference numbers and stored in the "Experiments" table. Related methods shared a reference number but may have had a "sub-experiment" reference letter. For example, Experiment 1b referred to the PCINAA method for Se analysis.

Details relating to the irradiation and counting of samples were stored in the "Irradiations" table. Each irradiation and count were assigned reference numbers. Thus, using experiment, irradiation and count reference numbers, each analysis of a

sample vial could be uniquely identified. For example, 2/163/2 (experiment/irradiation/count) uniquely identified the second count of irradiation number 163 of Experiment 2, referring to the analysis of vial 116, containing NIST SRM 1548 Total Diet, which was irradiated on 1993 May 31 for 1.5 min, allowed to decay for 1 h 55 min and counted for 30 min using Analyzer #1. The DUSR Irradiation Request Forms were also archived in binders according to method and irradiation numbers for reference to the original documentation on irradiation conditions.

Peak areas and background counts were entered into the "Results" table. If necessary, decay corrections were conveniently calculated by the database using the nuclide half-lives stored in the "Elements" table. The gamma-ray spectra were stored on disk, and printouts of the integrated peak areas were retained and archived with the Irradiation Request Forms in case reference to the original data was required.

The LIMS was used to print sorted hard copy summaries of the vials, irradiations and peak areas by vial, sample, irradiation number, or element according to need. Concentration calculations were performed using Microsoft Excel version 5.0a (Microsoft Corporation, 1985-1993). All spreadsheets were stored on disk. Calculated concentrations and daily intakes were then entered into the "Final_Results" table.

The storage of spectra, spreadsheets and data on computer disks, and the archive of original hard copies were invaluable for QC and traceability.

2.4 DIALYSIS OF DIET COMPOSITES

2.4.1 Dialysis Procedure

A dialysis procedure was applied to small portions of the duplicate diet composites. This was intended to reduce the salt content of the samples, thus lowering the Compton background of the gamma-ray spectra.

A small amount (about 2 g) of freeze-dried diet composite powder was weighed into a small beaker. About 10 mL DDW were added to the freeze-dried samples which were then dispersed by placing the beaker in an ultrasonic bath for 5 min. Blanks were prepared by adding about 10 mL of DDW to a beaker and repeating the procedure. A 25 cm x 5 cm length of dialysis tubing (Spectra/Por membrane MWCO 3 500, 34 mm diameter) was freeze-dried and then weighed. After moistening in DDW, a slip-knot was tied at one end. The diet composite slurry was then transferred to the dialysis tube, which was then sealed using a plastic closure. The dialysis tube was then suspended in a 3-L beaker filled with DDW. After about 12 h the DDW was replaced. Dialysis was stopped after 24 h.

Dialysis tubes and their contents were frozen at -40°C. They were then inserted into 1-L capacity conical freeze-drying flasks. The flasks were cooled with liquid nitrogen and connected to a freeze-dryer. After about 72 h, the flasks were disconnected. The dialysis tubes and contents were then weighed immediately. Since they were very brittle when dry, the tubes were exposed to the atmosphere for several hours in the flasks. After this period, the tubes were inserted into 7 mL polyethylene vials. A trimmed, inverted vial cap was placed inside each vial to ensure reproducible geometry. The vials

were then heat sealed.

2.4.2 Irradiations and Counting of Dialyzed Portions

Blanks and standards were irradiated at the inner sites while the dialyzed samples and standards were irradiated in the outer sites of the DUSR. Activation for the analysis of short-lived ^{77m}Se was performed by irradiating the samples for 30 s, allowing them to decay for 15 s before counting for 30 s. The medium-lived nuclides were analyzed using 5 min irradiations, 1-min decays, and 10-min counts. After a decay period of 1.5 h to 8 h, the samples were counted again for 30 min. The gamma-ray spectra were recorded using the "Analyzer #1".

2.5 *in vitro* ENZYMOLYSIS OF DIET COMPOSITES

2.5.1 Chemical Reagents

Duplicate diet composites were subjected to an enzymolytic digestion procedure intended to simulate human gastrointestinal digestion. Reagent-grade concentrated HCl and HNO_3 were supplied by BDH Chemicals and Caledon Laboratories Ltd., respectively. Ultrex grade HCl and HNO_3 were obtained from J.T. Baker Chemical Co. Ammonia solution in double quartz distilled water was supplied by Seastar chemicals. AnalaR grade NaCl and AristaR grade Na_2CO_3 were obtained from BDH Chemicals. α-Amylase (porcine, catalog # A-3176), Bile Salts (catalog #B-8756), Pancreatin (porcine, catalog #P-1750), Pepsin (porcine, catalog #P-7000), and HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]) (catalog #H-3375) were obtained

from Sigma Chemical Co.

The following solutions were prepared. (i) Gastric juice: pepsin was dissolved in saline hydrochloric acid (0.15 M NaCl, 0.02 M HCl prepared from Ultrex grade HCl) to give a 1% m/V solution. (ii) Intestinal juice: amylase (1% m/V) and pancreatin (3% m/V) were suspended (pancreatin appeared not to dissolve completely) in saline solution (0.15 M NaCl). Bile salts (1.5 g/L) were dissolved in saline solution. Intestinal juice was comprised of equal volumes of these two solutions. (iii) Sodium carbonate solution: a saturated solution of Na_2CO_3 was prepared in DDW. (iv) HEPES buffer: a 0.1% (m/V) solution was adjusted to pH 7.1 by adding ammonia solution.

2.5.2 Enzymolysis Procedure

The enzymolysis procedure was modified from that reported by McDowell (66) and is illustrated in Fig. 2.2. All containers were acid-washed using the method described previously. Either 10 g of homogenized diet composite slurry, or 2 g of freeze-dried diet composite powder was weighed into a beaker. It was transferred to a Teflon bottle using small amounts of DDW, and more DDW was added to give a final volume of about 20 mL. Blanks were prepared by adding about 20 mL of DDW to the Teflon bottles. Samples were allowed to mix on a temperature controlled (37°C) shaking water bath for about 5 min.

The Teflon bottles containing the mixed diet were removed from the shaker bath and 20 mL of gastric juice were added to each bottle. The initial pH was measured to be about 3.7 for most samples. Ultrex grade HCl was added dropwise to adjust the pH

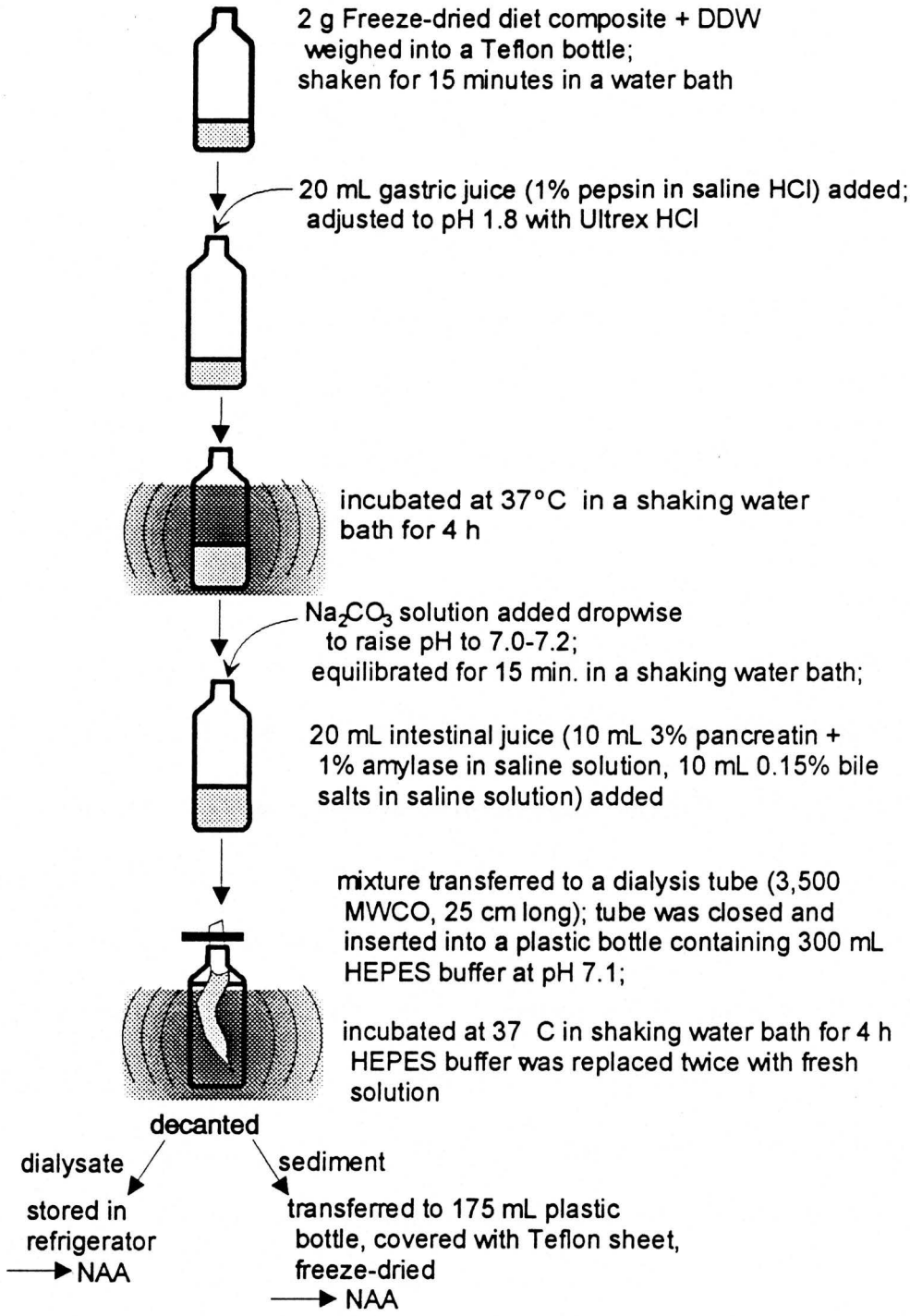


Fig. 2.2. Schematic of the *in-vitro* enzymolysis procedure

to 1.8 to 2.0. Samples were then placed in the shaking water bath and allowed to digest for 4 h. For the first few digestions, the pH was measured every 30 min in order to maintain the pH below 2.5 by adding extra HCl, if required. Since there was no significant change in pH for any of the samples, this practice was not continued.

After 4 h, the pH was measured to ensure that it had not changed. The samples were neutralized and adjusted to pH 7.0 to 7.2 by the dropwise addition of saturated Na_2CO_3 solution. The digestion mixtures were returned to the shaking water bath and allowed to equilibrate for 15 min.

Intestinal juice (10 mL pancreatin/amylase solution and 10 mL bile salt solution) was added to each Teflon bottle. Dialysis tubes (Spectra/Por membrane MWCO 3 500, 34 mm diameter) were prepared by immersing them in water for 5 min, tying a slip-knot in one end, and testing for leakage by filling with DDW. After emptying the DDW, each digestion mixture was transferred to its dialysis tube. Air was squeezed out of each tube, and then its top was folded over and sealed using a Spectra/Por closure. The tube was hung by its closure in an acid washed 500 mL plastic bottle (HDPE) containing 300 mL of HEPES buffer solution. The bottles were transferred to the shaking water bath and allowed to digest for 4 h. Buffer was changed every 80 min. Used buffer was stored in separate 1 L plastic bottles (LDPE). The pH was not checked during digestion due to the presence of the buffer.

At the end of the digestion period, the digestion mixture was emptied into an acid-washed pyrex beaker or acid washed 175 mL plastic (HDPE) bottle. The top of each beaker or bottle was covered with a small sheet of ultraclean Teflon held on with

an elastic band. Digestion mixtures were frozen at -40°C. When ready for freeze-drying, the Teflon sheets were pierced using the ends of a pair of tweezers. Digests were dried using a tray-type freeze-dryer in the laboratories of the Food Science and Technology Department of the Technical University of Nova Scotia. Samples remained in the dryer for one week.

Used dialysis buffer was emptied into Erlenmeyer flasks and made up to 1 000 mL. Two to three hundred mL of the diluted buffer was weighed into 1-L freeze-drying flasks, frozen by dipping the flask in liquid nitrogen, and then loaded onto an Edwards Modulyo freeze-dryer connected to an Edwards model E2M8 rotary high vacuum pump. Samples were dry after 4 d.

Dried samples were weighed and homogenized using a Technilab Instruments Micro Mill™ blender. They were then weighed into 7 mL polyethylene vials. A trimmed cap was inserted into each vial to ensure constant sample geometry. The vials were then heat sealed.

2.5.3 Irradiations and Counting of Enzymolyzed Portions

Blanks and standards were irradiated using the inner sites whereas enzymolyzed samples and standards were irradiated in the outer sites of the DUSR. Selenium was quantified through its short-lived ^{77m}Se by irradiating the samples for 5-15 s, allowing them to decay for 15 s before counting for 30 s. The dead time was below 10% using the "Analyzer #1". Medium-lived nuclides were detected after 5-15 s irradiations using the "Analyzer #4". A longer irradiation time was not possible because

of the high activities of ^{38}Cl and ^{24}Na in the irradiated diet samples. A 1-min decay was allowed before counting for 10 min. The dead time was below 10%. After a decay period of 1.5 h to 8 h, the samples were recounted for 30 min. Long-lived nuclides were analyzed using 7-h irradiations; after a few week's decay, they were counted for 12 h using the "Analyzer #4".

3. TRACE ELEMENTS IN DIET SAMPLES

Thirteen elements were analyzed in the duplicate diet composites by INAA using their short-, medium- and long-lived nuclides. These elements were Al, Br, Ca, Cl, I, Fe, Mg, Mn, K, Se, Na and Zn. In this chapter the current status of nutritional knowledge for each element is briefly reviewed from the available literature (1, 6, 12, 17, 134). The analytical method used is described along with its precision, accuracy and detection limits. External Quality Assessment (EQA) charts were prepared and are reported for quality assurance. Accuracy and precision of the analytical methods used were assessed by analysis of RMs and SRMs. Concentrations determined are reported and compared with certified or information values. The concentration and 24-h intake of each element in duplicate diets are presented and compared with 24-h intake determined from calculation from food composition tables and with literature values. The results for dialysis and enzymolysis of the duplicate diets are reported as well.

3.1 SELENIUM

3.1.1 Nutritional Functions of Selenium

Selenium is considered to be an essential element for humans although it may be toxic at high intake levels. In certain countries, such as China (135), New Zealand (136) and Finland (96, 100), Se levels in soils and diets as well as Se status of the population are low, leading to concerns about the potential effects of Se deficiency. Both China and Finland have initiated Se supplementation programs. (96, 100, 135).

In China, there has also been concern about excessive Se intakes (137). There are several review articles on nutritional aspects of Se (1, 6, 7, 12, 17, 134, 135, 139-148). The toxic effects of Se in animals was first noted in 1842. It was not until the mid-1950's that attention was drawn to the physiological role of Se when it was realized that many areas in the world were affected by Se deficiency in animals. The essentiality of Se in rats was first demonstrated in 1957 (149). Since then, the Se requirement in humans has been evaluated and some knowledge has been acquired on its nutritional role.

An important piece of evidence for the essentiality of Se is its presence in the active site of the enzyme glutathione peroxidase (GSH-Px) which catalyzes the reduction of hydrogen peroxide and organic peroxides by glutathione (7, 150, 151, 152). This prevents the production of oxygen free radicals which can damage cellular structures, and complements the free radical-scavenging activity of Vitamin E.

In addition to GSH-Px, the only other functional form of Se known in mammals is type I iodothyronine 5'-deiodinase which is a selenoprotein (152, 153, 154) involved in the control of thyroid hormone metabolism. Another selenoprotein, called Selenoprotein P, is a plasma protein found in humans and rodents; but its function is unknown. Other selenoproteins have also been reported (146, 150, 152, 155-158). Burk and Hill (156) speculated that up to a hundred selenoproteins may exist in animals.

In animals, deficiency of Se may be manifested by growth retardation, cataract formation, and inhibited spermatogenesis. Inadequate Vitamin E and Se may lead to necrotic and dystrophic symptoms. There have been reports of muscle pain and cardiomyopathy (necrotic disease of cardiac muscle) in humans deficient in Se (159-161).

Muscle pain was alleviated by supplementation of the diet with Se. Keshan and Kashin-Beck diseases have been reported to be associated with low dietary Se intake (135, 162-165). Both diseases were almost completely prevented by a diet supplemented with Se (135, 164-166). A hospital patient presenting "typical features" of Keshan disease has been reported in the U.S.A. (167). Selenium deficiency has also been associated with iodine deficiency disorders in China and Zaire (168-170).

There is evidence that Se is involved in sulphur amino acid metabolism and in preventing cardiac disorders (100, 171, 172). In other studies, no relation was found between Se status and risk factors for coronary heart disease (172 173). Some studies in China suggest that Se may be useful in the prevention of cancer (135), but studies in New Zealand, another country with low levels of Se in the diet, have shown no such link (136). Evidence from the U.S.A. has been conflicting (100). A number of studies related to Keshan disease and cancer indicate that Se can promote immunity (174, 175, 176). Selenium may also reduce the toxicity of heavy metals such as Cd and Hg, by binding to them and reducing their availability (177, 178).

The minimum requirement for humans has been proposed to be 17 $\mu g\ d^{-1}$ and 34 $\mu g\ d^{-1}$ recommended for safety in a Chinese study (135). Health Canada has set an RNI value of 50 $\mu g\ d^{-1}$ for adults (1). According to HC, this amount is exceeded by 2 to 5 times in normal Canadian diets. The U.S. Food and Nutrition Board has set RDA for Se as 55 $\mu g\ d^{-1}$ for females and 70 $\mu g\ d^{-1}$ for males (17, 144).

3.1.2 Toxicity of Selenium

High levels of Se may affect S metabolism and inhibit several enzymes. Agricultural feeds grown on seleniferous (Se-rich) soils have long been known to result in the following diseases in farm animals. "Blind Staggers" are characterized by blindness, muscle paralysis, abdominal pain, and respiratory failure. "Alkali Disease" causes hair loss, sore hoofs, liver damage, cardiac atrophy, cirrhosis, anemia, erosion of bone joints, and dry as well as dull coat.

High intake in humans (5 000 μg d^{-1}) has led to brittleness, loss, and disfigurement of hair and fingernails, and lesions of the nervous system (1, 34, 137, 143). Intake of 1 000 μg d^{-1} was found to produce symptoms of chronic toxicity (137). No toxic symptoms were observed for daily dietary intakes (DDI) of 326 μg in Venezuela (179), 724 μg in the U.S.A. (180) and 750 μg in China (137).

Therefore, a safe range of intake for Se was proposed. In 1980, the U.S. Food and Nutrition Board defined a safe and adequate daily dietary intake range (SADDIR) of 50 μg to 200 μg (181). Some diets in Canada and the U.S.A. do exceed this range (69, 180). Health Canada has not recommended an upper limit of safe intake. From their examinations of individuals experiencing Se toxicity due to dietary intake, Yang and Zhou proposed a maximum safe dietary Se intake of 400 ug d^{-1} (34).

3.1.3 Dietary Sources of Selenium

Hazell (6) has extensively reviewed the sources and availability of various minerals including Se, and Arthur (68) has reported Se content of various Canadian foods. Cereals and nuts are generally good sources of Se although the actual Se content depends on the Se levels of the soils on which the plants are grown. Fruits and vegetables generally contain little Se. Selenium concentrations tend to be higher and more constant in animal products than in plant materials. The concentration and forms of Se in meat can vary depending on the form and content in the animal feed. Since meats tend to be good sources of Se, deficiency is less likely to occur where meat products are a large proportion of the diet unless the meat has low Se to start with as in Australia and New Zealand.

Food preparation may have an effect on Se content. Higgs *et al.* (182) found that boiling mushrooms and asparagus, and drying cereals resulted up to 44% loss of Se. However, boiling of cereals and grains, and baking of poultry and seafood, and broiling of meat had no significant effect on Se content.

3.1.4 Availability of Selenium

Selenium absorption occurs mainly through the lower small intestine. Absorption is related to the solubility of the ingested compound, digestibility of selenoproteins, and on the dietary Se-to-sulphur ratio. Absorption of Se was 44-70% as selenite, 97% as selenomethionine (6), and 50-70% from food (7). Absorption of Se in pork was found to be 76-100%. Selenium in beef kidney (97%) and wheat flour (217%)

is highly bioavailable compared to tuna (22-57%), where all values were based on GSH-Px measurement and then normalized with respect to selenite (103).

3.1.5 Selenium Determination Techniques

Many techniques have been used to analyze for Se. These included flame atomic absorption spectrometry (AAS), graphite furnace AAS (GFAAS), hydride generation AAS (HGAAS), atomic fluorescence spectrometry (AFS), fluorometry with diaminonaphthalene (DAN), differential pulse anodic and cathodic stripping voltammetry, gas chromatography (GC) after derivatization with 4-nitro-O-phenylenediamine, X-ray fluorescence spectrometry (XRF), proton-induced X-ray emission (PIXE), and neutron activation analysis (NAA). The advantages and disadvantages of each technique have been discussed by Tolg (183).

The most common analytical techniques used are spectrofluorometry, HGAAS (184) and NAA (42, 185). Fluorometry and HGAAS have the disadvantages of requiring lengthy digestion procedures to dissolve the sample, and reagent blank correction. Instrumental NAA (INAA) is a sensitive, nondestructive multielement technique which is free of reagent blanks. Sample preparation of food for INAA is minimal, requiring only freeze-drying and weighing.

3.1.6 Development of INAA Methods for Selenium

Nuclides of Se that can be used for INAA have been discussed by Zhang and Chatt (186). There are seven nuclides (Table 3.1) which can be produced by thermal

Table 3.1. Nuclear data for selenium by NAA

Isotope (% abund.)	σ_{th}, b	Nuclide	Half-life	γ-ray, keV (% int.)
^{74}Se (0.87)	51.8 ± 1.2	^{75}Se	119.8 d	121.1 (17.1)
				136.0 (58.7)
				264.7 (58.5)
				279.5 (24.8)
				400.7 (11.4)
^{76}Se (9.02)	21 ± 1	^{77m}Se	17.4 s	161.9 (52.5)
^{77}Se (7.58)	0.733 ± 0.05*	^{77m}Se	17.4 s	161.9 (52.5)
^{78}Se (23.52)	0.33 ± 0.04	^{79m}Se	3.89 min	97.9 (8.9)
^{80}Se (49.82)	0.08 ± 0.01	^{81m}Se	57.28 min	102.9 (12.7)
^{80}Se (49.82)	0.53 ± 0.04	^{81}Se	18.45 min	275.9 (0.67)
				290.1 (0.55)
				828.4 (0.28)
^{82}Se (9.19)	0.039 ± 0.003	^{83}Se	22.3 min	225.2 (32.7)
				356.7 (70)
				510.2 (43)
				718.2 (15)
				836.8 (13)
^{82}Se (9.19)	0.006 ± 0.0004	^{83m}Se	70 s	356 (20)
				676 (17)
				989 (19)
				1031 (30)
				2054 (13)

* fast reactor neutron cross section.

or epithermal/fast neutron activation of the six stable isotopes of Se (187, 188, 189). The most commonly used nuclide is ^{75}Se. This nuclide has the advantage of a fairly high thermal neutron absorption cross section of 51.8 b. However, the target isotope ^{74}Se has a low abundance of only 0.87%. Because the half-life of ^{75}Se is 119.8 d, it requires long irradiations at a high neutron flux, and long decay and counting times to achieve an acceptable sensitivity. The total experimental time is typically two to three weeks, which can be time-consuming and expensive for the analysis of a large number of samples for Se.

Another drawback to the use of ^{75}Se is potential interferences due to overlapping gamma-rays from other nuclides (Table 3.2). One example is the 121.8 keV gamma-ray of ^{152}Eu, produced from highly abundant ^{151}Eu with a very high cross section, which interferes with the 121.1-keV gamma-ray of ^{75}Se. The same gamma-ray of ^{75}Se has a minor interference from ^{177m}Lu. The most widely used gamma-rays of ^{75}Se in INAA are 136.0 and 264.7 keV. These are interfered with by the 136.3-keV gamma-ray of ^{181}Hf and 264.1-keV gamma-ray of ^{182}Ta. The 279.5-keV gamma-ray of ^{75}Se is subject to interference from the 279.1-keV gamma-ray of ^{203}Hg. There is a gamma-ray of ^{75}Se which is free from interference, at 400.7 keV, but it has a low intensity and is therefore insensitive. The sensitivity of Se analysis using this peak can be enhanced using a well-type Ge detector, as reported by Makarewicz and Zeisler (190); however, limitations of high input count rate, pile-up losses, and decrease in resolution were also noted.

The ^{77m}Se nuclide can also be used to determine Se by INAA; it has a relatively short half-life of 17.4 s and a gamma-ray at 161.9 keV. Although the thermal

Table 3.2. Interfering nuclides and gamma-rays for selenium by NAA

Nuclide of Interest		Interfering Nuclide			
Nuclide (half-life)	γ-ray, keV (% intensity)	γ-ray, keV (% intensity)	Nuclide (half-life)	σ_{th} (b)	Isotope (% abund.)
^{75}Se (119.8 d)	121.1 (17.1)	121.8 (28.4)	^{152}Eu (13.54 a)	5900 ± 200	^{151}Eu (47.8)
		121.6 (5.9)	^{177m}Lu (161.4 d)	7 ± 2	^{176}Lu (2.6)
	136.0 (58.7)	136.3 (5.8)	^{181}Hf (42.4 d)	12.6 ± 0.7	^{180}Hf (35.1)
	264.7 (58.5)	264.1 (3.6)	^{182}Ta (114.4 d)	8200 ± 100	^{181}Ta (99.99)
	279.5 (24.8)	279.1 (33.5)	^{203}Hg (46.6 d)	4.9 ± 0.1	^{202}Hg (29.7)
^{77m}Se (17.4 s)	161.9 (52.5)	162.3 (34)	^{116m2}In (2.18 s)	92 ± 14	^{115}In (95.72)

neutron absorption cross section of ^{76}Se is 2.5 times lower than ^{74}Se, the isotopic abundance is 10 times higher (Table 3.1). Furthermore, the sensitivity can be enhanced because the short half-life of ^{77m}Se allows saturation activity to be reached in a short time. A potential interference is the 162.3-keV gamma-ray of $^{116m2}In$ (Table 3.2). However, the half-life of $^{116m2}In$ is only 2.18 s (Table 3.2), so that a 20s-decay time can eliminate this potential interference. Also, In is rarely detected in biological materials such as foods and diets so it is unlikely to cause problems in this study. Because of its short half-life, analysis time can be reduced considerably by the use of the ^{77m}Se nuclide in routine INAA.

Both single-cycle (*i.e.* conventional) INAA and cyclic INAA (CINAA) have previously been used in our laboratory to analyze for Se (42). The CINAA technique involves the irradiation of a sample for a short time, a quick transfer to a detector, counting, and then repetition of the process for a desired number of cycles. Improved precision and detection limits for some samples have been demonstrated by CINAA. Rather than acquiring a cumulative gamma-ray spectrum over a number of cycles within a short period as in CINAA, in pseudo-cyclic INAA (PCINAA) the sample is allowed to decay for several days before the next cycle. A separate gamma ray spectrum is acquired for each cycle, and peak areas are totalled later in PCINAA. It has the advantage of reducing background and major nuclide activities which otherwise would lead to an elevated Compton background.

An anti-coincidence (AC), sometimes referred to as "Compton Suppression," gamma-ray spectrometer has recently become available in our laboratory.

Anti-coincidence spectrometry is a gamma-ray counting technique used to lower the Compton background and to reduce the activities of radionuclides emitting coincident gamma-rays, such as activation products of Cl, Mn, and Na. Landsberger and co-workers (59, 60) have recently reviewed Compton suppression in NAA. Zhang (61) has developed methods for the determination of several elements using AC spectrometry. In this thesis, a method was developed for Se analysis using INAA and AC spectrometry.

The various INAA methods described above, namely INAA using ^{75}Se and conventional gamma-ray spectrometry, INAA using ^{77m}Se and conventional as well as AC gamma-ray spectrometry, and CINAA and PCINAA using ^{77m}Se with conventional gamma-ray spectrometry, were evaluated for their suitability to the analysis of Se in duplicate diets. Samples of the HMFS (the "in-house trial duplicate diet") were subjected to various irradiation, decay and counting times. The experimental conditions and results are shown in Table 3.3. Performance of the various methods was assessed based on three parameters: the ratio of signal (peak area) to background, sensitivity, and detection limit.

An irradiation time of 17 h, a decay time of 15.6 d, and a counting time of 12 h were employed for the detection of the 264.7-keV gamma-ray of ^{75}Se (Table 3.3). Although the sensitivity, 9066 counts/μg, was quite high, the background was also very high resulting in a low signal-to-background ratio of 0.051, indicating a very small peak, and poor peak shape and statistics. The detection limit of 190 ppb Se, was the highest among all the methods used.

Conventional INAA was also used for the detection of the 161.9-keV

Table 3.3. Evaluation of various instrumental methods for selenium analysis using duplicate diet HMFS

Vial	Method	t_i-t_d-t_c (s)	Counting Technique	Dead Time (%)	Peak Area (counts)	Background (counts)	Signal to Background Ratio	Sensitivity (counts/μg)	L_D (ppb)
154	INAA	17 h-15.6 d-12 h[a]	Normal	1.19	1071	20875	0.051	9066	190
36	INAA	30-10-30	Normal	<10	240	288	0.833	2780	110
36	INAA	30-15-30	Normal		197[b]		0.833	2280	
33	INAA	30-15-30	Anti-Co.	9.15	121	148	0.818	1470	120
34	INAA	20-15-30	Anti-Co.	7.55	109	149	0.732	1350	130
35	INAA	10-15-30	Anti-Co.	4.52	80	30	2.7	940	120
36	INAA	10-15-60	Anti-Co.	4.28	125	81	1.5	1450	120
29	INAA	10-15-60	Normal	5.44	86	315	0.27	1000	240
32	CINAA	20-5-20, 5 cycles	Normal		not detected				
157	PCINAA	30-10-30, 3 cycles	Normal	<10	796	1116	0.713	6900	60

[a]: 265-keV peak of ^{75}Se. All other results given are for the 162-keV peak of ^{77m}Se. [b]: calculated

gamma-ray of ^{77m}Se using only one cycle. As shown in Table 3.3, the HMFS sample was irradiated for 30 s, allowed to decay for 10 s, and counted for 30 s. The signal-to-background ratio was 0.833, sensitivity was 2785 counts/μg, and detection limit was 110 ppb.

The first alternative method to be considered is INAA using AC counting. This method has the advantage of Compton background suppression, and indeed the background counts were lower when the AC counting mode was used as shown in Table 3.3. Unfortunately, the net count under the 161.9-keV peak of ^{77m}Se was lowered mainly due to the placement of the sample at a different distance from the surface of the HPGe detector. Moreover, extra time was required to place the sample on the AC system, lengthening the decay time from 10 s to 15 s, and decreasing the sensitivity. It should also be noted that when the AC mode is used, calibration curves tend to deviate from linearity at dead times greater than 6-7%, whereas a cut-off of 10% is generally used for conventional INAA (61). In order to keep the dead time below 7%, irradiation time was reduced to 10 s, further reducing the sensitivity. Lengthening the counting time to 60 s increased the sensitivity slightly, but the detection limit remained unchanged. These results indicate that AC counting did suppress the background, but a lower sensitivity was obtained due to the restrictions imposed by irradiation and counting conditions. Thus, the use of AC counting was not an improvement over conventional INAA under the experimental conditions used.

When CINAA was used, there was no detectable peak of ^{77m}Se at 161.9 keV. This was likely due to the rapidly increasing background resulting from high

activities of comparatively long-lived nuclides such as ^{24}Na, ^{38}Cl and ^{56}Mn. Therefore, CINAA was not considered here as a viable method for the analysis of Se in duplicate diets.

The results of PCINAA were different from those of CINAA. In PCINAA, the same conditions were used as in INAA, except that the samples were allowed to cool for a week before the next cycle of irradiation-decay-counting. Peak areas and backgrounds from separate spectra collected during each cycle were summed after the third cycle. Although the signal to background ratio (0.713) did not change very much, both sensitivity (6900 counts/μg) and detection limit (60 ppb) were significantly improved. The main limitation of PCINAA is the time required to complete the analysis. It took two weeks to complete three cycles (with one week's decay between cycles). Because of the improved sensitivity and detection limit obtained using PCINAA, it was used to analyze the duplicate diets.

3.1.7 Quality Assurance of Selenium Measurement

In order to evaluate the precision and accuracy of Se determination using the long-lived ^{75}Se nuclide, five diet and biological reference materials were analyzed by conventional INAA. The results are shown in Table 3.4. It is evident that the quantitative determination limit of Currie, L_Q, (191) is significantly higher than the Se concentrations in these materials except that for NIST Rice Flour and Bovine Liver. In the latter cases, the measured values are in good agreement with the certified values. The agreement is poor for the remaining reference materials. The reasons for the poor

Table 3.4. Analysis of reference materials for selenium by INAA using ^{75}Se. Duplicate diet HMFS included for comparison

Reference Material	Ld (ppb)	Lq (ppb)	This Work (ppb)	Certified Value
IAEA H-4 Animal Muscle	190	580	400 ± 140[a]	280 ± 30
IAEA H-9 Human Diet	85	270	200 ± 110[a]	110 ± 10
NIST 1548 Mixed Diet	130	400	190 ± 20[b]	240 ± 5
NIST 1568 Rice Flour	86	270	410 ± 70[a]	400 ± 100
NIST 1577b	110	330	830 ± 110[c]	710 ± 70

[a]:average of two values; [b]:average of three values; [c]:average of four values

detection limits obtained in this work include comparatively low neutron flux and short irradiation time.

Precision and accuracy of Se determination were also evaluated using the short-lived nuclide ^{77m}Se (17.4 s). The samples were irradiated for 30 s and counted for 30 s after decay periods of 10 s. The 161.9-keV gamma-ray of ^{77m}Se was free from interference under these experimental conditions and was used. Nine NIST SRMs and RMs as well as three IAEA RMs were analyzed, and the results are presented in Table 3.5 and Fig. 3.1. Selenium content determined in this work agrees well with the certified values. The relative standard deviations (RSDs) of measurement were generally within $\pm$10% with a couple of exceptions where the levels were close to the detection limits. Detection limits ranged from 60 to 190 ppb. For IAEA H-9 Mixed Diet and NIST SRM 1548 the detection limits were 140 ppb and 120 ppb, respectively. The detection limits were high relative to the measured concentrations highlighting the need for more sensitive analytical methods to obtain better QA.

As noted earlier and in Table 3.3, the PCINAA method using ^{77m}Se was found to provide superior detection limits. The experimental conditions used were: t_i = 30 s, t_d = 10 s, t_c = 30 s, and three cycles, with a week's decay between the cycles. The results are presented in Table 3.6 and Fig. 3.2. Agreement between measured and certified values was generally very good, except for NIST SRM 1548 Total Diet. In this case, the measured value was slightly higher than the certified value. The precision of analysis was improved using PCINAA, indicated by lower RSDs. Comparisons of quantitative determination limits, L_Q, given in Tables 3.4, 3.5 and 3.6, for five SRMs and RMs reveal that the best limits were obtained by PCINAA using the short-lived nuclide

Table 3.5. Analysis of reference materials and HMFS diet for selenium by INAA using ^{77m}Se.

Reference Material	L_D (ppb)	L_Q (ppb)	This Work[a] (ppb)	Cert. Val.[b] (ppb)
IAEA A-13 Animal Blood	140	490	220 ± 40	(240)
IAEA H-4 Animal Muscle	77	290	310 ± 30[c]	280 ± 30
IAEA H-9 Human Diet	140	460	110 ± 30[c]	110 ± 10
NIST 1547 Peach Leaves	130	460	130 ± 50	120 ± 9
NIST 1548 Total Diet	120	400	290 ± 40[d]	245 ± 5
NIST 1549 Non-fat Milk Powder	90	330	110 ± 40[e]	110 ± 10
NIST 1567a Wheat Flour	64	240	1 120 ± 10[e]	1 100 + 200
NIST 1568 Rice Flour	60	240	340 ± 40	400 ± 100
NIST 1577b Bovine Liver	90	320	700 ± 40[f]	710 ± 70
Agr. Can. RM 8438 Wheat Flour	62	250	90 ± 20	76.0
Agr. Can. RM 8436 Wheat Flour	80	310	1 120 ± 70	1 230 ± 90
Agr. Can. RM 8415 Egg Powder	180	600	1 340 ± 30	1 390 ± 170
HMFS duplicate diet	120	440	320 ± 30	-

[a]:average of four values unless otherwise indicated; [b]: information values are in parentheses; [c]:average of seven values; [d]:average of eleven values; [e]:average of three values; [f]:average of eight values

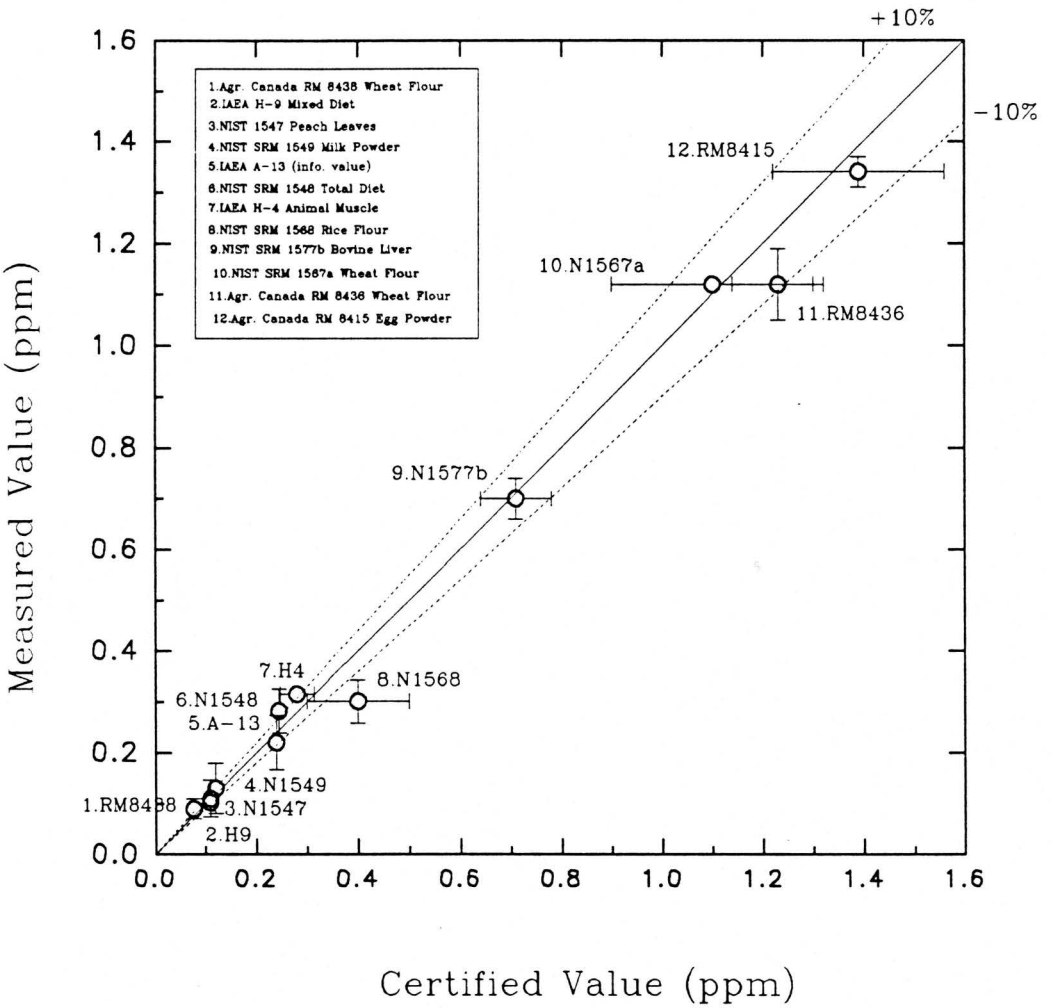


Fig. 3.1. Analysis of reference materials for selenium by INAA

Table 3.6. Analysis of reference materials and HMFS diet for selenium by PCINAA.

Reference Material	L_D (ppb)	L_Q (ppb)	This Work (ppb)	Certified Value (ppb)
Animal Muscle IAEA H-4	39	130	313 ± 0.007[a]	280 ± 30
IAEA H-9 Human Diet	74	240	110 ± 10[b]	110 ± 10
NIST 1548 Mixed Diet	63	210	290 ± 20[c]	245 ± 5
NIST 1568 Rice Flour	34	120	320 ± 20[d]	400 ± 100
NIST 1577b Bovine Liver	26	81	660 ± 30[d]	710 ± 70
HMFS Dupl. Diet[f]	55	180	296 ± 3[e]	-

[a]:average of seven values; [b]:average of six values; [c]:average of eight values; [d]:average of four values; [e]:average of three values; [f]: duplicate diet HMFS included for comparison

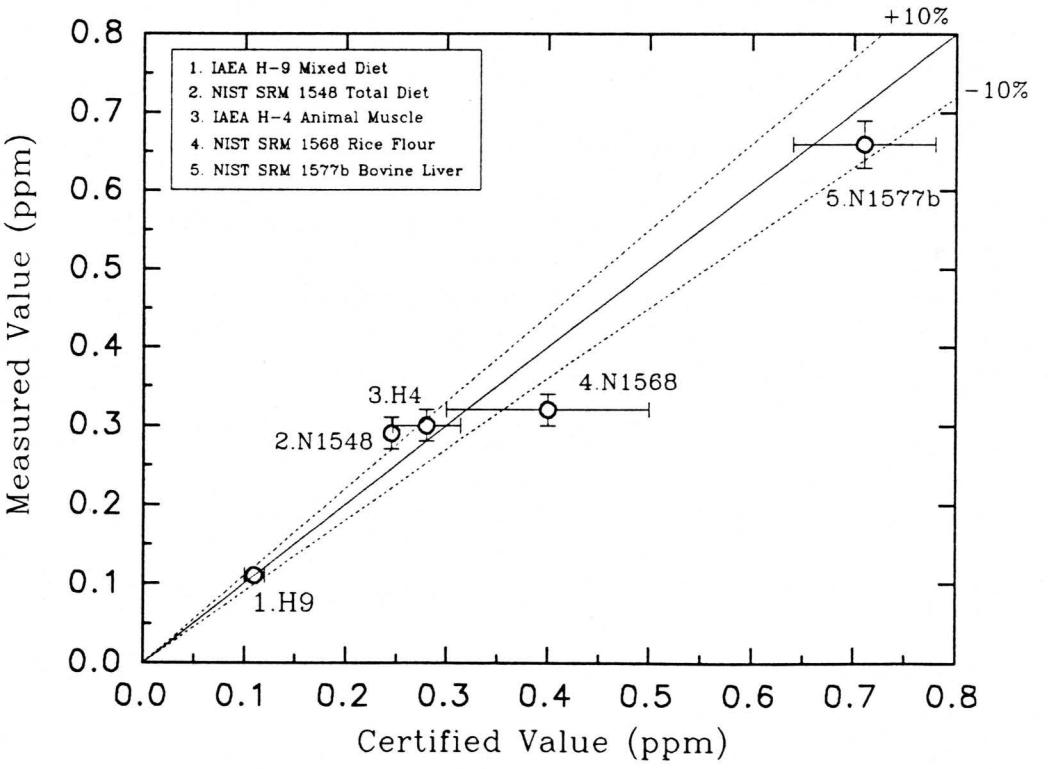


Fig. 3.2. Analysis of certified reference materials for selenium by PCINAA

^{77m}Se, followed by conventional INAA using the same nuclide, and conventional INAA using the long-lived nuclide ^{75}Se. In general, the L_Q values by PCINAA are about half of that by conventional INAA using ^{77m}Se. Both PCINAA and INAA methods employing ^{77m}Se were used in the present study to analyze the duplicate diet samples.

An external quality assessment chart (Fig. 3.3) for Se using conventional INAA using ^{77m}Se was prepared by replicate analysis of IAEA H-9 Mixed Diet. The data are within $\pm$2s which is considered as the warning limit (62). Within these limits, the data appear to be fairly scattered, due to the low Se level (110 ppb) of the reference material and relatively high detection limits (L_D = 140 ppb, L_Q = 460 ppb). Although agreement with the certified value is satisfactory, the scatter of the data highlights the need for a more sensitive method such as PCINAA.

Eight Se comparator standards were prepared and analyzed using identical conditions. These standards were used for IQA purposes. The individual values along with the average are shown in Fig. 3.4. Except one, all values were within $\pm$2s. It is evident that a good internal quality was maintained.

Further QA was done using the duplicate diets. Seven replicate analyses of the HMFS duplicate diet were performed by INAA and three by PCINAA, and the results are presented in Table 3.7. The relative standard deviations were 11% and 2%, respectively. Two other diet samples were selected at random for replicate analyses as a measure of IQA. Seven replicate INAA and four PCINAA analyses were performed on each of a diet composite collected from a female participant (HFMC) and from a male (HMNT). For INAA, the RSD were 28 and 8% for HFMC and HMNT, respectively.

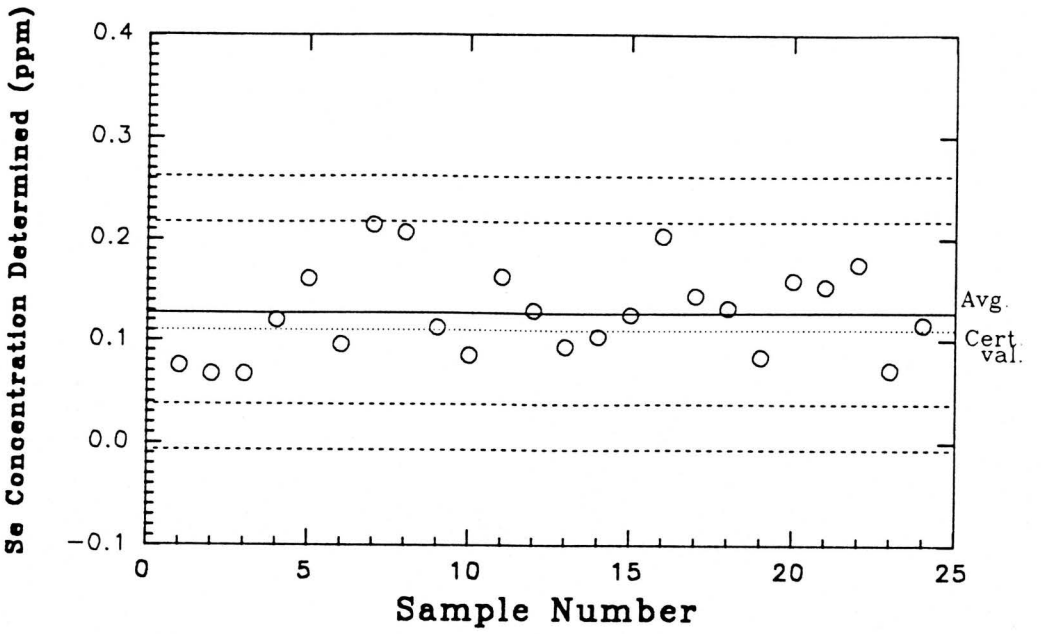


Fig. 3.3. External quality assessment chart for selenium by INAA using IAEA H-9

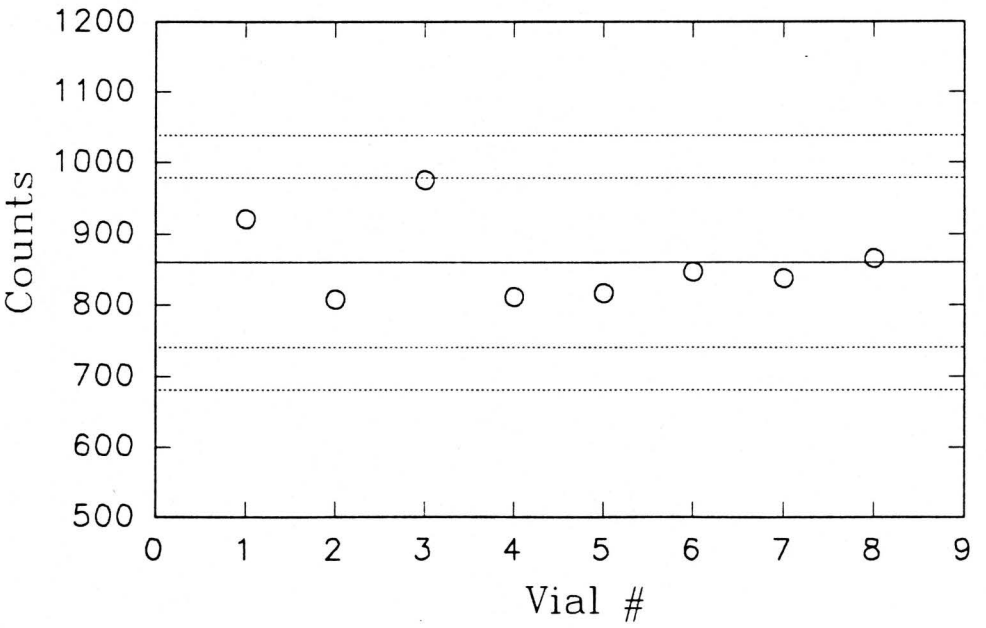


Fig. 3.4. Internal quality assessment chart for selenium using a 0.4 μg Se standard

Table 3.7. Selenium Content of Duplicate Diet Composites by INAA

Sample Code	INAA		PCINAA	
	Content±SD (ppb)	Daily Intake (μg d^{-1})	Content±SD (ppb)	Daily Intake (μg d^{-1})
HMFS	320 ± 40[a]	220 ± 30[a]	296 ± 3[b]	207 ± 2[b]
(a) Females				
HFFF	220 ± 70[c]	73 ± 20[c]	160	55
HFMC	250 ± 70[a]	89 ± 20[a]	220 ± 20[d]	76 ± 7[d]
HFNA	470 ± 20[c]	188 ± 7[c]	430	170
HFRR	300 ± 10[c]	132 ± 5[c]	290	120
HFSR	320 ± 10[c]	155 ± 6[c]	290	140
HFTC	320 ± 60[c]	120 ± 20[c]	240	88
HFWH	283 ± 5[c]	136 ± 2[c]	280	140
Mean	310	130	270	110
Median	300	130	280	120
Min. - Max.	220 - 470	73 - 188	160 - 430	55 - 170
RNI	50	50	50	50
RDA	55	55	55	55

Table 3.7 (continued). Selenium Content of Duplicate Diet Composites by INAA

Sample Code	INAA		PCINAA	
	Content±SD (ppb)	Daily Intake ($\mu g\ d^{-1}$)	Content±SD (ppb)	Daily Intake ($\mu g\ d^{-1}$)
(b) Males				
HMFF	350 ± 20[c]	160 ± 10[c]	360	170
HMMR	300 ± 20[c]	83 ± 5[c]	240	67
HMNT	310 ± 20[a]	240 ± 20[a]	280 ± 20[d]	200 ± 10[d]
HMRZ	110 ± 30[c]	66 ± 16[c]	120	75
HMSG	270 ± 20[c]	120 ± 10[c]	250	110
HMTH	240 ± 10[c]	118 ± 5[c]	250	130
HMWV	387 ± 9[c]	313 ± 7[c]	370	300
Mean	280	160	270	150
Median	300	120	260	130
Min. - Max.	110 - 390	66 - 310	120 - 370	67 - 300
RNI	50	50	50	50
RDA	70	70	70	70
(c) Overall				
Mean	300	140	270	130
Median	300	130	260	130
Min. - Max.	110 - 470	66 - 310	120 - 430	55 - 300

[a]: average of 7 values; [b]: average of 3 values; [c]:average of 2 values; [d]: average of 4 values.

The RSD values, 10% for HFMC and 6% for HMNT, were lower when PCINAA was used. In most cases, concentrations determined by PCINAA were slightly lower than values determined by INAA. Due to the improved precision obtained by PCINAA, the results determined by this method are considered to be more reliable than those of INAA.

3.1.8 Selenium Content of Duplicate Diets

The Se content of the 14 duplicate diet composites and that of the trial diet sample (HMFS) determined by INAA and PCINAA are reported in Table 3.7. The following discussion will focus on the PCINAA results.

The Se concentrations of the duplicate diet composites varied between 120 and 430 ppb. The highest values were 430 ppb for HFNA, 370 ppb for HMWV, and 360 ppb for HMFF (Table 3.7). As shown in Appendix 1, both HFNA and HMWV had high mass proportions of cereals. Curiously, HMFF's diet was low in cereal products (which are known to be a significant Se source in the Canadian diet) and highest in fluids (which are generally believed to be low in Se). The lowest Se concentrations were in HFFF (160 ppb) and HMRZ (120 ppb) composites. Both of these diets had low levels of cereals and nuts, but were relatively high in fruits and berries, and dairy products. The mean and median concentrations were 270 and 280 ppb for the females, 270 and 260 ppb for the males, and 270 and 260 ppb overall (Table 3.7). The close agreement between mean and median values is consistent with a normal distribution of Se in the diets.

Selenium dietary intakes (DI) varied from 55 to 300 $\mu g\ d^{-1}$ as shown in Table 3.7. The highest intakes were found in the HMWV and HMNT composites. These

were also the diets with the highest dry mass and were highest in cereals and vegetables. They were also high in meat and dairy products (Appendix 1). The HFFF and HMMR diets had the lowest intakes, namely 55 and 67 μg d^{-1} respectively. These were also the diets with the lowest dry masses, cereals and vegetables intakes. The HFFF diet did have high levels of meat and dairy product intakes as shown in Appendix 1. The mean and median DI for the females were 110 and 120 μg d^{-1}, respectively, and varied from 55 to 170 μg d^{-1}. The males had higher mean and median DI of 150 and 130 μg d^{-1}, respectively; they also had a wider range of DI, namely from 67 and 300 μg d^{-1}. The overall mean and median DI were 130 μg d^{-1}. All DI of Se calculated in this work exceeded the Canadian RNI of 50 μg d^{-1}. Almost all values exceeded the U.S. RDA of 55 and 70 μg d^{-1}. The participants do not appear to be at high risk of Se deficiency.

A survey of DDI of Se is presented in Table 3.8. In 1975, Se DI for Canadians was determined by Thompson *et al.* (69) in market baskets using fluorometry and the values ranged between 113 and 220 μg d^{-1}, that for Halifax residents being 220 μg d^{-1}. However, a study of normal hospital duplicate diets done by McDowell *et al.* in 1987 (42, 66) using cyclic INAA revealed the DI of Se in the Halifax area to be 95 μg d^{-1}. The median DI of the self-chosen duplicate diet composites of 90 free-living elderly Canadian women was 77.6 μg d^{-1} determined by INAA (76).

Mean Se DI in teen-age males in the United States between 1974 and 1982 was determined in market baskets to be 108 μg d^{-1} (81). For the 1982-89 period, the DI were 70 and 110 μg d^{-1} for women and men, respectively (86). Both of these values are lower than the measured DI of 120 for the women and 130 μg d^{-1} for men, as reported

Table 3.8. Survey of Daily Dietary Intake of selenium

Study Group	Composite	Analytical Method	DI ($\mu g\ d^{-1}$)	Reference
Australia				
Female, Male Adults	Equiv. composite	INAA/RNAA	57, 87	(198)
Belgium				
General population,	Market basket	HGAAS	41.1-55.5	(199)
Hosp. restaurant clients	Duplicate portion	HGAAS	52	(199)
Hospital patients (Liege)	Hospital diet	HGAAS	28.4	(201)
Macrobiotics	Duplicate portion	HGAAS	34	(200)
Military personnel	Cafeteria meals	HGAAS	61.1	(201)
Vegetarians	Duplicate portion	HGAAS	127	(200)
Benin				
General population	Equiv. composite	AAS	45	(202)
Brazil				
University students	Restaurant meals	RNAA	30.2-34.7	(203)
Residents, Santa Catarina & Manaus	Equiv. Composite	RNAA	45.1-146.0	(203)
Burundi				
Hospital patients/staff	Restaurant meals	HGAAS	66.6	(204)
University students/staff	Restaurant meals	HGAAS	63.9	(204)
Adult mothers, men	Market basket	HGAAS	38.4, 82.4	(204)
Rural population	Market basket	HGAAS	16.9	(204)
Canada				
General population	Market basket	FlUOR	113-220	(69)
Premenopausal women	Duplicate portion	INAA	77	(76)
Elderly women	Duplicate portion	INAA	77.6	(76)
Hospital patients	Hospital diet	CINAA	95	(66,42)
China				
Residents, Se-rich areas	Equiv. composite	Titr./FLUOR	750-4 990	(137)
Residents, Se-poor area	Equiv. composite	Titr./FLUOR.	11	(137)
Adult men	Duplicate portion	FLUOR	6	(205)
Residents of Beijing	Equiv. composite	Titr./FLUOR.	116	(137)
General population	Equiv. composite	INAA/RNAA	95.9	(206)
Adult farmers	Equiv. composite	FLUOR	15	(237)
Denmark				
Adult men	Duplicate portion	HGAAS	51	(207)
Finland				
General population	Equiv. composite	HGAAS	50-60	(208)
Middle-aged men	Equiv. composite	GFAAS	50-53	(209)
General population	Equiv. composite	GFAAS	110	(99)
Wt. reduction patients	Duplicate portion	GFAAS	89	(238)

Table 3.8 (continued). Survey of Daily Dietary Intake of selenium

Study Group	Composite	Analytical Method	DI ($\mu g\ d^{-1}$)	Reference
France				
Adult women, men	Equiv. composite	AAS	35, 43	(210)
Germany				
Adult men, women	Duplicate portion	PNAA, INAA	59, 55	(101, 102)
Adult women, men	Equiv. composite	HGAAS	38, 47	(236)
Adult women, men 1988	Duplicate portion	HGAAS	19.3, 25.0	(239)
Adult women, men 1991	Duplicate portion	HGAAS	24.8, 31.0	(239)
Adult women, men 1995	Duplicate portion	HGAAS	30.3, 42.0	(239)
Vegetarian women, men	Duplicate portion	HGAAS	29.6, 33.8	(239)
Greece				
Adult population	Equiv. composite	FLUOR	110	(212)
Adult female, male	Market basket	FLUOR	104, 122	(213)
Italy				
Residents, various cities	Duplicate portion	INAA	10.9	(214, 215, 216)
General population	Equiv. composite	ICP-AES	90	(217)
India				
Hospital patient	Hosp. vegetar. diet	INAA	123	(218)
Japan				
General population	Equiv. composite	FLUOR	208	(219)
Mexico				
Adults (Northern)	Equiv. composite	HGAAS	173	(220)
The Netherlands				
General population	Market basket	INAA	78	(231)
Teen-age males (18 y)	Market basket	FLUOR	69	(232)
New Guinea				
Gidra tribe (northern)	Equiv. composite	FLUOR	51.0-132.9	(221)
New Zealand				
Young women	Duplicate portion	FLUOR	6 - 70	(223,225)
Hamilton residents	Equiv. composite	?	56.2	(224)
Dunedin residents (M,F)	Equiv. composite	?	33,23	(141)
Hamilton residents	Equiv. composite	FLUOR	31.8	(226)
Norway				
Women (control)	Duplicate portion	INAA	91	(227)
Women (Se-rich bread)	Duplicate portion	INAA	137	(227)
Pakistan				
Islamabad residents	Equiv. composite	INAA	76-97	(228)
Gujranwala residents	Equiv. composite	INAA	120	(229)
Portugal				
Pinhel city residents	Duplicate portion	CINAA	36	(230)

Table 3.8 (continued). Survey of Daily Dietary Intake of selenium

Study Group	Composite	Analytical Method	DI ($\mu g\ d^{-1}$)	Reference
Scotland				
Glasgow residents	Equiv. composite	RNAA	234	(138)
Sweden				
Female, male Vegans	Duplicate portion	?	12.3, 6.9	(91)
Female, male lactoveg.	Duplicate portion	?	61, 68	(93)
General population	Market basket	GFAAS	44	(98)
Adult men and women	Duplicate portion	AAS	32	(2)
Taiwan				
University students/staff	Duplicate portion	INAA	90-120	(43)
Turkey				
Ankara-Lalahan residents	Duplicate portion	INAA	52-57	(233, 234)
Lalahan (rural) residents	Duplicate portion	GFAAS	23	(234)
U.S.A.				
Hospital patients, vegetarians	Hospital diet	PNAA	85.5, 118.1	(193)
Maryland residents	Duplicate portion	FLUOR	74	(194)
Females, males, ranchers, seleniferous area	Duplicate portion	GC-MS	128, 213, 288	(197, 180)
South Dakota, Wyoming residents	Duplicate portion	GC-MS	116	(180)
Teen-age male	Market basket	FLUOR, HGAAS, INAA	89-156	(78, 80, 81, 82, 12, 83, 85)
Teen-age female	Market basket	HGAAS, INAA	60-70	(83, 85, 86)
Adult male	Market basket	HGAAS, INAA	100-128	(83, 195, 85, 86)
Adult female	Market basket	HGAAS, INAA	60-70	(83, 85, 86)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	~200	(240)
General population	Equiv. composite	FLUOR	60	(211)
Venezuela				
Caracas residents	Equiv. composite	Fluorim.	220	(179)
Families	Family meals	INAA	300-400	(235)

in this thesis. Reported values in other studies in the United States tend to be about 70 to 150 μg d^{-1} (12, 78, 80, 82, 83, 85, 140, 192-196). In some seleniferous areas, median DI have been reported to be 213 (197) and 288 μg d^{-1} (180). Intake of an individual from one of these areas was 724 μg d^{-1}.

Other studies on the analysis of Se in diets worldwide have been reported for several countries, including Australia (198), Belgium (199, 200, 201), Benin (202), Brazil (203), Burundi (204), China (137, 205, 206), Denmark (207), Finland (99, 208, 209), France (210), Great Britain (211), Greece (212, 213), Italy (214, 215, 216, 216), India (218), Japan (219), Mexico, (220), New Guinea (221), New Zealand (141, 222, 223, 224, 225, 226), Norway (227), Pakistan (228, 229), Portugal (230), Scotland (138), Sweden (90, 91, 93, 98, 2), Taiwan (43), The Netherlands (231, 232), Turkey (233, 234), Venezuela (179, 235), and West Germany (101, 236). The DI of some selected studies are reported in Table 3.8. Daily intakes reported in the literature vary from 1.2 μg d^{-1} for Belgian patients receiving total parenteral nutrition (200) to 4.99 mg d^{-1} in a seleniferous area of China (137). Median DI for residents of a Se-poor area of China, where Keshan disease and Kashin-Beck disease were prevalent, was reported to be 6.0 μg d^{-1} (205). In most countries, the Se intake is between 40 and 100 μg d^{-1}. Countries with particularly low reported Se intakes (<40 μg d^{-1}) are China, Italy, New Zealand, and Sweden. High intake levels (near or above 200 μg d^{-1}) have been reported in Canada, China, Japan, Mexico, Scotland, the United States, and Venezuela. The overall median DI measured in this study was 130 μg d^{-1} which is comparable to the U.S. population groups (Table 3.8); these values are generally higher than those of most other countries, but significantly

lower than high values determined in countries with seleniferous soils.

3.1.9 Dialysis of Duplicate Diets for Selenium

The rather poor detection limits obtained for Se using ^{77m}Se by conventional INAA (Table 3.5) were mainly attributed to the interference from the high background activities of ^{24}Na and ^{38}Cl present in the diet samples. Removal of these elements from the matrix prior to irradiation would decrease the spectral background, lower the detection limits, and possibly allow the measurement of elements which were previously undetected. A simple dialysis method was used to minimize the effects of interfering elements. Dialysis could also provide speciation information, as elements which were bound to macromolecules, or were insoluble would be retained in the tubing, while soluble species and elements bound to small molecules could pass through. Freeze-dried duplicate diet composites and a few reference materials were mixed with a small amount of DDW, and transferred to dialysis tubes (MWCO 3 500). The tubes containing the slurry were immersed in 3 L DDW, and stirred for 24 h. This DDW was changed with fresh DDW after about 12 h. After 24 h, the dialysis tubes were removed, rinsed with fresh DDW, and freeze-dried. The freeze-dried sample was then analyzed by NAA together with the dialysis tubing.

The fractions of the mass retained and lost during dialysis were calculated. The results are reported in Tables 3.9 and 3.10. The masses retained by the two diet RMs, IAEA H-9 and NIST 1548, were 79 and 75%, respectively. The lowest percentage retained (58%) of the five RMs was by NIST SRM 1515 Apple Leaves. Retention was

Table 3.9. Mass dialyzed in RMs and SRMs

Reference Material	%non-dialyzable	%dialyzable[a]
IAEA H-9 Mixed Diet	79.0	21.0
NIST SRM 1515 Apple Leaves	58.1	41.9
NIST SRM 1548 Total Diet	75.4	24.6
NIST SRM 1568 Rice Flour	102.5	-2.48
NIST SRM 1577b Bovine Liver	95.4	4.60

[a]:calculated by difference

Table 3.10. Mass dialyzed in duplicate diet composites

Sample Code	%non-dialyzable	%dialyzable[a]
HMFS	65.9±0.2[b]	34.1±0.2[b]
(a) Females		
HFFF	56.3	43.7
HFMC	67.6±0.2[c]	32.4±0.2[c]
HFNA	70.7	29.3
HFRR	72.0	28.0
HFSR	79.4	20.6
HFTC	76.1	23.9
HFWH	55.1	44.9
Mean	68.3	31.7
Median	71.4	28.6
Min. - Max.	55.1 - 79.4	20.6 - 44.9
(b) Males		
HMFF	70.7	29.3
HMMR	88.2	11.8
HMNT	76.1±0.6[c]	23.9±0.6[c]
HMRZ	57.4	42.6
HMSG	70.4	29.6
HMTH	63.2	36.8
HMWV	72.4	27.6
Mean	70.4	29.6
Median	70.6	29.4
Min.-Max.	57.4-88.2	11.8-42.6
(c) Overall		
Mean	69.3	30.7
Median	70.7	29.3
Min.-Max.	55.1 - 88.2	11.8 - 44.9

[a]:calculated by difference; [b]:average of four values; [c]:average of two values

greater than 95% for both NIST SRM 1568 Rice Flour and NIST SRM 1577b Bovine Liver. For the diet samples (Table 3.10), 55-88% of the mass was retained. The median was 71%. In order to evaluate the precision, four replicate experiments using HMFS and two replicates using HFMC and HMNT were performed. The RSD was less than 1% in all cases, suggesting that the experiments were highly reproducible.

The samples were analyzed by NAA in order to evaluate the effectiveness of dialysis in reducing the Cl, K, and Na levels. Lower dead time allowed an irradiation time of 5 min whereas an irradiation time longer than 1.5 min was not possible for most undialyzed diet samples. Analysis of the samples by NAA revealed that less than 5% of the Cl, K, and Na contents of all diet composites and almost all RMs, were retained by the dialysis tubing. The only exception was NIST SRM 1568 Rice Flour, for which Cl, K, and Na retained were 12%, 9% and 16%, respectively. The overall results for Cl, K, and Na indicate that as expected, dialysis was effective in eliminating these elements from the diet samples.

The 161.9-keV peak of ^{77m}Se was again used for Se determination of the dialyzed samples. The irradiation time was 30 s, followed by a decay time of 15 s and a counting time of 30 s. The results are given in Tables 3.11 and 3.12. For the RMs and SRMs, the percent Se retained followed the same order as for the mass retained, although the percentages were lower. The RM with the lowest retention (33%) was NIST Apple Leaves. The two diet RMs, namely IAEA H-9 and NIST 1548, had Se retentions of 51 and 68%, respectively, whereas the percentages for NIST Rice Flour and Bovine Liver were 75 and 76%, respectively. Selenium retention of the duplicate diet composites

Table 3.11. Dialysis of reference materials for selenium

CRM	%non-dialyzable	%dialyzable[a]
IAEA H-9 Mixed Diet	51	49
NIST SRM 1515 Apple Leaves	33	67
NIST SRM 1548 Total Diet	68	32
NIST SRM 1568 Rice Flour	75	25
NIST SRM 1577b Bovine Liver	76	24

[a]:calculated by difference

Table 3.12. Dialysis of duplicate diet composites for selenium

Sample Code	%non-dialyzable	%dialyzable
HMFS	76 ± 5[b]	24 ± 5[b]
(a) Females		
HFFF	103.	-3.
HFMC	61 ± 10[c]	39 ± 10[c]
HFNA	71.	29.
HFRR	73.	27.
HFSR	72.	28.
HFTC	68.	32.
HFWH	84.	16.
Mean	78.	22.
Median	73.	28.
Min. - Max.	68 - 103	-3 - 32
(b) Males		
HMFF	65.	35.
HMMR	82.	18.
HMNT	64 ± 9.5[c]	36 ± 9.5[c]
HMRZ	8.	92.
HMSG	98.	2.
HMTH	107.	-7.
HMWV	70.	30.
Mean	72.	28.
Median	76.	24.
Min. - Max.	8 - 107	-7 - 92
(c) Overall		
Mean	75.	25.
Median	73.	28.
Min. - Max.	8 - 107	-7 - 92

[a]:calculated by difference; [b]:average of four values; [c]:average of two values

generally varied between 65 and 84%, while three values were near 100% and one was 8% only. The median for the whole group was 73% not much different from the mean of 75%. Relative standard deviations of HMFS, HFMC and HMNT were 6, 16 and 15%, respectively. Although dialysis was effective in minimizing the concentrations of Cl, K, and Na in the duplicate diets, the percentage of dialyzable Se varied from diet to diet.

Consequently, this dialysis procedure was considered not to be a suitable technique for the quantitative analysis of Se in the diets. However, it does suggest that different proportions of various Se species could be present in the diets. A correlation analysis of retained Se with the DI and mass fractions of the various food groups (Appendix 1) did not yield any significant relationships.

3.1.10 Enzymolytic Digestion of the Diets for Se

A simulated digestion procedure was developed for the purpose of estimating the potential availability of elements in the duplicate diet composites, and has been described in detail in section 2.6. Briefly, a small amount of the diet composite was mixed with gastric juice (1% pepsin in saline HCl) and incubated in a shaking water bath at 37° C for 4 h. The mixture was then neutralized with a saturated Na_2CO_3 solution, combined with intestinal juice (10 mL 3% pancreatin and 1% amylase in saline solution + 10 mL 0.15% bile salts in saline solution). This mixture was incubated for another 4 h. A sample of the trial diet, HMFS, was centrifuged using McDowell's method (66). The sediment and the supernatant were freeze-dried, and transferred to irradiation vials. Another sample of HMFS was subjected to dialysis instead of centrifugation. The

pancreatic digestion step took place in a dialysis tube (MWCO 3 500) immersed in DDW. Following digestion, the dialysate and the dialysis tube containing the residue, were freeze-dried and transferred to irradiation vials for analysis.

Separations by centrifuge and dialysis were evaluated by NAA using the 161.9-keV peak of ^{77m}Se. Irradiation conditions, dead time and peak areas are reported in Table 3.13. No Se was detected in the sediment fraction of the centrifuged sample, although a small peak at 162 keV was detected in the supernatant. However, the dead time was very high for both fractions. Even with an irradiation time as low as 10 s, the dead time was 30% for the supernatant. The dead times for the dialyzed sample were lower, being 13% in the dialysate and 6.7% in the residue. The 162- keV peak was observed during analysis of the residue, but no peak was detected in the gamma-ray spectrum of the dialysate. Since the digestion procedure involves gastric digestion in HCl and a pancreatic digestion step where the HCl is neutralized by Na_2CO_3, the Na and Cl levels were elevated compared with non-digested samples. The high dead times are attributed to the Na and Cl content of the samples. Since other researchers have indicated that dialysis is a better simulation technique of the *in vivo* gastrointestinal process than simple centrifugation (see section 1.3.3) and since the centrifuge method gave high dead times, it was not pursued further. The absence of the 161.9-keV peak in the dialyzate and high dead time indicate the need for chemical separation to remove the Na and Cl. It is suggested that an appropriate chemical separation method be developed in future. The results in the present work deal with NAA of the residue fraction of the enzymolytic digests of some RMs and duplicate diet composites.

Table 3.13. Centrifugation and dialysis of enzymolyzed of trial diet HMFS for Se

Separation	t_i-t_d-t_c (s)	Dead time	Peak Area (net counts)
Centrifuge			
sediment	30-10-30	17%	-
supernatant	10-10-30	30%	213
Dialysis			
residue	30-10-30	6.7%	322
dialysate	10-10-30	13%	-

Table 3.14. Mass lost through enzymolysis and dialysis of some SRMs

Reference Material	%Unavail.	%Avail
NIST SRM 1548 Total Diet	25	75
NIST SRM 1568 Rice Flour	1	99

Table 3.15. Mass lost through enzymolysis and dialysis of freeze-dried duplicate diets

Sample	%Unavail.± SD	%Avail+- SD
HMFS	81 ± 8 [a]	19± 8[a]
(a) Females		
HFFF	67	33
HFMC	72	28
HFNA	87	13
HFRR	81	19
HFSR	87	13
HFTC	87	13
HFWH	74	26
Avg.	79	21
Median	81	19
Min. - Max.	67 - 87	13 - 33
(b) Males		
HMFF	86	14
HMMR	82	18
HMNT	94 ± 1	6 ± 1
HMRZ	76	24
HMSG	91	9
HMTH	88	12
HMWV	89	11
Avg.	87	13
Median	88	12
Min. - Max.	76 - 94	6 - 33
(c) Overall		
Avg.	82	18
Median	84	16
Min. - Max.	67 - 94	6 - 33

[a]:average of seven experiments. [b]:average of three experiments

Table 3.16. Mass lost through enzymolysis and dialysis of wet duplicate diets

Sample	%Unavail.± SD	%Avail ± SD
HMFS	75 ± 4[a]	25 ± 4 [a]
(a) Females		
HFFF	60	40
HFMC	68 ± 10[b]	32 ± 10[b]
HFNA	66	34
HFRR	75	25
HFSR	76	24
HFTC	70	30
HFWH	72	27
Avg.	70	30
Median	70	30
Min.-Max.	60 - 76	24 - 40
(b) Males		
HMFF	87	13
HMMR	89	11
HMNT	82 ± 15[b]	18 ± 15[b]
HMRZ	62	38
HMSG	68	32
HMTH	71	29
HMWV	82	18
Males	77	23
Avg.	11	11
Median	82	18
Min.-Max.	62 - 89	11 - 40
(c)Overall		
Avg.	72	28
Median	70	30
Min.-Max.	60 - 89	11- 40

[a]:average of four experiments. [b]:average of three experiments

The mass retained and lost during the enzymolysis-dialysis procedure is reported in Tables 3.14-3.16. The dialyzable fraction, calculated by difference, may also be considered as "potentially available." About 25% of NIST SRM 1548 Total Diet was retained by the dialysis tubing, whereas only 1% of the mass of NIST 1568 Rice Flour was retained. A comparison of the dialysis results shown in Tables 3.9 and 3.14 shows that the enzymolysis increased the dialyzable mass significantly. For example, only 25% (Table 3.9) of the NIST SRM Total Diet was deemed "soluble" by dialysis alone whereas 75% (Table 3.14) of the same was considered "available" by enzymolysis-dialysis.

Both freeze-dried diet composite and wet slurry were subjected to digestion. Seven replicate experiments were performed on freeze-dried trial diet HMFS, and three on freeze-dried HMNT. The RSD of these experiments (based on % non-dialyzable) were 10% for HMFS and 1% for HMNT. Again, replicate experiments were performed on the wet slurry, including four replicates of HMFS and three each of HFMC and HMNT. The RSDs of the three diets were 5.4%, 15% and 18%, respectively. The mass lost during digestion of the freeze-dried diets (mean and median 18 and 16%, respectively) was generally lower than during dialysis. However, the percentage mass lost during enzymolysis of the wet diet slurry (mean and median 28 and 30%, respectively) was generally higher than freeze-dried diet and comparable to the results of dialysis of undigested diet.

Samples from the enzymolysis experiments were analyzed for Se using the 161.9 keV gamma-ray of ^{77m}Se. Unfortunately, the analyzer system on which these samples were analyzed became unserviceable before all of the diets could be analyzed.

Although other analyzers were tried, useable analytical signals were not obtained. Therefore, Se results (Tables 3.17 and 3.18) are only available for a small number of the diets. The undialyzed Se concentrations, percent non-dialyzed ("not potentially available") and percent potentially available (calculated by difference) are reported for a few RMs, freeze-dried diets and wet slurries. The percent dialyzable was higher in the enzymolysis experiments than in the dialysis experiments for all three RMs, although the increase for NIST Total Diet, 11%, was smaller than for NIST Rice Flour and NIST Bovine Liver. Significant limitations to these results were the large uncertainties. The relative uncertainty of non-dialyzable Se in freeze-dried HMFS and HMNT were 27 and 59%, respectively. The corresponding values for wet slurry were 12 and 35%, respectively. Because the percent potentially available was calculated by difference, and the differences were generally small, the relative uncertainties in these values were generally large. These results highlight the need for future work to determine the Se content in the dialysate of the enzymolysis experiments.

Table 3.17. Enzymolysis and dialysis of freeze-dried SRMs and duplicate diets for Se

Sample	Not Potentially Available		Potentially Available[a]	
	Conc. ± SD (ppb)	%Non-diayzed ± SD	Daily Intake (μg d^{-1})	%Dialyzed ± SD
Ref. Materials				
NIST 1548 Total Diet	170	58%		42%
NIST 1568 Rice Flour	110	35%		65%
NIST 1577b Bovine Liver	270	42%		58%
Duplicate Diets				
HMFS	170 ± 40[b]	58% ± 15%	88 ± 30	42% ± 15%
HMNT	100 ± 50[c]	36% ± 21%	130 ± 50	64% ± 21%
HMWV	330	91%	270	9

[a]:calculated by difference; [b]:average of four experiments; [c]:average of two experiments

Table 3.18. Enzymolysis and dialysis of wet duplicate diet composites for Se

Sample	Not Potentially Available		Potentially Available[a]	
	Conc. ± SD (ppb)	%Non-dialyzed ± SD	DI ± SD (μg d^{-1})	%Dialyzed ± SD
HMFS	210 ± 20[b]	72% ± 9%	59 ± 19	28% ± 9%
HFNA	98	23%	130	77%
HMFF	340	95%	84	5%
HMNT	220 ± 60[c]	78% ± 27%	45 ± 57	22% ± 27%
HMWV	76	21%	240	79%

[a]:calculated by difference; [b]:average of three experiments; [c]:average of two experiments

3.2 IODINE

3.2.1 Iodine in Health and Nutrition

Iodine has been known to be an essential trace element in humans, since the 19th century. Iodine deficiency is still considered to be a serious problem in many countries. The World Health Organization (WHO) has estimated (134) that about 1.6 billion people around the world are at risk of iodine deficiency diseases. Nutritional and health aspects of iodine have been the subject of several review articles (1, 12, 134, 147, 241, 242, 243, 244).

The human body contains 20-50 mg of iodine. Of this amount, 50% is found in muscle and 20% in the thyroid gland. The only known role of iodine is reported to be a component of thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). These hormones accelerate cellular reactions, increase oxygen consumption and basal metabolic rate and influence reproduction, growth and development, energy metabolism, differentiation, neuromuscular function and protein synthesis. Every step of the synthesis of these hormones is stimulated by thyroid stimulating hormone (TSH).

The term "goitre" was used for many years to refer to the effects of iodine deficiency. Goitre is the enlargement of the thyroid, observed as a swelling at the front of the neck. It is a common and obvious symptom of iodine deficiency. However, other effects have become known and the term Iodine Deficiency Disorders (IDD) is now commonly applied.

Iodine deficiency results in lowered production of T_3 and T_4, which leads to release of TSH, by the pituitary gland. This results in increased activity of the thyroid

gland and enlargement, so that the available iodine can be used more efficiently. The condition is common when intake drops below 50 μg d^{-1} and almost universal below 10 μg d^{-1}. Goitre can also be induced by substances called goitrogens which interfere with thyroid hormone metabolism. These substances can be found in various foods, including cabbage, turnips, rutabagas, cauliflower, turnips, peanuts, mustard seeds, soybeans, cassava, maize, bamboo shoots, sweet potatoes, lima beans, and millets. Since Se has been discovered to be part of the enzyme which converts T_4 to T_3 (152-154), a deficiency in this element may also hinder iodine utilization and lead to the development of IDD (147, 154, 168-170, 245).

Deficiency in pregnant women can lead to stillbirths, abortions and congenital abnormalities. Cretinism is a condition resulting from maternal deficiency, common to children born in iodine-deficient areas. It is characterized by mental deficiency, deaf mutism, poor muscle development, poorly formed bones, hypothyroidism and dwarfism. In children and adults, iodine deficiency can lead to reduced mental function. Effects observed have been reduced school performance, decision-making and initiative-taking.

Organizations such as WHO and UNICEF have made the correction and prevention of IDD a worldwide priority. The WHO has stated, "WHO shall aim at eliminating IDD as a major public health problem in all countries by the year 2000" (134). Iodine deficiency can be prevented or treated by supplementation of the diet with iodine (244). Treatment of existing IDD is generally more effective if iodine supplementation is accompanied with hormone therapy. Iodine can be supplemented as

iodized salt, as an additive to the feeds of farm animals, and as iodized oil, taken by mouth or by injection. Health Canada concluded that 160 μg d^{-1} of iodine is required to maintain positive balance in healthy adolescents and adults, and set this value as the RNI (1). The U.S. RDA for iodine is 150 μg d^{-1} (17).

Iodine is not highly toxic for people who have normal thyroid function. However, excessive iodine intake could induce hypothyroidism. In the case of previous iodine deficiency, high iodine intake can induce hyperthyroidism. Individuals over the age of 40 may be particularly susceptible to this latter condition. An upper limit of 2 000 μg d^{-1} has been suggested as a safe intake (134). Health Canada recommends that intakes not exceed 1 000 μg d^{-1} (1).

3.2.2 Dietary Intake of Iodine

The richest sources of iodine in diets are generally marine fish and seaweeds. Concentrations in other foods tend to be highly variable, subject to differences in soils, fertilization, and food processing and preparation. Milk can be a significant contributor to the daily diet, although its content can be quite variable, depending on the content of animal feeds and use of iodophor sanitizers. In some countries, iodate is used in the making of bread. Erythrosine, a red food coloring agent containing 57% iodine can be a significant source, as concluded by the U.S. F.D.A. in their Total Diet Study (81, 83, 85, 86). Discretionary use of iodized table salt may also contribute significantly to the diet. The iodine intake of Canadians was found to be 1 000 μg d^{-1} by Fischer and Giroux (246). Of this, 60% came from table salt and 25% was from dairy products.

Iodide is the most common form of iodine in foods. Other forms are reduced to iodide in the GI tract. This form is readily available for absorption. On the other hand, absorption of iodinated amino acids is slower and less complete. Absorption can also be inhibited by interaction with As, Co, Fe, and Mn. Most of the iodine in muscle meats and offal is bound to protein, although iodine in milk is not bound. However, in fish, most of the iodine is in inorganic form and is highly available. Since iodine is not known to be essential to plants, it is most likely present in the inorganic iodide form.

3.2.3 Iodine Analysis by EINAA with Anti-coincidence Counting

Iodine has previously been analyzed by a number of analytical techniques including colorimetry, ion-selective electrode, GC, and NAA (see citations in ref. 49). Neutron activation analysis has very good sensitivity for iodine. However, iodine cannot be measured easily in biological materials by NAA because of its low levels in biological materials as well as the high background resulting from the thermal neutron activation products of other elements in the material. This is particularly true of diet samples, which have high activities due to the ^{38}Cl, ^{56}Mn and ^{24}Na. Therefore, preconcentration NAA (49) or radiochemical NAA methods (88, 247, 248) have been used. Since iodine can be activated efficiently by epithermal neutrons (a resonance integral cross-section of 147 $\pm$ 6 b) while Cl, Mn and Na are not, epithermal irradiation can be used to lower the background activities so that iodine can be determined with a superior detection limit (42, 46, 249). Epithermal (more accurately, epi-cadmium in this case) activation can be

achieved conveniently in the Cd-shielded irradiation site of the DUSR (57) facility. Since the Cd shield has a hole in the bottom, there is some leakage of thermal neutrons. Extra shielding against this leakage can be provided by flexible boron carbide inserted as a disk on the bottom of the outer irradiation vial. Rao *et al.* showed that extra boron shielding can lower detection limits when conventional gamma-ray counting is used (46).

Anti-coincidence gamma-ray spectrometry can also be used to lower the Compton background arising from other activation products, and has been described earlier. The combination of EINAA and AC counting has the potential to achieve iodine analysis with high sensitivity and low detection limits, without the need for chemical separation. This combination was used by Landsberger to measure iodine in NIST soil samples (59). In the present work, a method involving EINAA and Compton suppression was developed for the analysis of iodine in human diet samples.

In order to evaluate the effects of counting methods, timing parameters, and shielding, subsamples of the HMFS duplicate diet composite were subjected to various epi-cadmium-irradiation, decay and counting conditions. The 442.9-keV peak of ^{128}I was used. Peak areas and background counts obtained are presented in Table 3.19. As the first line in Table 3.19 shows, a net peak area of only 89 counts was obtained after 5 min of irradiation and 17 min of counting using a conventional gamma-ray spectrometry system. The background activity of 1015 counts was also quite high. The same sample was subsequently transferred to the anti-coincidence spectrometry system (without any further irradiation of the sample) and counted for 30 min. Even after a 23-min decay time, a larger net peak area of 186 counts was obtained and the background counts were

Table 3.19. Preliminary experiments on EINAA-AC for iodine with boron shielding in Cd site.

Vial No.	Mass	System[a]	t_i-t_d-t_c (min)	Boron Shield	Pk. Area	Bkg.	Sig./ Bkg.	Counts/ g Sample
1	0.2883	Conv.	5-01-17	No	89	1015	0.088	309
2	0.2883	AC	5-23-30	No	186	524	0.355	645
3	0.2786	AC	10-01-17.6	No	316	912	0.346	117
3	(calculated)		5-01-17	No	164	456	0.360	589
4	0.2883	AC	15-2-20	No	280	744	0.376	971
4	(calculated)		15-2-30	No	371	1116	0.333	1289
5	0.2751	AC	15-03-30	Yes	563	1608	0.350	2047

[a]."Conv." denotes normal mode analyzer system. "AC" denotes anti-coincidence analyzer system

also lower. Another subsample was irradiated under similar conditions to the first one, but used for AC counting only. The results, shown in the third row of Table 3.19, were corrected for differences in irradiation and decay times for comparison purposes. The calculated values are reported in the fourth row of Table 3.19. The calculated net peak area (164 counts) was larger compared with the peak area obtained using the conventional system (89 counts). The peak area, signal-to-background ratio and peak area-to-sample mass ratios were all improved using AC counting. In order to obtain larger net peak areas (improved sensitivity), the irradiation time was lengthened to 15 min. Additionally, extra boron shielding was used for the sample counted using a conventional system and another sample counted using the AC system. A limitation to the use of the boron carbide shields presently available is caused by the presence of an Al impurity in the shield, requiring longer decay times for the ^{28}Al activity to subside. The highest signal-to-background ratio was obtained using conditions of t_i = 15 min, t_d = 2 min and t_c = 20 min and no extra boron shielding. Therefore, these conditions were selected for the analysis of the duplicate diets. However, a larger peak area-to-sample mass ratio suggests that future development of a method using boron shielding in combination with epi-cadmium irradiation may be worth pursuing.

3.2.4 Quality Assurance for Iodine by EINAA-AC

Iodine was measured using the 442.9-keV photopeak of ^{128}I and the experimental conditions noted above. The sensitivity was 3640 counts μg^{-1}. Precision and accuracy of measurements were evaluated by the analysis of RMs and SRMs (Table

3.20 and Fig. 3.5). Unfortunately, there are only a few biological materials available with certified values for iodine; furthermore, none of them are diets. Four materials were analyzed: IAEA H-9 Mixed Diet (information value only), and NIST SRM 1549 Milk Powder, RM 8435 Milk Powder and RM 8415 Egg Powder. There were good agreements between the measured and certified/information/literature values. The iodine content of the trial diet HMFS is also presented in Table 3.20 as an indication of the iodine levels measured in the duplicate diets. Detection limits calculated for these materials are also presented in the same table, and were found to vary between 90 and 375 ppb.

3.2.5 Iodine Content of Duplicate Diets

Iodine concentrations of the duplicate diets were determined using the EINAA-AC method. The results are reported in Table 3.21. It is evident that both iodine levels and DIs were quite variable for the female participants. The range was from non-detectable levels in diet HFWH, to 510 μg d^{-1} in HFSR. Variation was much smaller for the male participants, from 150 to 360 μg d^{-1}. Participants HFNA, HFRR, HFSR and HFTC had high DIs, while HFFF and HFWH had particularly low intakes of iodine. All except for HFFF and HFWH met or exceeded the U.S. RDA. Only these two as well as HMMR did not exceed the RNI. An examination of the foods consumed (Appendix 1) suggests that individuals with low intakes of milk also had low iodine content of the diet, while those having higher intakes of milk and fish had higher intakes. These relationships are discussed in detail in Chapter 4.

The median concentration and DIs for the female volunteers were 900 ppb

Table 3.20. Iodine content of some RMs, SRMs and trial diet HMFS (ppb).

Reference material	L_d	This work	Cert. value (Info. value)	Lit. values
NIST RM 8435 Milk Powder	255	2 200 ± 100[a]		2 377 ± 70[b]
NIST RM 8415 Egg Powder	200	1 800 ± 100[a]		1 875 ± 94[b]
IAEA H-9 Mixed Diet	260	400 ± 50[a]	(400)	372 ± 30[b], 370 ± 45[c]
NIST SRM 1549 Milk Powder	370	3 200 ± 200[d]	3 380 ± 20	3 150 ± 75[b] 3 115 ± 170[c]
HMFS	90	500 ± 110[a]	-	-

[a]. Average of four values; [b].From Rao and Chatt (ref. 49); [c].From Rao *et al* (ref. 46); [d]. Average of three values

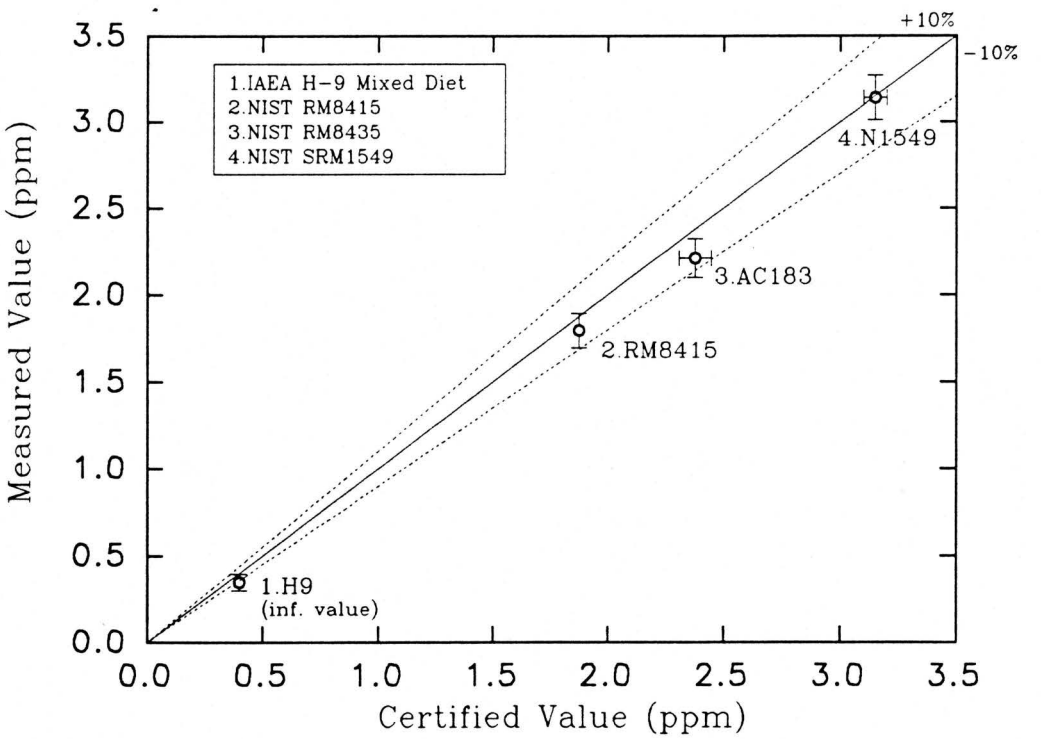


Fig. 3.5.Analysis of certified reference materials for iodine

Table 3.21. Iodine content of duplicate diets determined by the EINAA-AC method.

Sample Code	Content±SD (ppb)	Daily Intake (μg d^{-1})
HMFS	500 ± 110	280 ± 70
(a) Females		
HFFF	200	61
HFMC	490 ± 170	160 ± 50
HFNA	1000	360
HFRR	910	360
HFSR	1 200	510
HFTC	900	300
HFWH	38	17
Mean	670	250
Median	900	300
Min.-Max.	38 - 1 200	17 - 510
RNI		160
RDA		150
(b) Males		
HMFF	490	210
HMMR	600	150
HMNT	400 ± 40	270 ± 30
HMRZ	610	340
HMSG	580	240
HMTH	630	300
HMWV	480	360
Mean	540	270
Median	580	270
Min.-Max.	400 - 630	150 - 360
RNI		160
RDA		150
(c) Overall		
Mean	610	260
Median	590	280
Min.-Max.	38 - 1 200	17 - 510

and 300 μg d^{-1}, respectively. For the male volunteers, concentration and DIs were 580 ppb and 270 μg d^{-1}, respectively. The overall median concentration and intake were 590 ppb and 280 μg d^{-1}.

The only Canadian study on iodine content of the diet was performed in 1982 by Fischer and Giroux (246), who prepared a representative diet based on the Nutrition Canada survey. They reported a value of 1 046 μg d^{-1}, which is considerably higher than the median intakes reported in this study and exceeds all of the individual intakes determined in this study as well. Most of the iodine in this composite was contributed by the group which contained table salt. Perhaps recommendations to decrease salt intake have lead the volunteers of this present study (diets collected in 1987) to lower their salt intakes.

Similar to the studies of Fischer and Giroux in 1982, the DI for teen-age males in the U.S. Total Diet Study for the years 1982-1984 was also high, 710 μg d^{-1} (83). Later Total Diet studies reported lower values, such as 320 μg d^{-1} reported for teen-age males for the period 1985-1986 (85). It was suggested that the lower iodine intake was the result of a decrease in the use of iodine-containing food-coloring agents (red dye #3) and disinfectants by the food and agriculture industries. Other values of iodine intake surveyed in the literature are reported in Table 3.22. Daily intakes reported for Swedish pensioners and Dutch teenagers are comparable to the median intakes reported in this thesis. Iodine intakes reported for France and West Germany were particularly low.

Table 3.22. Survey of DDIs of iodine

Study Group	Composite Method	Analytical	Intake ($\mu g\ d^{-1}$)	Ref.
Canada				
General population	Equiv. composite	Colorimetry	1 046	(246)
France				
Adult women, men	Equiv. composite	AAS	109, 89	(210)
The Netherlands				
Teen-age males (18 y)	Market basket	Colorimetry	318	(232)
Sweden				
Female, Male pensioners	Duplicate portion	COLR.	318, 246	(90)
Female, Male Vegans	Duplicate portion	COLR.	58, 82	(91)
U.S.A.				
Adults,1980 (3 900 kcal/d)	Market basket	RNAA	388	(89)
Adults,1980 (2 900 kcal/d)	Market basket	RNAA	289	(89)
Teen-age male, 1974	Market basket	Colorimetry	780	(81)
Teen-age male, 1975	Market basket	Colorimetry	539	(81)
Teen-age male, 1978	Market basket	Colorimetry	528	(81)
Teen-age male, 1979	Market basket	Colorimetry	377	(81)
Teen-age male, 1980	Market basket	Colorimetry	320	(81)
Teen-age male,1981/1982	Market basket	Colorimetry	202	(81)
Teen-age female, male1982-84	Market basket	Colorimetry	420, 710	(83)
Adult female, male 1982-84	Market basket	Colorimetry	270, 520	(83)
Adult males (USDIET-1)	Market basket	INAA	300	(195)
Teen-age female, male1984-85	Market basket	Colorimetry	300, 490	(85)
Adult female, male 1984-85	Market basket	Colorimetry	250, 370	(85)
Teen-age female, male1985-86	Market basket	Colorimetry	210, 320	(85)
Adult female, male 1985-86	Market basket	Colorimetry	170, 250	(85)
Teen-age female, male1982-89	Market basket	Colorimetry	340, 550	(86)
Adult female, male1982-89	Market basket	Colorimetry	260, 410	(86)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	220	(240)
West Germany				
Adult women, men	Duplicate portion	AAS	51, 58	(250)

3.3 MAGNESIUM

3.3.1 Nutritional and Toxicological Aspects of Magnesium

There are several review articles on biological and nutritional aspects of Mg (1, 6, 12, 17, 241, 251, 252). The human body contains 20-28 g of Mg, of which 60-65% is in bone, 27% in muscles, 6-7% in other cells and 1% is in extracellular fluid (1, 252). More than 300 enzymes are activated by Mg as a result of its presence in an active site or induction of conformational changes. For example, Mg is a cofactor in the transfer of phosphate groups in the production of adenosine triphosphate (ATP), and is required for synthesis, transcription and degradation of DNA. It is also involved in muscle relaxation and neuromuscular transmission, and aids the binding of calcium to tooth enamel.

Magnesium deficiency is rare because of the homeostatic mechanisms of the human body. The deficiency is only produced by disease states such as severe malabsorption, chronic alcoholism, prolonged parenteral nutrition, kidney dysfunction, and childhood malnutrition. Deficiency symptoms include weakness, confusion, personality change, muscle tremor, anorexia, nausea, lack of coordination and gastrointestinal disorders. Low Mg status has been linked to heart failure and hypertension.

Magnesium balance in men has been maintained at intakes of 210-320 mg d^{-1}. Therefore, the U.S. FNB set the Mg RDA of 280 mg d^{-1} for women and 350 mg d^{-1} for men (17). Because of concern about the accuracy of Mg balance studies, Health Canada determined Mg requirements somewhat differently. They concluded that the minimal requirement may be less than 50 mg d^{-1} (1) and requirements to maintain normal

levels in plasma were estimated to be 220 mg d^{-1} in Mg-replete men and 180 mg d^{-1} in women. Consequently, the Canadian RNI values, 200 mg d^{-1} for women and 250 mg d^{-1} for men, are somewhat lower than the American RDA values.

Toxic levels of DI are difficult to obtain, because Mg is not particularly known to be toxic. However, impaired kidney function can lead to inhibition of Mg excretion, causing increased Mg levels and toxic symptoms. This occurs most commonly in elderly people who consume Mg-containing laxatives and antacids. Early symptoms are drowsiness, nausea, vomiting and hypotension. This is followed by bradycardia (slowness of heartbeat), vasodilation, electrocardiographic changes, hyporeflexia, and central nervous system depression. Eventually, respiratory depression, coma and asystolic arrest (cardiac standstill) may lead to death.

3.3.2 Dietary Sources, Intakes, and Bioavailabiliy of Magnesium

Varying amounts of Mg are found in most foods. Good sources of Mg are whole seeds, such as nuts, legumes and unmilled grains, cereal products such as bread, soybeans and green vegetables. Except for bananas, fruits commonly eaten in North America contain relatively little Mg. Refining and cooking can significantly reduce the Mg levels in foods. Such processes as the refining of whole wheat to flour and polishing of rice may remove >80% of the original Mg content, while boiling vegetables can remove >50%. The duplicate portion technique used in this work to collect the diets is advantageous in that foods are analyzed in the forms as they are consumed, thus taking into account possible loss of Mg during cooking or processing. Drinking water contains

variable levels of Mg. Studies in the U.S.A. and Canada estimated that drinking water supplied 9-27% of the DI although this was much lower in soft-water areas (251).

Hazell (6) has extensively reviewed the literature on Mg bioavailability. Absorption of Mg in man varies from 20 to 70% depending on bodily status, amount ingested and other components in the diet. Since bioavailability varies with Mg species, the actual forms in which Mg exists in food is of interest. Mg salts are generally poorly absorbed when ingested and tend to cause diarrhea. In milk, most of the Mg is in true solution with the remainder bound to casein. In cereal grains, most is believed to be bound to phytate which is known to reduce its absorption (108). Brink *et al.* (253) have demonstrated that Mg bound to casein is more available than when it is bound to soybean protein or phytate. Therefore, fortification of cereal products with Mg has been suggested (6). In green stem and leafy vegetables, Mg is primarily bound to chlorophyll which is believed to be more available than phytate-bound Mg. It has been shown that Mg absorption is inversely related to the concentrations of calcium and phosphorous in the diet (366).

3.3.3 Determination of Magnesium by INAA

Magnesium was determined in this work using ^{27}Mg produced by the reaction $^{26}Mg(n,\gamma)^{27}Mg$. The most intense photopeak of ^{27}Mg is located at 843.8 keV, but it was interfered with by the 846.8-keV peak of ^{56}Mn produced by the reaction $^{55}Mn(n,\gamma)^{56}Mn$. There is considerable spectral overlap of the two photopeaks. The alternative photopeak of ^{27}Mg at 1014.4 keV was free from interference and thus used for

the determination of Mg. The 1014.4-keV photopeak is less intense than the 843.8-keV peak, hence the detection limit of Mg obtained by the former is poorer. The sensitivity obtained using the 1014.4-keV photopeak was only 2.69 counts μg^{-1}.

There is a possibility of a contribution to the ^{27}Mg activity from ^{27}Al via the reaction $^{27}Al(n,p)^{27}Mg$. Correction for ^{27}Al contribution can be made using a combination of thermal and epithermal INAA methods. However, experiments with Al comparator standards demonstrated that this was not a significant interference at the levels of Al in the duplicate diets.

Four RMs and SRMs were analyzed for Mg. Results are shown in Table 3.23 and Fig. 3.6. Agreement between measured and certified values was satisfactory. However, RSD of analysis was relatively large. For all RMs except for IAEA H-4 Animal Muscle (which also had the largest concentration), RSD was greater than 12%. An EQA chart, prepared by replicate analysis of IAEA H-9 Mixed Diet is shown in Fig. 3.7.

The data are widely scattered, reflecting the insensitivity of the 1014.4-keV photopeak. All points, however, lie within the warning limits. Although most of the measured values lie above the certified value there is no statistical difference between the average and certified value.

3.3.4 Magnesium Content of Duplicate Diets

Concentrations and daily intakes of Mg in the duplicate diets are given in Table 3.24. Precision was evaluated by replicate analysis of HMFS, HFMC and HMNT.

Table 3.23. Mg content of some RMs, SRMs and trial diet HMFS

Reference material	L_D (ppm)	This work (ppm)	Certified value (ppm)
IAEA H-4 Animal Muscle	320	1150 ± 70[a]	1050 ± 50
IAEA H-9 Mixed Diet	550	890 ± 120[b]	785 ± 55
NIST SRM 1548 Total Diet	480	620 ± 120[b]	556 ± 27
NIST SRM 1577b Bovine Liver	150	540 ± 70[c]	601 ± 28
HMFS Duplicate Diet	510	800 ± 80[a]	-

[a]. Average of three values; [b]. Average of five values; [c]. Average of six values

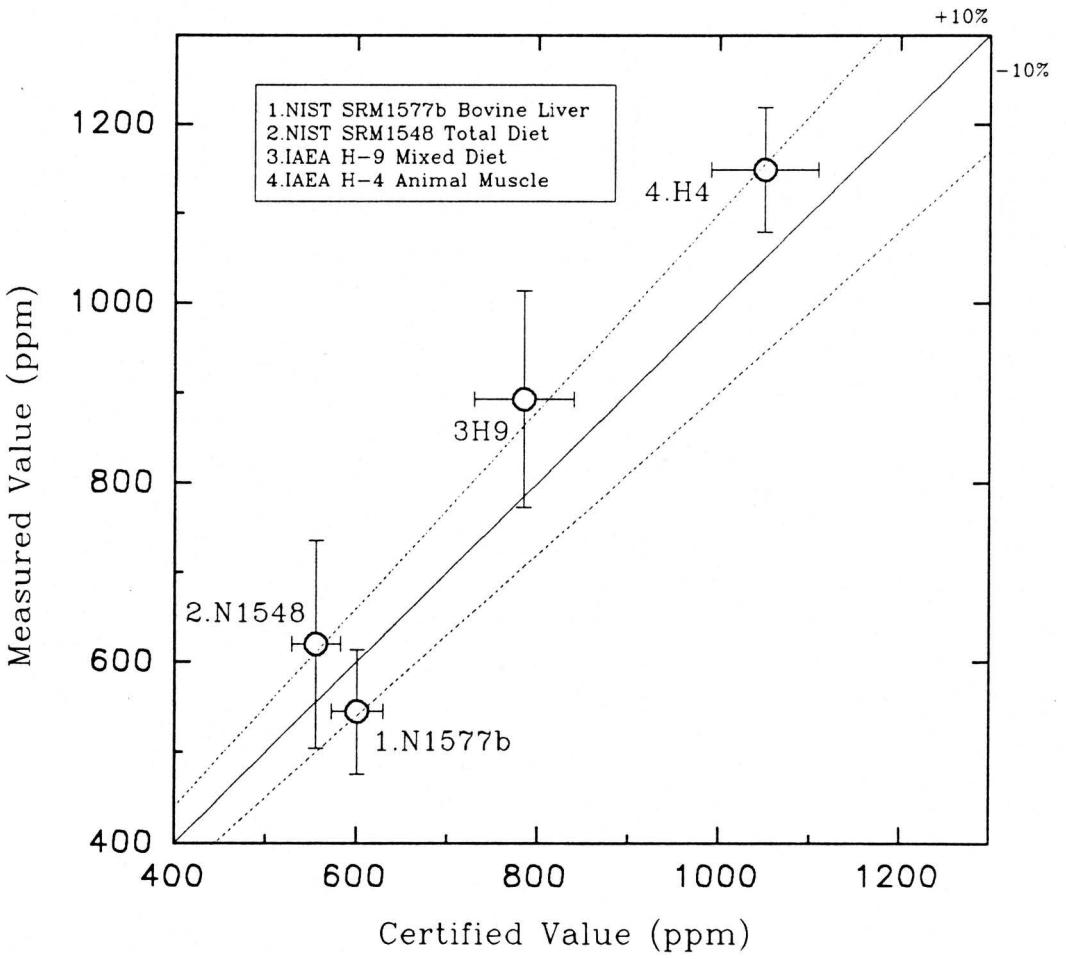


Fig. 3.6. Analysis of RMs and SRMs for magnesium

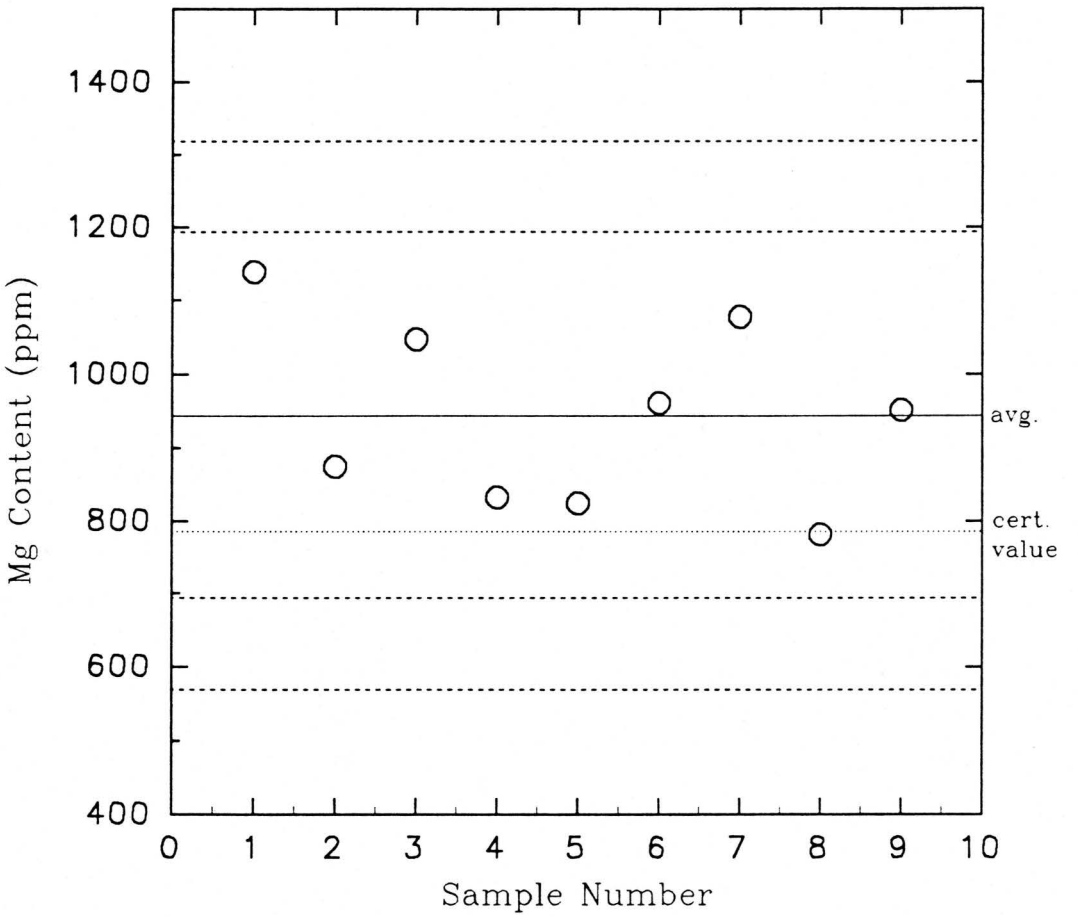


Fig. 3.7. External quality assessment chart for INAA of magnesium using IAEA H-9

Table 3.24. Mg content of duplicate diets determined by INAA and FCT

Sample Code	Content±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})
HMFS	800 ± 80[a]	560 ± 60[a]	
(a) Females			
HFFF	640	220	84
HFMC	460 ± 40[b]	160 ± 20[b]	99
HFNA	720	290	235
HFRR	940	410	255
HFSR	740	350	177
HFTC	1210	430	137
HFWH	660	316	125
Mean	770	310	159
Median	720	320	137
Min.-Max.	460-1210	160-430	84-255
RNI		200	200
RDA		280	280
(b) Males			
HMFF	480	220	156
HMMR	770	210	116
HMNT	1200 ± 40[a]	860 ± 30[a]	394
HMRZ	580	350	225
HMSG	920	420	170
HMTH	830	440	290
HMWV	1420	1140	414
Mean	890	520	252
Median	830	420	225
Min.-Max.	480-1420	210-1140	116-414
RNI		250	250
RDA		350	350
(c) Overall			
Mean	830	420	206
Median	760	350	174
Min.-Max.	460-1420	160-1140	84-414

[a]:average of three values

The RSD values of replicate analyses of the HMFS and HFMC samples were 10%. Since the HMNT sample had a higher Mg content, the RSD value of 4% was lower.

The Mg concentrations in the diets of most of the female participants were between 500 and 700 ppm. The range of concentrations in the men's diets was somewhat broader, from 480 to 1420 ppm. Particularly high concentrations of 1200, 1210 and 1420 ppm were determined in the HFTC, HMNT and HMWV composites, respectively. These diets appear to be relatively high in cereals including whole wheat products, vegetables, and fish (Appendix 1). Median Mg concentrations for the men, women and the whole group were 720, 830 and 760 ppm, respectively.

The mean and median DIs, 310 and 320 mg d^{-1}, of the female participants were lower than the mean and median, 520 and 420 mg d^{-1}, of the male participants. The mean and median Mg intakes agreed more closely for the females, than for the males, suggesting a more symmetric distribution of intakes. The overall mean and median intake were 420 and 350 mg d^{-1}, respectively, with a range of 160 - 1140 mg d^{-1}. Obviously, most participants had DIs in excess of the RNI and RDA values. Only one female, HFMC, and two males, HMFF and HMMR, had DIs below both the RNI and RDA.

Magnesium DI was calculated from FCT by Chatt and Pegg (27). Their results, with means, medians and ranges are tabulated in Table 3.24. Measured DIs are plotted against the calculated values in Fig. 3.8. All calculated values are below the measured values (Table 3.24). Mean and median intakes calculated from FCT are about half of the measured values. A paired t-Test was performed; the t statistic calculated was 2.72 and was greater than the critical t-value of 1.74 at an α of 0.05. This indicates a

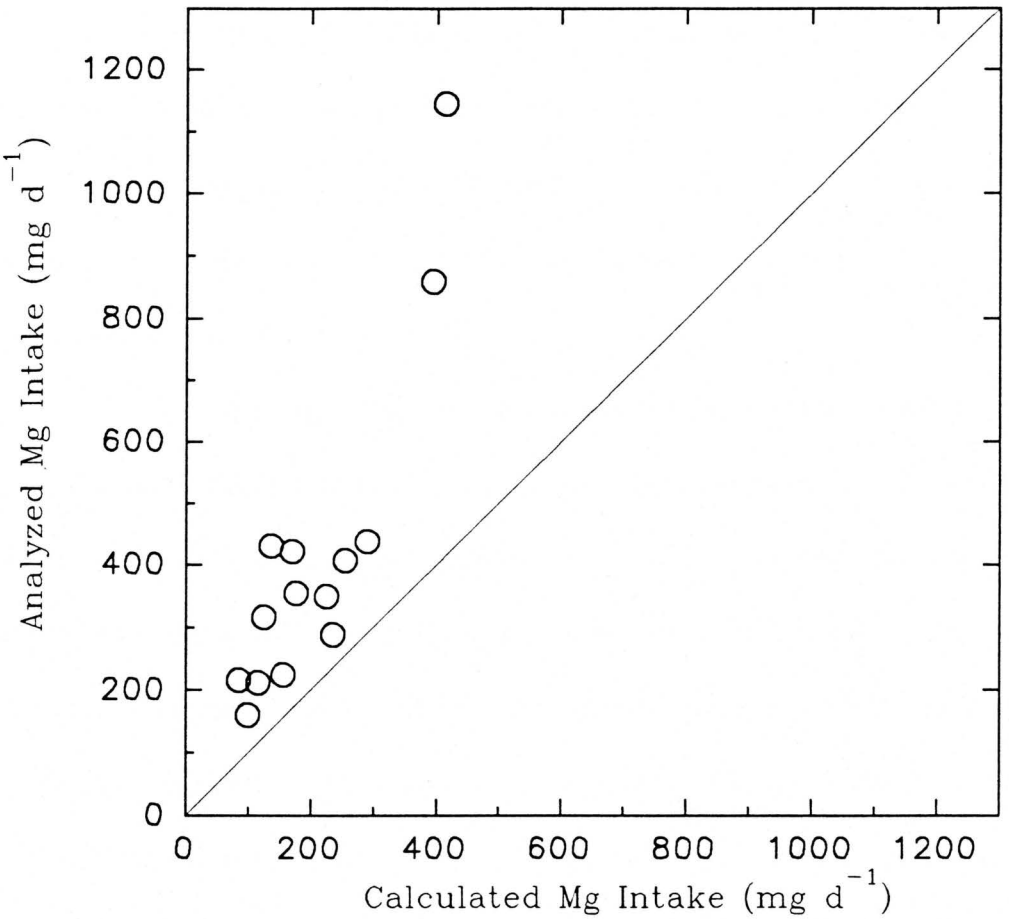


Fig. 3.8 Daily intake of magnesium determined by INAA and calculated using FCT

difference between the means. An ANOVA calculation, performed on the datasets produced an F-value of 7.38 compared to a critical F-value of 3.23 and a P-value of 0.0116, indicating a difference between measured and calculated values. Since the average concentrations determined in three RMs were slightly higher than the certified values, a systematic error is possible, but would only account for about 10% of the discrepancy. Despite the differences, there was a high correlation (correlation coefficient 0.88) between measured and calculated values. These results indicate that the FCT data predicted general trends and relative levels of Mg between diets, but produced values for daily intake which were too low.

According to Health Canada, the average intakes for adults aged 20-39 were 205 mg d^{-1} and 303 mg d^{-1} for females and males, respectively (1). A survey of Mg intakes for several countries is reported in Table 3.25. The main sources of Mg were determined to be cereals (27%), fruits and vegetables (27%) and dairy products (17%). Shah *et al.* determined the Mg content of a "representative" Canadian diet to be 247 mg d^{-1}, based on the analysis of foodstuffs. This value is about 100 mg d^{-1} lower than the value determined in this study. In the U.S., Mg intake has been reported to be about 195 mg d^{-1} for women and 301 mg d^{-1} for men (86). Thus, most North Americans tend to have intakes below the U.S. RDA, but many do meet the Canadian RNI. In the U.S.A., typical intakes for adult men and women are 300 mg d^{-1} and 200 mg d^{-1} (83, 85, 86, 254) with values as low as 125 mg d^{-1} for female college and high school students (255) and as high as 580 mg d^{-1} for male and female college students (256).

In most countries, values range from 200-400 mg d^{-1}. Magnesium intake

Table 3.25. Survey of DDI of Mg

Study Group	Composite	Analytical Method	Intake (mg d^{-1})	Ref.
Belgium				
Military personnel	Cafeteria meals	HGAAS	306	(258)
Hospital patients Antwerp, Liege	Hospital diet	HGAAS	258, 214	(258)
Canada				
General population	Equiv composite	AAS	247	(259)
Finland				
Hospital patients	Hospital diet	GFAAS	360	(97)
Wt. reduction patients	Dupl. portion	AAS	243	(238)
France				
Adult women, men	Equiv. composite	AAS	264, 324	(210)
Germany				
Adult women, men 1988	Market basket	AAS	287, 389	(260)
Adult women, men 1988	Dupl. portion	AAS	193, 247	(260, 261)
Adult women, men 1991	Market basket	AAS	278, 360	(260)
Adult women, men 1991	Dupl. portion	AAS	211, 259	(260, 261)
Adult women, men 1995	Dupl. portion	AAS	214, 296	(261)
Vegetarian women, men	Dupl. portion	AAS	376, 474	(261)
India				
Bombay residents	1/8 Dupl. portion	AAS	740	(262)
Japan				
Adult men	Dupl. portion	AAS/GFAAS, ICP-AES	186, 200	(257)
The Netherlands				
Teen-age males (18 y)	Market basket	AAS	435	(232)
Spain				
Residents, var. regioms	Equiv. composite	AAS	379-388	(263)
Sweden				
Female, male pensioners	Dupl. portion	AAS	170, 210	(90)
Women (high b.p.)	Dupl. portion	AAS	270	(264)
Women (low b.p.)	Dupl. portion	AAS	243	(264)
Female, male Vegans	Dupl. portion	AAS	468, 615	(91)
Female, male lactoveg.	Dupl. portion	AAS	350, 505	(93)
Adult men and women	Dupl. portion	AAS	243	(2)
General population	Market basket	AAS	300	(98)
Taiwan				
University faculty, staff, students	Dupl. portion	INAA	160, 190, 140	(43)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	250	(240)
U.S.A.				
Female students	Dupl. portion	emission spec.	125	(255)
Hospital patients-summer, winter	Hospital diet	emission spec.	30, 385	(265)

Table 3.25. (continued) Survey of DDI of Mg

Study Group	Composite	Analytical Method	Intake (mg d^{-1})	Ref.
Female univ. students	Metabolic diet	emission spec.	202	(266)
50-60 yr. old men and women	planned menu (25% calories from fat)	emission spec.	199	(267)
50-60 yr. old men and women	planned menu (35% calories from fat)	emission spec.	277	(267)
Teen-age males	Equiv. composite	INAA	<495	(78)
Male & female students	Cafeteria meals	AAS	580	(256)
Hospital patients	Hospital diet	AAS	302	(268)
Hospital patients	Ovo-lacto veg. hospital diet	AAS	316	(268)
Adult male volunteers	Metabolic diet	GFAAS	447	(269)
Adult male volunteers	Low- fibre metabol. diet w/spinach	AAS	304	(254)
Adult male volunteers	High-fibre metabol. diet w/spinach	AAS	362	(254)
Male volunteers	High-fibre metabol. diet no spinach	AAS	336	(254)
Teen-age male, 1976	Market basket	AAS	354	(81)
Teen-age male, 1977	Market basket	AAS	328	(81)
Teen-age male, 1980	Market basket	AAS	326	(81)
Teen-age male, 1981/82	Market basket	AAS	343	(81)
Teen-age female, male1982-84	Market basket	ICP-AES	193, 296	(83)
Adult female, male 1982-84	Market basket	ICP-AES	187, 288	(83)
Adult males (USDIET-1)	Market basket	FAAS/ICP-AES/INAA	305	(195)
Teen-age female, male1984-85	Market basket	ICP-AES	196,298	(85)
Adult female, male 1984-85	Market basket	ICP-AES	193, 301	(85)
Teen-age female, male1985-86	Market basket	ICP-AES	199, 305	(85)
Adult female, male 1985-86	Market basket	ICP-AES	196, 303	(85)
Adult men	Dupl. portion	AAS	304	(270)
Teen-age female, male1982-89	Market basket	ICP-AES	197, 302	(86)
Adult female, male 1982-89	Market basket	ICP-AES	195, 301	(86)

of Bombay, India residents (740 mg d^{-1}), Swedish Vegans (females 468 mg d^{-1}, males 615 mg d^{-1}) and lactovegetarians (females 350 mg d^{-1}, males 505 mg d^{-1}) and Dutch teen-age males (435 mg d^{-1}) were reported to be particularly high. On the other hand, Taiwanese Mg intake appears to be quite low, with values of 140-190 mg d^{-1} for three study groups within a university (43). Intakes for Japanese men, 200 mg d^{-1} (257), and Swedish pensioners, females 170 mg d^{-1} and males 210 mg d^{-1} (90), were also lower than most others.

3.3.5 Dialysis of Duplicate Diets for Magnesium

The dialysis procedure for the duplicate diets was previously described in Chapter 2 and in section 3.1.10. Dialyzed diet samples were analyzed for Mg using the 1014.4-keV gamma-ray of ^{27}Mg. A 5-min irradiation was followed by 1-min decay and 5-min counting. The sensitivity was 3.55 counts μg^{-1}. The detection limit for dialyzed HMFS was 60 ppm, almost one tenth of the detection limit of the undialyzed diet. The improvements in sensitivity and detection limit were attributed to the lower spectral background and increased irradiation time made possible by the removal of Cl and Na by dialysis. Blank dialysis tubes immersed in DDW for 24 h, contained about 40 ppm Mg.

The measured Mg concentration in the residue, and calculated percent non-dialyzable and percent dialyzable are reported for one RM and three SRMs, and the diets in Tables 3.26 and 3.27, respectively. Of the RMs, NIST SRM 1548 Total Diet had the lowest percent non-dialyzable, 21% and NIST SRM 1568 Rice Flour had the highest, 70%. The other two materials, IAEA H-9 Mixed Diet and NIST SRM 1577b Bovine

Table 3.26. Dialysis of some RMs and SRMs for magnesium

Material	**Conc. (residue)**	**% non-dialyzable**	**% dialyzable[a]**
IAEA H-9 Mixed Diet	370	42	58
NIST SRM 1548 Total Diet	128	21	79
NIST SRM 1568 Rice Flour	330	70	30
NIST SRM 1577b Bovine Liver	296	54	46

[a] calculated by difference.

Table 3.27. Dialysis of duplicate diet composites for magnesium

Sample Code	Conc. in residue (ppm)	%non-dialyzable	%dialyzable[a]
HMFS	370 ± 30	46±9	54±9
(a) Females			
HFFF	140	22	78
HFMC	250 ± 40	54±14	46±14
HFNA	290	40	60
HFRR	220	24	76
HFSR	290	39	61
HFTC	560	47	53
HFWH	230	35	65
Mean	280	37	63
Median	250	39	61
Min. - Max.	140-560	22-54	46-78
(b) Males			
HMFF	340	71	29
HMMR	160	20	80
HMNT	580	49±8	51±8
HMRZ	290	50	50
HMSG	90	10	90
HMTH	290	35	65
HMWV	430	30	70
Mean	310	38	62
Median	290	35	65
Min. - Max.	90-580	10-71	29-90
(c) Overall			
Mean	298	38	62
Median	290	37	63
Min. - Max.	91-583	10-71	29-90

Liver had values of percent non-dialyzable of 42 and 54%. Lowest of the diets in terms of percent Mg in the residue was HMSG, 10%, whereas the highest was HMFF, 71%. The median value of the whole group was 37%. A correlation analysis of the percent dialyzable food group mass fractions (Appendix 1) yielded no strong correlations (Table 3.28). There were two very weak correlations (0.4) with fish, seafoods and seafood products, and with fruits and berries. Although an attempt was made to measure Mg in the enzymolyzed/dialyzed samples, no gamma-ray peaks were detected at 1014.4 keV.

Table 3.28. Correlation of percent non-dialyzable Mg with mass fractions of various food groups.

	Correlation Coefficient
%Mg non-dialyzable	1
Cereal Products/Nuts	0.0519
Vegetables	-0.25989
Fruits & Berries	0.3867
Meat & Meat Products	0.1891
Fish, Seafoods & Products	0.4118
Milk products (liquids)	-0.08243
Cheese	-0.29274
Food Fats	-0.09021
Fluids (excl. fr. & veg. juices)	-0.1709
Other Foods	-0.0664
Tea/Coffee	-0.00404
Soft Drinks	0.0525
Mineral Water	-0.20644
Wine	-0.06086

3.4 MANGANESE

3.4.1 Nutritional and Toxicological Aspects of Manganese

Several review articles on the subject of Mn in nutrition and health have been published (1, 6, 12, 17, 134, 241, 252). Manganese is considered an essential element for animals and humans. It is a constituent of metalloenzymes and a non-specific activator of enzymes. The enzymes arginase, pyruvate carboxylase, glutamine synthetase and Mn superoxide dismutase all contain and require Mn for normal function. Manganese can activate many of the same enzymes also activated by Mg including kinases, decarboxylases, hydrolases and transferases. Processes in which Mn is involved include mucopolysaccharide synthesis, collagen formation, synthesis of fatty acids and cholesterol, synthesis and digestion of proteins, and bone formation and development.

Deficiency of Mn in humans and animals consuming natural diets is uncommon since Mn is abundant in edible plant materials. Deficiency has been induced in experimental animals. Symptoms of deficiency include impaired growth, skeletal abnormalities, disturbed reproductive function, and defects in lipid and carbohydrate metabolism. Manganese-deficient rats have a tendency toward convulsions and electroencephalographic abnormalities.

Definitive evidence of Mn deficiency has not been found in humans although possible cases of deficiency in individuals who were fed low-Mn diets have been cited (134, 252). Saltman and Strause (271, 272) concluded that Mn was essential to

bone development and maintenance, suggesting that Mn status may be related to bone mineral loss in osteoporosis. Due to insufficient data, requirements for Mn have not been firmly established. The requirement has been estimated to be as low as 0.74 mg d^{-1}, although there are higher estimates of about 3.5 mg d^{-1} as well. Therefore, Health Canada was not able to establish firm recommendations (1). Similarly, the U.S. FNB has not established an RDA, but did set a provisional SADDI of 2.0 - 5.0 mg d^{-1} (17).

Very high levels of Mn , *i.e.* > 1 mg/g (4), are required to produce toxic symptoms. Much smaller amounts are toxic by inhalation of dust or injection. Toxic symptoms include neuropsychiatric abnormalities such as hallucinations, delusions and compulsions, and Parkinson's disease-like symptoms. In addition, growth and appetite may be depressed and Fe metabolism may be impaired. Excessive Mn intake can also cause anemia due to interferences with Fe absorption.

3.4.2 Dietary Sources, Intakes, and Bioavailability of Manganese

The highest levels of Mn are found in tea, whole grains and cereal products. Fruits and vegetables contribute smaller amounts whereas drinking water contributes very little. Dairy products, meat, fish and poultry are poor sources of Mn. Most Mn in the Canadian diet is derived from fruit and grain products (73, 273). Mean DI of adult Canadians is about 3-3.8 mg d^{-1} while for the U.S. it is 2.14-2.81 mg d^{-1} (86).

After ingestion, Mn is absorbed throughout the small intestine. Absorption is about 2-8% (1, 274, 275). The actual fractional amount absorbed depends on a number of factors. Lactose, ascorbic acid, ethanol, and meat in the diet appear to enhance Mn

availability. At high Mn intakes, however, ascorbic acid may decrease availability. Iron, Ca and dietary fibre may also decrease Mn availability. Therefore, Mn in cereals may have low availability since it is primarily bound to phytate. However, Davidsson *et al.* (274) found that phytate, phosphate and ascorbic acid added to infant formula and Fe and Mg added to wheat bread had no affect on absorption of Mn. The relatively large amounts of Mn in tea are largely unavailable to humans.

3.4.3 Determination of Manganese by INAA

In the present work, the INAA of the duplicate diets for short and medium-lived nuclides was originally performed using both irradiation and decay times of 1 min and counting time of 10 min. These conditions represented a compromise and were generally not optimized for specific elements. In these experiments, Mn was determined using the 1810.7-keV photopeak of ^{56}Mn (half-life = 2.58 h) produced by the reaction ^{55}Mn(n,γ)^{56}Mn. This peak is free from interference under the experimental conditions used, but is not as intense as the 846.8-keV peak of ^{56}Mn. Unfortunately, the latter peak is subject to interference from the 843.8-keV peak of ^{27}Mg (half-life = 9.5 min). However, a decay time of more than 1.5 h allows the 843.8-keV peak of the shorter-lived ^{27}Mg to decay almost completely permitting integration of the area under the 846.8-keV peak of ^{56}Mn without interference and for a. longer counting time. A drawback to this approach is increased time and effort required for the analysis, since an extra gamma-ray spectrum must be collected and a decay correction must be calculated for each sample. However, this limitation is more than compensated by a substantial improvement in

sensitivity, *viz.* 172 counts μg^{-1} using the 1810.7-keV peak and a 10-min count compared to 3094 counts μg^{-1} using the 846.8-keV peak and a 30-min count. The Mn concentrations of the duplicate diets reported here were determined using 1.5-min irradiations, followed by 1.5-7h decays, and 30-min counts employing the 846.8-keV peak of ^{56}Mn. The detection limit for duplicate diet HMFS was 0.45 ppm.

The Mn content and detection limits of four RMs and SRMs are shown in Table 3.29 and Fig. 3.9. Agreement between measured and certified values was very good, except for NIST SRM 1568 Rice Flour, in which the measured value was slightly lower than the certified value. A careful examination of the original data and documented irradiation conditions was performed, but no experimental error was found to account for this discrepancy. The very good agreement between measured and certified values for the other four RMs does suggest that the accuracy of the method is high. Precision of analysis was good as indicated by the low RSD (10% or less).

An EQA chart for Mn analysis, prepared by replicate analysis of IAEA H-9 Mixed Diet is shown in Fig. 3.10. All values lie within the warning limits of ±2s. Precision is good. All values lie above the certified value, but well within the confidence limits of the certified value. The EQA chart results indicate the good quality of the analytical procedures maintained during the analysis of the duplicate diet samples.

3.4.4 Manganese Content of Duplicate Diets

The Mn content of duplicate diets determined by INAA is presented in Table 3.30. The RSD for replicate analyses of diets HMFS, HFMC and HMNT were

Table 3.29. Manganese content of some RMs, SRMs and trial diet HMFS

Reference material	L_d (ppm)	This work (ppm)	Certified value (ppm)
IAEA H-4 Animal Muscle	0.21	0.59 ± 0.06[a]	0.52 ± 0.037
IAEA H-9 Mixed Diet	0.45	12.2 ± 0.1[a]	11.8 ± 0.6
NIST SRM 1548 Total Diet	0.39	5.0 ± 0.4[a]	5.2 ± 0.4
NIST SRM 1568 Rice Flour	0.089	18.23 ± 0.09[a]	20.1 ± 0.4
NIST SRM 1577b Bovine Liver	0.54	9.4 ± 0.2[b]	10.5 ± 1.7
Duplicate diet HMFS	0.45	9.0 ± 0.9[a]	-

[a]. Average of three values; [b]. Average of six values

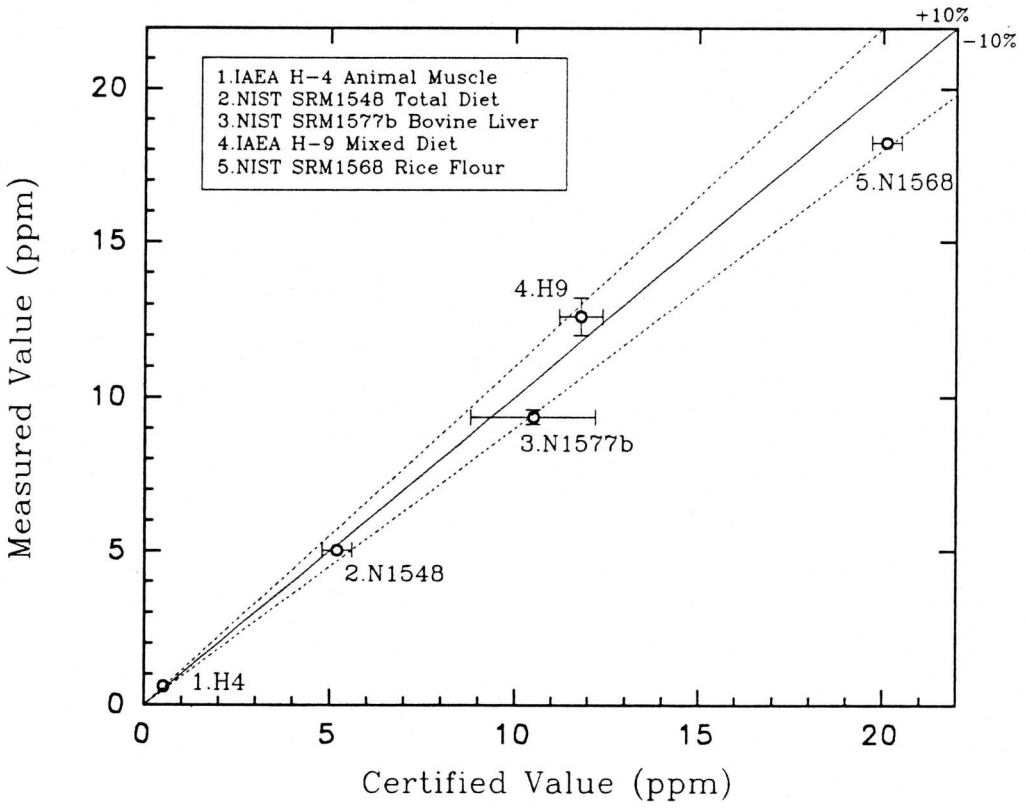


Fig. 3.9.Analysis of RMs and SRMs for manganese

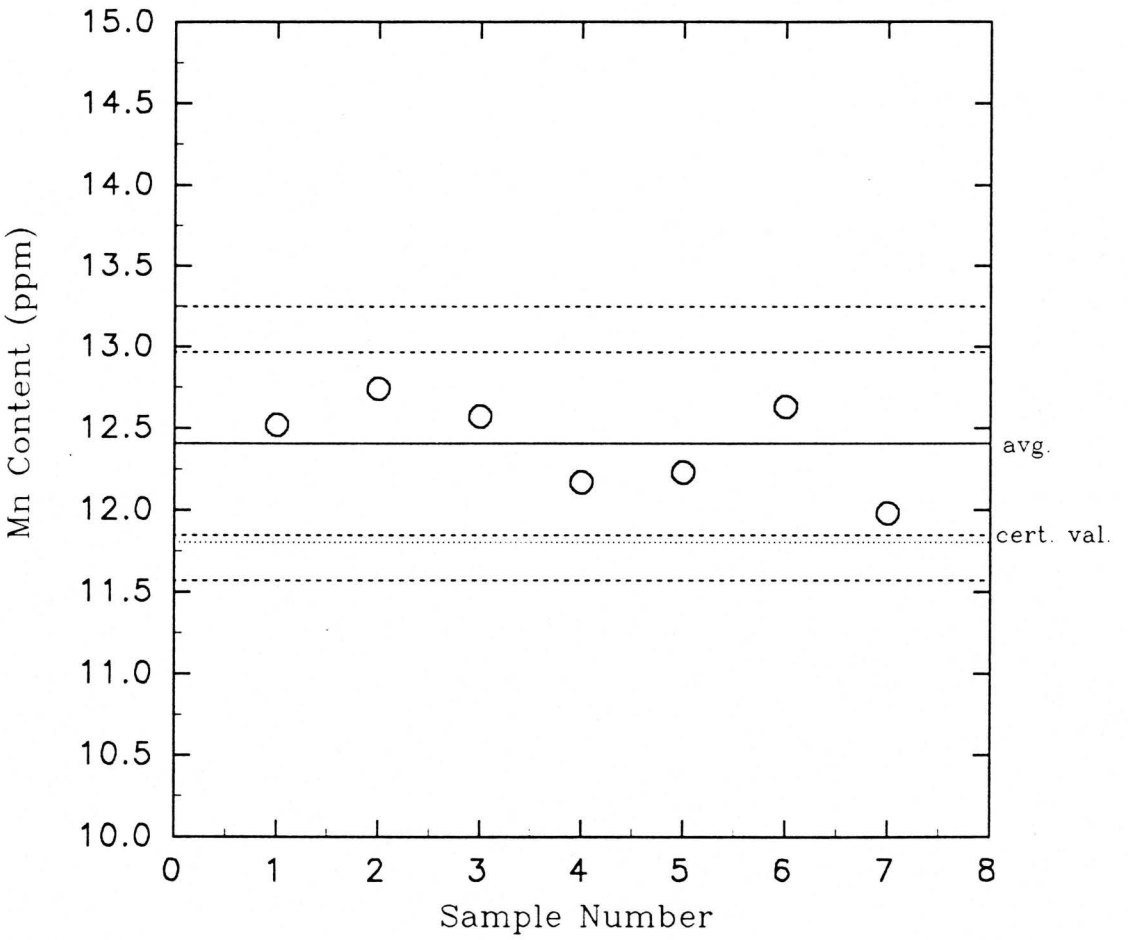


Fig. 3.10. External quality assessment chart for INAA of manganese

Table 3.30. Mn Content of Duplicate Diet Composites determined by INAA

Sample Code	Content±SD (ppm)	DI (mg d^{-1})
HMFS	9.0 ± 0.9[a]	6.3 ± 0.6[a]
(a) Females		
HFFF	8.6	2.9
HFMC	10.3 ± 0.1[a]	3.6 ± 0.05[a]
HFNA	7.1	2.8
HFRR	8.1	3.5
HFSR	7.6	3.6
HFTC	11.8	4.2
HFWH	8.0	3.8
Mean	8.8	3.5
Median	8.1	3.6
Min. - Max.	7.1 - 11.8	2.8 - 4.2
(b) Males		
HMFF	4.0	1.9
HMMR	5.8	1.6
HMNT	16.1 ± 0.5[a]	11.5 ± 0.4[a]
HMRZ	5.8	3.5
HMSG	9.2	4.2
HMTH	8.9	4.7
HMWV	10.9	8.8
Mean	8.7	5.2
Median	8.9	4.2
Min. - Max.	4.0 - 16.1	1.6 - 11.5
SADDI (U.S.)		2.0 - 5.0
(c) Overall		
Mean	8.7	4.3
Median	8.4	3.6
Min. - Max.	4.0 - 16.1	1.6 - 11.5

[a]:Average of three values

10%, 1% and 3%, respectively. Precision, as assessed by RSD of these three diets, is considered to be very good.

Most of the diets had Mn concentrations within the range of 7 to 11 ppm (Table 3.30). The male diets had the most variability, with low concentrations of 4.0, 5.8 and 5.8 ppm in HMFF, HMMR and HMRZ diets, respectively, and a high concentration of 16.1 ppm in the HMNT composite. The highest concentrations were 10.3, 11.8 and 16.1 ppm for HFMC, HFTC and HMNT, respectively. These diets tended to be high in cereals (especially whole grain), vegetables, and tea and coffee (see Appendix 1). There was little difference between mean and median values. The median concentrations were 8.1, 8.9 and 8.4 ppm, for the women, men and overall, respectively.

The range of intakes was quite wide, with a minimum of 1.6 mg d^{-1} and a maximum of 11.5 mg d^{-1}. In general, the female diets were more consistent than the male diets. The range for the women, only 1.4 mg d^{-1}, was much smaller than the 9.9 mg d^{-1} range for the men. Mean and median DI were in fairly close agreement. Median Mn intake for men and women were 3.2 mg d^{-1} and 3.6 mg d^{-1}, respectively. The overall median intake was 3.6 mg d^{-1}.

A survey of DDI values for Mn is reported in Table 3.31. Canadians' Mn intakes have been reported from 1.877 mg d^{-1} (259) to 7.7 mg d^{-1} (70). Most values lie between 2.9 and 3.8 mg d^{-1} (70, 73-77, 259, 276). Kirkpatrick and Coffin reported DI of Mn for Halifax residents to be 2.954 mg d^{-1} in 1974 (75). The intake determined in this study falls in this range.

Most values in the U.S.A. fall between 2 and 4 mg d^{-1}, as shown in Table

Table 3.31. Survey of Average DDI of Mn

Study Group	Composite Method	Analytical	Intake (mg d^{-1})	Ref.
Australia				
Adult female (w/o tea, w/ tea)	Equiv. composite	INAA	4.06, 6.65	(280)
Belgium				
General population	Hospital meals & Dupl. portion	AAS	3.1	(281)
Hosp. patients/militar. personnel	Hospital/military	AAS	3.1	(282)
Benin				
General population	Equiv. composite	AAS	3.122	(202)
Canada				
General Population	Market basket	AAS	4.091	(74)
General Population	Market basket	AAS	2.930	(75)
Vancouver residents	Market basket	AAS	3.648	(75)
Halifax residents	Market basket	AAS	2.954	(75)
Winnipeg residents	Market basket	AAS	2.930	(77)
Female university students	Duplicate portion	AAS	2.4	(276)
Elderly women	Duplicate portion	INAA	3.8	(76)
Elderly women	Duplicate portion	INAA	7.7	(70)
Elderly women	Duplicate portion	INAA	3.8	(73)
General population	Equiv composite	AAS	1.877	(259)
Denmark				
Adult men	Duplicate portion	ICP-MS	3.9	(207)
Finland				
Hospital patients	Hospital diet	GFAAS	6.0	(97)
Wt. reduction patients	Duplicate portion	AAS	3.6	(238)
France				
Adult women, men	Equiv. composite	AAS	1.7	(210)
India				
Bombay residents	1/8 Dupl.. portion	AAS	8.3	(262)
Hospital patient	Hosp. vegetar. diet	INAA	17.3	(218)
Japan				
Adult men	Duplicate portion	AAS/GFAAS	3.75	(257)
Adult men	Duplicate portion	ICP-AES	3.4	(302)
The Netherlands				
Men & women, 1976-78	Duplicate portion	AAS/GFAAS	3.8	(283)
Men & women, 1984-85	Duplicate portion	AAS/GFAAS	3.3	(283)
New Guinea				
Female Wosera residents	Equiv. composite	INAA	7.1-10.1	(277)
Male Wosera residents	Equiv. composite	INAA	8.0-11.8	(277)
New Zealand				
Female students	Metabolic Diet	AAS	2.78	(284)
Female students	Duplicate portion	AAS	2.7	(285)

Table 3.31. (continued) Survey of Average DDI of Mn

Study Group	Composite Method	Analytical	Intake (mg d^{-1})	Ref.
Pakistan				
Islamabad residents	Equiv. composite	INAA	5.8-6.2	(228)
Scotland				
General population	Equiv. composite	PNAA	5.4	(279)
Spain				
Company employees	Cafeteria lunches	AAS	4.0	(286)
University students	Cafeteria meals	FAAS	2.19	(287)
Sweden				
General population	Market basket	AAS	3.7	(98)
Taiwan				
Univ. faculty,staff,students	Duplicate portion	INAA	2.29, 3.03, 2.35	(43)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	2.674	(240)
General population	Equiv. composite	AAS	4.6	(278)
U.S.A.				
Female students	Duplicate portion	emission spec.	<0.24-1.53	(255)
Hosp. Patients- summer, winter	Hospital diet	emission spec.	0.88, 1.78	(265)
Female university students	Metabolic diet	emission spec.	1.22	(266)
Men and women planned menu (25, 35% cal. fr. fat)		emission spec.	3.14, 3.69	(267)
Teen-age males	Market basket	INAA	<6.8	(78)
Male & female students	Cafeteria meals	AAS	3.35	(256)
Male metabolic unit patients	Metabolic diet	AAS	2.130	(288)
Military personnel	Dining hall meals	AAS	2.5	(289)
Hospital patients	Normal, vegetar. diet	AAS	2.77, 3.22	(268)
Teen-age male, 1976-1982	Market basket	AAS	3.43-3.68	(81)
Teen-age female, male1982-84	Market basket	ICP-AES	1.76, 2.74	(83)
Adult female, male 1982-84	Market basket	ICP-AES	2.05, 2.72	(83)
Adult males (USDIET-1)	Market basket	FAAS/ICP-AES/INAA	2.740	(195)
Teen-age female, male1984-85	Market basket	ICP-AES	1.83, 2.86	(85)
Adult female, male 1984-85	Market basket	ICP-AES	2.16, 2.86	(85)
Teen-age female, male1985-86	Market basket	ICP-AES	1.82, 2.82	(85)
Adult female, male 1985-86	Market basket	ICP-AES	2.22, 2.90	(85)
Teen-age female, male1982-89	Market basket	ICP-AES	1.78, 2.76	(86)
Adult female, male 1982-89	Market basket	ICP-AES	2.14, 2.81	(86)
Adult men	Duplicate portion	AAS	4.0	(270)
West Germany				
Adult men	Duplicate portion	PNAA	2.7	(101)
Adult female	Duplicate portion	INAA	3.56	(102)
Karlsruhe/Stuttgart residents				
Adult women, men	Duplicate portion	AAS	2.2, 3.0	(250)

3.31. The U.S. F.D.A. estimated the Mn intake in teen-age males to range from 3.43 mg d^{-1} to 3.68 mg d^{-1} in studies from 1976 to 1981/82 (81). Lower values, of 2.8 mg d^{-1} and 1.8 mg d^{-1} were obtained for teen-age males and females after the reorganization of the Total Diet Study (86). For adult males and females, the Mn intake estimated in the U.S. Total Diet Study was 2.8 mg d^{-1} and 2.1 mg d^{-1} (86). Low values of 0.88 mg d^{-1} and 1.78 mg d^{-1} were reported by Gormican *et al* (265) for hospital diets, perhaps representing the use of highly processed foods. The highest intakes are generally reported for teen-age males, although one of the highest intakes was reported by Holbrook *et al.* who determined the content in duplicate portions of adult men to be 4.0 mg d^{-1} (270).

Manganese intakes in many countries outside North America also fall between 2 and 4 mg d^{-1} as shown in Table 3.30. In countries where plant foods and tea comprise a large portion of the diet, the DIs can be relatively high, *e.g.* 17.3 mg d^{-1} in India (262), 7.1-11.8 mg d^{-1} in New Guinea (277), and 6 mg d^{-1} in Pakistan (228). Manganese intake can also be relatively high in other countries where tea drinking is common, 6.65 mg d^{-1} in Australia (280), 4.6 mg d^{-1} in Great Britain (278), and 5.4 mg d^{-1} in Scotland (279). Manganese intake in France (women: 1.7 mg d^{-1} and men: 2.1 mg d^{-1}) has been reported to be lower than in most countries (210), perhaps due to the decreased consumption of plant foods.

3.4.5 Dialysis and Enzymolysis of Duplicate Diets for Manganese

The duplicate diet samples were subjected to dialysis and enzymolysis. Manganese was measured in dialyzed samples using an irradiation time of 5 min,

followed by a decay time of 1.5-7 h and a counting time of 30 min. The sensitivity was 6588 counts μg^{-1} and the detection limit of HMFS was 0.043 ppm. The sensitivity was increased due to a longer irradiation time, and the detection limit was improved over un-dialyzed samples due to lower background activity in the spectra.

The results of dialysis of RMs, SRMs, and duplicate diets are shown in Tables 3.32 and 3.33, respectively. The highest percent dialyzed Mn was 69% in NIST SRM 1548, whereas the lowest value of 30% was in NIST SRM 1568 Rice Flour (Table 3.32). The duplicate diet with the most dialyzable Mn of 81% was HFFF and the lowest was HFTC with 28%. The overall median was 44%. A correlation analysis between the percent dialyzable Mn and the mass fractions of the various food groups was performed Table 3.34). The only significant correlation of 0.51 was with vegetables.

The duplicate diet samples were also subjected to enzymolysis/dialysis. The samples were then analyzed using 5-15s irradiations, 1.5-7h decays and 30-min counts. Longer irradiation times were not used due to high dead times caused by ^{38}Cl and ^{24}Na activities. Under these conditions, the sensitivity was 259 counts μg^{-1}. The digestion blanks contained 4 ± 1 µg of Mn. Since the samples typically contained about 11 µg, the blank was significant.

Table 3.35 shows the results for NIST SRM 1548 Total Diet, 1568 Rice flour, and 1577b Bovine Liver. The percent potentially available Mn was very high for the Rice Flour, but was very low for the Total Diet and Bovine Liver SRMs. Results for freeze-dried duplicate diet and wet diet slurry are presented in Table 3.36 and 3.37, respectively. The RSD of the Mn concentrations of the dialysis residue were quite high,

Table 3.32. Dialysis of RMs and SRMs for manganese

Material	Conc. in residue (ppm)	%non-dialyzable	%dialyzable[a]
IAEA H-9 Mixed Diet	5.83	47.8	52.2
NIST SRM 1548 Total Diet	1.54	30.6	69.4
NIST SRM 1568 Rice Flour	12.8	70.1	29.9
NIST SRM 1577b Bovine Liver	5.52	59.0	41.0

Table 3.33. Dialysis of duplicate diets for manganese

Sample Code	Conc. in residue (ppm)	%non-dialyzable	%dialyzable[a]
HMFS	6.1 ± 0.5	68±12	32±12
(a) Females			
HFFF	1.7	19	81
HFMC	5.0 ± 1.3	49±13	51±13
HFNA	2.9	41	59
HFRR	2.7	33	67
HFSR	3.0	38	62
HFTC	8.5	72	28
HFWH	4.8	60	40
Mean	4.1	45	55
Median	3.0	41	59
Min. - Max.	1.7-8.5	19-72	28-81
(b) Males			
HMFF	2.7	67	33
HMMR	2.4	41	59
HMNT	10 ± 1	64±10	36±10
HMRZ	2.8	47	53
HMSG	3.5	38	62
HMTH	3.6	41	59
HMWV	6.8	63	37
Mean	4.6	52	48
Median	3.5	47	53
Min. - Max.	2.4-10	38-72	33-62
(c) Overall			
Mean	4.3	48	52
Median	3.2	44	56
Min. - Max.	1.7-10	19-72	28-81

Table 3.34. Correlation analysis between %non-dialyzable Mn and food group mass fractions.

	Mn non-dial.
Mn non-dial.	1
Cereal Products/Nuts	-0.0017
Vegetables	0.51455
Fruits & Berries	-0.232
Meat & Meat Products	0.11782
Fish, Seafoods & Products	-0.0001
Milk products (liquids)	-0.3231
Cheese	-0.0685
Food Fats	0.10921
Fluids (excl. fr. & veg. juices)	0.26832
Other Foods	-0.1626
Tea/Coffee	-0.0093
Soft Drinks	-0.2285
Mineral Water	0.31429
Wine	0.44182

Table 3.35. Enzymolysis of some RMs and SRMs for manganese

Sample	Not Potentially Available		Potentially Available[a]	
	Conc. ± SD (ppm)	%Non-diayzed±SD	Daily Intake (mg d^{-1})	%Dialyzed ± SD
NIST SRM 1548 Total Diet	5.7	114%		-14%
NIST SRM 1568 Rice Flour	2.0	11%		89%
NIST SRM 1577b Bovine Liver	9.4	100%		0%

[a]:calculated by difference

Table 3.36. Enzymolysis of freeze-dried duplicate diet composites for manganese

Sample	Not Potentially Available		Potentially Available[a]	
	Conc. ± SD (ppm)	%Non-diayzed±SD	Daily Intake (mg d^{-1})	%Dialyzed ± SD
HMFS	6.7 ± 0.7	75 ± 15	1.6 ± 1.1	25 ± 15
(a) Females				
HFFF	5.9	69	0.90	31
HFMC	10.5	101	-0.04	-1
HFNA	5.7	81	0.55	19
HFRR	4.4	55	1.6	45
HFSR	4.6	60	1.4	40
HFTC	13.6	116	-0.66	-16
HFWH	5.8	72	1.0	27
Mean	7.2	79	0.69	21
Median	5.8	73	0.90	27
Min.-Max.	4.4-13.6	55-120	-0.66-1.6	-20-45
(b) Males				
HMFF	1.8	45	1.0	55
HMMR	2.4	41	0.93	59
HMNT	11.5 ± 2.5	71 ± 18	3.3 ± 2.1	29 ± 18
HMRZ	5.2	89	0.38	11
HMSG	5.1	55	1.9	45
HMTH	5.2	58	1.9	42
HMWV	7.4	68	2.8	32
Mean	5.5	61	1.8	39
Median	5.2	58	1.9	42
Min.-Max.	1.8-13.6	41-116	0.38-3.3	11-59
(c) Overall				
Mean	6.4	70	1.2	30
Median	5.5	68	1.0	32
Min.-Max.	1.8-13.6	41-120	-0.6-3.3	-16-59

[a]:calculated by difference

Table 3.37. Enzymolysis of wet duplicate diet composite slurry for manganese

Sample	Not Potentially Available		Potentially Available[a]	
	Conc. ± SD (ppm)	%Non-diayzed±SD	Daily Intake (mg d^{-1})	%Dialyzed ± SD
HMFS	4.3 ± 0.7	48 ± 13%	3.3 ± 1.2	52 ± 13
(a) Females				
HFFF	6.5	76	0.70	24
HFMC	9.5 ± 3.8	92	0.28 ± 1.4	8 ± 38
HFNA	3.4	47.7	1.5	52
HFRR	5.4	66	1.2	34
HFSR	6.1	79	0.75	21
HFTC	6.5	55	1.9	45
HFWH	13.4	168	-2.6	-68
Mean	7.2	84	0.53	16
Median	6.5	76	0.75	24
Min.-Max.	3.4-13.4	48-168	-2.6-1.9	68-52
(b) Males				
HMFF	2.2	55	0.85	45
HMMR	4.5	79	0.34	21
HMNT	12.6 ± 4.2	78 ± 29	2.5	22 ± 29
HMRZ	5.0	86	0.50	14
HMSG	8.0	87	0.54	13
HMTH	6.7	76	1.1	24
HMWV	6.4	59	3.6	41
Mean	6.5	74	1.4	26
Median	6.4	78	0.85	22
Min.-Max.	2.2-13.4	55-168	0.34-2.52	13-45
(c) Overall				
Mean	6.9	79	0.94	21
Median	6.4	77	0.80	29
Min.-Max.	2.2-13.4	48-168	-2.6-3.6	-68-52

[a]:calculated by difference

from 10 to 40%. Thus, the uncertainties calculated for the %non-dialyzed and the %dialyzed were very high. These results, then should only be considered to be qualitative. Some diets had negative values for %dialyzed representing a higher concentration in the residue after digestion/dialysis than was in the original sample. This was most likely due to variability in the amount of Mn contributed by the reagents. Most of the diets had %potentially available in the range of 20-50%, the overall median values for freeze-dried powder and wet slurry being 32 and 29%, respectively. Most of the %dialyzed values were higher for the freeze-dried diet samples than for the slurry, perhaps due to smaller particle size resulting from the extra homogenization step used after freeze-drying.

3.5 ALUMINUM

3.5.1 Aluminum and Health

Aluminum is not known to be an essential element for humans. Furthermore, there has been public concern about Al exposure due to its adverse biological effects. There has been considerable concern, debate and study about the role of Al in the initiation and progression of Alzheimer's disease (1, 134, 290, 291). The nutritional and toxicological aspects of Al have been reviewed by Health Canada (1), Greger (292) and WHO (134). Aluminum toxicity has been reported to be related to osteomalacia (porous, uncalcified bone) and encephalopathy (brain disease), among others. Exposure of the lung to Al can result in fibrosis. Aluminum is toxic to the GI tract, inhibiting smooth muscle contraction and interfering with muscle calcium metabolism.

Reviews of Al bioavailability have been published by Priest (291), and Powell and Thompson (293). Aluminum is absorbed poorly in the GI tract. Average absorption from the diet has been estimated to be about 0.1% (293) and its availability depends on a number of factors. Some food components such as citrate may increase absorption. Ligands such as silicic acid, phosphate, phytate and polyphenols (from tea) may decrease absorption. Interaction with other elements, such as Ca, Fe, Mg, and P could decrease the absorption of Al as well as these elements. Deficiencies of divalent cations and fluoride content of food may also affect absorption. Since it is poorly absorbed and efficiently excreted by the kidneys, Al does not accumulate at the normal amounts consumed. However, individuals having impaired kidney function are at greater

risk of toxicity. Severe toxic reactions have been reported in dialysis patients when Al-contaminated dialysis solutions were used.

3.5.2 Aluminum in Food and Diet

Aluminum is one of the most common elements in the human environment. It is naturally present in water and most foods, and its levels vary with geographic location. Pennington (84) and Sherlock (30) have reviewed the content of Al in foods and diets. Typical concentrations of Al in foods are 0.02-5 mg/kg (wet weight). Its content in plant foods can be high, as can the concentration in some fish and shellfish. Tea infusions contain relatively high levels of Al (31, 294), but Al from tea does not appear to result in accumulation of this element in rats (295). Food processing, preparation and storage can also lead to the addition of Al to the diet. Aluminum salts are sometimes added to foods as emulsifiers, in preserving and pickling, as anticaking agents and in some baking powders and leavening agents. Processed cheese and products made with Al-containing baking powders can be particularly high in Al. Soil remaining on vegetables can contribute quite high levels of Al to the diet. The use of Al containers and utensils in cooking and storage may also increase levels in food (31, 32, 30). Aluminum content of drinking water can be increased by the use of Al sulphate in the water purification process. Acid rain can also increase Al concentration of surface water, which may be used for drinking.

Typical dietary intakes of Al range between 1-30 mg d^{-1}. Milk, other dairy products, and cereal products account for over half the intake. Consumption of Al-rich

products can lead to 2 or 3 times normal DI. Furthermore, several non-prescription drugs such as antacids, buffered aspirin and antidiarrheals contain large amounts of Al and may contribute significantly to the intake. However, bioavailability of Al varies with the species consumed.

There are relatively few studies of Al in diet, particularly in Canada. Because of the health concerns noted above and of the lack of reliable data, there is a need for information on the total content and availability of Al in foods and diets. One of the objectives of the present work is to address this problem.

3.5.3 Determination of Aluminum by INAA and EINAA

Aluminum is a rather difficult element to determine by almost all analytical techniques. In the past both AAS and NAA have been used for this purpose. In the present work, INAA and a combination INAA-EINAA method were used.

The 1778.9-keV gamma-ray of ^{28}Al (half-life = 2.24 min) produced by the reaction $^{27}Al(n,\gamma)^{28}Al$ has been used in this thesis for the determination of Al. This nuclide has a potential interference resulting from the P present in the diets. Unwanted ^{28}Al activity can be produced by the reaction $^{31}P(n,\alpha)^{28}Al$. The contribution to the ^{28}Al activity due to a second interfering reaction $^{28}Si(n,p)^{28}Al$ was considered negligible because of the very low levels (*viz.* <1 ppm) of Si generally present in biological materials, foods in particular (296, 297). The interference from P can be corrected for using a combination INAA-EINAA method (298, 299).

The ^{28}Al activity arising from the irradiation in reactor flux neutrons (which

include thermal and epithermal neutrons) is due to both ^{27}Al and ^{31}P target isotopes and can be given by the following equation:

$$A_R = {}^{Al}A_R + {}^{I}A_R$$

where, A_R = total activity after reactor flux neutron irradiation,

${}^{Al}A_R$ = activity produced from parent isotope ^{27}Al,

${}^{I}A_R$ = activity produced by the interfering parent isotope, *i.e.* ^{31}P.

Similarly, $A_E = {}^{Al}A_E + {}^{I}A_E$

where the respective activities are produced after irradiations with epithermal neutrons.

The cadmium ratios of Al and the interfering elements are defined as:

$$^{Al}CR = \frac{{}^{Al}A_R}{{}^{Al}A_E} \quad \text{and} \quad {}^{I}CR = \frac{{}^{I}A_R}{{}^{I}A_E}$$

If ${}^{I}CR$ approaches 1 and ${}^{Al}CR \gg 1$, it can be shown that

$${}^{Al}A_R = A_R - A_E$$

As described previously, epi-cadmium neutron irradiation may be performed conveniently at the DUSR facility using the cadmium-shielded irradiation site (57). This correction method was applied to the Al content measured in this work. Irradiation conditions for both thermal and epi-cadmium irradiations were t_i=2 min, t_d=1 min and t_c=5 min. The sensitivity for Al analysis by INAA was 123 counts μg^{-1}.

A difficulty encountered during the analysis was the presence of Al in the sample vials used for irradiations in the DUSR facility. An analysis of eleven acid-washed blank vials gave an Al content of 0.5 $\pm$ 0.2 μg (mean $\pm$ SD). The duplicate diet samples contained as little as 0.6 μg Al, so the blank values were significant as well as

variable. Furthermore, blank vials exposed to the laboratory environment through open storage and repeated use tended to have very high Al blanks, most likely due to contamination. The results presented in this thesis were obtained by measurement of freshly prepared samples stored in acid-washed plastic bags until analyzed. Internal spacers, used to assure sample geometry of most vials, were not used in vials prepared for Al analysis in order to reduce the Al blanks.

Detection limits for various RMs and a duplicate diet composite are given in Table 3.38. Detection limits were below the measured values except for NIST SRM 1549 Milk Powder. Quantitatve determination limits were 7 to 9 ppm for all RMs except 1 ppm for NIST SRM 1567a Wheat Flour, and 15 ppm for NIST SRM 1548 Total Diet. The measured values of the diet samples lie between the detection and determination limits.

3.5.4 Quality Assurance of Aluminum Analysis

Accuracy of Al determination was assessed by the analysis of RMs and SRMs (Table 3.38, Fig. 3.11). Presently, there are no diet RMs certified for Al. The IAEA H-9 mixed diet and NIST SRM 1548 Total Diet have been analyzed but both only have information values. Three other biological SRMs with certified Al values have been analyzed. These were NIST SRM 1515 Apple Leaves, SRM 1547 Peach leaves, and SRM 1567a Wheat Flour. The first two of these SRMs have Al concentrations which are much higher than that reported for most diets. The concentration of Al in SRM 1567a is closer to the levels usually found in diets such as the HMFS diet (Table 3.38)

Table 3.38. Aluminum Content of RMs, SRMs and trial diet HMFS (ppm)

Reference material	L_D	This work (uncorrected)	This work (corrected)	Certified value (Info. val.)
IAEA H-9 Mixed Diet	2.6	6.7±0.8[a]	5.1±1.3[a]	(10)
NIST SRM 1515 Apple Leaves	2.6	278±8[b]	281±9[b]	286±9
NIST SRM 1547 Peach Leaves	2.6	245±4[b]	246±5[b]	249±8
NIST SRM 1548 Total Diet	4.5	44±8	42±10	(33)
NIST SRM 1549 Milk Powder	2.3	4.5±1.5[b]	1.0±2.0[b]	(2)
NIST SRM 1567a Wheat Flour	0.31	5.0±0.5[b]	4.5±0.5[b]	5.7±1.3
HMFS Duplicate Diet	2.3	6.7±1.0[a]	5.2±0.07[a]	-

[a]. Average of three values; [b]. Average of four values; [c]. Average of eight values

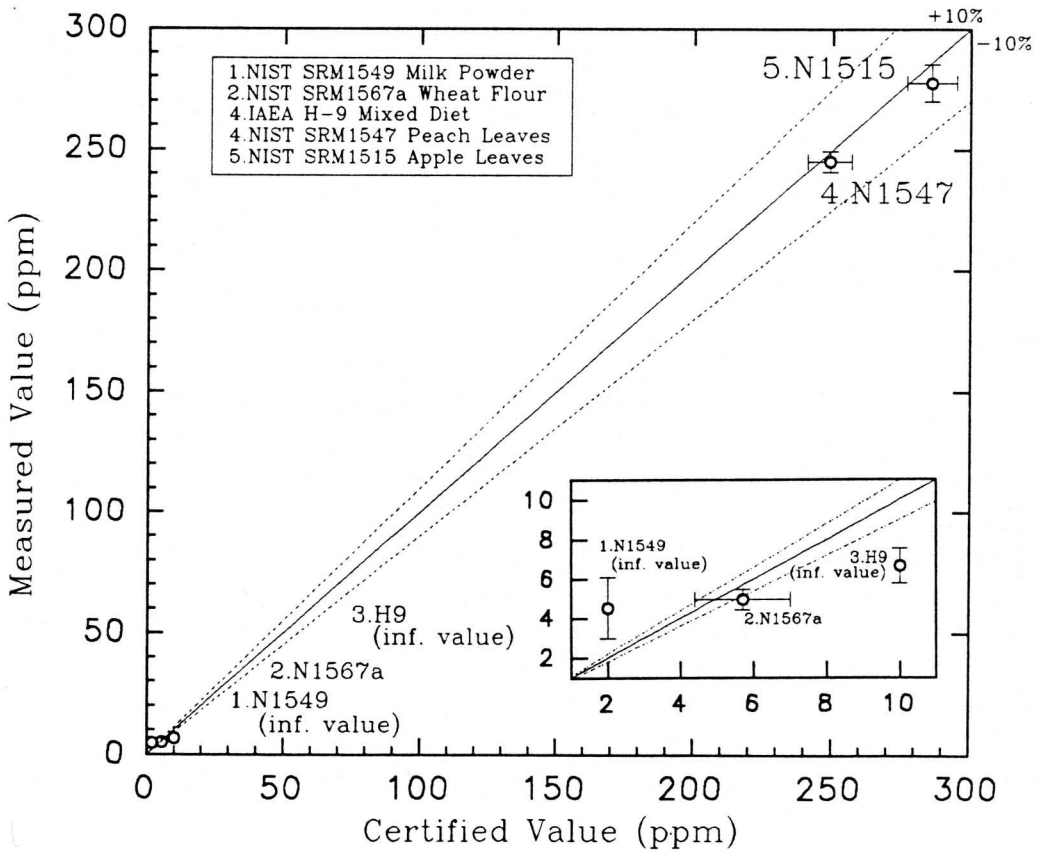


Fig. 3.11. Analysis of RMs and SRMs for Aluminum

The average value determined in NIST SRM 1549 Milk Powder was higher than the information value. The experimental value determined for IAEA H-9 Mixed Diet is somewhat lower than the information value and lower than a previous determination by other researchers in our laboratory (42). However, there was good agreement between the measured and certified values for the SRMs which are certified for Al. These results suggest that the accuracy of the analytical method developed here was high. It should be noted that the blank Al levels in the irradiation vials were relatively high and also variable; the correction for the blanks lead to a decreased precision of the final result. Consequently, low Al values (<10 ppm) should be interpreted with caution.

Epithermal corrections for ^{31}P interference were small for all RMs and SRMs except for H-9 Mixed Diet and 1549 Milk Powder. Even in these two cases, the differences between the corrected and uncorrected values were not statistically significant. Unfortunately, neither of these RMs is certified for Al, and only information values are given.

3.5.5 Aluminum Content of Duplicate Diets

The Al content of the diet and DIs determined for each participant are reported in Table 3.39. A comparison of the results by INAA (*i.e.* without correction for ^{31}P) and by EINAA/INAA (corrected) show that the corrected values were lower by about 1 ppm, but these differences were not significant. Concentrations of Al varied widely from 1.9 to 67 ppm although most values were near 10 ppm. The overall mean and median Al concentrations were 15 and 9.4 ppm.

Table 3.39. Aluminum Content of Duplicate Diet Composites determined by INAA and EINAA/INAA (correction for ^{31}P)

Sample Code	INAA		EINAA/INAA	
	Conc.±SD (ppm)	DI (mg d^{-1})	Conc.±SD (ppm)	DI (mg d^{-1})
HMFS	6.7±1.0[a]	4.6±0.7[a]	5.2±0.07[a]	3.6±0.05[a]
(a) Females				
HFFF	7.2	2.4	5.8	2.0
HFMC	11±1[a]	3.8±0.5[a]	9.9±1.6[b]	3.5±0.5[b]
HFNA	6.5	2.6	4.4	1.8
HFRR	5.8	2.5	4.9	2.1
HFSR	7.1	3.4	5.9	2.8
HFTC	18	6.4	16	5.9
HFWH	67	32	65	32
Mean	17	7.6	16	7.1
Median	7.2	3.4	5.9	2.8
Min.-Max.	5.8-67	2.4-32	4.4-65	1.8-32
(b) Males				
HMFF	5.9	2.8	4.3	2.0
HMMR	1.9	0.53	0.30	0.08
HMNT	9.4±2.0[a]	6.7±1.4[a]	7.5±2.2[b]	5.4±1.6[b]
HMRZ	37	22	36	21
HMSG	19	8.6	17	7.9
HMTH	11	5.8	10	5.3
HMWV	9.5	7.6	7.4	5.9
Mean	13	7.7	12	7.0
Median	9.5	6.7	7.5	5.4
Min.-Max.	1.9-37	0.53-22	0.30-36	0.08-21
(c) Overall				
Mean	15	7.7	14	7.0
Median	9.4	4.8	7.4	4.4
Min.-Max.	1.9-67	0.53-32	0.30-66	0.08-32

[a]. average of three values

Aluminum intakes were also quite variable, from less than 1 to 32 mg d^{-1}. The mean and median intakes for the female participants were 7.6 and 3.4 mg d^{-1} (Table 3.39). Since Al is not an essential element, its content in food may vary significantly and not follow a normal distribution. This may explain why the mean and median are not close to each other. The range of Al intakes for the female participants was large, from 2-32 mg d^{-1}. The mean and median intakes for the male participants were closer than those for the females, at 7.7 and 6.7 mg d^{-1}, respectively. However, the range was still large, varying from 0.5-22 mg d^{-1}.

The overall median intake of Al was 4.8 mg d^{-1}. Individual intakes varied widely from 0.5 to 32 mg d^{-1}. Participants with high Al intake may have consumed large amounts of foods and beverages (such as tea) with high Al levels or used Al cooking utensils from which the element could have leached out. The individuals who had the highest intakes HFWH (32 mg d^{-1}) and HMRZ (22 mg d^{-1}) were also the greatest coffee and tea drinkers (see Appendix 1 for list of foods consumed).

As previously stated, Al is a rather difficult element to determine in diets. There are relatively few studies on the Al content of diet reported in the literature (Table 3.40). Some studies from the 1960's and 1970's have reported fairly high Al values (78, 300). Tipton *et al.* reported the DI from duplicate diets of two American male volunteers to be 17 mg d^{-1} (300). Tanner and Friedman reported the DI calculated for U.S. teen-age males from INAA of a market basket study to be 20 mg d^{-1} (78). More recent values (269, 87) are lower, perhaps reflecting improvements in analytical methodology and minimization of contamination and blanks. Other studies in the U.S.A. have also reported

Table 3.40. Survey of Average Daily Dietary Intake of Al

Study Group	Composite	Analytical Method	DI (mg d^{-1})	Ref.
Germany				
Adult women, men 1988	Duplicate portion	GFAAS	5.4, 6.5	(303)
Adult women, men 1991	Duplicate portion	GFAAS	4.6, 4.9	(303)
Adult women, men 1995	Duplicate portion	GFAAS	3.1, 3.2	(303)
Japan				
Adult men	Dupl. portion	AAS/GFAAS	4.37	(257)
Adult men	Dupl. portion	ICP-AES	4.0	(302)
The Netherlands				
Men & women, 1976-78	Dupl. portion	AAS/GFAAS	4.0	(283)
Men & women, 1984-85	Dupl. portion	AAS/GFAAS	2.8	(283)
Poland				
Hospital patients/staff	Hospital canteen meals	AAS/Colorim.	21.31	(301)
Taiwan				
University faculty, staff, students	Dupl. portion	INAA	8.0, 9.9, 12.3	(43)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	2.330	(240)
U.S.A.				
Adult male volunteer	Dupl. portion	arc em. spec.	17	(300)
Adult male volunteer	Dupl. portion	arc em. spec.	17	(300)
Female students	Dupl. portion	AES	<1.53-33.3	(255)
Hospital Patients- Summer	Hospital diet	AES	6.974	(265)
Hospital Patients- Winter	Hospital diet	AES	5.324	(265)
Men & women planned menu (25% cal fr. fat)		AES	4.95	(267)
Men & women planned menu (35% cal fr. fat)		AES	6.15	(267)
Teen-age males	Market basket	INAA	20	(78)
Male volunteers	Metabolic diet	GFAAS	4.6	(269)
Adult males (USDIET-1)	Market basket	ICP-AES/INAA	14.3	(195)
Teen-age females, males1984	Market basket	ICP-AES	9, 7.7	(87)
Adult females, males 1984	Market basket	ICP-AES	9, 14	(87)
Teen-age females, males1993	Market basket	ICP-AES	7.7, 11.5	(87)
Adult females, males 1993	Market basket	ICP-AES	7.1, 8.2	(87)

lower values, *e.g.* 5-7 mg d^{-1} in hospital diets (265, 267). The DIs calculated from the U.S. Food and Drug administration's Total Diet Study for male and females in 1993 were 7.1 and 8.2 mg d^{-1} respectively. However, a Polish investigation of hospital canteen meals published in 1995 reported a relatively high DI of 21 mg d^{-1} (301).

Two research groups have used INAA for measuring Al levels in the diet. In the U.S.A., Tanner and Friedman reported an intake of 20 mg d^{-1} for teen-age males, using the market basket diet collection approach (78). Liu *et al.* used the duplicate portion technique to determine Al intake of three study groups within a university community (43); the intake varied from 8.0-12.3 mg d^{-1}. Neither group appeared to make corrections for the interfering reaction of ^{31}P to ^{28}Al.

There do not appear to be any previous studies on Al intake in Canada. The median DI for the female volunteers of this study are lower than most of the literature values Table 3.40). For the males, the median DI is slightly higher than results reported in Japan (257, 302), the Netherlands (283) and some studies in the U.S.A. (265, 269), but lower than DIs reported for Poland (301), Taiwan (43) and the U.S.A. (78, 87, 300).

3.5.6 Aluminum in Dialyzed and Digested Diets

The dialyzed diet samples were analyzed for Al using the 1778.9-keV peak of ^{28}Al. Unfortunately, the Al content of the residue was determined in most cases to be significantly higher than that in the original sample. This was possibly due to contamination.

Samples subjected to enzymolysis were also analyzed using the same gamma-ray of ^{28}Al described above. The irradiation time was only 5-15 s because of the high dead time. A 1-min decay and 10-min count followed the irradiation. The blanks contained 8 ± 4 μg of Al. Since the undigested diet samples typically contained about 12 μg, the blank correction was quite large. Consequently, results are only reported in this thesis for the materials with the highest concentrations of Al, namely NIST SRM 1548 Total Diet, and duplicate diets HFWH and HMRZ (Table 3.41). The Al fraction calculated to be potentially available was quite high for HMRZ, where the percent available in the freeze-dried diet and wet slurry were 89% and 78%, respectively. For HFWH, the percent potentially available was lower, the values being 10% in freeze-dried diet and 7% in wet slurry. The main differences between these diets were higher milk and soft drink intakes by HMRZ (see Appendix 1). Perhaps the acidity of soft drinks could solubilize Al although one might have suspected that this effect would be compensated by the alkalinity of the milk.

Table 3.41. Enzymolysis of some selected duplicate diets and a SRM for Al

Sample Code	Conc. (residue) (ppm)	%non-dialyzable	%potentially available[a]
NIST SRM 1548 Total Diet	23	53	47
(a) Females			
HFWH (dry)	60	90	10
HFWH (wet)	62	93	7
(b) Males			
HMRZ (dry)	4	11	89
HMRZ (wet)	8	22	78

3.6 CALCIUM

3.6.1 Calcium in Nutrition and Health

In the Nutrition Canada survey conducted in the early 1970's, it was concluded that Ca intake was not sufficient to meet the recommendations for adult women (619 mg d^{-1}) and males (709 mg d^{-1}) over 65 years of age (65). There has been considerable interest in Ca nutrition, largely due to concern about osteoporosis. The recent trend is toward the development of recommendations for "optimal" nutrient intakes which not only will prevent acute deficiency but also chronic diseases such as osteoporosis (304-307). There are several review articles on nutritional and health aspects of Ca (1, 6, 12, 17, 241, 308-311).

Calcium is a major as well as an essential element in the human body which contains about 1200 g of it. Most Ca is located in the bones and teeth, where it is an important structural component. The remaining 1% of body Ca is involved in several important functions, such as enzyme activation, nerve transmission, membrane transport, blood clotting, muscle contraction, and hormone function.

Calcium deficiency is not observed in otherwise healthy humans. The human body is capable of adapting to a range of Ca intakes. A decrease in intake stimulates an increase in absorption and decrease in excretion. However, low intake of Ca associated with vitamin D deficiency can result in rickets and osteomalacia. In recent years, there has been concern about osteoporosis (post-menopausal bone loss leading to weakness and risk of fracture) in women. There is evidence that a high Ca intake might be beneficial in the prevention of the disease (1, 304, 308, 310) although this subject is

somewhat controversial due to the complex dependence of Ca uptake on various components of the North American diet (312). There is some evidence to suggest that Ca intake is inversely related to blood pressure (1), and increased Ca intake may reduce the risk of certain types of cancers (304).

Because the human body can adapt to low Ca intakes, determination of its requirements is difficult. In some areas of the world, an intake of 200 mg d^{-1} is considered adequate to maintain a good health (1). However, in the U.S.A., osteoporosis may still develop with an intake of 600 mg d^{-1}. The U.S. R.D.A. for Ca is 800 mg d^{-1} for both adult men and women. Health Canada set the RNI to be 800 mg d^{-1} for adult men and 700 mg d^{-1} for adult women.

Since the establishment of the RDA and RNI for Ca, proposals have been made to increase the recommended intakes with the objective to prevent osteoporosis and chronic diseases. The Osteoporosis Society of Canada has recommended a DI for adults (19-49 a) to be 1000 mg d^{-1} (305). The U.S. National Institutes of Health (307) estimated the optimal Ca intake to be 1 000 mg d^{-1} for adult men and women (25-50 a) and 1 200-1 500 mg d^{-1} for adolescents/young adults (11-24 a). Achieving these aims may be difficult because most women in the U.S. have inadequate Ca intakes which are well below the RDA (313, 314). Many men have intakes below the RDA as well (311). High intakes of Ca generally do not result in toxic effects, because the body regulates absorption and excretion. If Mg is deficient, overdoses of parathyroid hormone, vitamin D or Ca can result in calcification of soft tissues.

3.6.2 Dietary Sources and Bioavailability of Calcium

Milk, yogurt, hard cheeses, cottage cheese, dark green leafy vegetables and broccoli are rich in Ca. Citrus fruits, canned fish with edible bones and dried peas and beans are also good sources. Butter, sour cream, cream cheese and other high-fat dairy products, meat and nuts contain little Ca. Hard water can also provide some Ca.

Absorption of Ca through the intestine occurs via passive and homeostatically controlled active processes (310, 315). Vitamin D and parathyroid hormone are involved in the regulation of intake and excretion. Absorption requires bile salts, bile and dietary fat, although excess fat inhibits absorption. About 10-40% of Ca is typically absorbed, the remainder being lost to the faeces.

Calcium absorption can be reduced or enhanced by a number of factors (308, 309, 316). Acids, protein and some amino acids, and sugars such as lactose, sorbose, cellobiose, xylose, raffinose and mannitol can increase Ca uptake. Fatty acids, phytates, oxalates and phosphorus reduce solubility and absorption. Calcium from milk is highly available due to its association with milk proteins and Vitamin D, and probably lactose and citrate. In general, Ca from plant foods is less available because of the lower protein content and other factors.

3.6.3 Determination of Calcium by INAA

Calcium levels were measured by INAA using the 3084.1-keV gamma-ray of ^{49}Ca (Table 2.2) which was free from interferences under the conditions of t_i=1.5 min, t_d=1 min and t_c=10 min. and it gave a sensitivity of 0.679 counts μg^{-1}.

The Ca content determined in IAEA H-9 Mixed Human Diet RM and NIST SRM 1548 Total Diet agreed well with the certified values (Table 3.42, Fig. 3.12). The results for IAEA H-4 animal muscle were slightly high, the result of one high value in three determinations (340, 240, 240 μg/g). It should be noted that the γ-ray peaks were very small (less than 100 counts) and close to the detection limits for IAEA H-4, NIST SRM 1568 Rice Flour and 1577b Bovine Liver (Table 3.42). This led to reduced precision of analysis (RSD of about 20%) although there was good agreement between analyzed and certified values for the latter two SRMs. These results suggest that the analytical method yielded good accuracy and precision at the Ca levels typically found in human diets. It should be noted here that the Ca levels of the duplicate diets analyzed in this work were all well above the detection limits.

An external QA chart for Ca, prepared from replicate analysis of IAEA H-9 Mixed Diet over several days, is shown in Fig. 3.13; all values lie within the control limits. However, the analysis of sample number 5 gave an extremely low value for Ca which fell outside the warning limits. Despite a careful investigation, the reason for this low value was not found. None of the other elements analyzed from the same irradiation or spectrum gave low values (Cl, K, Mg, Mn, Na). An analysis of NIST SRM 1568 performed on the same day did not yield any values which were below the certified value for that RM. However, an analysis of an IAEA H-4 sample immediately preceding the sample number 5 also gave a low value for Ca. No other samples were analyzed for Ca after sample number 5 on that day. Since analyses on following days and months did not yield low values (as indicated by samples 6-9 in Fig. 3.13) and there was generally good

Table 3.42. Calcium content of RMs, SRMs, and the HMFS trial diet

Reference material	L_D (ppm)	This work (ppm)	Certified value (ppm)
IAEA H-4 Animal Muscle	100	280 ± 50[a]	254 ± 66
IAEA H-9 Mixed Diet	310	2200 ± 100[b]	2310 ± 160
NIST SRM 1548 Total Diet	270	1600 ± 100[a]	1740 ± 70
NIST SRM 1568 Rice Flour	33	120 ± 25[a]	120 ± 7
NIST SRM 1577b Bovine Liver	270	91 ± 22[c]	116 ± 4
HMFS Duplicate Diet	290	2400 ± 200[a]	-

[a]. Average of three values; [b]. Average of four values; [c]. Average of six values

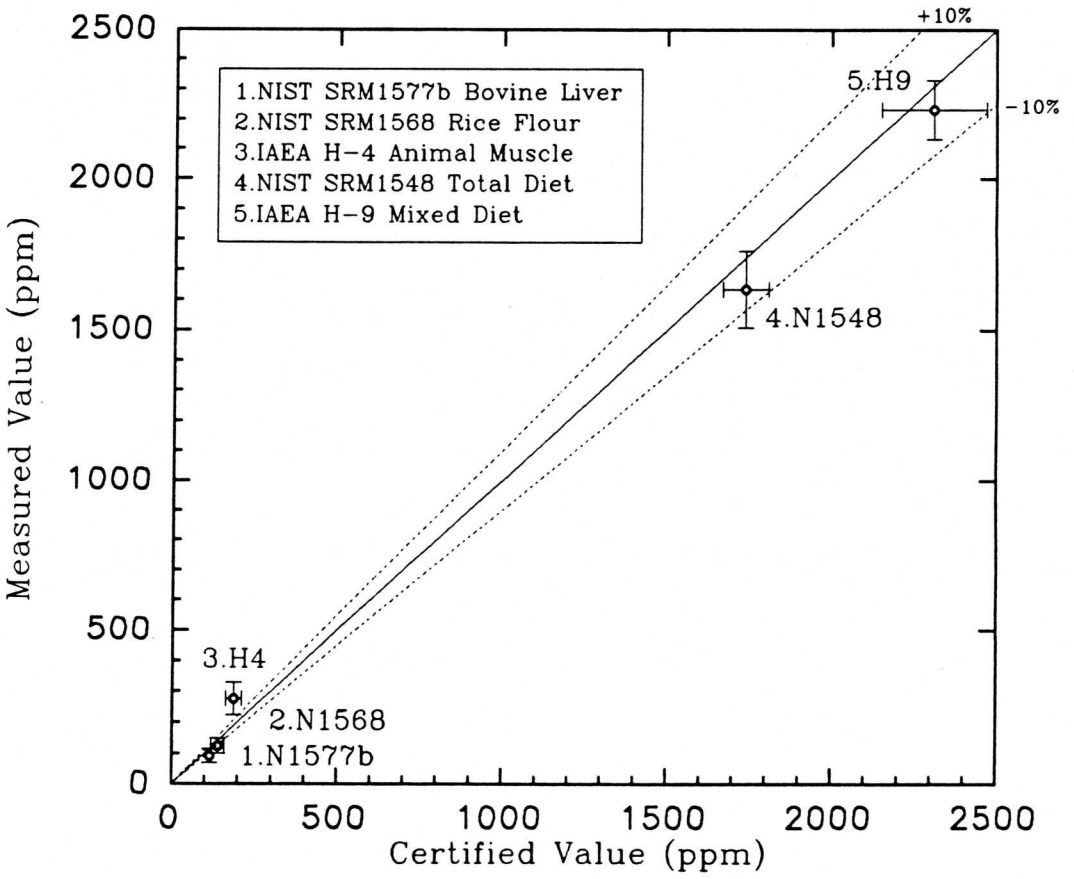


Fig. 3.12. Analysis of RMs and SRMs for Ca

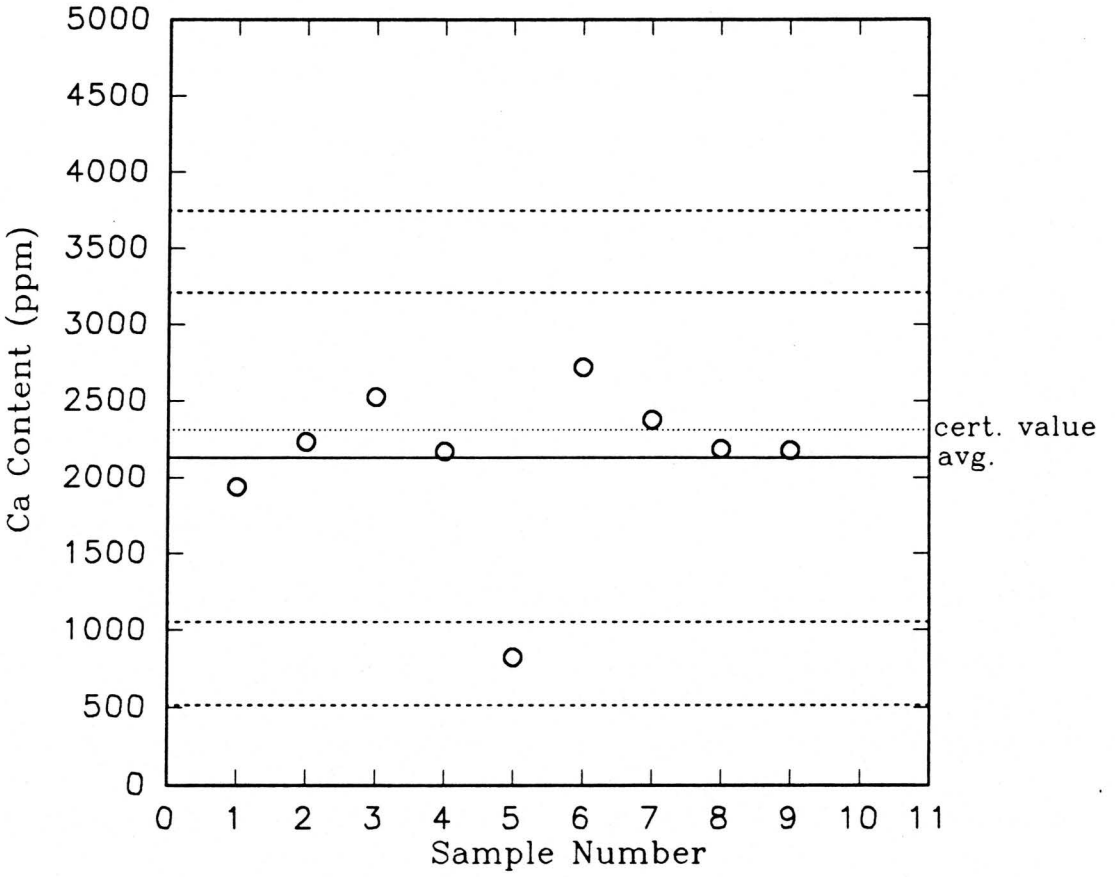


Fig. 3.13. External quality assessment chart for the analysis of Ca

agreement between analyzed values and the certified value, it was concluded that the analytical method was in statistical control. The value for sample number 5 was not incorporated into the measured value for IAEA H-9 reported in this thesis.

3.6.4 Calcium Content of Duplicate Diets

Concentration and DI of Ca in the duplicate diet samples are shown in Table 3.43. The RSD of the five replicate analyses of the HMFS diet was 9%, but was higher (19%) for the three replicates of the HFMC diet sample. The reduced precision is most likely due to the relatively low Ca content of this sample. It is also possible that the HFMC diet is not relatively homogeneous with respect to Ca. Precision of analysis for HMNT diet was excellent (RSD 3%).

The mean and median intakes were quite close, suggesting a normal distribution (Table 3.43). The median Ca intake was 900 mg d^{-1} for the females and 1 100 mg d^{-1} for the males. The overall median Ca intake was 1 080 mg d^{-1}. The median DIs of most individuals exceed the Canadian RNI and the U.S. R.D.A.. Four individuals, namely HFMC, HFWH, HFTC and HMMR, had DIs which were well below the recommended levels. Three of these were women, perhaps suggesting that women are more likely to have low Ca intakes. The most recent trend in nutrition recommendations for Ca is toward DIs above the RNI and RDA in order to prevent osteoporosis (305, 307). Four of the seven women in this study did not meet these new recommendations (1 000 mg d^{-1}) for adult women.

Daily intakes of the duplicate diets calculated from food composition tables

Table 3.43. Calcium content of duplicate diet composites determined by INAA

Sample Code	Content±SD (ppm)	DI from NAA (mg d^{-1})	DI from FCT (mg d^{-1})
HMFS	2400±200[a]	1700±200[a]	
(a) Females			
HFFF	3560	1200	1100
HFMC	1700±300[a]	580±110[a]	299
HFNA	3580	1420	1520
HFRR	2070	900	1170
HFSR	2910	1390	936
HFTC	1910	680	417
HFWH	1190	570	674
Mean	2410	960	875
Median	2070	900	936
Min.-Max.	1190-3580	570-1420	299-1520
RNI		700	700
RDA		800	800
(b) Males			
HMFF	2750	1280	991
HMMR	1750	480	499
HMNT	2210±70[a]	1590±50[a]	1700
HMRZ	1840	1100	770
HMSG	2310	1060	533
HMTH	1640	870	851
HMWV	2060	1660	1470
Mean	2080	1150	974
Median	2060	1100	851
Min.-Max.	1640-2750	480-1659	499-1700
RNI		800	800
RDA		800	800
(c) Overall			
Mean	2240	1050	924
Median	2060	1080	894
Min.-Max.	1190-3580	480-1660	299-1700

[a]. Average of three values

(FCT) by Chatt and Pegg (27) are reproduced in Table 3.43. The analyzed and calculated values (Fig. 3.14) show a linear relationship, although the calculated DIs were generally lower than the analyzed DIs (Table 3.43). The correlation coefficient calculated using for analyzed and calculated DIs was 0.84. Median intake calculated from FCT was close to the analyzed median intake for women, but was somewhat lower for the men and lower overall. Both ANOVA and a paired t-Test were performed on the analyzed and FCT data. The t-value calculated was 0.84 and was lower than the critical t-value of 2.1, at α=0.05. The F-value calculated from ANOVA, namely 0.70, was lower than the critical F-value of 4.2 with P=0.41. No difference was detected between the two sets of data. It was concluded that the FCT calculations gave a reasonably good estimate of the Ca DI.

The median intakes determined in this work are higher than those reported for most other Canadian studies (72, 259, 317). Food disappearance data and memory recall studies indicated that the Ca intake of Canadians may vary from 580 mg d^{-1} to 1 160 mg d^{-1} (1). Calcium intake was determined from duplicate portion studies of Canadian university students and elderly women to be about 880 mg d^{-1} and 770 mg d^{-1}, respectively (72, 317). Shah *et al.* prepared a representative Canadian diet based on food consumption patterns observed in the Nutrition Canada Survey and reported Ca intake to be 875 mg d^{-1} (259). Dairy products provided 72% of the DI of Ca.

There have been many studies on the Ca content in diets of several countries, a summary of the literature being presented in Table 3.44. The Ca content of the duplicate diets determined in this study are higher than those of the U.S.total diets for

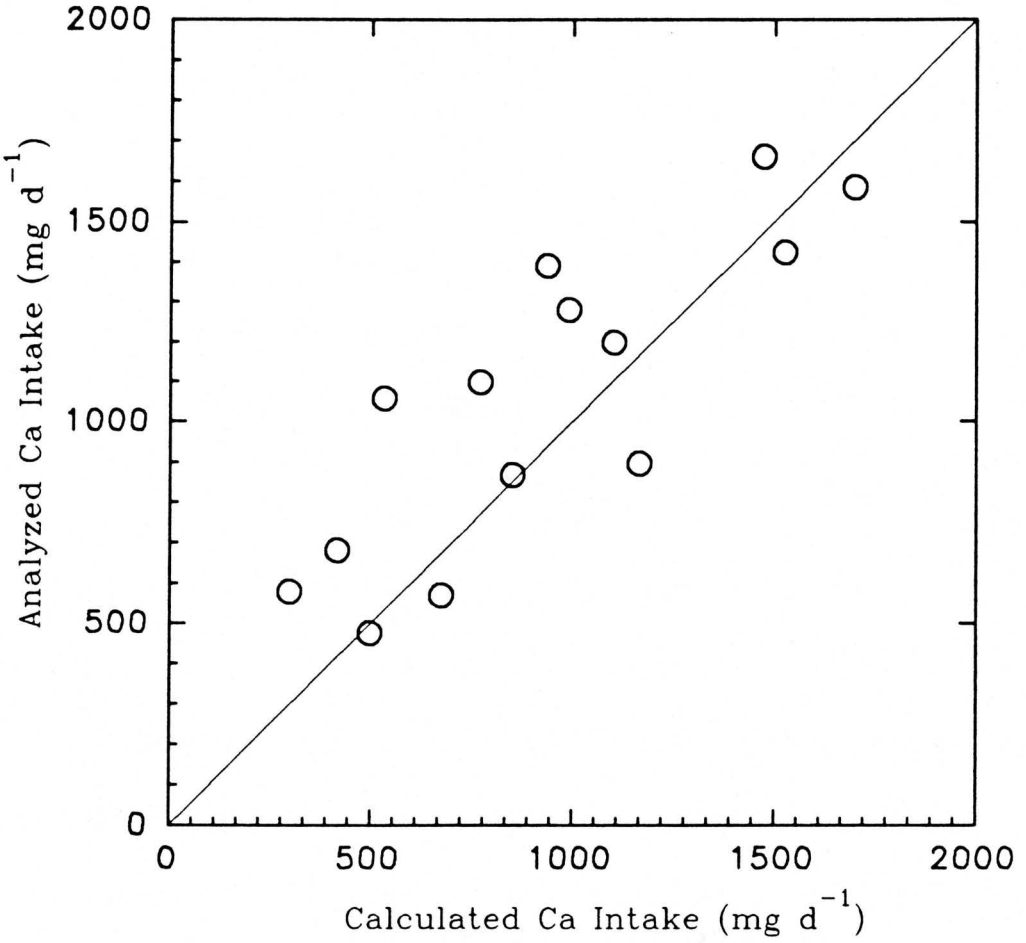


Fig. 3.14. DI of calcium determined by INAA and calculated from FCT

Table 3.44. Survey of DDI of calcium

Study Group	Composite	Analytical Method	Intake (mg d^{-1})	Ref.
Belgium				
General population	Hospital meals & Dupl. portions	AAS	624	(281)
Military students, personnel	Cafeteria meals	GFAAS	674, 665	(258)
Hosp. patients Antwerp, Liege	Hospital diet	GFAAS	701, 432	(258)
Canada				
Female, male students	Cafeteria meals	AAS	858, 901	(317)
Post-menopausal women	Dupl. portion	AAS	769	(72)
General population	Equiv composite	AAS	875	(259)
Denmark				
Female osteoporosis patients	Metabolic diet	?	1 118	(312)
Finland				
Hospital patients	Hospital diet	GFAAS	1 400	(97)
Wt. reduction patients	Duplicate portion	AAS	776	(238)
France				
Adult women, men	Equiv. composite	AAS	836, 1 026	(210)
India				
Bombay residents	1/8 Dupl. portion	AAS	710	(262)
Japan				
Adult men	Dupl. portion	ICP-AES	560	(302)
New Guinea				
Wosera resident, women, men	Equiv. composite	INAA	500-814, 536-878	(277)
Spain				
Madrid, Valencia, Galicia, Andalucia residents	Equiv. compos.	AAS	690-980	(263)
Sweden				
Female, male pensioners	Dupl. portion	Flame photom.	610, 726	(90)
Dalby resident 1, 2	Dupl. portion	not stated	610, 840	(318)
Adult women (high, low b.p.)	Dupl. portion	AAS	577, 577	(264)
Female, male lactovegetarians	Dupl. portion	AAS	1024, 703	(93)
Female, male Vegans	Dupl. portion	AAS	527, 725	(91)
Dalby residents	Dupl. portion	AAS	680	(92)
Adult men and women	Dupl. portion	AAS	800	(2)
General population	Market basket	AAS	1 180	(98)
Taiwan				
Univ. faculty, staff, students	Dupl. portion	INAA	640, 190, 390	(43)
Thailand				
General Population	Market basket	AAS	113	(319)
The Netherlands				
Teen-age males (18 y)	Market basket	AAS	1 320	(232)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	1 370	(240)

Table 3.44. (continued) Survey of DDI of calcium

Study Group	Composite	Analytical Method	Intake (mg d^{-1})	Ref.
U.S.A.				
Metabolic Unit Patients	Normal Test Diet	gravimetry	1 030	(320)
Metabolic Unit Patients	Prepared Diet	flame photo	1 868	(321)
Female students	Dupl. portion	FES	780	(255)
Adult male volunteer 1, 2	Dupl. portion	flame photo.	940, 2 300	(300)
Hosp. Patients-summer, winter	Hospital diet	FES	1 304, 1 390	(265)
Female university students	Metabolic diet	FES	978	(266)
Men & women planned menu (25% cal fr. fat)		FES	450	(267)
Men & women planned menu (35% cal fr. fat)		FES	724	(267)
Teen-age males	Market basket	INAA	<884	(78)
Male & female students	Cafeteria meals	AAS	1 216	(256)
Male metabolic unit patients	Metabolic diet	AAS	196	(288)
Hospital patients	Hospital diet	AAS	1 282	(268)
Hospital patients	Ovo-lacto vegetarian hosp. diet	AAS	1 567	(268)
Adult male volunteers	Metabolic diet	GFAAS	813	(269)
Adult male volunteers	Low- fibre diet w/spinach	AAS	964	(254)
Adult male volunteers	Higher fibre diet w/spinach	AAS	1077	(254)
Adult male volunteers	Metabolic diet no spinach	AAS	1058	(254)
Teen-age male, 1974	Market basket	AAS	1 184	(81)
Teen-age male, 1975	Market basket	AAS	1 152	(81)
Teen-age male, 1978	Market basket	AAS	1 055	(81)
Teen-age male, 1979	Market basket	AAS	1 162	(81)
Teen-age female, male1982-84	Market basket	ICP-AES	726, 1 141	(83)
Adult female, male 1982-84	Market basket	ICP-AES	564, 836	(83)
Adult males (USDIET-1)	Market basket	FAAS/ICP-AES/INAA	840	(195)
Teen-age female, male 1984-85	Market basket	ICP-AES	730, 1 152	(85)
Adult female, male 1984-85	Market basket	ICP-AES	577, 854	(85)
Teen-age female, male 1985-86	Market basket	ICP-AES	742, 1 169	(85)
Adult female, male 1985-86	Market basket	ICP-AES	581, 863	(85)
Adult men	Dupl. portion	ICP-AES	920	(270)
Teen-age female, male 1982-89	Market basket	ICP-AES	725, 1 141	(86)
Adult female, male 1982-89	Market basket	ICP-AES	577, 858	(86)
Adult men	Metabolic diet	AAS	1 011	(322)
West Germany				
Adult men	Dupl. portion	PNAA	380	(101)

adult men and women (83, 85, 86) and duplicate diets of adult men (270). Higher intakes in the U.S. were reported for cafeteria meals, hospital diets and older studies (see Table 3.44). The largest intake reported was 2 300 mg d^{-1} (300) in the duplicate diet of an adult male volunteer. Some reports cited particularly low Ca intakes, such as 380 mg d^{-1} in West Germany (101), 190 mg d^{-1} among Taiwanese University staff and families (43), and 113 mg d^{-1} in Thailand (318).

3.6.5 Dialysis and Enzymolysis of Duplicate Diets for Calcium

Calcium was analyzed in dialyzed duplicate diet samples using experimental conditions of t_i=5 min, t_d=1 min, and t_c=10 min. Under these conditions, the sensitivity was 0.950 counts μg^{-1}. The results for three RMs and SRMs are shown in Tables 3.45 and 3.46. Calcium in NIST SRM 1568 Rice Flour had the lowest percent dialyzed (19%), whereas both mixed diet materials, namely IAEA Mixed Diet (57%), and NIST SRM 1548 Total Diet (65%), were higher. For the duplicate diets, the percent dialyzed ranged from 30 to 80%, the overall median being 54%. The relative uncertainty in percent dialyzed was 12-34%.

Duplicate diets, and some SRMs subjected to the enzymolysis/dialysis procedure were analyzed using irradiation times between 5 and 15 s, decays of 1 min, and counts for 10 min. The sensitivity was 0.053 counts μg^{-1}. Results for the SRM samples were found to be unreliable and are therefore not reported. Reproducibility of the digestion experiments was assessed by conducting replicate experiments using diets HMFS, HFMC and HMNT. The RSD of the concentration in the non-dialyzed residue

Table 3.45. Dialysis of reference materials for calcium.

CRM	Conc. in residue (ppm)	% non-dialyzable	% dialyzable[a]
IAEA H-9 Mixed Diet	1 052	43	57
NIST SRM 1548 Total Diet	574	35	65
NIST SRM 1568 Rice Flour	100	81	19

[a]:calculated by difference

Table 3.46. Dialysis of duplicate diet composites for calcium.

Sample Code	Conc. in residue (ppm)	% non-dialyzable	% dialyzable[a]
HMFS	1 290±50	53±7[b]	47±7[b]
(a) Females			
HFFF	730	20	80
HFMC	820 ± 120	50±17[c]	50±17[c]
HFNA	1 400	39	61
HFRR	780	38	62
HFSR	900	31	62
HFTC	1 330	70	30
HFWH	580	49	51
Mean		42	58
Median		39	61
Min. - Max.		20-70	30-80
(b) Males			
HMFF	1 320	48	52
HMMR	670	38	62
HMNT	1 320 ± 60	60±5[c]	40±5[c]
HMRZ	1 110	60	40
HMSG	580	25	75
HMTH	730	44	56
HMWV	1 170	57	43
Mean		47	53
Median		48	52
Min. - Max.		25-60	40-75
(c) Overall			
Mean		45	55
Median		46	54
Min. - Max.		20-70	30-80

[a]:calculated by difference; [b]:average of four values; [c]:average of two values

was 3-10% for HMFS and HMNT and about 20% for wet HFMC slurry. Because thedifferences between the concentrations before and after enzymolysis/dialysis were small, the uncertainties in percent dialyzed were high. As the results in Tables 3.47 and 3.48 show, the percent dialyzed was also very small. For the males, the percent dialyzed of freeze-dried diet was generally higher than the wet slurry, although the difference may not be statistically significant.

Curiously, the percent dialyzed appears to be smaller for the digested diet samples, than for those subjected to dialysis without prior digestion (Table 3.45). One possible explanation for this observation was a coat of "slime" noticed on the interior surface of the dialysis tubing, which could have blocked the pores. The origin of this slime was not clear, although there are a number of possiblities: it could have arisen from solubilization of fats by the bile salts and deposition on the tubing, degradation of the cellulose in the tubing by the digestive enzymes, precipitated carbonate compounds (*e.g.* $CaCO_3$) produced by the addition of saturated Na_2CO_3 solution during the enzymolysis procedure, or microbial growth occuring while the mixtures were incubated. The lower dialyzability for Ca in the digested diets in comparison with undigested diets may suggest a problem with the digestion procedure. However, Se dialyzability was increased after enzymolysis (Section 3.1.11, p. 100), suggesting that the pores of the dialysis tubing were not blocked. More work is required to determine the source of the slime and whether it hinders dialysis.

Table 3.47. Enzymolysis and dialysis of freeze-dried duplicate diets for calcium.

Sample	Not Potentially Available		Potentially Available[a]	
	Conc.±SD (ppm)	% Non-diayzed±SD	Daily Intake (mg d^{-1})	% Dialyzed±SD
HMFS	2621±272	107±22	-135±360	-7.9±22
(a) Females				
HFFF	3839	108	-95	-8
HFMC	2179	132	-185	-32
HFNA	3081	86	197	14
HFRR	1861	90	90	10
HFSR	1664	57	596	43
HFTC	2006	105	-34	-5
HFWH	1390	117	-98	-17
Mean		99	67	1
Median		105	-34	-5
Min-Max		57-132	-185-596	-32-43
(b) Males				
HMFF	1954	71	370	29
HMMR	2119	121	-102	-21
HMNT	1829±190	83±11	270±190	17±11
HMRZ	1269	69	340	31
HMSG	1638	71	307	29
HMTH	1285	78	190	22
HMWV	1611	78	359	22
Mean	1672	91	248	18
Median	1638	83	307	22
Min-Max	1269-2119	69-121	-102--370	-21-31
(c) Overall				
Mean	1980	75	158	10
Median	1845	78	193	16
Min-Max	1269-3839	24-105	-185-596	-32-43

[a] :calculated by difference.

Table 3.48. Enzymolysis and dialysis of wet duplicate diet slurry for calcium.

Sample	Not Potentially Available		Potentially Available[a]	
	Conc.±SD (ppm)	%Non-diayzed±SD	Daily Intake (mg d^{-1})	%Dialyzed±SD
HMFS	2300±100	93±15	120±260	7±15
(a) Females				
HFFF	3990	110	-140	-12
HFMC	1900±400	110±40	410±1200	-14±45
HFNA	2220	62	540	38
HFRR	2120	100	-23	-3
HFSR	2930	100	-9	-1
HFTC	1770	93	50	7
HFWH	2100	180	-440	-77
Mean	2430	110	55	-9
Median	2120	100	-9	-3
Min-Max	1770-3990	62-180	-440-540	-77-38
(b) Males				
HMFF	2550	93	90	7
HMMR	1230	71	140	29
HMNT	2570±90	120±8	-260±110	-16±8
HMRZ	1980	110	-80	-8
HMSG	2250	98	25	2
HMTH	1480	90	86	10
HMWV	2160	110	-87	-5
Mean	2030	97	-13	3
Median	2160	98	25	2
Min-Max	1230-2570	71-120	-260-140	-16-29
(c) Overall				
Mean	2230	100	21	-3
Median	2140	100	8	-2
Min-Max	1230-3990	62-180	-440-540	-77-38

[a] : calculated by difference.

3.7 SODIUM, POTASSIUM AND CHLORINE

3.7.1 Nutritional and Toxicological Aspects of Sodium, Potassium and Chlorine

The major as well as essential elements Na, K and Cl are important electrolytes which maintain the human body's acid-base balance and osmotic equilibrium. The Na^+ and Cl^- ions are the major extracellular electrolytes while K^+ is the main intracellular cation. Chloride ion is associated with Na^+ and water in foods, body fluids and tissues, and is required for digestion as stomach hydrochloric acid. Reviews of the nutritional aspects of Na, K and Cl have been published (1, 6, 12, 17, 241). As these three elements are closely related in terms of their nutritional significance and biological function, they have been considered together in this section.

Since these elements (or ions) are abundant in the food supply, deficiency is rare, and is usually caused by disease or trauma such as starvation, excess vomiting, diarrhea or excessive sweating. Potassium deficiency can also be caused by the chronic use of diuretics and laxatives. Loss of Na without associated water loss allows water to move into cells, resulting in water intoxication which leads to mental apathy, muscle twitching, and anorexia. Chloride deficiency has only been observed in humans in cases where Cl^- was unintentionally excluded from infant formula. The result included alkalosis, memory loss, and growth retardation. Inadequate K^+ may cause inhibited growth, paralysis, sterility, muscle weakness, diminished heart rate and death.

Acute toxicity resulting from large intakes of these elements is rare, since the kidney can efficiently remove them with sufficient water intake. Excessive DI combined with impaired excretion due to renal failure can lead to elevated body levels

and toxicity. Hyperkalemia, or highly elevated K levels, can lead to cardiac arrest. Sustained levels of high Na intake are associated with an increased risk of hypertension in some individuals. The risk of hypertension may also be related to the Na to K ratio.

3.7.2 Dietary Sources, Intakes, and Bioavailability of Sodium, Potassium and Chlorine

Health Canada concluded that 115 mg d^{-1} of Na, 175 mg d^{-1} of Cl, and 1 170 mg d^{-1} of K are adequate to meet the nutritional needs of sedentary adults in a temperate climate (1). Since Canadian intakes far exceed these levels, no RNI was set. However, a decrease in Na intake and increase in K intake were recommended in order to reduce the risk of hypertension. The U.S. FNB has recommended a safe minimum intake for Na of 500 mg d^{-1} and Cl of 750 mg d^{-1} (17). The minimum K requirement was concluded to be 1 600-2 000 mg d^{-1} for adults. Recommendations for increased consumption of fruits and vegetables would increase that level to 3 500 mg d^{-1}.

Foods and beverages containing NaCl are the main sources of Na and Cl intake. Most of the consumed Na and Cl in North America is from salt added during manufacture and processing. The natural levels of NaCl in food account for about 10% of the intake, drinking water about 10%, and salt added during cooking or prior to consumption about 15% (17). Non-NaCl sources of Na (*e.g.* $NaHCO_3$ and monosodium glutamate) generally supply less than 10% of the daily Na intake. A small amount of Cl is supplied in the form of KCl.

There are few studies on Na, Cl and K in Canadian diet. In one such study

There are few studies on Na, Cl and K in Canadian diet. In one such study (323), where no salt was added during or after cooking, 70% of the Na intake (1 142 mg d^{-1}) was determined to originate from dairy products, meat, poultry and cereal products. Salt content of fruits, vegetables and legumes were generally low. A diet high in processed foods may be associated with high NaCl intakes. Some individuals have very high discretionary NaCl (*i.e.* salt added before consumption) intakes. Sodium intake in western countries varies from 1.8 to over 10 g d^{-1} (1, 17, 86).

Since K is an essential element to living cells, it is found in a wide variety of foods. Fruits, vegetables and fresh meats are good sources. Potatoes, bananas, orange juice, apricots, dried and fresh fruits, and fruit juices are excellent sources of K. However, processing of food often lowers K content due to leaching. Drinking water generally contributes little to the K intake. In the Canadian study cited above (323), 50% of the K intake was from dairy products, meat, fish, poultry, and cereal products. The Canadian intake of K is about 2.8 g d^{-1} while the K intake in the U.S. ranges from 1 to 11 g d^{-1} (17, 86).

Virtually all of the ingested Na, K, and Cl is absorbed from the intestines. Some electrolytes are lost in sweat and faeces. The body levels of each element are regulated by excretion *via* the kidneys.

In view of the importance of these elements to human health, concern related to NaCl intake and hypertension, and the limited Canadian data available on the intakes of these elements, more information on the DI of these elements would be useful. Therefore, one of the objectives of this thesis is to determine the Na, Cl, and K levels in

the duplicate diets collected.

3.7.3 Determination of Sodium, Potassium, and Chlorine by INAA

Sodium, K, and Cl were determined using the 1368.6-keV gamma-ray of ^{24}Na (half-life=15 h), 1524.7-keV gamma-ray of ^{42}K (half-life=12.4 h), and 1642.7-keV gamma-ray of ^{38}Cl (half-life=37.2 min). All gamma-rays were free from interference. Samples were analyzed for Na and Cl by irradiation for 1.5 min, decay for 1 min and counting for 10 min. The sensitivities of Na and Cl by this procedure were 15.1 and 10.9 counts μg^{-1}, respectively. Initially, the analysis for K was performed using the same conditions as above; however, the gamma-ray peaks were very small. In order to analyze for ^{56}Mn, a second 30-min count was performed on each sample after 1.5-7h decay, as discussed previously in section 3.4.3. The 1524.7-keV peak of ^{42}K was also measured in these spectra, giving improved sensitivity. Sensitivity using the first set of conditions was 0.212 counts μg^{-1}, whereas it was 0.818 counts μg^{-1} using the 30-min count. The results reported in this thesis were obtained using t_i=1.5 min, t_d=1.5-7 h, and t_c=30 min.

Four RMs and SRMs were analyzed for Na, K, and Cl using the above conditions. The results and calculated detection limits are shown in Table 3.49 and Figs. 3.15-3.17. The HMFS trial diet composite has been included for comparison purposes. For Na, the measured values appear to be slightly low which may suggest a systematic error, possibly in the comparator standards. However, these values are not low enough to be statistically different from the certified values. Therefore, the accuracy was considered to be satisfactory for the measurement of Na in the duplicate diets. An EQA

Table 3.49. Sodium, potassium and chlorine content of RMs, SRMs and trial diet HMFS determined by INAA

RM	Na			K			Cl		
	L_D	This work	Cert. value	L_D	This work	Cert. value	L_D	This work	Cert. value
IAEA H-4	57	1900 ± 40[a]	2060 ± 130	640	15800±700[a]	15800 ± 600	54	1840±50[a]	1890±83
Animal Muscle									
IAEA H-9	120	7900 ± 200[b]	8100 ± 70	1160	8500 ± 300[c]	8300 ± 700	128	12700±200[b]	12500±150
Mixed Diet									
NIST 1548	113	5940 ± 30[a]	6250 ± 260	983	6400 ± 600[a]	6060 ± 280	116	8200 ± 100[a]	8700 ± 400
Total Diet									
NIST 1568	13	ND	6 ± 1.5	242	1100 ± 200[a]	1120 ± 20	18	230±20[a]	-
Rice Flour									
NIST 1577b	10	2390 ± 20[d]	2420 ± 60	280	10070 ± 80[d]	9940±20	44	2680±60[d]	2780±60
Bovine Liver									
HMFS	114	6000 ± 100[a]	-	1140	6400 ± 200[a]	-	118	9000 ± 100[a]	-
Duplicate Diet									

All values in ppm. [a]. Average of three values; [b]. Average of five values; [c]. Average of two values; [d]. Average of six values

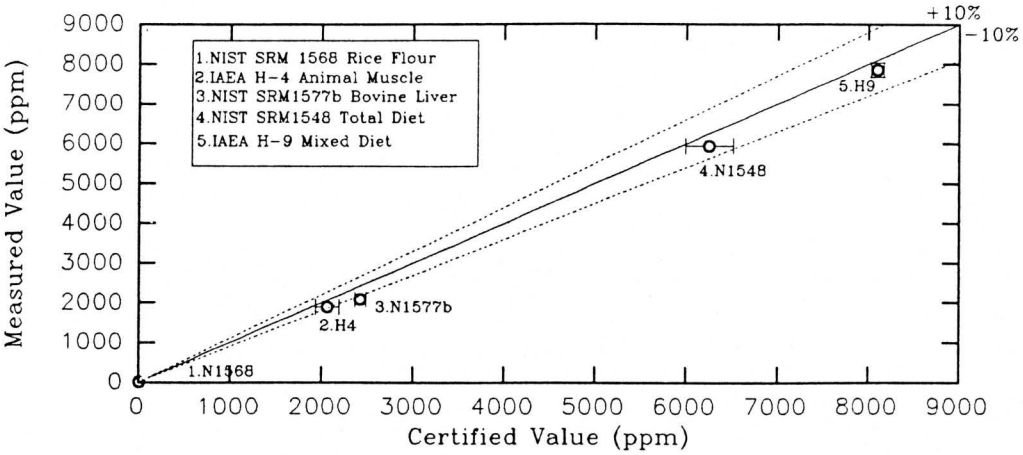


Fig. 3.15. Analysis of RMs and SRMs for sodium

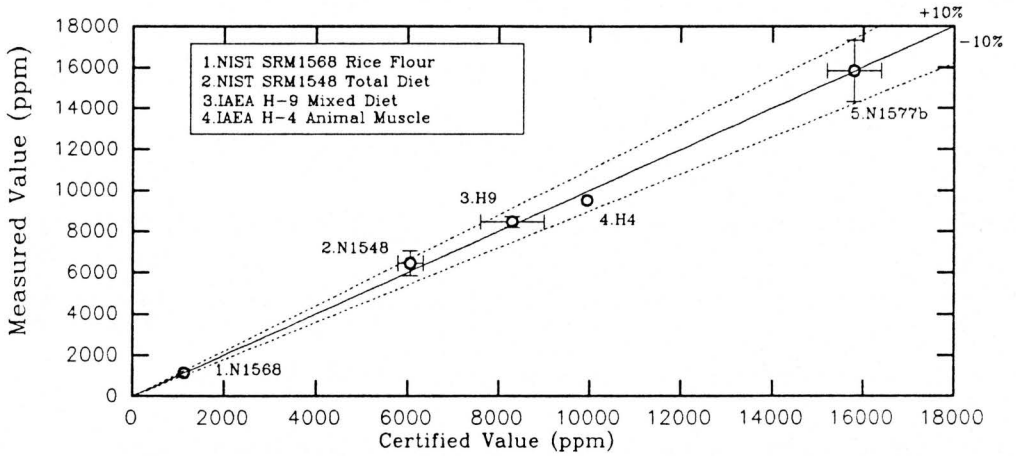


Fig. 3.16. Analysis of RMs and SRMs for potassium

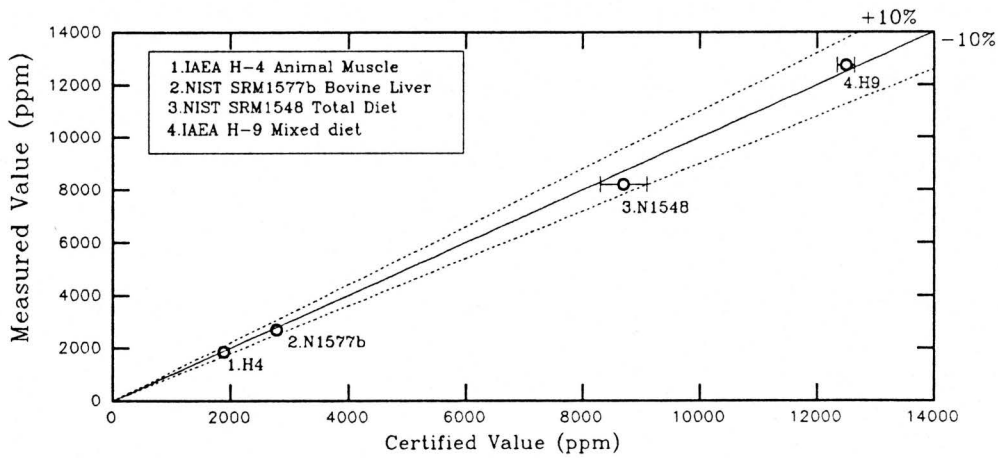


Fig. 3.17. Analysis of RMs and SRMs for chlorine

chart for Na obtained by replicate analysis of IAEA H-9 Mixed Diet is shown in Fig. 3.18. The first measured value (*i.e.* sample number 1) lies outside the upper warning limit but within the control limit. Since this result was obtained from the very first experiments, it might have occurred due to inexperience. All subsequent measurements were within the warning limits and close to the certified value.

It is evident from Table 3.49 and Fig. 3.16 that the agreement between analyzed and certified values for K is good. The precision of measurements varied from 5 to 20%. Detection limits were between 240 and 1160 ppm.. All measured concentrations were well above the detection limits. The EQA chart for K, prepared by analyzing the IAEA H-9 Mixed Diet, is shown in Fig. 3.19. All values lie within the warning limits of ±2s. The average of all determinations agrees well with the certified value. Datapoints for sample numbers 4, 5 and 7 were obtained by counting for 30 min after 1.5-7h decay. All other samples were measured using a 10-min count after 1-min decay. The increased precision of samples 4, 5 and 7 is a result of better peak statistics from the larger peaks.

The values of the four RMs and SRMs analyzed for Cl are shown in Table 3.49 and graphically presented in Fig. 3.16. The agreement between the measured and certified values is very good. The detection limits varied from 44 to 130 ppm. All measured concentrations were well above the detection limits. The values for the HMFS trial diet are also shown in Table 3.49 for comparison purposes. An EQA chart for Cl was prepared by analyzing IAEA H-9 Mixed Diet and is shown in Fig. 3.19. The variation between samples is fairly large although all points are within the warning limits.

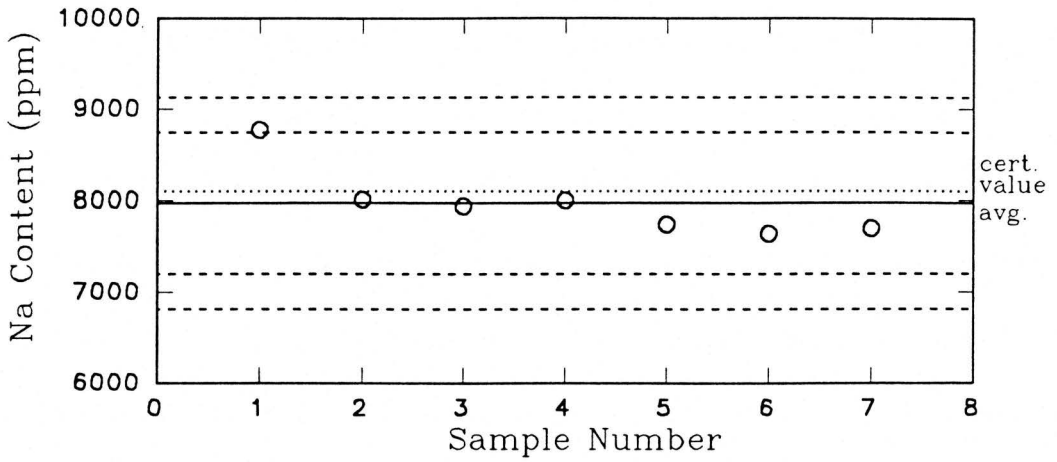


Fig. 3.18. External quality assessment chart for the analysis of sodium

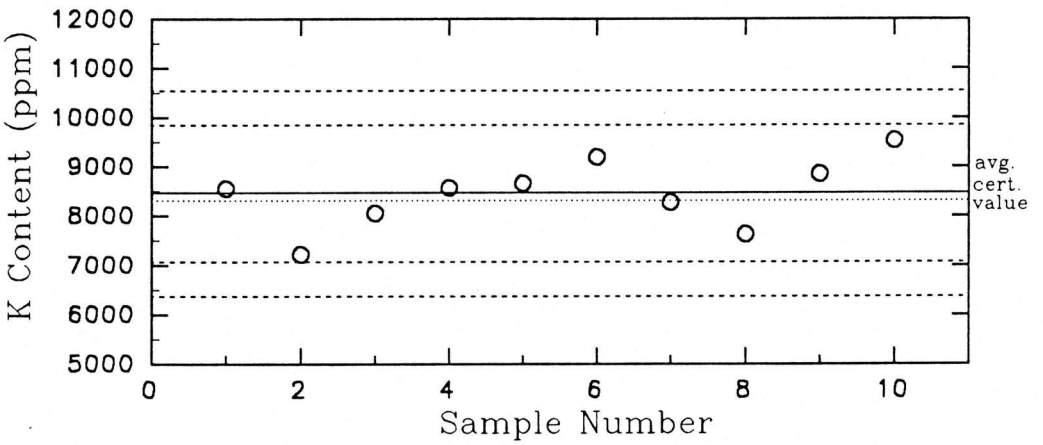


Fig. 3.19. External quality assessment chart for the analysis of potassium

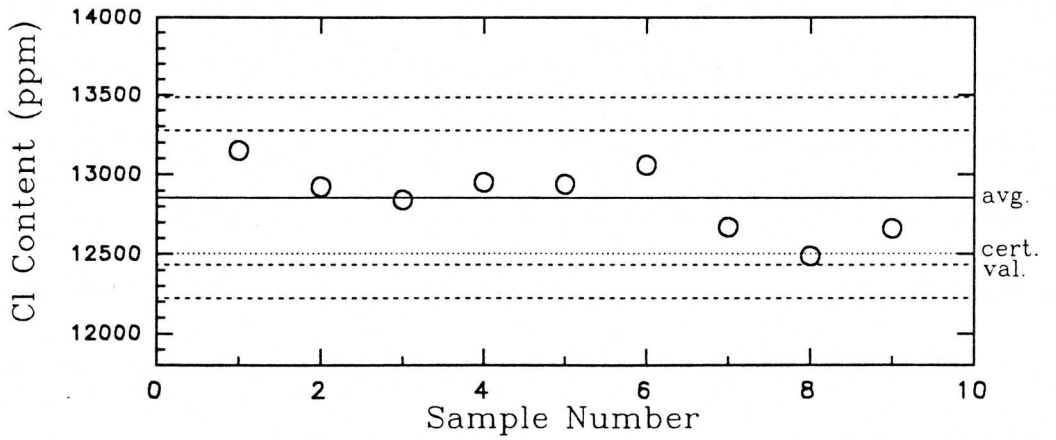


Fig. 3.20. External quality assessment chart for the analysis of chlorine

3.7.4 Sodium, Potassium, and Chlorine Content of Duplicate Diets

Fifteen duplicate diets were analyzed in this work for Na, K, and Cl, and the results are given in Table 3.50. The RSD for three replicate analyses of HMNT and HMFS for Na was relatively low, *viz.* 0.1% and 2%. For HFMC, the RSD was somewhat higher of about 14%. The RSD values for three replicate analyses of HMFS, HFMC, and HMNT for K were 2%, 7% and 7%, respectively. This level of precision is considered to be good and was a significant improvement over the preliminary work in which a 10-min counting time was used. The precision of measurement for Cl, assessed by RSD of the replicate analyses of the same diets was 1.1%, 9.9% and 0.8%, respectively. The results of analyses of the duplicate diets for Na, K, and Cl are discussed below in turn.

There was a fairly wide range of Na concentrations and intakes. The concentrations for HFFF, HFTC, and HMMR were particularly high (10 014 ppm, 12 420 ppm and 11 310 ppm, respectively), suggesting that they consumed high-Na foods. After examining the contents of these diets (Appendix 1), it was discovered that HFFF consumed Lipton Cup-a-Soup, HFTC consumed pickles, and HMMR consumed potato chips and hash browns, all of which are known to contain high levels of Na. The largest Na intake (7 300 mg d^{-1}) was observed for HMNT; since he consumed the most food (by mass), this is not surprising. A fairly high Na concentration in his diet also contributed to the large intake. The lowest DI, 1 300 mg d^{-1}, was measured in HFMC's duplicate diet. The median Na intakes were 3 070 mg d^{-1} and 3 600 mg d^{-1} for men and women, respectively. The overall median of 3 230 mg d^{-1} is well above the U.S. SADDI of

Table 3.50. Sodium, potassium and chlorine content of duplicate diet composites determined by INAA and FCT

Sample Code	Na			K			Cl	
	Conc.±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})	Conc.±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})	Conc.±SD (ppm)	DI from (mg d^{-1})
HMFS	6000±100[a]	4220±90[a]		6400±200[a]	4500±100[a]		9000±100[a]	6290 ± 70[a]
(a) Female Participants								
HFFF	10014	3380	3760	6600	2200	2000	14000	4730.00
HFMC	3300±500[a]	1300±200[a]	1510	6000±400[a]	2100±200[a]	1280	6200±600[a]	2200±200[a]
HFNA	5394	2150	3890	9200	3600	3130	9570	3810
HFRR	4270	1850	1760	8600	3700	3970	6450	2790
HFSR	6430	3070	1700	9200	3100	2960	9990	4760
HFTC	12420	4430	3710	8500	4400	2510	19100	6830
HFWH	8965	4310	4060	6000	4300	2480	11600	5560
Mean	7300	2920	2910	8000	3200	2620	11000	4380
Median	6430	3070	3710	8500	3000	2510	9990	4731
Min.-Max.	3580-12420	1250-4430	1510-4060	6000-10800	2100-4400	1280-3970	6226-19100	2200-6830
SADDI		500	500		2 000	2 000		750

Table 3.50. (continued) Sodium, potassium and chlorine content of duplicate diet composites determined by INAA and FCT

Sample Code	Na			K			Cl	
	Conc.±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})	Conc.±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})	Conc.±SD (ppm)	DI from (mg d^{-1})
(b) Males								
HMFF	4810	2240	2200	8400	3900	3120	7600	3540
HMMR	11310	3090	2810	8000	2200	1870	17100	4680
HMNT	5304±6[a]	3809±4[a]	4710	11700±800[a]	8400±600[a]	5630	8980±70[a]	6450±50[a]
HMRZ	6020	3600	1910	6800	4100	2570	8790	5250
HMSG	8950	4100	3760	7400	3400	2340	13600	6230
HMTH	4040	2130	2040	9000	4700	3800	6980	3680
HMWV	9040	7300	6640	8700	7000	5060	14040	11300
Mean	7070	3750	3440	8600	4800	3480	11000	5880
Median	6020	3600	2810	8400	4100	3120	8980	5250
Min.-Max.	4040-11300	2130-7300	1910-6640	6800-11700	2200-8400	1870-5630	6980-17100	3540-11300
SADDI		500	500		2 000	2 000		750
(c) Overall								
Mean	7180	3340	3180	8300	4000	3050	11000	5130
Median	6230	3230	3260	8500	3800	2760	9780	4750
Min.-Max.	3580-12420	1250-7300	1510-6640	6000-11700	2100-8400	1280-5630	6220-19100	2180-11300

[a]. Average of three values

0.500 g d^{-1}. In fact, none of the intakes fell below the SADDI.

Daily Na intakes of the duplicate diets calculated from FCT (27) are given in Table 3.50 and compared with measured values in Fig. 3.21. As shown in Fig. 3.21, measured and calculated values agree reasonably well. However, in FCT, the median intake for the female participants was overestimated while that for the male participants was underestimated. The overall median intake agreed well with the measured intake. Correlation coefficient for measured and calculated intakes was 0.83, suggesting a fairly high correlation. The t-statistic calculated for a paired t-test was 0.282 compared with a critical t-value of 2.06 at α=0.05. The F-value calculated using ANOVA was 0.0798, which was much lower than the critical F-value, 4.22, with P=0.78. Neither ANOVA nor the t-test indicated a difference between the values obtained using INAA and FCT. These results suggest that the FCT calculations performed well in estimating the intakes of the study participants.

A survey of the Na content of diets from different countries is given in Table 3.51. Sodium intake for adult men and women, and populations from various Canadian cities was reported by Shah *et al.* in 1982, to be 3 510 mg d^{-1}, 2 380 mg d^{-1}, and 2 740 mg d^{-1}, respectively (71). The DIs of the male participants of our work are quite close to the intake determined by Shah *et al.* Intakes for the female participants and the overall intake are larger than the values obtained for adult women and various cities determined by Shah *et al.* Early determinations of intakes by teen-age males in the U.S. F.D.A. Total Diet Study were relatively high, at 4 700-4 900 mg d^{-1} (81). After the reorganization of the Total Diet Study, in 1982, the intakes calculated for

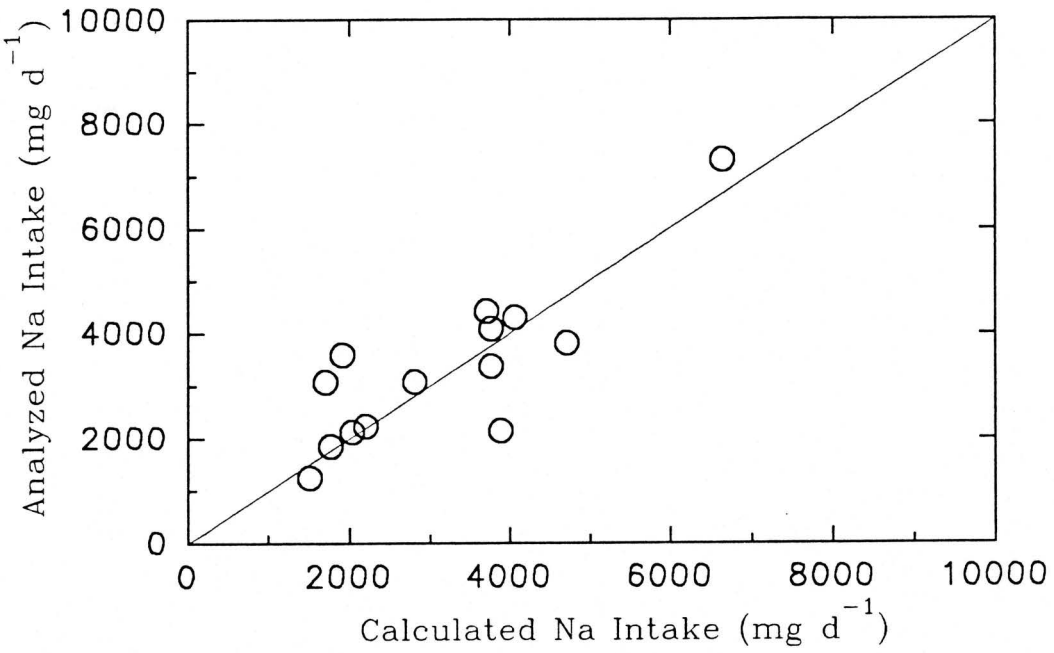


Fig. 3.21. DI of sodium determined by INAA and calculated using FCT

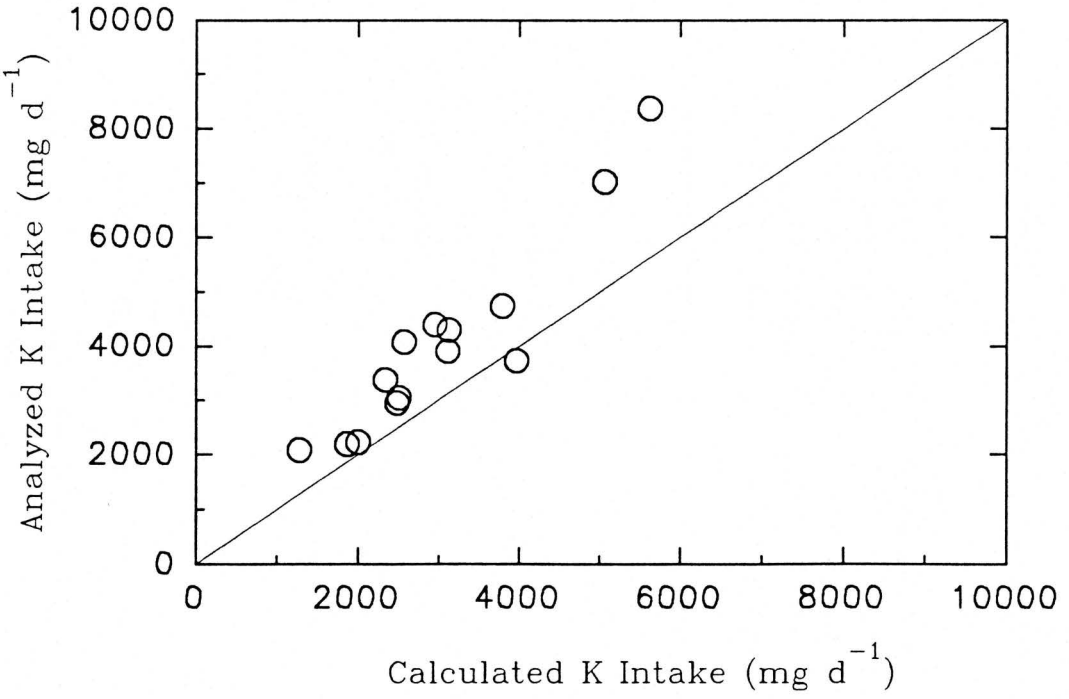


Fig. 3.22. DI of K determined by INAA and calculated from FCT

Table 3.51. Survey of DI (mg d^{-1}) of Na, K and Cl.

Study Group	Composite	Anal. Method	Na	K	Cl	Refs.
Canada						
Adult women, men	Equiv. composite	AAS	2 380-3 510	2 430-3 580		(71)
Denmark						
Adult men	Dupl. portion	AAS	3 274	3 429		(207)
Finland						
Hospital patients	Hospital diet	GFAAS	3 800	4 200		(97)
Wt. reduction patients	Duplicate portion	AAS	2 346	2 873		(238)
India						
Bombay residents	1/8 Dupl. portion	AAS	6 700	4 100		(262)
Hospital patient	Hosp. vegetar. diet	INAA	8 170	7 320	17 00	(218)
Japan						
Adult men	Dupl. portion	AAS/GFAAS	4 390	1 640		(257)
Adult men	Dupl. portion	ICP-AES	4 500	1 900		(302)
Pakistan						
Islamabad residents	Equiv. composite	INAA	878-1 168	2 278-2 490	1 420-1582	(228)
Spain						
Residents of various cities	Equiv. composite	FES	2 245-2 828	2 491-3 500		(263)
Sweden						
Female, male pensioners	Dupl. portion	Flame photo.	1 800-2 000	1 700, 2 100		(90)
Women; high-b.p., low-b.p.	Dupl. portion	AAS	2 415, 2 200	2 200, 2 000		(264)
Female, male vegans	Dupl. portion	Flame photo.	1 725, 2 714	3 170, 4 850		(91)
Dalby residents	Dupl. portion	Flame photo.	2 350	2 000		(92)
Female, male lactovegetarians	Dupl. portion	Flame photo.	2 024,2 390	3 400, 4 890		(93)
Adult men and women	Dupl. portion	Flame photo.	2 530	2 150		(2)
Taiwan						
Univ. faculty, staff, students	Dupl. portion	INAA	2 010-2 800	1 170-1 370	2 750-3 320	(43)

Table 3.51 (continued). Survey of DI (mg d^{-1}) of Na, K and Cl.

Study Group	Composite	Anal. Method	Na	K	Cl	Refs.
Turkey						
Ankara & Lalahan residents	Dupl. portion	INAA	4 340	2 380		(233)
The Netherlands						
Teen-age males (18 y)	Market basket	AAS	4 390	4 340		(232)
United Kingdom						
General population	Equiv. composite	MS/XRF/NAA	4 640	2 800	5 400	(240)
General population	Equiv. composite	not stated	3 200	2 510		(324)
U.S.A.						
Metabolic Unit Patients	Normal, prepared diet	Flame photo.	3 447, 2 815	3 240, 2 848	4 640	(320)
Female students	Dupl. portion	FES	2 258	1 914		(255)
Adult male volunteer 1, 2	Dupl. portion	arc ES	3 500, 4 600	1 700, 3 800		(300)
Hosp. Patients-summer,winter	Hospital diet	FES	5 912, 7 158	3 942, 4 881		(265)
Female university students	Metabolic diet	FES	4 100-4 650	2 440		(266)
Women & men	planned menu (25%, 35% cal fr. fat)	FES	2 784, 4 985	3 992, 4 985		(267)
Teen-age males	Market basket	INAA	<4 704	4 728	6 542	(78)
Male & female students	Cafeteria meals	flame photo.	7 540	5 940		(256)
Teen-age male, 1977	Market basket	AAS	4 701-4 974	3 366-3 485		(81)
Teen-age female, male1982-84	Market basket	ICP-AES	2 210, 3 359	1 998, 2 977		(83)
Adult female, male 1982-84	Market basket	ICP-AES	2 016, 3 097	1 938, 2 889		(83)
Adult males (USDIET-1)	Market basket	FAAS/ICP-AES/INAA	3 185	3 120	4 620	(195)
Teen-age female, male1982-89	Market basket	ICP-AES	2 162, 3 263	2 044, 3 030		(85, 86)
Adult female, male 1982-89	Market basket	ICP-AES	1 980, 3 040	2 017, 2 998		(85, 86)
West Germany						
Adult men	Dupl. portion	PNAA	4 500	2 400	6 000	(101)

teen-age declined to about 3300 mg d^{-1} (83, 85, 86). With the reorganization, intakes were calculated for various age groups, including adult men and women, giving values of about 3 100 mg d^{-1} for men and 2 200 mg d^{-1} for women. Earlier studies in the U.S. reported higher intakes. For example, in a study of male and female college students, a very high value of 7 540 mg d^{-1} was reported (256). An early study performed by Soman *et al*, published in 1969, reported a fairly large intake of 6 700 mg d^{-1} for Bombay, India residents (262). Sodium intakes larger than those of Canadians have been reported for adult Japanese men, Dutch teen-age males, and West German adult men (see Table 3.51). Intakes in Sweden and Taiwan were lower than North American intakes, at 1 800-2 714 mg d^{-1} and 2 010-2 800 mg d^{-1}, respectively. Quite low Na intakes of 878-1 168 mg d^{-1} have been reported for diets in Pakistan (228).

The K content and DIs of the fifteen duplicate diets are presented in Table 3.50. Median daily K intakes for men and women were 4 100 and 3 000 mg d^{-1}, respectively. The overall median intake was 3 800 mg d^{-1}. A relatively wide range of K intakes (2 100-8 500 mg d^{-1}) was noted for all participants. Two individuals, HMWV and HMNT, had relatively high intakes of 7 000 and 8 400 mg d^{-1}, respectively. The U.S. FNB and Health Canada recommend a K-rich diet because of the potential benefit in preventing hypertension (1, 17). All intakes exceeded the U.S. SADDI.

The DIs, calculated using FCT as shown in Table 3.50 and Fig. 3.22, tended to be lower than the measured values. Only in the case of HFRR was the calculated value greater than the measured value. However, neither t-test nor ANOVA indicated a difference between the two sets of data. The calculated t-value was 1.7

compared with a critical t-value of 2.1 at α=0.05. For ANOVA, the calculated F-value was 3.0 compared with a critical F-value of 4.3 at P=0.095. The two groups did have a strong Pearson-product correlation (0.94). These results suggest that FCT provided reasonably good estimates of K intake.

A summary of literature values for K intake is presented in Table 3.51. Shah *et al.* reported intakes from populations of Canadian cities (Table 3.11) to be 2 865 mg d^{-1} (71). They also reported intakes of adult men and women to be 3 580 and 2 430 mg d^{-1}, respectively. The K intakes determined from INAA in the present work are generally higher than many literature values. Total diets collected in the U.S. generally contain 2 000 mg d^{-1} for adult women and 3 000 mg d^{-1} for adult men. These values are about 1 000 mg d^{-1} lower than the duplicate diets of this study. Intakes of adult Danish men, Finnish hospital patients, Swedish vegetarians and vegans, Dutch teen-age males, U.S. hospital diet, a planned U.S. menu, and one study of U.S. teen-age males exceeded the intakes reported in this work (see Table 3.44). The lowest K intakes were reported from Japan (1 640 mg d^{-1}) and Taiwan (1 170 mg d^{-1}).

The Cl content of the duplicate diets for Cl are shown in Table 3.50. The median intake for the female participants was 4 730 mg d^{-1} while it was 5 250 mg d^{-1} for the men, and 4 750 mg d^{-1} overall. Individual intakes ranged from 2 180 to 11 300 mg d^{-1}. The intakes are all well below the SADDI, except for HMWV whose Cl intake, 11 300 mg d^{-1} was very high indeed. His Na intake was also the highest of the participants, which would account for the high Cl intake. The ratio of median Cl concentration to median Na concentration was 1.57. For NaCl salt, the Cl-to-Na ratio by

mass is 1.54. The close agreement of these values suggests that most of the Na and Cl in the diets was supplied by NaCl.

There are few studies on the Cl content of diet. The results of six studies are summarized in Table 3.51. No data on Cl in Canadian diet is available. Tanner and Friedman reported Cl intake of 6 542 mg d^{-1} for teen-age males in the U.S.A. (78). In Taiwan, Cl intake ranged from 2 750 to 3 320 mg d^{-1} (43). The intake for West German men was reported to be 6 000 mg d^{-1} (101). Chlorine intake in Pakistan is lowest of the literature surveyed, ranging from 1 420 to 1 582 mg d^{-1}.

3.7.5 Dialysis of Duplicate Diets for Sodium, Potassium and Chlorine

One of the objectives of the dialysis experiments was to minimize the effects of interfering elements such as Na and Cl. Dialyzed diet samples were analyzed using 5-min irradiations, 1-min decays, and 10-min countings. The same nuclides and gamma-rays discussed above were used. As discussed previously in section 3.1.10, dialysis was effective in eliminating almost all of these elements from the duplicate diets.

However, this was not the case in enzymolysis/dialysis experiments where the gamma-ray spectra had intense photopeaks of ^{24}Na and ^{38}Cl. It appeared that not all of the Na and Cl from the HCl and Na_2CO_3 used during the digestion procedure were removed. This limited irradiation times to only 5-15 s. Due to contamination from the reagents, Na and Cl were not analyzed in the enzymolyzed samples. The 1524.7-keV gamma-ray of ^{42}K could hardly be detected. The detection limit for the trial diet HMFS was 1 400 ppm which was higher than the detection limit of 1 140 ppm obtained after the

analysis of HMFS without enzymolysis. The poor detection limit was most likely due to the elevated background caused by the NaCl in contamination. A calculation of percent potentially available based on the detection limit indicated that more than 78% of the K in the diet was potentially available. There is no reason to suspect that the Na, K, and Cl availability in the samples subjected to enzymolysis/dialysis would be less than for samples subjected to dialysis alone, so the actual percent available is probably greater than 95%.

3.8 IRON

3.8.1 Iron in Health and Nutrition

Iron is an essential element required for humans. Its deficiency is considered to be the most common nutritional deficiency affecting man (6, 325, 326). The Nutrition Canada survey (65) indicated that the DIs of teen-age girls and adult women did not meet the RNI for Fe (13 mg d^{-1}). In the U.S.A., studies have shown that blacks, preschool-aged children, adolescents, menstruating women and the elderly are particularly at risk of Fe deficiency (325). Fairly serious problems with Fe deficiency exist in Africa and southern Asia (327). Obviously, assessment and monitoring of Fe intake are of high priority.

Nutritional aspects of Fe have been discussed in a number of review articles (1, 12, 241, 242, 325, 328). The adult human body contains about 3-4 g of Fe, of which 2.5 g are in functional forms such as haemoglobin and myoglobin, 3 mg in enzymes, 3 mg in transport species, and 0.3-1 g in storage species. The proteins haemoglobin and myoglobin distribute to and store oxygen in cells. Enzymes containing Fe include cytochromes involved in electron transport, catalase involved in metabolism of peroxides and ribonucleotide reductase involved in RNA synthesis.

Iron deficiency can result from a lack of bioavailable Fe in the diet, losses through bleeding, or increased demand (*e.g.* in pregnancy) leading to impaired production of red blood cells and anemia; symptoms include lethargy, reduced brain function, heart enlargement, and a depressed immune system. Iron toxicity (overload) results in damage to the liver, heart, pancreas, and possibly other organs, but it is not that common. The

only reported cases for a population consuming a "normal" diet occurred in a South African tribe, who had an Fe intake of approximately 115 mg d^{-1} (about ten times the normal Canadian intake) from beer brewed in Fe vessels. In Canada, the most common cases involve the accidental ingestion of supplements by children.

3.8.2 Dietary Sources, Intakes, and Bioavailability of Iron

Good sources of Fe are liver and other organ meats, beef, and other meats. Milk and eggs are poorer sources. Dried fruits, lima beans, legumes, dark green leafy vegetables, sardines, prune juice are also rich in Fe. Wholegrain cereals and breads are significant contributors to the DI of Fe. Many other vegetables, fruits, and food cooked in Fe utensils can add Fe to the diet as well (33, 35, 329). Processing and cooking can have significant effects on the levels and bioavailability of Fe in foods (29).

Absorption of Fe from diet varies with the Fe status of the person. In Fe-replete populations, about 10-15% of Fe consumed is absorbed (1, 241). Although many foods may contribute to the DI of Fe, not all foods are equally well absorbed. Heme Fe, which is bound to haemoglobin/myoglobin and is highly available, constitutes only 10-15% of the western diet. Most Fe in the diet is non-heme, sources of which include cereals, vegetables, fruits, eggs and Fe-fortified foods. The bioavailability of non-heme Fe is greatly affected by other components of the diet which enhance or inhibit absorption (6, 325, 326, 330). Meat, ascorbic acid, sucrose, fructose, lactic acid, succinic acid, and fats can enhance absorption of non-heme Fe. Phytates and fiber components from cereal foods, such as bread, and from legumes, such as soya, can bind Fe in insoluble form,

rendering it unavailable. Tannins in tea, polyphenol compounds in coffee, phosvitin in egg yolk, oxalates in spinach, and lactoferrin in milk are all inhibitors of Fe absorption. Other dietary minerals such as Ca, Cd, Co, Mn and Sn may interfere with Fe availability as well (6, 112, 326).

Currently, the Canadian RNI for Fe is 9 mg d^{-1} for men and 13 mg d^{-1} for women (1). The U.S. R.D.A. are higher, at 10 mg d^{-1} for men and 15 mg d^{-1} for women (17). A study in Canada (259) and others in the U.S. (*e.g.* 81, 83, 85) have reported the intake of teen-age and adult women to be about 10-11 mg d^{-1}, which is below the RNI and RDA. In fact, without supplementation or fortification of the diet, it may not be possible for many women to meet the RDA.

It is evident that Fe is important in human nutrition and that many individuals may not meet the recommended Fe intakes. It is also evident that there is limited information on Fe content of the Canadian diet perhaps due to the lack of analytical data. One of the objectives of the present work is to develop an INAA method for the determination of Fe in diet and to measure the DIs of Fe by the selected Canadian volunteers. Since Fe bioavailability varies with components in the diet, the enzymolysis/dialysis procedure was also used to attempt to estimate the potential bioavailability of Fe in the duplicate diets.

3.8.3 Determination of Iron by NAA

Iron was determined using the 1099.3-keV gamma-ray of ^{59}Fe (half-life=44.5 d). Since Fe is not very sensitive by INAA, the long irradiation and counting

times were desired. For safety reasons, the irradiation time in the DUSR facility was limited to less than 22 h. Most samples were irradiated for 17-18 h. Various counting times were evaluated, including 0.5, 1, 4, and 12 h. Twelve hours was the longest counting time practical for routine use, as it allowed overnight counting, making the analyzer available for other researchers during the working day. The restrictions on irradiation and counting times limited the sensitivity achievable. Even with a 12-h count, only a few counts were detected at the 1099.3-keV photopeak. The results reported in this section were obtained using irradiation times of 17-21.8 h, decay times of 14-60 d, and counting times of 12 h. The sensitivity obtained was 31.5 counts μg^{-1}.

The Fe concentrations of three RMs and SRMs are reported in Table 3.52 and Fig. 3.23. Precision of analysis was relatively poor with RSD of 29% for NIST SRM 1548, 28% for IAEA H-9, 12% for IAEA H-4, and 10% for NIST SRM 1577b. The poor precision can be explained in terms of poor count rates and relatively high detection limits. Although all measured values were above detection limits, they were close to or below the quantitative determination limits which varied from 58 ppm for IAEA H-9 to 170 ppm for NIST 1577b. There was satisfactory agreement between measured and certified values for most materials except for NIST SRM 1577b where the background around the 1099.3-keV peak of ^{59}Fe was relatively high due to the presence of a large 1115.5-keV peak of ^{65}Zn..

An EQA chart for Fe was prepared by replicate analysis of IAEA H-9 and is shown in Fig. 3.24. Precision of analysis was quite poor due to low count rates. All values were within the warning limits. Agreement between the average result and the

Table 3.52. Iron content of RMs, SRMs and the HMFS trial diet (ppm)

Reference material	L_D	This work	Certified value
IAEA H-4 Animal Muscle	20	53 ± 6[a]	49.0 ± 4.2
IAEA H-9 Mixed Diet	18	40 ± 11[b]	33.5 ± 2.5
NIST SRM 1548 Total Diet	19	32 ± 9[c]	32.6 ± 3.6
NIST SRM 1577b Bovine Liver	54	220 ± 20[c]	184 ± 15
HMFS Duplicate Diet	19	46 ± 7[a]	-

[a] average of three values; [b] average of five values; [c] average of four values

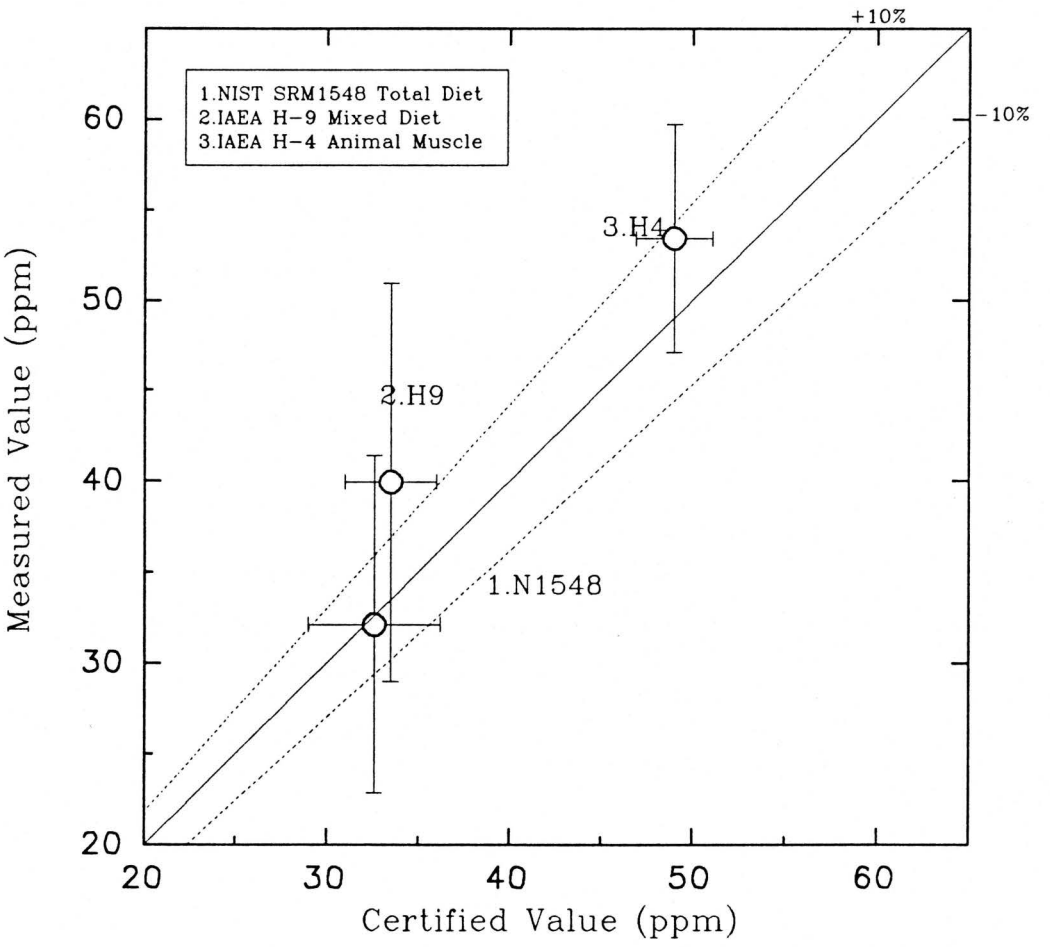


Fig. 3.23.Analysis of certified RMs and SRMs for iron

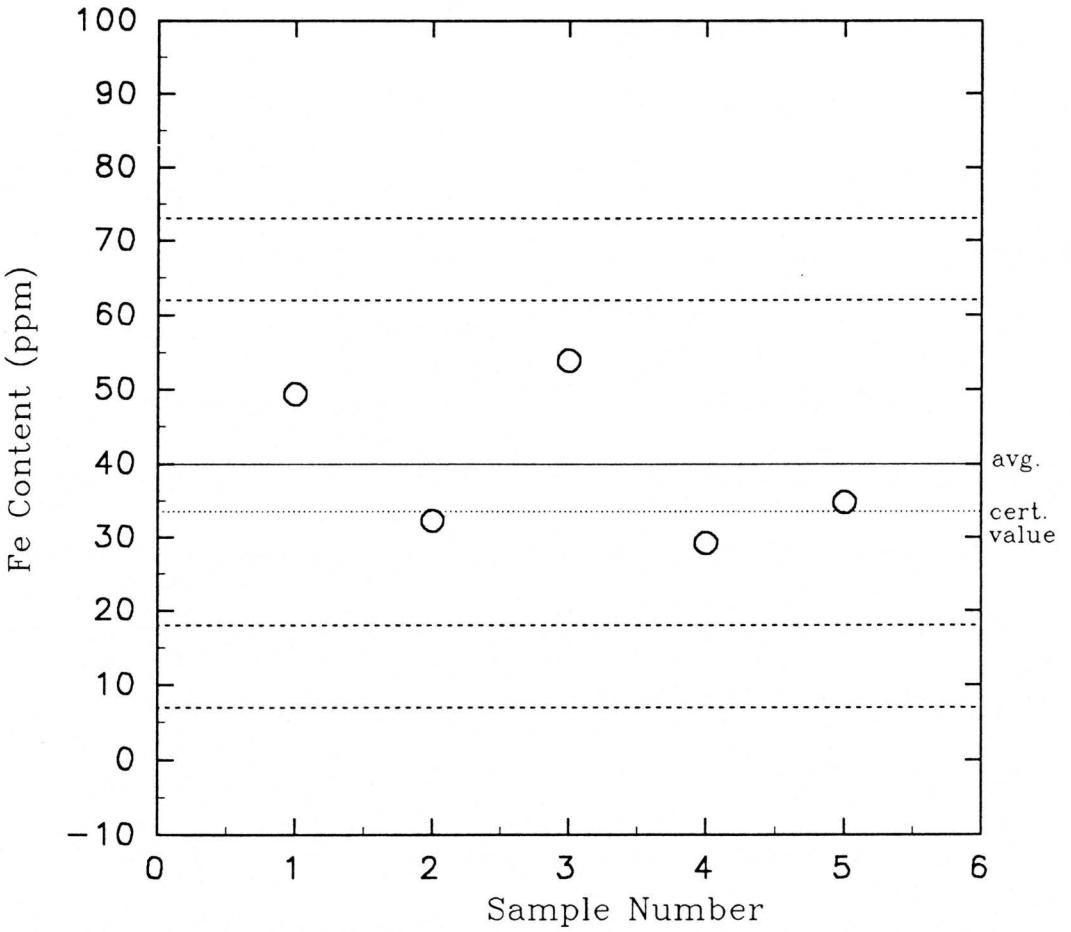


Fig. 3.24. External quality assessment chart for the analysis of iron

certified value was satisfactory.

3.8.4 Iron Content of Duplicate Diets

The concentrations of Fe in the duplicate diets and the DIs are reported in Table 3.53. The RSDs were calculated from three replicates of each of HMFS (14%), HFMC (26%), and HMNT (13%) diets; there is considerable uncertainty in these results.

For the women, the median concentration and DI of Fe were 49 ppm and 17 mg d^{-1} (Table 3.53). The concentration and DI of the men were higher than the women, the values being 59 ppm and 27 mg d^{-1}. Overall median concentration and intakes were 52 ppm and 21 mg d^{-1}, respectively. There is close agreement between the means and medians. There is a fairly broad range of intakes. The lowest intake, 9 mg d^{-1} was for HFMC, while the highest was 61 mg d^{-1}, determined for HMWV.

Daily intakes of Fe, calculated from FCT by Chatt and Pegg (27) are shown in Table 3.29 and Fig. 3.25. The calculated intakes are generally lower than the measured intakes. Median intakes for men, women and for both sexes were 17.7, 13.8 and 15.1 mg d^{-1}, respectively. A paired t-Test and ANOVA were performed. The F-value calculated for ANOVA was 2.48, while the F_{crit} was 3.26 at $P < 0.129$. The t-statistic calculated for the data sets was 1.63, while the critical t value was 1.72 for $P < 0.05$. Neither the t-Test nor ANOVA indicated a difference between the data sets. The Pearson-Product correlation coefficient calculated for the measured and calculated results was 0.83, indicating a high positive correlation between the two sets of data. These results suggest that the calculations based on FCT performed well in estimating the DI of Fe for the

Table 3.53. Iron content of duplicate diet Composites determined by INAA

Sample Code	Content±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})
HMFS	46±7[a]	32±5[a]	
(a) Females			
HFFF	49	16	12.6
HFMC	25±7[a]	9±2[a]	8.81
HFNA	70	27	13.8
HFRR	55	24	16.3
HFSR	65	31	28.4
HFTC	34	12	10.9
HFWH	23	11	13.9
Mean	46	19	14.9
Median	49	17	13.8
Min.-Max.	23-70	9-31	8.81-28.4
RNI		13	13
RDA		15	15
(b) Males			
HMFF	59	27	17.7
HMMR	67	18	12.3
HMNT	38±5[a]	27±4[a]	27.6
HMRZ	31	19	17.1
HMSG	32	15	10.8
HMTH	68	36	19.8
HMWV	75	61	32.1
Mean	53	29	19.6
Median	59	27	17.7
Min.-Max.	31-75	15-61	10.8-32.1
RNI		9	9
RDA		10	10
(c) Overall			
Mean	49	24	17.3
Median	52	21	15.1
Min.-Max.	23-75	9-61	8.81-32.1

[a]. Average of three values

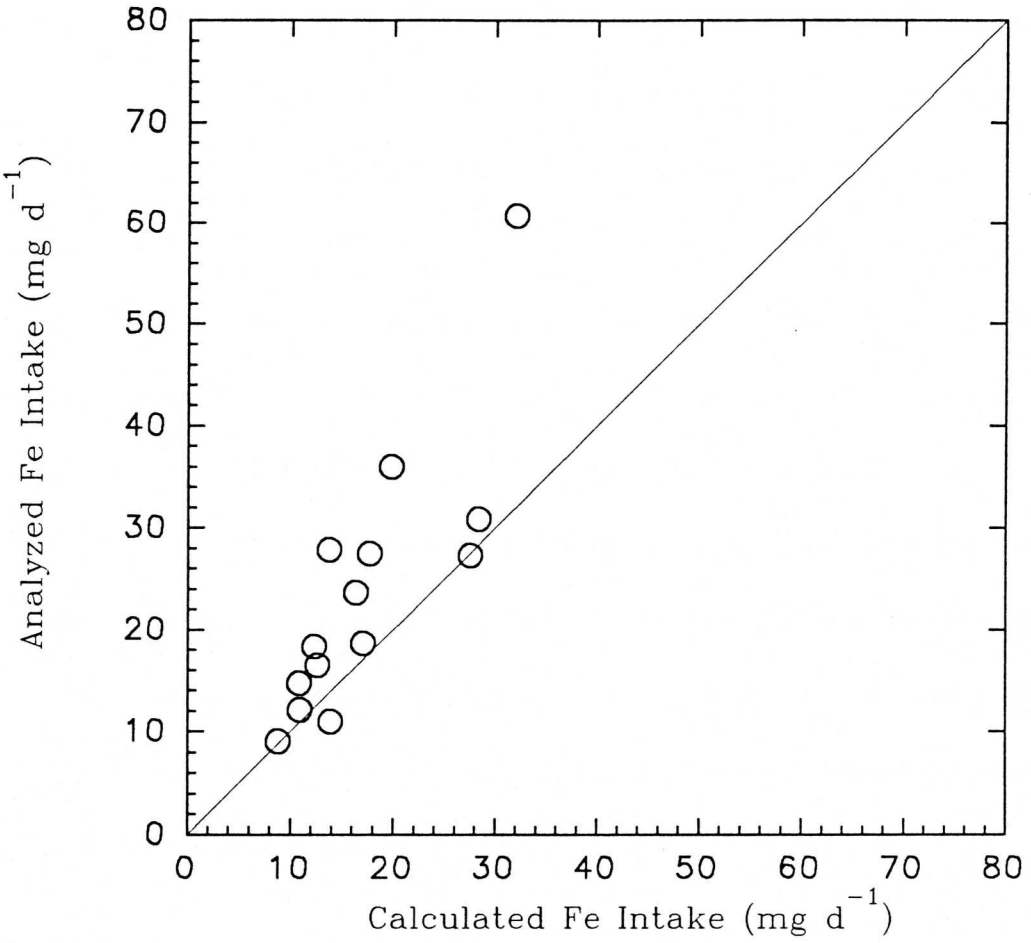


Fig. 3.25. DI of iron determined by INAA and calculated from FCT

study group.

A survey of the literature on Fe DI is summarized in Table 3.54. The DIs determined by INAA in this study agree reasonably well with DIs of 19.4 and 17.4 mg d^{-1}, determined by Meranger and Smith (74) and Kirkpatrick and Coffin (77) for Canadians, respectively. In a more recent study by Shah *et al.* (259), however, a lower value of 10.4 mg d^{-1} was reported for a "representative Canadian diet". In the U.S.A., a wide range of intakes have been reported, from a low value of 6.41 mg d^{-1} for female college and high school students (255) to a high value of 28 mg d^{-1} for a male volunteer (300). Both of these values were reported in 1969. More recent intakes in the U.S.A. are typically 11 mg d^{-1} for teen-age and adult females, 16 mg d^{-1} for adult males, and 18 mg d^{-1} for teen-age males (81, 83, 85, 86). The intakes in most countries are in the range of 8 to 14 mg d^{-1}. Particularly low Fe intakes, 5 mg d^{-1} in Italy (215, 216) and 6 mg d^{-1} in Taiwan (43) have been reported. The highest intake in the literature survey was 62.4 mg d^{-1} obtained for Indian vegetarian diet (218).

3.8.5 Enzymolysis of Duplicate Diets for Iron

The diet samples subjected to enzymolysis were analyzed for Fe by irradiation for about 7 h, decay for up to 250 d and counted for 12 h. The digestion blanks contained 36±11 μg of Fe, which was relatively large compared with the 29-130 μg contained in most of the digested diets. The high and variable blanks led to large RSD values of 21-48% for the Fe concentrations of digested HMFS, HFMC and HMNT diets. The percent potentially available was calculated from the differences between the

Table 3.54. Survey of DDI of iron

Study Group	Composite	Analytical Method	Intake (mg d^{-1})	Refs.
Canada				
General population	Market basket	AAS	19.443	(74)
Winnipeg residents	Market basket	AAS	17.398	(77)
General population	Equiv composite	AAS	10.4	(259)
China				
Adult farmers	Equiv. composite	AAS	18.0	(237)
Denmark				
Adult men	Duplicate portion	AAS	11.3	(207)
Finland				
Hospital patients	Hospital diet	GFAAS	13	(97)
Wt. reduction patients	Duplicate portion	AAS	8.8	(238)
France				
Adult women, men	Equiv. composite	AAS	8.1, 10	(210)
India				
Bombay residents	1/8 Dupl. portion	AAS	39.4	(262)
Hospital patient	Hosp. vegetar. diet	INAA	62.4	(218)
Italy				
Residents of various cities	Dupl. portion (?)	INAA	5.1-13.3	(215, 216)
Japan				
Adult men	Duplicate portion	AAS/GFAAS	11.4	(257)
New Zealand				
Female students	Metabolic diet	AAS	12.6	(222)
Pakistan				
Islamabad residents	Equiv. composite	INAA	9.2-18.3	(228)
Spain				
University students	Cafeteria meals	FAAS	15.37	(287)
Residents, various regions	Equiv. composite	AAS	10.79-14.97	(263)
Sweden				
Female, male vegans	Duplicate portion	AAS	16, 17	(91)
Female, male lactovegetarians	Duplicate portion	AAS	12.7, 16	(93)
Adult men and women	Duplicate portion	AAS	12.1	(2)
General population	Market basket	AAS	16	(98)
Taiwan				
University staff, faculty, students	Duplicate portion	INAA	9.4, 6.0, 6.7	(43)
The Netherlands				
Teen-age males (18 y)	Market basket	AAS/GFAAS	14.1	(232)
Turkey				
Ankara & Lalahan	Duplicate portion	INAA	16.7	(233)
U.S.A.				
Nursing Students	1/4 Dupl. portion	Colorimetry	9.2	(331)

Table 3.54. (continued) Survey of DDI of iron

Study Group	Composite	Analytical Method	Intake (mg d^{-1})	Refs.
Adult male volunteer	Duplicate portion	arc em. spec.	15	(300)
Adult male volunteer	Duplicate portion	arc em. spec.	28	(300)
Female students	Duplicate portion	FES	6.41	(255)
Hosp. Patients-summer,winter	Hospital diet	FES	7.6, 9.2	(265)
Female university students	Fe-fortified metabol. diet	FES	22.9	(266)
Men & women planned menu	(25%, 35% cal fr. fat)	FES	13.6, 18.6	(267)
Teen-age males	Market basket	INAA	30	(78)
Male & female students	Cafeteria meals	AAS	16	(256)
Hospital patients	Hospital diet	AAS	13.4	(268)
Hospital patients	Vegetarian Hosp. diet	AAS	14.3	(268)
Male volunteers	Metabolic diet	GFAAS	15.8	(269)
Teen-age male, 1974	Market basket	AAS	19.9	(81)
Teen-age male, 1975	Market basket	AAS	18.3	(81)
Teen-age male, 1976	Market basket	AAS	15.5	(81)
Teen-age male, 1977	Market basket	AAS	15.8	(81)
Teen-age male, 1978	Market basket	AAS	16.7	(81)
Teen-age male, 1979	Market basket	AAS	17.5	(81)
Teen-age male, 1980	Market basket	AAS	19.8	(81)
Teen-age male,1981/1982	Market basket	AAS	18.0	(81)
Teen-age female, male1982-84	Market basket	ICP-AES	10.7, 17.2	(83)
Adult female, male 1982-1984	Market basket	ICP-AES	10.4, 15.9	(83)
Adult males (USDIET-1)	Market basket	FAAS/ICP-AES/INAA	15.74	(195)
Teen-age female, male1984-85	Market basket	ICP-AES	10.86, 17.53	(85)
Adult female, male 1984-85	Market basket	ICP-AES	10.43, 15.89	(85)
Teen-age female, male1985-86	Market basket	ICP-AES	10.78, 17.48	(85)
Adult female, male 1985-86	Market basket	ICP-AES	10.50, 15.87	(85)
Adult men	Duplicate portion	AAS	16.0	(270)
Teen-age female, male1982-89	Market basket	ICP-AES	11.0, 18.1	(86)
Adult female, male 1982-89	Market basket	ICP-AES	10.7	(86)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	23.25	(240)
West Germany				
Adult men	Duplicate portion	PNAA	13.9	(101)

Fe concentrations of the undigested diets and the residue of the digested diets. Since these differences were small and the SDs of the concentrations were large, the calculated uncertainties of the percent potentially available values were very large (Tables 3.55 and 3.56). Consequently, the percent available values are suitable only for a qualitative interpretation. All diets had low calculated percent availability except for HMFF. This diet contained beef from a stew, a pork chop, orange juice, a nectarine, and orange soda pop containing orange juice, but no coffee. As previously discussed in Section 3.8.2, meat and ascorbic acid (from the juice, nectarine and soda pop) are promoters of Fe absorption, whereas whole-grain cereals and tea/coffee in many of the other diets contain compounds which inhibit Fe absorption. These factors may explain the relatively high percent Fe potentially available of the HMFF diet. A few of the other samples, namely freeze-dried HFMC and HFTC, and HFWH wet slurry, had higher iron concentration in the digested samples than in the undigested diets suggesting contamination. The problems of the large uncertainties of the percent potentially available would likely be solved by direct measurement of the potentially available iron in the dialysate. The development of an analytical method for this purpose could be the subject of future work.

Table 3.55. Enzymolysis and dialysis of freeze-dried duplicate diets for iron.

Sample	Not Potentially Available		Potentially Available[a]	
	Conc.±SD (ppm)	% Non-diayzed±SD	DI (mg d^{-1})	% Dialyzed±SD
HMFS	36±18	78±49	7±17	22±49
(a) Females				
HFFF	42	85	2.5	15
HFMC	47	181	-7.4	-81
HFNA	36	51	14	49
HFRR	34	63	8.8	37
HFSR	76	120	-5.4	-18
HFTC	260	770	-81	-666
HFWH	21	88	1.3	12
Mean	73	193	-9.6	-93
Median	42	88	1.3	12
Min-Max	20-260	51-770	-80-14	-670-49
(b) Males				
HMFF	30	34	18	66
HMMR	76	110	-2.5	-14
HMNT	30±6	78±25	6.0±7.6	22±25
HMRZ	22	69	5.8	31
HMSG	32	101	-0.14	-1.0
HMTH	42	62	14	38
HMWV	63	84	9.8	16
Mean	41	77	7.2	23
Median	32	78	6.0	22
Min-Max	20-76	34-110	-2.5-18	-14-66
(c) Overall				
Mean	57	135	-1.2	-38
Median	42	85	2.5	15
Min-Max	20-260	34-770	-81-18	-670-66

[a] :calculated by difference.

Table 3.56. Enzymolysis and dialysis of wet duplicate diet slurry for iron.

Sample	Not Potentially Available		Potentially Available[a]	
	Conc.±SD (ppm)	%Non-diayzed±SD	DI (mg d^{-1})	%Dialyzed±SD
HMFS	34±15	74±42	8.5±15	26±42
(a) Females				
HFFF	43	88	2.0	12
HFMC	29±9.3	110±65	-0.94±5.6	-10±65
HFNA	46	66	9.5	34
HFRR	44	81	4.5	19
HFSR	53	82	5.4	18
HFTC	13	38	7.5	62
HFWH	40	180	-8.4	-77
Mean	38	92	2.8	8.3
Median	43	82	4.5	18
Min-Max	13-53	38-180	-8.4-9.5	-77-62
(b) Males				
HMFF	9.9	17	23	83
HMMR	69	100	-0.52	-2.8
HMNT	28±6	75±26	6.8±8.0	25±26
HMRZ	17	56	8.2	44
HMSG	33	100	-0.61	-4.2
HMTH	62	91	3.2	9.1
HMWV	59	78	13	22
Mean	40	75	7.6	25
Median	33	78	6.8	22
Min-Max	9.9-69	71-100	-0.61-23	-4.2-83
(c) Overall				
Mean	39	83	5.2	17
Median	43	81	5.4	19
Min-Max	9.9-69	17-180	-8.4-23	-77-83

[a] : calculated by difference.

3.9 ZINC

3.9.1 Nutritional and Toxicological Aspects of Zinc

Zinc is an essential trace element for man and animals. Zinc deficiency is a serious worldwide problem, even in affluent countries (327, 332, 1, 134). The nutritional and toxicological significance of Zn has been the subject of a number of review articles (12, 241, 242, 1, 332, 134). All tissues and fluids of the human body contain Zn, the total content being 2 to 3 g. Zinc plays a role in a wide range of biochemical processes, such as bone development (272), the synthesis and degradation of lipids, proteins and nucleic acids, male reproductive function, and in the process of gene expression. Over 200 enzymes are known to contain Zn, including Cu-Zn superoxide dismutase, alcohol dehydrogenase, carboxypeptidase, and alkaline phosphatase. Other enzymes are also activated by Zn.

Marginal or mild Zn deficiency leads to reduced growth rate and impaired immune function. Low Zn intake may also cause impaired taste and slower wound healing, and be involved in the development of osteoporosis (272). Severe deficiency leads to growth retardation, delayed sexual and skeletal maturation, orificial and acral dermatitis, diarrhea, alopecia, and behavioral changes.

Since it is one of the least toxic trace metals, Zn toxicity occurs only rarely in humans. Toxicity by ingestion (12) occurs at very high intakes (>2 g) only. There are a few reported cases of accidental or intentional ingestion of therapeutic forms and poisonings due to consumption of soured food stored in Zn-plated metal containers (12, 242, 332). Toxic effects include nausea, vomiting, diarrhea, fever, muscle incoordination,

dizziness, lethargy, renal failure, and anemia. Excessive Zn intakes (150 mg d^{-1}) have also been reported to impair immune response (see citations in refs. 1 and 332) and interfere with the metabolism of other trace elements, especially Cu.

The basal requirement of adult men and women has been estimated to be 5.7 and 4.0 mg d^{-1} (134), respectively. Health Canada has set RNIs for men and women to be 12 and 9 mg d^{-1}, respectively (1). The U.S. R.D.A. for Zn is 15 mg d^{-1} for adult men and 12 mg d^{-1} for adult women. The WHO has proposed an upper limit of 45 mg d^{-1} as the safe range of population mean intake for adults (134).

3.9.2 Dietary Sources, Intakes, and Bioavailability of Zinc

Hazell has given a detailed review of the dietary sources and bioavailability of Zn (6). Foods derived from animals, such as meats, shellfish, and egg yolks are high in bioavailable Zn. Although dairy products and whole grains are rich in Zn, the bioavailability of both sources is low. Fruits, vegetables, and fats are generally poor sources. The Zn intake in industrialized countries from diets which are relatively high in fat, refined sugar and animal protein is about 10-12 mg. A number of estimates of Zn intake, 7 to 20 mg d^{-1}, in Canada have previously been made using various diet collection methods and AAS (74, 77, 276, 259, 333).

Absorption of Zn by the small intestine is usually 10-40%, although it has been reported to be as low as 1% and as high as 90% (see refs. cited in 6). Absorption and excretion vary with intake and Zn status. Several factors can enhance or inhibit absorption. Meat, protein, oxalate, and citric acid enhance Zn absorption whereas alcohol

inhibits it (6, 332). Phytate, present in bran, whole grain cereals, legumes and in other vegetables forms a precipitate with Zn rendering it unavailable. Minerals such as Ca, Cd, Cu, Fe, Hg, Mo, and Se can interfere with Zn uptake. Calcium can form a Ca-phytate precipitate, which co-precipitates Zn more effectively than Zn-phytate. Dietary fibre may also bind Zn.

In view of the concerns about Zn deficiency, potential effects on Cu metabolism, and the fairly wide range of intakes previously reported in Canada, more data on the Zn intake from the diet would be valuable. In order to address this problem, an INAA method was developed in this thesis for the analysis of Zn in diet. Because of the influence of other food components on Zn bioavailability, the enzymolysis/dialysis experiments were used in an attempt to estimate the potential bioavailability of Zn in the duplicate diets.

3.9.3 Determination of Zinc by INAA

Zinc can be determined by INAA using the 1115.5-keV gamma-ray of ^{65}Zn (half-life=244.3 d). This peak can be interfered with by 1112.2-keV gamma-rays of ^{152}Eu (half-life=13 a), 1115.5-keV of ^{65}Ni (2.52 h), 1120.0-keV of ^{214}Bi, 1120.5-keV of ^{46}Sc (83.8 d) and 1121.2-keV of 182 Ta (115 d). Because ^{65}Ni has a short half-life and poor sensitivity, and the concentrations of Eu and Ta tend to be low in biological materials, no interference from these interfering nuclides was expected. Scandium is present in many biological samples at fairly high concentrations while the 1120.0-keV gamma-ray of ^{214}Bi is present in the natural background. The 1115.5-keV peak of ^{65}Zn was

overlapped somewhat by a peak at 1112 keV, attributed to a ^{152}Eu source used in the DUSR facility, and a peak at 1120 keV due to ^{214}Bi and ^{46}Sc. Fortunately, the degree of overlap of the interfering photopeaks was small relative to the 1115.5-keV gamma-ray of ^{65}Zn, and was not considered to be significant. All of the above assumptions have proven to be valid as there was a good agreement between the measured and certified values of the RMs and SRMs analyzed in this work (Table 3.57).

The ^{65}Zn nuclide was used for INAA of the duplicate diets. The longest irradiation time available, 17-21.8 h was used. Sufficient decay time was permitted to allow shorter-lived ^{82}Br and other short-lived radionuclides to decay. One week was generally sufficient for this purpose although decay times of up to 60 d were used for convenience. Most samples were counted for 1 h. The sensitivity was 32.30 counts μg^{-1}. The detection limit for the trial diet HMFS was 4.5 ppm (Table 3.57).

In order to assess the precision and accuracy of Zn analysis, four RMs and SRMs were analyzed. The results for the RMs, along with the results for the HMFS trial diet, are presented in Table 3.57 and Fig. 3.26. The average measured value was higher than the certified value for each RM. This may have been a result of systematic error although agreement between measured and certified values was acceptable. The RSD values were 15% for H-9 Mixed Diet, 12% for 1568 Rice Flour, 9.7% for 1548 Total Diet, and 1.8% for 1577b Bovine Liver. The imprecision of the first three likely results from uncertainty in the net peak areas due to the interfering photopeaks.

An EQA chart for Zn was prepared by replicate analysis of IAEA H-9 Mixed Diet is shown in Fig. 3.27. All measured values are within the warning limits.

Table 3.57. Zinc Content of RMs, SRMs and the HMFS trial diet determined by INAA.

Reference material	L_d (ppm)	This work (ppm)	Certified value (ppm)
IAEA H-9 Mixed Diet	4.9	31 ± 5[a]	27.5 ± 1.8
NIST SRM 1548 Total Diet	4.8	34 ± 3[b]	30.8 ± 1.1
NIST SRM 1568 Rice Flour	4.6	23 ± 3[c]	19.4 ± 1.0
NIST SRM 1577b Bovine Liver	3.3	130 ± 2[c]	127 ± 17
HMFS Duplicate Diet	4.5	22 ± 2[c]	-

[a] average of seven values; [b] average of five values; [c] average of four values.

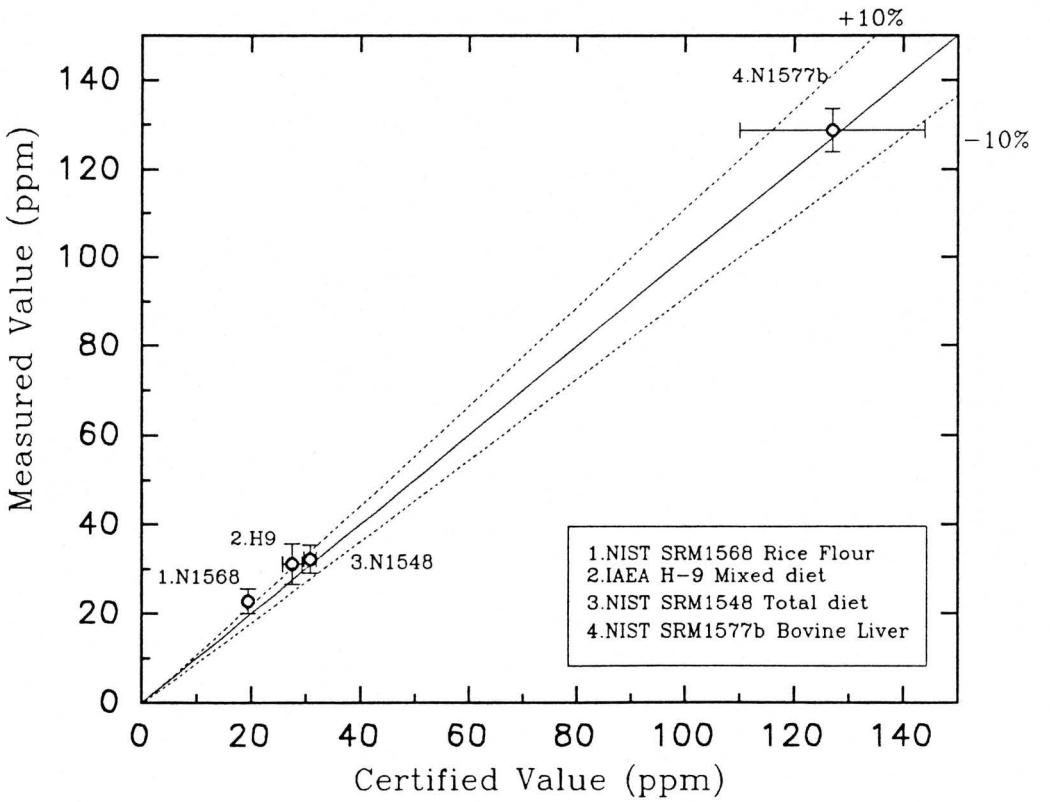


Fig. 3.26.Analysis of certified reference materials for Zinc

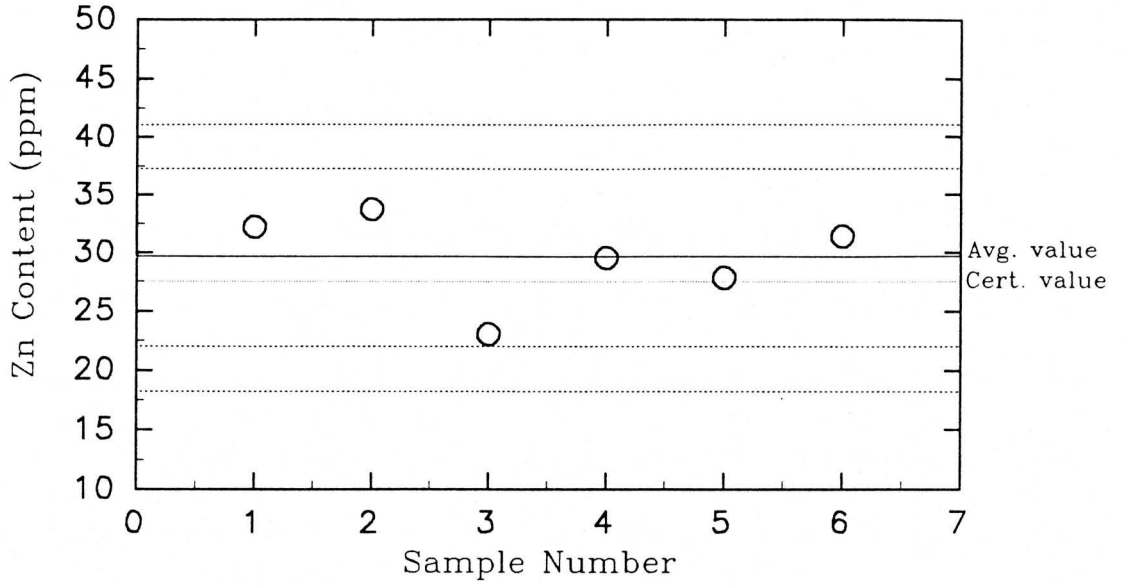


Fig. 3.27. External quality assessment chart for the analysis of zinc

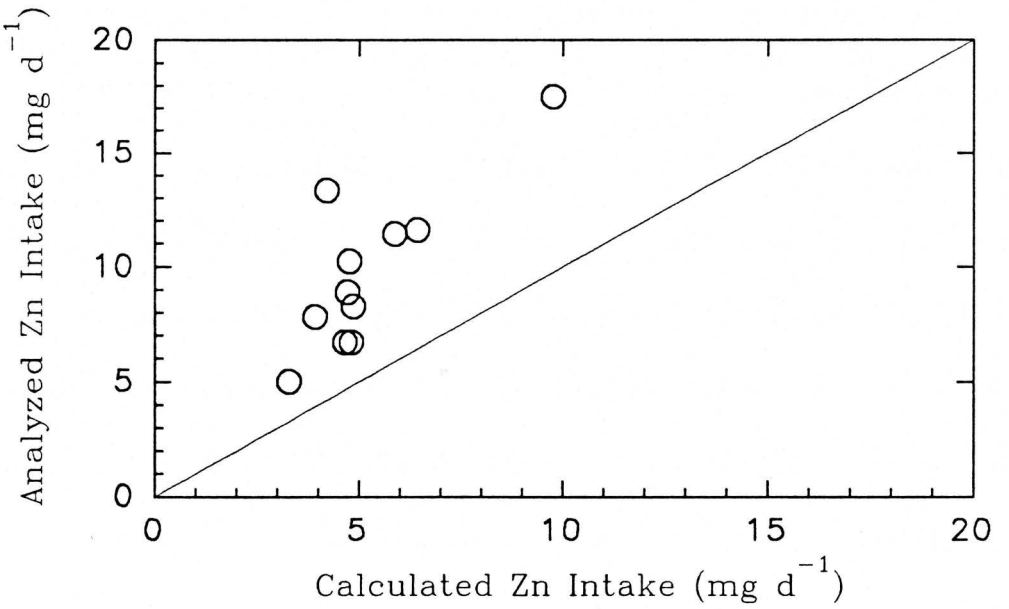


Fig. 3.28. DI of zinc determined by INAA and calculated from FCT

The large SD (one half of the warning limits in Fig. 3.27) is indicative of the relatively poor precision of the method. However, the method did appear to be in statistical control throughout the analysis.

3.9.4 Zinc Content of Duplicate Diets Determined by INAA

The results of INAA of the duplicate diets for Zn are presented in Table 3.58. Precision was evaluated by analyzing four replicates each of the HMFS, HFMC and HMNT diets, the RSDs of analysis being 9%, 8% and 10%, respectively. The Zn concentrations in the diet varied from 14 to 47 ppm. The concentrations for men and women were very similar. The female, male, and overall mean concentrations were all 26 ppm, and the median concentrations were either equal or very close to the mean. These results suggest similar diet compositions with respect to Zn for the men and women and a symmetric distribution of the concentrations. The highest concentrations were 44 ppm for HFSR, 47 ppm for HMFF, and 37 ppm for HMNT. An inspection of the food records revealed that all three of these composites included beef, known to be a good source of Zn. Other diets which included beef were HFRR and HMFS. The HFRR diet had a fairly high concentration of 31 ppm, while HMFS had a below average concentration of 22 ppm, perhaps because the quantities of beef in a casserole and pizza may have been small. The diets with the lowest Zn concentration were HFMC (14$\pm$1 ppm) and HMSG (15 ppm). These diets were high in fruits and berries and food fats, and HFMC was particularly high in fluids. Neither diet contained whole grains and both were relatively low in milk products.

Table 3.58. Zinc content of duplicate diet composites determined by INAA

Sample Code	Content±SD (ppm)	DI from INAA (mg d^{-1})	DI from FCT (mg d^{-1})
HMFS	22 ± 2[a]	15 ± 1[a]	
(a) Females			
HFFF	26	8.9	4.71
HFMC	14 ± 1[a]	5.0 ± 0.4[a]	3.28
HFNA	29	11	5.87
HFRR	31	13	4.20
HFSR	44	21	3.79
HFTC	22	7.8	3.92
HFWH	17	8.3	4.85
Mean	26	11	4.37
Median	26	8.9	4.20
Min.-Max.	14 - 44	5.0 - 21	3.28-5.87
RNI		9	9
RDA		12	12
(b) Males			
HMFF	47	22	6.12
HMMR	25	6.7	4.66
HMNT	37 ± 4[a]	27 ± 3[a]	9.68
HMRZ	17	10	4.76
HMSG	15	6.7	4.81
HMTH	22	12	6.43
HMWV	22	18	9.76
Mean	26	14	6.60
Median	22	12	6.12
Min.-Max.	15 - 47	6.2 - 27	4.66-9.76
RNI		12	12
RDA		15	15
(c) Overall			
Mean	26	13	5.49
Median	22	11	4.79
Min.-Max.	14 - 47	5.0 - 27	3.28-9.76

[a]:average of four values

The Zn DI varied from 5.0 to 27 mg d^{-1}. The overall mean and median intakes were 13 and 11 mg d^{-1}. Intakes for the men (mean 14 and median 12 mg d^{-1}) were slightly higher than those of the women (mean 11 and median 8.9 mg d^{-1}), reflecting the higher overall diet mass of the men. The diets with the highest DIs were HFSR (21 mg d^{-1}), HMFF (22 mg d^{-1}) and HMNT (27 mg d^{-1}), a result of the high Zn concentrations in these diets. The diets with the lowest concentrations of Zn were also those with lowest intakes of 5.0 mg d^{-1} for HFMC and 6.7 mg d^{-1} for HMSG.

Zinc daily intakes calculated from FCT (27) are also reported in Table 3.58 and depicted graphically in Fig. 3.28. In all cases, the values calculated from FCT are lower than those calculated from measured concentrations. The correlation coefficient calculated for the two sets of data was 0.64, which was the weakest correlation of all elements discussed in this thesis. A paired t-Test gave a t-value of 3.9, compared with a critical t-value of 2.1 at α=0.05. An ANOVA calculation was also performed, giving an F-value of 15.0, compared with a critical F-value of 4.22 at P=0.00064. Both t-Test and ANOVA indicate a difference between these data sets. Calculations based on FCT significantly underestimated the Zn intake of the study participants. The overall median of the DIs calculated using FCT was 44% of the DI obtained using INAA. It is possible that the FCT used do not have reliable as well as exhaustive food data. In contrast to the results of this work, Gibson and Scythes (276) calculated Zn DIs from FCT which were 138% higher than DIs obtained using AAS. Hakala *et al.* (238) also reported high Zn DI values from FCT-based calculations of duplicate diets of Finnish weight reduction patients.

Table 3.59. Survey of DDI of zinc

Study Group	Composite	Analytical Method	DI (mg d^{-1})	Ref.
Belgium				
General population	Hosp. meals & Dupl. portions	AAS	14.7	(281)
Benin				
General population	Equiv. composite	AAS	8.047	(202)
Burundi				
Hospital patients, univ. students/staff	Restaurant meals	AAS	13.5, 14.4	(204)
Adult mothers, men	Market basket	AAS	10.4, 12.5	(204)
Rural population	Market basket	AAS	10.6	(204)
Canada				
General population	Market basket	AAS	19.863	(74)
Winnipeg residents	Market basket	AAS	15.550	(77)
Male & female students	Cafeteria meals -	AAS	11.57	(333)
Female university students	Duplicate portion	AAS	7.3	(276)
General population	Equiv composite	AAS	12.0	(259)
China				
General population	Equiv. composite	INAA/RNAA	8.136	(206)
Adult farmers	Equiv. composite	AAS	9.5	(237)
Denmark				
Adult men	Duplicate portion	ICP-MS	11.3	(207)
Egypt				
General population	Equiv. composite	AAS	8.54	(335)
Finland				
Hospital patients	Hospital diet	GFAAS	14	(97)
Wt. reduction patients	Duplicate portion	AAS	7.9	(238)
France				
Adult women, men	Equiv. composite	AAS	9.6, 11.7	(210)
Germany				
Adult men	Duplicate portion	PNAA	11.8	(101)
Adult women	Duplicate portion	INAA	10.6	(102)
Adult women, men	Duplicate portion	AAS	7.6, 9.8	(250)
Adult women, men 1988	Duplicate portion	AAS/ICP-AES	7.6, 10.1	(337)
Adult women, men 1991	Duplicate portion	AAS/ICP-AES	7.1, 9.0	(337)
Adult women, men 1995	Duplicate portion	AAS/ICP-AES	6.3, 8.2	(337)
Vegetarian women, men	Duplicate portion	AAS/ICP-AES	8.4, 9.5	(337)
India				
Bombay residents	1/8 Dupl. portion	AAS	16	(262)
Hospital patient	Hosp. vegetar. diet	INAA	25.6	(218)
Italy				
Residents, towns A, B, C, D	Dupl. portion (?)	INAA	8.1, 4.7, 6.7, 11.3	(215)
Residents, towns A,B,C,D,E	Dupl. portion	INAA	12.5, 7.2, 10.3, 14.9, 4.2	(216)

Table 3.59. (continued) Survey of DDI of zinc

Study Group	Composite	Analytical Method	DI (mg d^{-1})	Ref.
Japan				
Adult men	Duplicate portion	AAS/GFAAS	7.24	(257)
Adult men	Duplicate portion	ICP-AES	7.1	(302)
The Netherlands				
Teen-age males (18 y)	Market basket	AAS/GFAAS	13.5	(232)
Men & women 1976-78	Duplicate portion	AAS/GFAAS	9.1	(283)
Men & women 1984-85	Duplicate portion	AAS/GFAAS	8.3	(283)
New Guinea				
Female, male Wosera residents	Equiv. composite	INAA	6.1-7.7, 8.0-8.8	(277)
New Zealand				
Female students	Metabolic diet	AAS	18.4	(222)
Female students	Duplicate portion	AAS	10.0	(285)
Norway				
Women-control, Se-rich bread	Duplicate portion	INAA	11.3, 8.7	(227)
Pakistan				
Islamabad residents	Equiv. composite	INAA	11.4-13.9	(228)
Scotland				
Glasgow females, males	Equiv. composite	AAS	7.6, 10.1	(336)
Spain				
Company employees	Cafeteria lunches	AAS	14	(286)
University students	Cafeteria meals	FAAS	12.10	(287)
Residents of various regions	Equiv. composite	AAS	9.05-12.77	(263)
Sweden				
Female, male pensioners	Duplicate portion	AAS	7.2, 8.2	(90)
Female- high b.p., low b.p.	Duplicate portion	AAS	8.1, 10	(264)
Female, male vegans	Duplicate portion	AAS	10, 13	(91)
Female, male lactovegetarians	Duplicate portion	AAS	10.1, 11.5	(93)
Adult men and women	Duplicate portion	AAS	7.8	(2)
General population	Market basket	AAS	12	(98)
Taiwan				
Univ. faculty, staff, students	Duplicate portion	INAA	5.71, 8.69, 6.09	(43)
Turkey				
Ankara & Lalahan	Duplicate portion	INAA	11.3	(233)
U.S.A.				
Adult male volunteers 1, 2	Duplicate portion	flame photo.	11, 18	(300)
Female students	Duplicate portion	FES	13.25	(255)
Hosp. Patients-summer, winter	Hospital diet	FES	13.34, 14.49	(265)
Female university students	Metabolic diet	FES	11.5	(266)
Hospital patients	Hospital diet	AAS	11.3	(338)
Metabolic unit patients	Metabolic diets	AAS	11.3-14.5	(338)

Table 3.59. (continued) Survey of DDI of zinc

Study Group	Composite	Analytical Method	DI (mg d^{-1})	Ref.
Men & women	planned menu (25%, 35% cal fr. fat)	FES	7.30, 10.87	(267)
Hospital patients	Regular, vegetarian Hosp. diet	AAS	14.6, 12.2	(339)
Teen-age male	Market basket	INAA	17.2	(78)
Male & female students	Cafeteria meals	AAS	11.0	(256)
Hospital Patients	Hospital diet	AAS	11.1	(340)
Research institute employees	Duplicate portion	AAS	8.6	(341)
Male metabolic unit patients	Metabolic diet	AAS	12.4	(288)
Military personnel	Dining hall meals	AAS	19.7-20.3	(289)
Hospital patients	Normal, Vegetar. Hosp. diet	AAS	12.2, 9.7	(268)
Adult male volunteers	Metabolic diet	GFAAS	13.7	(269)
Adult male volunteers	Low-fibre, higher-fibre diet	AAS	11.4, 11.5	(254)
New mothers,	Duplicate portion	AAS	5.6	(334)
Teen-age male,1976-79	Market basket	AAS	16.8-18.0	(80, 82, 192)
Teen-age male, 1974-1982	Market basket	AAS	12.5-13.6	(81)
Adult men	Duplicate portion	AAS	13.6	(363, 270)
Teen-age female, male1982-84	Market basket	ICP-AES	9.90, 15.61	(83)
Adult female, male 1982-84	Market basket	ICP-AES	9.56, 16.15	(83)
Adult males (USDIET-1)	Market basket	FAAS/ICP-AES/INAA	16.30	(195)
Teen-age female, male1984-85	Market basket	ICP-AES	10.14, 15.88	(85)
Adult female, male 1984-85	Market basket	ICP-AES	9.78, 16.54	(85)
Teen-age female, male1985-86	Market basket	ICP-AES	10.37, 16.28	(85)
Adult female, male 1985-86	Market basket	ICP-AES	9.98, 16.81	(85)
Teen-age female, male1982-89	Market basket	ICP-AES	10.1, 15	(86)
Adult female, male 1982-89	Market basket	ICP-AES	9.7, 16.2	(86)
Adult male	Metabolic diet	AAS	13.8	(322)
United Kingdom				
General population	Equiv. composite	MS/XRF/NAA	14.250	(240)

Literature values of Zn DI are reported in Table 3.59. There have been four previous determinations of Zn in Canadian diets. Two values, 11.57 mg d^{-1} determined by Srivastava *et al.* (333) and 12.0 mg d^{-1} determined by Shah *et al.* (259) are close to the overall median intake of 11 mg d^{-1} determined in this study. The only other study using duplicate portions of free-living individuals performed by Gibson and Scythes (276) determined an intake of 7.3 mg d^{-1} in female university students. This value is slightly lower than the intake of 8.9 mg d^{-1} determined in this study. Two relatively high intakes of 19.9 mg d^{-1} and 15.6 mg d^{-1} were determined by Meranger and Smith (74) and Kirkwood and Coffin (77).

Many studies on Zn content of diet have been performed in the U.S.A. (see Table 3.59). Intakes are in the vicinity of 12 mg d^{-1}. Teen-age males and adult males tend to have higher intakes (78, 80-83, 85, 86). The lowest reported intake for adults in the U.S.A. was determined for new mothers who consumed only 5.6 mg d^{-1} of Zn (334). The highest intake reported was for military personnel who had an intake of 20 mg d^{-1} (289).

The DIs of non-North American countries vary from 4.7 mg d^{-1} (215) determined for the residents of an Italian industrial city to 18.4 mg d^{-1} for a metabolic diet prepared for female New Zealand students (222). Most intakes fall within the range 7 to 14 mg d^{-1}. The diet of the population of Papua New Guinea (277), of which the main food sources are roots, tubers and leaves had low intakes (6 to 9 mg d^{-1}). Diets having a high content of unrefined cereals or legumes, such as the diet of Indian vegetarians (262), can have fairly high intakes (although the bioavailability may be low). Other

countries with relatively low reported intakes (<10 mg d^{-1}) are Egypt (335), Japan (257), New Guinea (277), Scotland (336), Sweden (90), and West Germany (250). Relatively high intakes (>13 mg d^{-1}) were reported for Belgium (281), Burundi (204), Finland (97), India (218), Italy (216), India (262), Spain (286), and the United Kingdom (240).

3.9.5 Enzymolysis of Duplicate Diets for Zinc

Zinc was analyzed in the diet samples subjected to enzymolysis using conditions of t_i=6-7 h, t_d<250 d, and t_c=12 h. As for Fe, the uncertainties calculated for the percent potentially available were very high, rendering only a qualitative interpretation of the data possible. The percent available (Tables 3.60 and 3.61) was low for all diets except freeze-dried HMWV and HMFF wet slurry. However, the corresponding HMWV wet slurry and freeze-dried HMFF had very low availability, so no conclusions could be drawn as to the availability of Zn in these diets. The large uncertainties in potential availability, which were calculated by difference, point to the need for a method to directly measure the dialyzable Zn. Future work could involve the development of an analytical method for the measurement of Zn in the digestion dialysate. As noted in the discussion of the enzymolysis for Ca, the very low percent availability for both Fe and Zn may suggest that future work on modification or optimization of the enzymolysis procedure may be required.

Table 3.60. Enzymolysis and dialysis of freeze-dried duplicate diets for zinc.

Sample	Not Potentially Available		Potentially Available[a]	
	Conc.±SD (ppm)	% Non-diayzed±SD	DI (mg d^{-1})	% Dialyzed±SD
HMFS	21±2	98±18	0.36±2.7	2.3±18
(a) Females				
HFFF	21	80	1.8	20
HFMC	19	135	-1.7	-35
HFNA	28	99	0.10	0.9
HFRR	23	74	3.4	26
HFSR	41	94	1.24	5.8
HFTC	240	110	-0.90	-12
HFWH	17	96	0.31	3.7
Mean	25	99	0.60	1.4
Median	23	96	0.31	3.7
Min-Max	17-41	74-130	-1.7-3.4	-35-26
(b) Males				
HMFF	44	93	1.4	6.6
HMMR	25	100	-0.19	-2.8
HMNT	34±4.3	91±20	2.5±5.7	9.4±20
HMRZ	16	91	0.88	8.6
HMSG	16	110	-0.82	-12
HMTH	22	96	0.44	3.8
HMWV	10	47	9.4	53
Mean	24	90	1.9	9.5
Median	22	93	0.88	6.6
Min-Max	20-44	47-110	-0.82-9.4	-12-53
(c) Overall				
Mean	24	94	1.3	5.5
Median	23	94	1.2	5.8
Min-Max	10-44	47-130	-1.7-9.4	-35-53

[a] :calculated by difference.

Table 3.61. Enzymolysis and dialysis of wet duplicate diet slurry for zinc.

Sample	Not Potentially Available		Potentially Available[a]	
	Conc.±SD (ppm)	%Non-diayzed±SD	DI (mg d^{-1})	%Dialyzed±SD
HMFS	23±0.3	100±11	-0.27±1.6	-1.7±11
(a) Females				
HFFF	36	140	-3.3	-37
HFMC	19±1.3	130±20	-1.6±0.8	-33±20
HFNA	27	92	0.87	7.7
HFRR	27	89	1.5	11
HFSR	42	97	0.61	3.0
HFTC	32	150	-3.6	-46
HFWH	34	200	-8.1	-98
Mean	31	130	-1.9	-27
Median	32	130	-1.7	-33
Min-Max	19-42	89-200	-8.1-1.5	-98-11
(b) Males				
HMFF	21	44	12	56
HMMR	27	110	-0.70	-10
HMNT	26±8	69±29	8.2±8.4	31±29
HMRZ	15	88	1.2	12
HMSG	20	130	-2.31	-34
HMTH	18	84	1.9	16
HMWV	21	98	0.31	1.8
Mean	21	90	3.0	10
Median	21	88	1.2	12
Min-Max	15-27	44-130	-2.3-12	-34-56
(c) Overall				
Mean	26	110	0.52	-8.5
Median	27	98	0.31	1.8
Min-Max	15-42	44-200	-8.1-12	-98-56

[a] : calculated by difference.

3.10 BROMINE AND RUBIDIUM

3.10.1 Bromine and Rubidium in Health

There are a few brief review articles on the nutritional and toxicological aspects of Br (11,342, 343) and Rb (4, 344). Bromine and Rb are not known to be essential to humans although there are a few studies which are suggestive. In humans, Br intake has been associated with quality of sleep. Bromine toxicity is usually found as a result of industrial exposure or drug intake, leading to a condition called bromism. Dietary intake is generally not high enough to cause toxicity.

The adult man contains approximately 360 mg of Rb. Because of its physicochemical similarity to K, it is also somewhat physiologically related. In some cases, it has been shown that Rb can act as a nutritional substitute for K. Some studies suggest that Rb intake may have beneficial health effects (345), but more work is required before Rb can be unequivocally declared essential. No Canadian DI data on Rb were found during this literature survey also little from other countries. Because of the lack of data on Br and Rb in diet and because of the sensitivity of INAA for these elements, they were measured in the duplicate diets.

3.10.2 Bromine and Rubidium in Foods and Diets

Diet is the major source of Br intake. There is little data on the natural content in foodstuffs. However, there has been concern about Br-containing food additives and pesticide sprays. Bromopropylate and methyl bromide have been used to control mites on various crops. Bromine additives were used as food preservatives but

discontinued due to concern over toxicity. Dietary intake is generally in the range 2 - 8 mg d^{-1}. Bread and milk were the largest contributors to the DI in Scotland (342). Interestingly, in this survey of the literature, no data for Canadian diets were found, and only one reported value of a DI of 6.82 mg d^{-1} in U.S. Total Diet (195). As noted above, not much information is available on Rb in foods and diets.

3.10.3 Determination of Bromine by INAA

Bromine was analyzed in this study using the 554.3-keV gamma-ray of ^{82}Br (half-life =35.3 h). Samples were irradiated for 17-21.8 h, allowed to decay for 3-7 d and counted for 30 min-1 h. The sensitivity was 48100 counts μg^{-1}. For Rb, the samples were allowed to decay for 7-60 d and counted for 12 h. The 1077-keV peak of ^{86}Rb was free from interference under these conditions and was used, giving a sensitivity of 1380 counts μg^{-1}.

The Br and Rb content of three RMs and SRMs are reported in Table 3.62 and shown graphically in Fig. 3.29. Duplicate diet composite HMFS has been included in Table 3.62 for comparison. There is good agreement for Br between the analyzed and certified values for IAEA H-9 Mixed Diet and H-4 Animal Muscle. The measured Br content of NIST SRM 1568 was slightly lower but very close to the information value. Detection limits of Br for the RMs and HMFS are also presented in Table 3.62. All measured Br concentrations were well above the detection limits. An EQA chart for Br analysis of IAEA H-9 Mixed Diet is shown in Fig. 3.30. All values were within the

Table 3.62. Bromine and rubidium content of RMs, SRMs and HMFS trial diet.

RM	Br			Rb		
	L_D (ppm)	This work (ppm)	Cert. value (Info. value) (ppm)	L_D (ppm)	This work (ppm)	Cert. value (Info. value) (ppm)
IAEA H-4 Animal Muscle	0.099	3.4 ± 0.2^a	4.1 ± 0.62	0.44	19.8 ± 0.2^b	-
IAEA H-9 Mixed Diet	0.12	7.34 ± 0.07^c	7.5 ± 0.7	0.35	8.8 ± 0.5^a	8.0 ± 0.6
NIST SRM 1568 Rice Flour	0.025	0.87 ± 0.03^a	(1)		ND[d]	-
NIST SRM 1548 Total Diet	0.096	8.3 ± 0.1^c	-	0.44	7.5 ± 4.2^c	-
HMFS Duplicate Diet	0.12	9.9 ± 0.5^a	-	0.36	3.9 ± 0.1^e	-

[a] average of 4 values; [b] average of 3 values; [c] average of 6 values; [c] average of 3 values; [d] not done; [e] average of 2 values

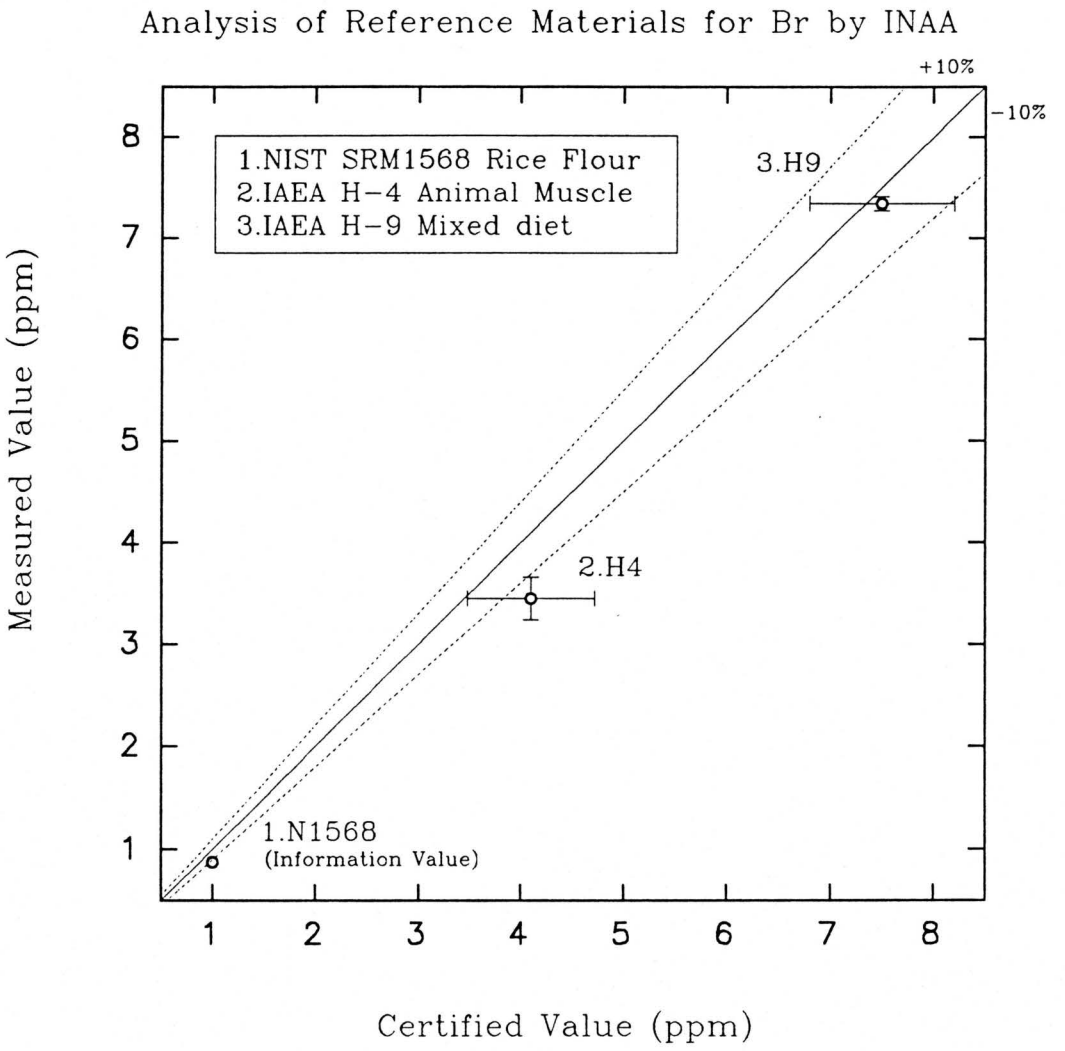


Fig. 3.29. Analysis of certified RMs and SRMs for bromine

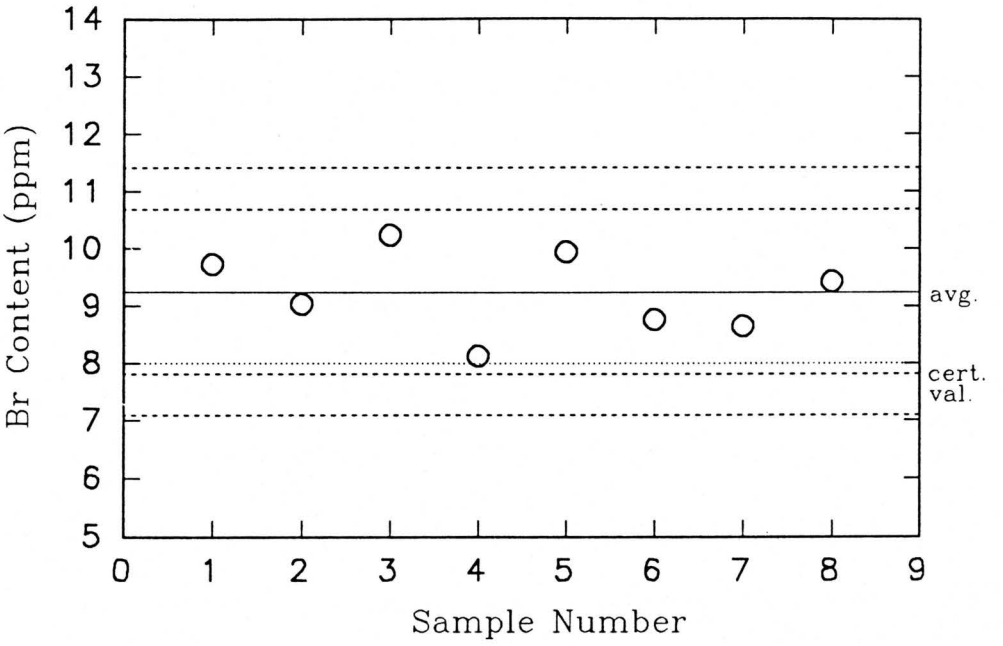


Fig. 3.30. External quality assessment chart for analysis of bromine

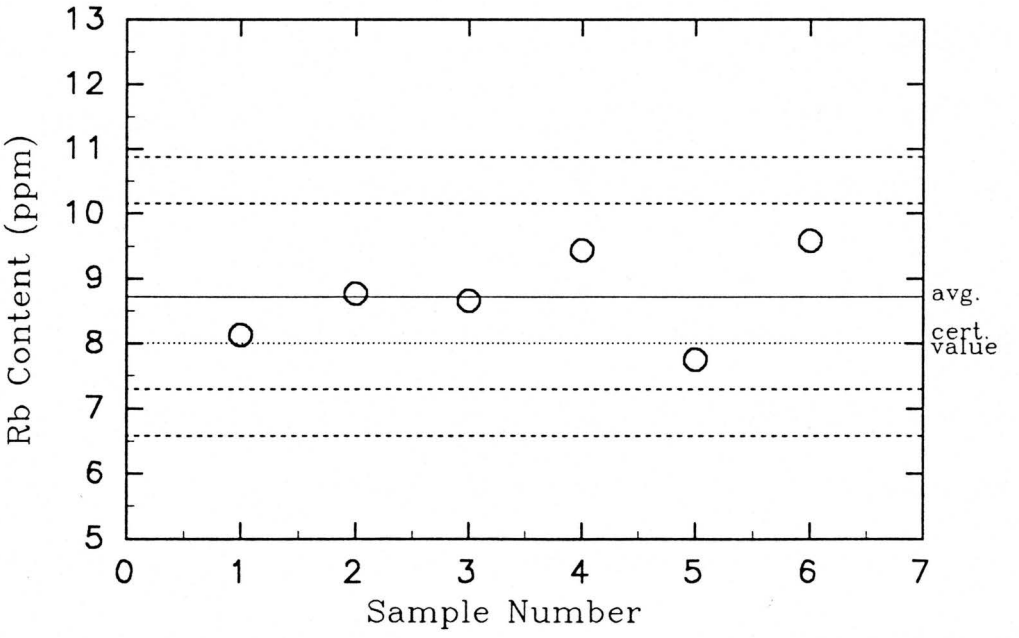


Fig. 3.31. External quality assessment chart for analysis of rubidium

warning limits. Although all data points lie above the certified value (7.5±0.7 ppm), they fall within its confidence limits (see Fig. 3.31).

For the HMFS diet, the detection limit of Rb was 0.36 ppm. The Rb concentration of 3.9 ppm in HMFS was well above the detection limit. Rubidium content of three RMs and SRMs are reported in Table 3.62, but only IAEA H-9 Mixed Diet is certified for this element. In that case, there was good agreement between the measured and certified values. An EQA chart for Rb analysis, prepared by replicate analysis of IAEA H-9 along with the diet samples, is shown in Fig. 3.31. All values lie within the warning limits. The certified value agrees with the average of all values. The analytical method appears to be in statistical control.

The results for the analysis of duplicate diets are reported in Table 3.63. The mean and median intakes are relatively close, suggesting a symmetric distribution of the data. The median Br intake for the female participants was 3.72 mg d^{-1} and the range was 1.87-8.09 mg d^{-1}. The median intake for the males was higher at 6.00 mg d^{-1}. The range of intakes for the men was 1.96-10.6 mg d^{-1}. The overall median DI of Br was 5.07 mg d^{-1}.

The Rb content of the duplicate diet composites is also shown in Table 3.63. Precision of determination was quite good, the RSD being less than 6% for three replicate determinations of each of HMFS, HFMC and HMNT. The concentrations differed only by about a factor of two between the minimum and maximum, but DIs varied by a factor of seven. The highest DI was 7.5 mg d^{-1} by HMNT, while the lowest were 1.4 mg d^{-1}, 1.5 mg d^{-1} and 1.6 mg d^{-1} for HFFF, HMMR and HFTC, respectively.

Table 3.63 Bromine and Rubidiumcontent of duplicate diet composites determined by INAA

Sample Code	Br		Rb	
	Content±SD (ppm)	DI (mg d^{-1})	Content±SD (ppm)	DI (mg d^{-1})
HMFS	9.9 ± 0.5	6.9 ± 0.3	3.9 ± 0.1[a]	2.8 ± 0.1[a]
(a) Females				
HFFF	7.12	2.40	4.3	1.4
HFMC	5.36 ± 0.03	1.87 ± 0.01	6.0 ± 0.3[b]	2.1 ± 0.1[b]
HFNA	19.5	7.75	9.6	3.8
HFRR	8.59	3.72	8.6	3.7
HFSR	17.0	8.09	9.2	4.4
HFTC	11.2	3.99	4.6	1.6
HFWH	5.04	2.43	4.8	2.3
Mean	10.5	4.32	6.7	2.8
Median	8.59	3.72	6.0	2.3
Min.-Max.	5.04 - 19.5	1.87 - 8.09	4.3 - 9.6	1.4 - 4.4
(b) Males				
HMFF	11.2	5.20	7.1	3.3
HMMR	7.16	1.96	5.6	1.5
HMNT	14.7 ± 0.7	10.6 ± 0.5	10.4 ± 0.7[b]	7.5 ± 0.5[b]
HMRZ	10.0	6.00	4.5	2.7
HMSG	10.8	4.95	5.4	2.5
HMTH	15.1	7.94	6.9	3.6
HMWV	11.5	9.31	7.5	6.0
Mean	11.5	6.56	6.8	3.9
Median	11.2	6.00	6.9	3.3
Min.-Max.	7.16 - 15.1	1.96 - 10.6	4.5 - 10.4	1.5 - 7.5
(c) Overall				
Mean	11.0	5.44	6.8	3.3
Median	11.0	5.07	6.4	3.0
Min.-Max.	5.04 - 19.5	1.87 - 10.6	4.3 - 10.4	1.4 - 7.5

The mean and median intakes for the female volunteers were 2.8 and 2.3 mg d^{-1}, respectively, while for the men these values were 3.9 and 3.3 mg d^{-1}. The overall mean and median intakes were 3.3 and 3.0 mg d^{-1}.

Literature values for the intake of Br and Rb are presented in Table 3.64. The median Br intake determined in this study is lower than 16.3 mg d^{-1} reported for Indian vegetarian diet (218), 8.0 and 9.4 mg d^{-1} for the Netherlands (231, 232) and 8 mg d^{-1} for Scotland (342). It is very close to 6.82 mg d^{-1} determined by Iyengar *et al.* for U.S. Total Diet (195). Values reported for other countries, including China (206), Pakistan (228), Taiwan (43), Turkey (233) and West Germany (101) were lower than the median value reported in this work for the duplicate diets. A particularly low value of 0.812 mg d^{-1} has been reported for the DI in China (206).

A survey of DI values for Rb from the literature is shown in Table 3.64. Most diets were analyzed for Rb by INAA due to its sensitivity and ease of determination rather than for reasons of nutritional or toxicological significance. There are no data available in the literature for Canada and only one value for the U.S. In most countries for which data are available, the intakes were about 2 mg d^{-1}; this is lower than the mean and median intake determined in this study. The highest intakes of 6.6 and 7 mg d^{-1} were reported for a large Italian city (215) and industrial town (216). The lowest intakes reported were for university faculty/families (0.87 mg d^{-1}) and students (1.11 mg d^{-1}) in Taiwan (43).

Table 3.64. Survey of DDI of bromine and rubidium

Study Group	Composite	Analytical Method	Br DI (mg d^{-1})	Rb DI (mg d^{-1})	Ref.
China					
General population	Equiv. composite	INAA/RNAA	0.812	-	(206)
India					
Bombay residents	1/8 Dupl. portion	AAS	-	2.7	(262)
Hospital patient	Hosp. vegetar. diet	INAA	16.3	6.4	(218)
Italy					
Residents of var. cities	Dupl. portion(?)	INAA	-	1.4-5.7	(215)
Japan					
Adult men	Duplicate portion	AAS/GFAAS		1.89	(257)
The Netherlands					
General population	Market basket	INAA	9.4	-	(231)
Teen-age males (18 y)	Market basket	INAA	8.0	-	(232)
Norway					
Women (control, Se-rich)	Duplicate portion	INAA	-	2.6, 2.5	(227)
Pakistan					
Islamabad residents	Equiv. composite	INAA	2.6-3.6	-	(228)
Gujranwala residents	Equiv. composite	INAA	5.35	-	(229)
Scotland					
Glasgow residents	Equiv. composite	INAA	8	-	(342)
Taiwan					
Univ. faculty/families	Duplicate portion	INAA	2.73	0.87	(43)
Univ. staff/families	Duplicate portion	INAA	3.26	1.50	(43)
Univ. students	Duplicate portion	INAA	4.22	1.11	(43)
Turkey					
Ankara & Lalahan residents	Dupl. portion	INAA	2.71	1.89	(233)
United Kingdom					
General population	Equiv. composite	MS/XRF/NAA	8.4	4.35	(240)
U.S.A.					
Adult males (USDIET-1)	Market basket	INAA	6.820	2.80	(195)
West Germany					
Adult men	Duplicate portion	PNAA	2.5	1.9	(101)

3.11 VANADIUM AND OTHER ELEMENTS

Vanadium is of some interest to human health (1, 11, 134, 346). Vanadium-activated enzymes have been discovered in bacteria, algae and lichens, vanadium-containing proteins have been discovered in animals and humans (347, 155, 157), and there is some evidence of deficiency symptoms in vanadium-deprived animals (348). The evidence does suggest that V may have an essential function in humans (349). However, a biochemical function in higher animals or humans has not yet been discovered. Vanadium is relatively toxic at high intakes (>25 mg d^{-1}); however, typical intakes (about 15 μg d^{-1}) are not high enough to produce toxic effects. Vanadium can be analyzed by INAA using the 1434.2-keV gamma-ray of ^{52}V (half-life=3.75 min). Unfortunately, this peak was not observed during analysis of the duplicate diets using t_i=1.5 min, t_d= 1 min, and t_c=10 min. This is due to the low level of V in the diet samples, as well as the elevated Compton background resulting from ^{24}Na, ^{38}Cl and ^{56}Mn

Some effort was directed towards the search for an optimal INAA method for detecting ^{52}V and other short-lived radionuclides. Both epi-cadmium irradiation and AC gamma-ray spectrometry were independently attempted because of the lower background that may be achieved using these techniques. No nuclides previously undetected using conventional INAA were detected using either of these techniques, although AC counting demonstrated potential for improving detection limits of elements that have already been quantified using conventional gamma-ray spectrometry. The INAA methods do not appear to be sensitive enough to determine V; therefore chemical separation methods appear to be warranted. A dialysis procedure was applied as a

convenient way to remove Na and Cl from the diet composites, leading to a lower background, and allowing previously undetected elements to be determined.

The dialyzed duplicate diets were analyzed using t_i=5 min, t_d=1 min, and t_c=10 min. The 1434.2-keV gamma-ray of ^{52}V was detected in the dialyzed duplicate diets. One potential difficulty was the finding of V in the markers (Black Sanford Sharpie Fine Point) used to identify the sample vials. The degree of contamination varied with the marker and the amount of ink used. Some comparator standard blanks, on which extra ink was used to modify the identification codes, had peak areas of over 700 counts, compared with 150-1700 counts for the dialyzed diets. Recently prepared comparator standard blanks gave net peak areas of <120 counts. Sensitivities calculated for recently prepared standards agreed well with the old standards. The 1434.2-keV gamma-ray was not detected in any of the dialysis blanks. Therefore, V contamination from the markers was not considered to be a significant interference.

The sensitivity for V was 780 counts μg^{-1} and the detection limit of the HMFS trial diet was 0.024 ppm. The measured value of 0.055$\pm$0.018 ppm was below the quantitative determination limit of 0.070 ppm, which explains the relatively large SD of the former. The results for RMs, SRMs, and the duplicate diets are reported in Table 3.65. The V concentration of dialyzed NIST SRM 1515 Apple Leaves and 1577b Bovine Liver were 51 and 67% of their certified and information values, respectively. The highest V concentration of 0.221 ppm was found in the HFFF diet, while the lowest of 0.031 and 0.032 ppm were in the HFSR and HFTC diets. There was little difference

Table 3.65. Dialysis of RMs, SRMs and duplicate diets for vanadium and titanium

Sample	V		Ti	
	Conc.±SD (ppm)	DI±SD (mg d^{-1})	Conc.±SD (ppm)	DI±SD (mg d^{-1})
IAEA H-9 Mixed Diet	0.019		<3.6	
NIST 1515 Apple Leaves	0.133[a]		11	
NIST 1548 Total Diet	0.038		8.6	
NIST 1568 Rice Flour	0.025		<3.9	
NIST 1577b Bovine Liver	0.082[b]		<4.2	
HMFS	0.055±0.018	0.039±0.013	3.9±0.4	2.8±0.3
(a) Females				
HFFF	0.221	0.074	<1.6	<0.57
HFMC	0.034±0.013	0.012± 0.004	61±1	21.5±0.4
HFNA	0.057	0.023	<3.1	<1.2
HFRR	0.039	0.017	<2.7	<1.2
HFSR	0.031	0.015	<2.9	<1.4
HFTC	0.032	0.011	12	4.3
HFWH	0.033	0.016	<3.8	<1.8
Mean	0.064	0.024		
Median	0.034	0.016		
Min. - Max.	0.031-0.221	0.011-0.074		
(b) Males				
HMFF	0.081	0.038	4.7	2.2
HMMR	0.105	0.029	<3.2	<0.86
HMNT	0.034±0.008	0.024±0.005	<2.6	<1.9
HMRZ	0.041	0.024	23	14
HMSG	0.078	0.036	<3.5	<1.6
HMTH	0.061	0.032	<3.1	<1.6
HMWV	0.062	0.050	<3.6	<2.9
Mean	0.066	0.033		
Median	0.062	0.032		
Min. - Max.	0.034-0.105	0.024-0.038		
(c) Overall				
Mean	0.065	0.029		
Median	0.049	0.024		
Min. - Max.	0.031-0.221	0.011-0.074		

[a] certified value=0.26; [b] information value=0.123

between the mean V concentrations of 0.064 and 0.062 ppm of the men and women, respectively. The overall median concentration was 0.065 ppm. The overall mean and median DIs were 0.029 and 0.024 mg d^{-1}.

Tipton *et al.* (300) reported DI values for V of 0.061±0.005 and 0.17±0.01 mg d^{-1}, determined by arc emission spectrometry, for two volunteers who collected duplicate diets over a 50-d period. Vanadium in a general hospital diet was determined using AAS by Myron *et al.* (350), who calculated the DI to be 0.0280±0.7 mg d^{-1}. Pennington and Jones (84) reported V intakes in total diets for adult men and women, of 0.0183 and 0.0081 mg d^{-1}. Uthus and Seaborn (346) reported that typical V intakes are about 0.015 mg d^{-1}. Recently, Illing-Gunther *et al.* (351) reported DIs for duplicate diets of German men and women, collected in 1992, to be 0.036 and 0.025 mg d^{-1}, respectively. These DI values reported in the literature are of approximately the same magnitude as V in the dialyzed duplicate diets of this thesis work.

Copper is an essential trace element involved in the function of several enzymes, and cases of Cu deficiency are known (1, 134). Copper can be analyzed by INAA using the 1039.2-keV gamma-ray of ^{66}Cu (half-life=5.1 min). However, this peak was not detected using t_i=1.5 min, t_d= 1 min, and t_c=10 min for the same reasons described above for V. Again, both epi-cadmium irradiation and AC gamma-ray spectrometry were independently applied but the 1039.2-keV peak was not detected.

The dialysis procedure was then applied to the duplicate diets, and the residues were analyzed using t_i=5 min, t_d=1 min, and t_c=10 min. The 1039.2-keV gamma-ray of ^{66}Cu was detected. The net peak areas of the blanks were equal to or larger than

(in many cases about two times) those of the diet samples. The origin of the Cu contamination of the blanks was investigated. Both the stock dialysis tubing and the DDW used were analyzed using INAA; neither contained significant amounts of Cu. A piece of dialysis tube which had been analyzed was subjected to the dialysis procedure. Prior to immersion in the DDW, 30 counts were obtained from integration of the 1039.2-keV peak. Following immersion and freeze-drying, the net peak area increased to 1168 counts. It is postulated that the contamination may arise from the accidental release of the vapour from pump oil to the freezing flask containing the sample during the freeze-drying step. Due to the Cu contamination discovered in the blanks, no results are reported for Cu in the dialyzed diets.

Another gamma-ray detected in some of the dialyzed diets, which was not previously detected in the undialyzed diets, was the 320.1-keV gamma-ray of ^{51}Ti (half-life=3.74 min). The sensitivity for Ti was 830 counts μg^{-1}. None of the RMs or SRMs are certified for Ti. The detection limit of the HMFS trial diet was 3.0 ppm. Five diets, HFMS, HFMC, HFTC, HMFF and HMRZ had detectable Ti levels. The results for these as well as the detection limits for the other diets are presented in Table 3.65. In particular, HFMC had a very high concentration of 61 ppm Ti. It seems unlikely that Ti concentrations of this magnitude represent the natural content of the diet. Perhaps this high Ti content arises from the Ti blades of the blender used to homogenize the diets during preparation of the composites. Krushevska and Barnes (352) reported the Ti concentration of IAEA H-9 to be 0.34 ppm, which is well below the detection limit of 3.6 ppm obtained in this work. Tipton *et al.* reported DIs of two volunteers to be 0.75 and

2.0 mg d^{-1} (300), while Hamilton and Minski reported a DI of about 0.8 mg d^{-1} for a prepared U.K. diet (240). These values are near the detection limits of the INAA method used in this work for the dialyzed diets. The diets with detectable levels of Ti, namely HMFS, HFMC, HFTC, HMFF, and HMRZ all had higher DIs than the literature values reported above.

The elements Au, Ba, Co, Cr, Cs, Sb, and Sc were detected in some diets through their long-lived nuclides: ^{198}Au (412 keV, half-life=2.7 d), ^{131}Ba (124 keV, half-life=11.8 d), ^{60}Co (1173, 1332 keV, half-life=5.27 a), ^{51}Cr (320 keV, half-life=27.7 d), ^{17}Cs (796 keV, half-life=2.062 a), ^{124}Sb (603 keV, half-life=60.2 d) and ^{47}Sc (160 keV, half-life=3.42 d). Except for Sb, the precision and accuracy of determination using these nuclides were not satisfactory. Of these elements, only Co and Cr have nutritional or toxicological importance (12, 1).

The nuclide ^{51}Cr was detected after irradiation for 17 to 24 h, less than 30 d of decay, and counting for 12 h. Unfortunately, the precision of analysis of IAEA H-9, and HMFS, HFMC and HMNT diets was very poor (100% RSD), a result of the low Cr levels in the samples and relatively high levels in the blank vials. Because irradiation time was restricted for safety reasons, the only ways to increase the sensitivity of analysis would have been to keep the decay time to two to three weeks, increase the counting time, or increase the sample size. Twelve hours were chosen as the longest convenient counting time, because the samples could be counted overnight when there is a decreased demand for the detectors. The routine use of counting times longer than 17 h (4:30 p.m.-

8:00 a.m.) are inconvenient because they make the detector unavailable during the work day.

Cobalt can be analyzed by its short-lived ^{60m}Co (58.6 keV, half-life=10.5 min) as well as long-lived ^{60}Co (half-life=5.27 a) nuclides. The net peak areas of the 58.6-keV peak was small, but the background at that low energy was high. The small peaks on high, sloped backgrounds led to poor precision of analysis. The long-lived 1173.5-keV and 1332.5-keV photopeaks of ^{60}Co were subject to interference from a ^{60}Co source used in the DUSR facility to test alarms. Although both the source and detector were shielded, the gamma-rays from the source was still detected, giving significant background counts. Furthermore, it was discovered that the ink used in some markers to label the samples contained significant levels of Co, leading to quite variable net peak areas. For these reasons, the Co concentrations were not quantified.

Although the Sb results had acceptable precision (RSD of 15% for IAEA H-9 Mixed Diet) the calculated Sb concentration in IAEA H-9 Mixed Diet was 0.24$\pm$0.04 ppm, which was much higher than the value of 2.5$\pm$0.4 ppb determined previously by Chatt *et al.* (42). This RM is not certified for Sb. There may be an error in preparation of the comparator standards, although a careful examination of the documented procedure did not reveal it. The analyzer system became unserviceable before this problem could be resolved, so no results are available for Sb at this time. Further investigation into this problem could be the subject of future work.

4. EXPLORATORY DATA ANALYSIS

4.1 INTRODUCTION

In the previous chapter, concentrations and daily intakes were measured for thirteen elements in fourteen duplicate diet composites. The focus of that chapter was to glean as much knowledge as possible from measurements of individual elements. However, more information may be made available by considering the measurements together as a multivariate dataset. Exploratory data analysis is the computation and graphical display of patterns of association in multivariate sets of data. The objective of this chapter is to determine whether exploratory data analysis can provide useful information on the duplicate diets and mineral intake which was otherwise unavailable, suspect or difficult to discern. The statistical tools in this chapter have been used to investigate interactions between elements and foods consumed, and the influence of food sources and dietary habits on the intake of trace elements by the study participants.

4.1.1 Correlation, Hierarchical Cluster Analysis (HCA), Principal Components Analysis (PCA)

Chemometrics has provided powerful tools for the extraction of chemical information from analytical data. These tools are particularly suited to the analysis of large multivariate datasets. Because of their size and complexity, interactions between samples and variables may be difficult to detect. Multivariate data reduction procedures can reveal new previously unavailable information. These techniques have found

application to a number of analytical problems, such as the determination of the sources of trace elements in atmospheric precipitation (353), the characterization of soil depth profiles of trace elements (354), classification of wines by trace element content (355), identification of the animal origin of milk from its mineral content (356) and interactions between trace elements in daily diets (357). In this chapter, the statistical tools applied to the duplicate diet data are correlation, hierarchical cluster analysis (HCA) and principal component analysis (PCA). Each is described briefly below. A more detailed treatment may be found in the review by Currie (357) and the textbooks of Sharaf *et al* (358), Massart *et al* (359) and Haswell (360).

Covariance is a measure of the extent to which two sets of data vary together. For example, large values in one group may be associated with large values in the other group. It is calculated by determining the average of the product of the deviations from the means. A drawback to the covariance parameter is that it is sensitive to the unit of measure. The correlation statistic has the advantage of being independent of the scale. It is obtained by dividing the covariance by the product of the standard deviations of the two datasets. The statistic so calculated is called the correlation coefficient and can vary from -1 to +1. A correlation coefficient of +1 suggests a perfect positive linear relationship between the two groups (y=ax). A correlation coefficient of -1 implies a negative correlation, in which large values in one group are associated with small values in the other. Non-related or uncorrelated variables have a correlation coefficient close to zero. Correlation coefficients were calculated using the data analysis correlation tool in Microsoft Excel (Microsoft Excel 5.0a, Microsoft Corp. 1985-1993).

One could represent a set of data by plotting the measured values in a graph, where each variable had its own axis. If there are *n* variables, then they would be plotted in an *n*-dimensional space. Each sample is represented by a single point in *n*-space. When $n=2$,the graph is simply an "x-y" plot, which is easy to produce and relatively simple to interpret. However, when $n>3$, the space becomes difficult to visualize and interpret. Both HCA and PCA are methods for simplifying the representation of the space.

In HCA, distances between points are calculated. Points separated by small distances are likely to be similar, whereas points separated by large distances are likely to be dissimilar. Points grouped closely together are called clusters. A dendrogram is a figure used to visually represent the distances between samples, linking samples and clusters of samples as a function of distance. Algorithms used usually calculate the distances between all points and then find the smallest distance. These two points are linked and considered to be one point. The process is then repeated until all clusters are linked. HCA was performed using the Einsight program (Infometrix, 1991).

PCA is a method for reorganizing the multivariate data. Consider that the data is plotted in an *n*-dimensional space, where each axis represents a separate variable and *n* represents the total number of variables. In PCA, the data is transformed such that a new series of data axes are constructed. The new axes (components) are linear combinations of the original variables in the data set. They are orthogonal (*i.e.* uncorrelated) and are prioritized, based on how much variation in the data that they account for. The data is then plotted in 2 or three dimensions against the components

accounting for the largest variation in the data (hence the term principal components). Thus, a high-dimensional plot (in this study thirteen elements were analyzed, giving thirteen dimensions) can be reduced to two or three dimensions and visually plotted, allowing patterns in the data to be more clearly seen. Two statistics, scores and loadings are calculated. Scores represent the projections of the data on each axis. Loadings represent the contribution the variables make to the principal components. Thus, by plotting scores and loadings, one can obtain information on both the samples and variables. PCA was performed using the Einsight program (Infometrix).

4.1.2 Preparation of Data for Analysis

The first step in exploratory data analysis is to prepare a data matrix containing all of the data. Usually, the samples are placed in rows and the variables in columns. However, the primary objective of the analysis was to discover how the elements (variables) relate with each other, and Einsight has certain features which aid studying the row parameters (usually samples). Therefore, the data matrix was transposed, putting variables (minerals, food groups and nutrients) in rows, and samples (duplicate diet composites) in columns.

The next step is to preprocess the data, if necessary. Since correlation is independent of the unit of measurement, preprocessing is unnecessary. However, in HCA and PCA, a wide range in values (*e.g.* concentrations) can skew the data, such that the highest values account for all of the variance. For example, Na and Cl are at much higher concentrations in the duplicate diet composites than Se. Any relationships with

Se would likely be obscured by Na and Cl. Scaling ensures that all the variables have equal weight. Mean-centering is another form of preprocessing which translates the data set so that the origin coincides with the mean of the data set. In this study, "autoscaling", which performs both mean centering and scaling, was used. Autoscaling is performed by calculating the mean and standard deviation for each variable. Then the difference between each datum and its mean is divided by the standard deviation. Autoscaling can be performed in Einsight, but it is designed to perform autoscaling on columns. Since it was desirable to have the variables in rows, the autoscaling calculation was performed in Excel and the scaled data was later imported into Einsight.

Exploratory data analysis (correlation, HCA and PCA) were performed on trace element (Chapter 3), food group (Appendix A) and nutrient (Appendix A) data. Both concentrations and daily intakes were investigated. The daily intakes give the total amounts consumed. This information relates to the health of the participants. The concentrations and food group mass fractions adjust the data for total food mass consumed, thus providing more information on the balance of elements and foods in the diet. Thus, the concentrations and mass fractions may give insight into the sources and dietary habits of the participants.

4.2 CORRELATION

In this section, results of correlation calculations are presented for the elements Al, Br, Ca, Cl, Fe, I, K, Mg, Mn, Na, Rb, Se and Zn. Daily intakes and concentrations of these elements were compared with daily intakes and concentrations of

the elements, food groups and some nutrients calculated by Chatt and Pegg (27). Since correlations among the elements may relate to food intake patterns, correlation results are first presented for the food groups. It should be noted that the intakes and mass fractions of many food items were not measured directly, but were estimated from the diet records and duplicate food items weighed after return to the lab (for a more detailed discussion, lists of food items, and estimated and measured masses, see Appendix A).

4.2.1 Correlation Between Food Groups: Daily Intake

Correlation coefficients calculated for the daily intakes of the various food groups for all participants, the women and the men are presented in Tables 4.1, 4.2 and 4.3, respectively. The strongest correlations, ≥0.8 for the elements and ≥0.7 for the food groups, are given in boldface and enclosed in boxes.

Correlations involving the whole group (Table 4.1) are discussed first. Both dry mass and moisture were strongly correlated with the total mass. This shows that the intake of both dry and wet food items increased with increasing quantity of foods consumed. Dry mass was correlated strongly with vegetables, suggesting that a large proportion of the dry mass may have come from vegetables. There were strong correlations of wine (alcohol) with total mass, dry mass and vegetables. However, these results are based on only two diets which contained alcoholic beverages, so the sample is very small. These two diets also were the heaviest and contained large masses of vegetables, which explains the correlations.

Table 4.1. Correlation coefficients of food group daily intakes for men and women

	Total mass	Dry mass	Moist-ure	Cereals/ Nuts	Vegs.	Fruits& Berries	Meat & Prods.	Fish & Seafood	Milk Prods.	Cheese	Food Fats	Fluids, no juice	Other Foods
Total mass	1.000												
Dry mass	**0.856**	1.000											
Moisture	**0.989**	0.768	1.000										
Cereals	0.365	0.625	0.269	1.000									
Vegetables	0.697	**0.817**	0.625	0.537	1.000								
Fruits&Berries	0.504	0.450	0.493	-0.008	0.092	1.000							
Meat & Prods.	0.443	0.309	0.459	0.277	0.157	0.280	1.000						
Fish&Seafood	-0.032	0.025	-0.047	-0.043	0.126	0.290	-0.064	1.000					
Milk Prods.	0.052	0.231	-0.003	0.356	0.292	-0.263	-0.361	-0.337	1.000				
Cheese	0.358	0.089	0.417	-0.279	-0.107	0.178	0.118	-0.383	-0.260	1.000			
Food Fats	0.285	0.361	0.248	0.415	0.577	-0.279	-0.024	-0.166	0.617	-0.357	1.000		
Fluids(no juice)	0.548	0.175	0.628	-0.261	0.114	0.151	0.386	-0.067	-0.590	0.652	-0.209	1.000	
Other Foods	0.165	0.071	0.184	-0.223	-0.071	-0.076	-0.327	-0.275	-0.145	0.445	-0.260	0.503	1.000
Tea/Coffee	0.394	0.268	0.410	-0.025	-0.133	0.464	0.188	-0.247	-0.358	0.298	-0.257	0.523	0.566
Soft Drinks	0.122	-0.097	0.179	-0.430	-0.132	0.129	-0.014	0.260	-0.356	0.417	-0.257	0.461	0.350
Water	-0.161	-0.452	-0.067	-0.390	-0.055	-0.506	0.128	-0.004	-0.302	0.169	-0.113	0.416	-0.056
Wine	**0.724**	**0.828**	0.655	0.617	**0.763**	0.225	0.347	-0.070	0.199	0.166	0.523	0.173	-0.103

Table 4.2. Correlation coefficients of food group daily intakes for women

	Total mass	Dry mass	Moist-ure	Cereals/ Nuts	Vegs.	Fruits& Berries	Meat& Prods.	Fish & Seafood	Milk Prods.	Cheese	Food Fats	Fluids, no juice	Other Foods
Total mass	1.000												
Dry mass	0.135	1.000											
Moisture	**0.985**	-0.036	1.000										
Cereals/	-0.385	0.697	-0.508	1.000									
Vegetables	-0.404	0.183	-0.439	0.461	1.000								
Fruits&Berries	**0.704**	0.310	0.657	-0.446	-0.319	1.000							
Meat & Prods.	**0.709**	0.662	0.602	0.179	-0.271	0.512	1.000						
Fish & Seafood	0.019	-0.284	0.068	-0.001	**0.744**	-0.284	-0.215	1.000					
Milk Prods.	-0.537	0.059	-0.552	0.452	0.049	-0.398	-0.484	-0.280	1.000				
Cheese	0.175	-0.628	0.284	**-0.838**	**-0.740**	0.231	-0.109	-0.385	-0.326	1.000			
Food Fats	-0.454	-0.088	-0.443	0.483	0.213	-0.608	-0.533	0.073	**0.847**	-0.474	1.000		
Fluids(no juice)	0.671	-0.383	**0.743**	-0.621	-0.385	0.254	0.385	0.217	**-0.835**	0.545	-0.599	1.000	
Other Foods	0.296	**-0.740**	0.427	-0.616	-0.606	-0.166	-0.050	0.012	-0.287	**0.739**	-0.153	**0.724**	1.000
Tea/Coffee	**0.826**	0.310	**0.780**	-0.250	-0.532	0.624	**0.845**	-0.274	-0.687	0.275	-0.638	0.698	0.249
Soft Drinks	0.075	-0.696	0.195	**-0.807**	-0.697	0.110	-0.204	-0.341	-0.245	**0.989**	-0.395	0.489	**0.769**
Water	0.123	-0.621	0.231	-0.317	0.336	-0.351	-0.224	**0.848**	-0.462	0.067	-0.053	0.594	0.507
Wine													

Table 4.3. Correlation coefficients of food group daily intakes for men

	Total mass	Dry mass	Moist-ure	Cereals/ Nuts	Vegs.	Fruits& Berries	Meat& Prods.	Fish & Seafood	Milk Prods.	Cheese	Food Fats	Fluids, no juice	Other Foods
Total mass	1.000												
Dry mass	**0.934**	1.000											
Moisture	**0.994**	**0.889**	1.000										
Cereals/	**0.812**	**0.886**	**0.768**	1.000									
Vegetables	**0.913**	**0.894**	**0.895**	**0.835**	1.000								
Fruits&Berries	0.448	0.581	0.396	0.448	0.250	1.000							
Meat & Prods.	0.278	0.113	0.320	0.435	0.264	-0.072	1.000						
Fish & Seafood	-0.209	-0.089	-0.240	-0.031	-0.280	0.654	-0.081	1.000					
Milk Prods.	0.471	0.487	0.454	0.260	0.686	-0.055	-0.184	-0.419	1.000				
Cheese	0.506	0.328	0.546	0.255	0.205	0.091	0.366	-0.470	-0.159	1.000			
Food Fats	0.369	0.393	0.352	0.463	**0.705**	-0.256	0.110	-0.268	**0.715**	-0.409	1.000		
Fluids(no juice)	0.499	0.255	0.560	0.201	0.250	-0.118	0.284	-0.486	-0.172	**0.859**	-0.191	1.000	
Other Foods	0.106	0.092	0.107	-0.17	-0.078	-0.094	-0.555	-0.384	-0.126	0.437	-0.293	0.589	1.000
Tea/Coffee	0.369	0.478	0.327	0.187	0.212	0.295	-0.626	-0.229	0.131	0.352	-0.151	0.367	**0.862**
Soft Drinks	-0.021	-0.221	0.040	-0.256	-0.229	0.088	-0.019	0.381	-0.488	0.103	-0.299	0.442	0.268
Water	-0.343	-0.582	-0.262	-0.450	-0.327	-0.699	0.495	-0.487	-0.115	0.275	-0.153	0.223	-0.231
Wine	**0.732**	**0.831**	0.683	**0.954**	**0.829**	0.272	0.426	-0.228	0.395	0.221	0.548	0.107	-0.176

The women's diets had a number of correlations between food groups (Table 4.2). Total mass was correlated with moisture, fruits and berries, tea/coffee and meat and meat products. Dry mass was not correlated with total mass. Fluids and tea/coffee were strongly correlated with moisture. The cereal products group was negatively correlated with cheese and soft drinks. Vegetables and seafood were positively correlated. The vegetables group was negatively correlated with cheese. Meat and tea/coffee were strongly correlated. Water and fish were correlated. Milk was positively correlated with food fats and negatively correlated with fluids (probably indicating competition between beverages for intake). Cheese, other foods and soft drinks appear to be mutually correlated, perhaps due to their choices as snack foods.

There were some differences between the men and women in terms of food group correlations. Total mass of the men's diets (Table 4.3) was strongly correlated with dry mass, moisture, cereals, vegetables and wine, but were not correlated with fruits and berries or meat. Dry mass and moisture were correlated with cereal products and vegetables. Cereal products were correlated with vegetables. Vegetables were correlated with fats (perhaps due to the consumption of butter or margarine with the vegetables). Milk products were also correlated with fats. Cheese was strongly correlated with fluids. The other foods group was strongly correlated with tea/coffee (probably due to the inclusion of sugar and coffee whitener in the other foods group).

4.2.2 Correlation Between Food Groups: Concentrations and Mass Fractions

Elemental concentration discussed in this section is daily intake adjusted for total wet mass of the diet composite. Mass fractions of the food groups are simply the mass in each group divided by the total wet mass. Adjusting intakes for the mass of the diet should give more of an indication of the balance of foods and elements in the diet which should yield information on the dietary sources and food intake habits. For example, a diet which is weighted towards milk should have higher concentrations of elements associated with milk such as Ca. Before considering the correlations between elements, as for the daily intakes, correlations between food groups are considered first.

The mass fractions of the various food groups are shown in Tables 4.4, 4.5 and 4.6. As before, the mass fractions of the whole study group are discussed first, before comparing the diets of the women and men.

The only noteworthy correlations for the whole group are a strong negative correlation between percent dry mass and percent moisture (as expected) and a negative correlation between milk and fluids (probably reflecting competition between beverages).

The percent dry mass of the females' diets had a strong correlation with cereal products. The cereal products were negatively correlated with percent moisture. Not surprisingly fluids were positively correlated with percent moisture and negativelycorrelated with percent dry mass. Meat and meat products were correlated with tea/coffee, but fish and seafood was strongly correlated with water. Milk products were strongly correlated with fats, but negatively correlated with fluids and tea/coffee. Cheese,

Table 4.4. Correlation coefficients of food group mass fractions (g food group/g wet diet) for men and women

	%Dry mass	%Moist -ure	Cereals/ Nuts	Vegs.	Fruits& Berries	Meat & Prods.	Fish & Seafood	Milk Prods.	Cheese	Food Fats	Fluids, no juice	Other Foods
%Dry mass	1.000											
%Moisture	**0.955**	1.000										
Cereals/Nuts	0.615	-0.574	1.000									
Vegetables	0.473	-0.522	0.287	1.000								
Fruits & Berries	-0.003	-0.154	-0.321	-0.158	1.000							
Meat & Prods.	-0.103	0.202	0.020	-0.235	-0.069	1.000						
Fish & Seafood	0.045	-0.052	-0.079	0.253	0.356	-0.088	1.000					
Milk Prods.	0.454	-0.391	0.550	0.195	-0.271	-0.456	-0.279	1.000				
Cheese	-0.466	0.448	-0.507	-0.470	-0.009	-0.087	-0.433	-0.338	1.000			
Food Fats	0.249	-0.158	0.428	0.444	-0.457	-0.213	-0.170	0.604	-0.430	1.000		
Fluids (no juices)	-0.649	0.670	-0.613	-0.377	-0.230	0.328	-0.061	**0.788**	0.576	0.441	1.000	
Other Foods	-0.168	0.169	-0.281	-0.293	-0.342	-0.195	-0.338	-0.167	0.412	0.252	0.528	1.000
Tea/Coffee	-0.192	0.077	-0.243	-0.553	0.233	0.164	-0.277	-0.459	0.262	0.441	0.446	0.489
Soft Drinks	-0.359	0.356	-0.426	-0.259	0.178	-0.060	0.301	-0.411	0.446	0.311	0.396	0.199
Water	-0.474	0.588	-0.310	-0.027	-0.566	0.281	-0.009	-0.308	0.183	0.106	0.639	0.219
Wine	0.246	-0.247	0.113	0.489	-0.022	0.038	-0.142	-0.034	0.000	0.398	-0.122	-0.217

Table 4.5. Correlation coefficients of food group mass fractions (g food group/g wet diet) for women

	%Dry mass	%Moist -ure	Cereals/ Nuts	Vegs.	Fruits& Berries	Meat& Prods.	Fish & Seafood	Milk Prods.	Cheese	Food Fats	Fluids, no juice	Other Foods
%Dry mass	1.000											
%Moisture	**-0.979**	1.000										
Cereals/Nuts	**0.875**	**-0.810**	1.000									
Vegetables	0.531	-0.589	0.525	1.000								
Fruits & Berries	-0.243	0.100	-0.551	-0.273	1.000							
Meat & Prods.	0.021	-0.095	0.084	-0.322	0.262	1.000						
Fish & Seafood	-0.246	0.171	-0.049	0.659	-0.240	-0.206	1.000					
Milk Prods.	0.590	-0.460	0.655	0.261	-0.371	-0.458	-0.256	1.000				
Cheese	-0.448	0.474	-0.685	-0.581	0.175	-0.215	-0.378	-0.379	1.000			
Food Fats	0.413	-0.267	0.635	0.317	-0.606	-0.527	0.008	**0.895**	-0.473	1.000		
Fluids (no juices)	**-0.740**	**0.705**	-0.687	-0.433	0.003	0.203	0.207	**-0.859**	0.590	-0.653	1.000	
Other Foods	-0.588	0.690	-0.466	-0.523	-0.406	-0.188	-0.044	-0.289	**0.721**	-0.142	**0.720**	1.000
Tea/Coffee	-0.412	-0.354	-0.419	-0.677	0.361	**0.750**	-0.311	**-0.745**	0.295	**-0.698**	0.653	0.228
Soft Drinks	-0.426	-0.464	-0.643	-0.538	0.094	-0.267	-0.345	-0.320	**0.993**	-0.415	0.564	**0.758**
Water	-0.547	0.526	-0.336	0.258	-0.393	-0.249	**0.819**	-0.477	0.106	-0.140	0.654	0.508
Wine												

Table 4.6. Correlation coefficients of food group mass fractions (g food group/g wet diet) for men

	%Dry mass	%Moist -ure	Cereals/ Nuts	Vegs.	Fruits& Berries	Meat& Prods.	Fish & Seafood	Milk Prods.	Cheese	Food Fats	Fluids, no juice	Other Foods
%Dry mass	1.000											
%Moisture	**-0.907**	1.000										
Cereals/Nuts	0.415	-0.330	1.000									
Vegetables	0.182	-0.346	0.544	1.000								
Fruits & Berries	0.449	-0.579	0.118	-0.003	1.000							
Meat & Prods.	-0.445	**0.754**	0.135	-0.339	-0.492	1.000						
Fish & Seafood	0.268	-0.214	0.093	-0.239	**0.803**	-0.078	1.000					
Milk Prods.	0.284	-0.380	-0.216	0.538	-0.078	-0.462	-0.344	1.000				
Cheese	-0.521	0.380	-0.171	-0.220	-0.369	0.172	-0.617	-0.290	1.000			
Food Fats	0.131	-0.078	0.434	**0.779**	-0.371	0.005	-0.248	0.490	-0.487	1.000		
Fluids (no juices)	-0.593	**0.701**	-0.340	-0.610	-0.690	0.525	-0.430	-0.529	0.597	-0.314	1.000	
Other Foods	-0.018	-0.038	-0.343	-0.396	-0.383	-0.255	-0.441	-0.169	0.426	-0.289	0.640	1.000
Tea/Coffee	0.445	-0.571	-0.208	-0.177	0.052	-0.665	-0.231	0.110	0.172	-0.274	0.122	**0.830**
Soft Drinks	-0.508	0.388	-0.170	-0.286	0.254	0.022	0.514	-0.642	-0.012	-0.276	0.256	0.057
Water	-0.492	**0.744**	-0.392	-0.492	**-0.745**	**0.769**	-0.447	-0.086	0.332	-0.088	0.709	0.144
Wine	0.368	-0.392	**0.861**	**0.774**	-0.048	-0.047	-0.304	0.183	0.041	0.530	-0.410	-0.289

soft drinks and other foods were mutually correlated, probably reflecting snack food choices.

Again, there were differences between the male and female diets. Percent dry mass was not strongly correlated with cereals, or with any other food group. Percent moisture was correlated with fluids, but the meat and meat products group and water were also mutually correlated. The vegetables group was correlated with food fats. The fruits and berries group was correlated with fish and seafoods and negatively correlated with water.

In summary, the content of the women's diets appeared to have three identifiable factors: cereals, fluids (particularly tea/coffee), and snack foods. The men's diets had mutual correlations between water and meat, and fruits and berries and fish, and tea/coffee and other foods.

4.2.3 Correlation Between Elements: Daily Intakes

Correlation coefficients of the measured daily intakes of the elements for the whole study group, the women and the men are shown in Tables 4.7, 4.8 and 4.9, respectively. There appear to be two groups of correlations overall (Table 4.7). Bromine, K, Rb and possibly Se are mutually correlated. Sodium and Cl are also correlated.

An examination of the correlation coefficients of the content of the women's diets again appears to suggest two groups (Table 4.8). Sodium and Cl were strongly correlated. There also appears to be a mutual correlation between Fe, iodine, K,

Table 4.7. Correlation coefficients of elemental daily intakes for men and women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Al	1.000												
Br	-0.295	1.000											
Ca	-0.500	0.781	1.000										
Cl	-0.068	0.062	0.003	1.000									
Fe	-0.508	0.703	0.602	0.239	1.000								
I	-0.409	0.735	0.686	0.120	0.635	1.000							
K	-0.362	**0.892**	0.644	0.111	0.726	0.666	1.000						
Mg	-0.194	0.472	0.180	0.484	0.508	0.309	0.670	1.000					
Mn	-0.117	0.387	0.076	0.106	0.126	0.120	0.590	0.751	1.000				
Na	0.078	-0.093	-0.119	**0.976**	0.116	-0.005	-0.048	0.387	0.003	1.000			
Rb	-0.369	**0.822**	0.689	-0.080	0.686	0.672	**0.941**	0.565	0.564	-0.218	1.000		
Se	-0.268	0.707	0.640	0.142	0.744	0.538	0.774	0.537	0.311	0.017	**0.800**	1.000	
Zn	-0.445	0.654	0.644	-0.121	0.583	0.600	0.700	0.255	0.181	-0.202	0.782	0.560	1.000

Table 4.8. Correlation coefficients of elemental daily intakes for women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Al	1.000												
Br	-0.338	1.000											
Ca	-0.525	0.791	1.000										
Cl	0.402	0.085	-0.060	1.000									
Fe	-0.450	**0.882**	**0.878**	-0.160	1.000								
I	-0.569	**0.836**	0.562	-0.100	**0.805**	1.000							
K	-0.165	**0.913**	0.637	0.047	**0.885**	**0.832**	1.000						
Mg	0.081	0.349	0.006	0.506	0.315	0.524	0.580	1.000					
Mn	0.397	-0.232	-0.692	0.439	-0.438	0.035	-0.108	0.518	1.000				
Na	0.563	-0.074	-0.162	**0.969**	-0.255	-0.272	-0.062	0.443	0.442	1.000			
Rb	-0.234	**0.808**	0.598	-0.330	**0.887**	**0.805**	**0.904**	0.328	-0.199	-0.394	1.000		
Se	0.218	0.713	0.381	-0.043	0.634	0.502	**0.857**	0.376	-0.123	-0.085	0.799	1.000	
Zn	-0.251	0.779	0.694	0.026	**0.885**	0.774	**0.812**	0.443	-0.089	-0.024	**0.833**	0.514	1.000

Table 4.9. Correlation coefficients of elemental daily intakes for men

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Al	1.000												
Br	0.193	1.000											
Ca	0.201	**0.812**	1.000										
Cl	0.133	0.486	0.635	1.000									
Fe	-0.137	0.613	0.575	0.695	1.000								
I	0.668	0.708	0.581	0.552	0.596	1.000							
K	0.089	**0.958**	**0.852**	0.567	0.596	0.569	1.000						
Mg	0.063	**0.820**	0.786	**0.882**	0.774	0.637	**0.871**	1.000					
Mn	0.073	**0.901**	0.764	0.633	0.502	0.509	**0.960**	**0.896**	1.000				
Na	0.182	0.405	0.589	**0.994**	0.653	0.542	0.485	**0.827**	0.554	1.000			
Rb	-0.019	**0.930**	**0.849**	0.553	0.593	0.477	**0.993**	**0.860**	**0.952**	0.467	1.000		
Se	-0.186	0.738	**0.850**	0.754	**0.868**	0.479	0.789	**0.876**	0.716	0.696	**0.813**	1.000	
Zn	-0.173	0.687	0.774	0.172	0.383	0.146	0.778	0.485	0.628	0.095	**0.821**	0.669	1.000

o, Zn and possibly Se. Calcium was correlated with Fe but did not have strong correlations ith any of the other elements.

The content of the men's diets had several correlations (Table 4.9) and was slightly ore complicated than the women's diets. Sodium, Cl and Mg were mutually correlated. agnesium was also included with Ca, K, Mn, Rb, Se and possibly Zn in a correlated group. was correlated strongly only with Se.

4.2.4 Correlation Between Elements: Concentrations

A scan of correlation coefficients for the whole study group (Table 4.10) suggests two mutual correlations. Sodium and Cl are strongly correlated. Bromine, K, Rb and possibly Se are also mutually correlated.

The content of the female diets shows several strong correlations (Table 4.11). Again, Na and Cl are strongly correlated. Bromine, Ca, Fe, iodine, K, Rb, Se and Zn appear to be mutually correlated. Magnesium and Mn had no noteworthy correlations.

Correlations in the male diets (Table 4.12) were somewhat different from the female diets. Na and Cl, and K and Rb were strongly correlated. Magnesium and Mn were strongly correlated. Manganese and possibly Mg were also correlated with K and Rb.

The correlation between Na and Cl reflects the salt content. Correlation between Rb and K is not unexpected, since the two belong to the same family and would be expected to show similar behavior. The differences between the female and male diets

Table 4.10. Correlation coefficients of elemental concentrations for men and women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Al	1.000												
Br	-0.295	1.000											
Ca	-0.500	0.781	1.000										
Cl	-0.068	0.062	0.003	1.000									
Fe	-0.508	0.703	0.602	0.239	1.000								
I	-0.409	0.735	0.686	0.120	0.635	1.000							
K	-0.362	**0.892**	0.644	0.111	0.726	0.666	1.000						
Mg	-0.194	0.472	0.180	0.484	0.508	0.309	0.670	1.000					
Mn	-0.117	0.387	0.076	0.106	0.126	0.120	0.590	0.751	1.000				
Na	0.078	-0.093	-0.119	**0.976**	0.116	-0.005	-0.048	0.387	0.003	1.000			
Rb	-0.369	**0.822**	0.689	-0.080	0.686	0.672	**0.941**	0.565	0.564	-0.218	1.000		
Se	-0.268	0.707	0.640	0.142	0.744	0.538	0.774	0.537	0.311	0.017	0.800	1.000	
Zn	-0.445	0.654	0.644	-0.121	0.583	0.600	0.700	0.255	0.181	-0.202	0.782	0.560	1.000

Table 4.11. Correlation coefficients of elemental concentrations for women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Al	1.000												
Br	-0.383	1.000											
Ca	-0.588	**0.904**	1.000										
Cl	0.114	0.322	0.077	1.000									
Fe	-0.501	**0.919**	**0.930**	0.045	1.000								
I	-0.559	**0.869**	0.747	0.202	**0.882**	1.000							
K	-0.370	**0.938**	**0.825**	0.151	**0.943**	**0.923**	1.000						
Mg	-0.219	0.528	0.301	0.436	0.553	0.747	0.685	1.000					
Mn	-0.246	0.208	-0.043	-0.006	0.135	0.541	0.343	0.458	1.000				
Na	0.344	0.086	-0.142	**0.944**	-0.151	-0.042	-0.074	0.311	-0.189	1.000			
Rb	-0.366	**0.852**	**0.788**	-0.121	**0.934**	**0.886**	**0.948**	0.553	0.371	-0.311	1.000		
Se	-0.100	**0.846**	**0.714**	0.039	**0.820**	0.727	**0.926**	0.520	0.252	-0.151	**0.886**	1.000	
Zn	-0.378	**0.815**	**0.757**	0.131	**0.900**	**0.870**	**0.828**	0.593	0.214	0.009	**0.863**	0.635	1.000

Table 4.12. Correlation coefficients of elemental concentrations for men

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Al	1.000												
Br	0.026	1.000											
Ca	-0.252	0.258	1.000										
Cl	-0.273	-0.341	-0.067	1.000									
Fe	-0.534	0.276	-0.189	0.362	1.000								
I	0.298	0.194	-0.709	0.255	0.378	1.000							
K	-0.322	**0.804**	0.301	0.021	0.358	-0.026	1.000						
Mg	-0.183	0.561	0.456	0.473	0.466	0.038	0.732	1.000					
Mn	-0.088	0.758	0.516	0.118	0.120	-0.094	**0.905**	**0.845**	1.000				
Na	-0.212	-0.427	-0.105	**0.994**	0.304	0.254	-0.080	0.398	0.032	1.000			
Rb	-0.379	0.764	0.494	-0.057	0.300	-0.257	**0.967**	0.728	**0.898**	-0.155	1.000		
Se	-0.686	0.355	0.591	0.274	0.674	-0.278	0.510	0.699	0.460	0.199	0.614	1.000	
Zn	-0.616	0.218	0.364	-0.407	0.091	-0.682	0.469	0.007	0.235	-0.460	0.602	0.404	1.000

may have been due to the higher contribution of cereal products to the dry mass in the female diets. The male diets seemed to be more balanced in that no single food group dominated the dry mass content. These hypotheses are tested in the next two sections by examining correlations between the elemental content and the content of the various food groups.

4.2.5 Correlation Between Elements and Food Groups: Daily Intakes

The correlations between daily intakes of elements and food groups are shown in Tables 4.13, 4.14 and 4.15. Correlations of the elements with the food groups might have allowed the sources of the daily intake to be determined.

Correlation coefficients for both the men and women are shown in Table 4.13. Dry mass, cereals, vegetables, Br, K, Mg, Mn, Rb, Se and possibly Ca and Zn were mutually correlated. Aluminum was correlated only with tea and coffee. This is consistent with the high levels of Al reported in tea (84, 30, 31), but a number of the duplicate diets with large amounts of coffee but no tea, *e.g.* HFTC, HMRZ and HMSG, also had high levels of Al as well (Appendix A). Sodium and Cl were weakly correlated with total, dry and wet mass, suggesting a distribution throughout the diet. Iron was correlated with dry mass, but there were no other strong correlations. Iodine was weakly correlated with cereals.

Table 4.13. Correlation coefficients of elemental and food group daily intakes for men and women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Total mass	0.398	0.525	0.604	0.652	0.476	0.003	0.689	0.707	0.682	0.645	0.576	0.612	0.549
Dry mass	0.297	**0.785**	0.668	0.656	**0.709**	0.358	**0.892**	**0.873**	**0.813**	0.598	**0.828**	**0.791**	0.627
Moisture	*0.406*	*0.421*	*0.553*	*0.616*	*0.382*	*-0.102*	*0.592*	*0.621*	*0.607*	*0.624*	*0.472*	*0.527*	*0.496*
Cereals/Nuts	0.022	**0.714**	0.660	0.551	0.595	0.646	**0.702**	0.694	0.600	0.483	**0.758**	**0.774**	0.582
Vegetables	0.039	**0.721**	0.544	0.510	0.513	0.416	**0.872**	**0.797**	**0.797**	0.409	**0.798**	0.676	0.680
Fruits & Berries	0.525	0.016	0.020	0.298	0.241	-0.278	0.151	0.347	0.261	0.371	0.104	0.272	-0.083
Meat& Prods.	0.220	0.083	0.229	0.448	0.244	-0.204	0.207	0.286	0.193	0.503	0.224	0.425	0.438
Fish & Seafood	-0.056	0.069	-0.133	0.255	0.013	0.143	-0.013	0.193	0.090	0.227	-0.125	-0.014	-0.30
Milk Prods.	-0.349	0.620	0.603	-0.221	0.358	0.473	0.527	0.158	0.257	-0.325	0.589	0.329	0.558
Cheese	0.009	-0.253	0.115	0.087	0.101	-0.360	-0.161	-0.035	-0.094	0.121	-0.169	-0.005	-0.071
Food Fats	-0.156	0.565	0.490	0.139	0.085	0.146	0.675	0.420	0.690	0.042	0.677	0.341	0.587
Fluids (no juice)	0.399	-0.220	-0.045	0.404	-0.145	-0.448	-0.093	0.072	0.060	0.471	-0.237	-0.099	-0.066
Other Foods	0.358	-0.121	-0.046	0.111	-0.128	0.029	-0.163	-0.111	-0.169	0.159	-0.275	-0.352	-0.280
Tea/Coffee	**0.807**	-0.107	-0.142	0.285	-0.092	-0.299	-0.050	0.067	0.099	0.399	-0.131	-0.104	-0.184
Soft Drinks	0.117	-0.259	0.002	-0.121	-0.362	-0.224	-0.309	-0.299	-0.261	-0.084	-0.364	-0.363	-0.275
Water	-0.317	-0.388	-0.311	-0.085	-0.283	-0.268	-0.347	-0.301	-0.330	-0.086	-0.408	-0.271	-0.054
Wine	-0.027	0.648	0.629	0.746	0.694	0.178	**0.849**	**0.932**	**0.891**	0.664	**0.812**	**0.812**	0.590

Table 4.14. Correlation coefficients of elemental and mass food group daily intakes for women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Total mass	0.676	-0.467	-0.266	0.639	-0.429	-0.682	-0.404	0.052	0.167	**0.799**	-0.555	-0.298	-0.210
Dry mass	0.526	0.396	0.107	0.099	0.430	0.30	0.621	0.436	0.238	0.181	0.658	0.720	0.637
Moisture	0.591	-0.540	-0.287	0.628	-0.507	-0.739	-0.514	-0.022	0.127	**0.775**	-0.673	-0.424	-0.322
Cereals/Nuts	-0.032	**0.891**	0.482	0.135	**0.753**	**0.817**	**0.940**	0.555	0.17	0.025	**0.858**	**0.825**	**0.793**
Vegetables	-0.127	0.240	-0.228	0.122	0.144	0.584	0.448	**0.811**	0.585	0.010	0.308	0.315	0.172
Fruits & Berries	0.675	-0.647	-0.435	-0.010	-0.391	-0.694	-0.393	-0.083	0.036	0.230	-0.295	-0.128	-0.248
Meat & Prods.	0.677	-0.050	-0.099	0.490	-0.051	-0.219	0.020	0.168	0.364	0.632	0.004	0.063	0.334
Fish & Seafood	-0.113	-0.090	-0.358	0.536	-0.255	0.192	-0.024	0.680	0.629	0.424	-0.310	-0.228	-0.186
Milk Prods.	-0.544	**0.711**	**0.829**	-0.352	**0.832**	0.576	0.688	0.059	**-0.740**	-0.461	0.678	0.573	0.480
Cheese	-0.139	-0.651	-0.201	-0.351	-0.530	-0.654	**-0.846**	**-0.864**	-0.324	-0.261	-0.628	**-0.775**	-0.550
Food Fats	-0.417	**0.739**	0.683	0.042	0.626	0.528	0.651	0.205	-0.504	-0.17	0.447	0.568	0.262
Fluids (no juice)	0.374	-0.642	-0.528	0.520	**-0.795**	-0.699	**-0.789**	-0.275	0.387	0.591	**-0.879**	-0.737	-0.562
Other Foods	-0.249	-0.321	0.046	0.236	-0.398	-0.433	-0.652	-0.550	-0.201	0.211	**-0.702**	**-0.780**	-0.396
Tea/Coffee	**0.806**	-0.420	-0.369	0.441	-0.487	-0.671	-0.423	-0.218	0.280	0.613	-0.428	-0.197	-0.187
Soft Drinks	-0.284	-0.577	-0.119	-0.379	-0.454	-0.550	**-0.799**	**-0.846**	-0.362	-0.320	-0.586	**-0.792**	-0.500
Water	-0.156	-0.276	-0.387	0.550	-0.515	-0.098	-0.399	0.238	0.507	0.440	-0.653	-0.575	-0.451
Wine													

Table 4.15. Correlation coefficients of elemental and mass food group daily intakes for men

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Total mass	0.403	**0.816**	**0.964**	0.585	0.573	**0.723**	**0.819**	0.733	0.698	0.550	**0.790**	**0.765**	**0.715**
Dry mass	0.388	**0.916**	**0.896**	**0.712**	0.693	**0.835**	**0.901**	**0.888**	**0.838**	0.665	**0.858**	**0.796**	0.594
Moisture	0.398	**0.765**	**0.959**	0.531	0.523	0.670	**0.773**	0.667	0.638	0.501	**0.749**	**0.736**	**0.733**
Cereals/Nuts	0.099	**0.775**	**0.883**	**0.890**	0.685	0.575	**0.856**	**0.966**	**0.871**	0.845	**0.854**	**0.889**	0.561
Vegetables	0.217	**0.893**	**0.932**	0.518	0.470	0.541	**0.952**	**0.777**	**0.880**	0.452	**0.945**	**0.744**	**0.845**
Fruits & Berries	0.287	0.546	0.413	0.475	0.593	**0.784**	0.348	0.525	0.341	0.458	0.288	0.484	-0.057
Meat & Prods.	-0.559	0.030	0.470	0.377	0.325	-0.286	0.183	0.285	0.141	0.362	0.276	0.608	0.470
Fish & Seafood	-0.024	-0.005	-0.131	0.063	-0.044	0.136	-0.176	0.015	-0.035	0.064	-0.197	-0.057	-0.475
Milk Prods.	0.036	**0.717**	0.433	-0.140	0.177	0.248	**0.715**	0.310	0.600	-0.228	**0.710**	0.287	**0.762**
Cheese	0.267	0.015	0.404	0.348	0.458	0.339	0.048	0.158	-0.140	0.394	0.037	0.388	0.230
Food Fats	-0.090	0.573	0.470	0.114	-0.075	-0.044	**0.706**	0.424	**0.757**	0.037	**0.734**	0.280	0.674
Fluids (no juice)	0.505	-0.078	0.401	0.245	0.045	0.230	-0.008	0.019	-0.138	0.308	-0.027	0.144	0.197
Other Foods	**0.821**	-0.170	-0.121	0.050	-0.161	0.390	-0.191	-0.142	-0.222	0.124	-0.279	-0.358	-0.333
Tea/Coffee	**0.840**	0.281	0.140	0.299	0.216	0.759	0.203	0.259	0.168	0.332	0.095	-0.022	-0.163
Soft Drinks	0.498	-0.366	-0.052	-0.199	-0.566	-0.016	-0.446	-0.428	-0.418	-0.127	-0.479	-0.406	-0.351
Water	-0.582	-0.565	-0.275	-0.472	-0.231	**-0.762**	-0.438	-0.549	-0.558	-0.456	-0.348	-0.173	0.179
Wine	-0.063	**0.767**	**0.808**	**0.824**	**0.724**	0.457	**0.888**	**0.954**	**0.887**	**0.765**	**0.901**	**0.888**	0.646

The Al intake of the women's diets was correlated with tea/coffee. Bromine, Ca, Fe, iodine, K, Rb Se and Zn were correlated with cereals. Bromine, Ca and Fe were also correlated with milk. Sodium was correlated with total mass and withmoisture. There were no noteworthy correlations for Cl. Manganese only had one noteworthy correlation, a negative correlation with milk. Iron, K, Rb and Se were negatively correlated with fluids, indicative of the low levels of these elements in this group.

The men's diets had several strong correlations. Aluminum was correlated with tea/coffee and other foods (probably because of the inclusion of sugar and coffee whitener in the other foods category). Bromine, Ca, K, Mg, Mn, Rb, Se, and possibly Cl, iodine and Zn appeared to have similar correlations with total mass, dry mass, moisture, cereals, vegetables and milk. K, Mg, Rb and Se were correlated with food fats. Wine had several strong correlations, but this was likely due to the large total mass, cereals and vegetables in the two diets, namely HMNT and HMWV, which contained alcoholic beverages (Appendix A).

4.2.6 Correlation Between Elements and Food Groups: Concentrations and Mass Fractions

Correlation coefficients calculated for the measured concentrations of the elements and the calculated mass fractions for the entire group, the women and the men are shown in Tables 4.16, 4.17 and 4.18, respectively.

Table 4.16. Correlation coefficients of elemental concentrations and mass fractions of food groups for men and women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
%Dry mass	-0.132	**0.722**	0.428	0.090	**0.703**	0.691	**0.817**	0.585	0.477	-0.028	**0.821**	0.670	0.469
%Moisture	-0.032	-0.652	-0.307	0.065	-0.576	-0.637	**-0.737**	-0.571	-0.503	0.153	-0.768	-0.576	-0.417
Cereals/Nuts	-0.158	0.696	**0.714**	0.220	0.494	**0.786**	0.668	0.429	0.324	0.128	**0.740**	**0.741**	0.588
Vegetables	-0.207	0.416	0.084	0.035	0.228	0.368	0.624	0.664	0.580	-0.082	0.535	0.387	0.378
Fruits & Berries	0.436	-0.274	-0.384	-0.331	-0.152	-0.363	-0.258	0.058	0.052	-0.224	-0.185	-0.145	-0.375
Meat & Prods.	0.059	-0.201	-0.123	0.401	0.043	-0.151	-0.152	-0.024	-0.211	0.487	-0.099	0.009	0.268
Fish & Seafood	-0.005	0.072	-0.163	0.267	-0.071	0.097	-0.015	0.299	0.176	0.233	-0.171	-0.080	-0.330
Milk Prods.	-0.395	**0.720**	**0.795**	-0.256	0.569	0.607	**0.702**	0.117	0.111	-0.377	**0.713**	0.608	0.568
Cheese	-0.101	-0.582	-0.278	-0.329	-0.317	-0.490	-0.619	-0.507	-0.306	-0.285	-0.468	-0.423	-0.382
Food Fats	-0.278	0.527	0.509	0.058	0.131	0.229	0.672	0.356	0.567	-0.060	0.611	0.413	0.448
Fluids (no juice)	0.169	**-0.709**	-0.635	0.250	-0.573	-0.579	**-0.752**	-0.435	-0.347	0.334	**-0.799**	**-0.712**	-0.527
Other Foods	0.169	-0.272	-0.186	0.163	-0.190	-0.058	-0.353	-0.333	-0.374	0.202	-0.425	-0.523	-0.365
Tea/Coffee	**0.699**	-0.356	-0.477	0.023	-0.361	-0.379	-0.466	-0.293	-0.138	0.162	-0.438	-0.499	-0.382
Soft Drinks	0.077	-0.369	-0.136	-0.237	-0.535	-0.304	-0.539	-0.459	-0.286	-0.198	-0.483	-0.499	-0.468
Water	-0.324	-0.407	-0.333	0.361	-0.157	-0.143	-0.333	-0.251	-0.347	0.352	-0.441	-0.285	-0.109
Wine	-0.133	0.213	0.056	0.222	0.236	-0.174	0.426	**0.740**	**0.702**	0.159	0.397	0.357	0.203

Table 4.17. Correlation coefficients of elemental concentrations and mass fractions of food groups for women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
%Dry Mass	-0.132	**0.722**	0.428	0.090	**0.703**	0.691	**0.817**	0.585	0.477	-0.028	**0.821**	0.670	0.469
%Moisture	-0.032	-0.652	-0.307	0.065	-0.576	-0.637	**-0.737**	-0.571	-0.503	0.153	**-0.768**	-0.576	-0.417
Cereals/Nuts	-0.221	**0.937**	**0.737**	0.288	**0.869**	**0.911**	**0.953**	0.665	0.417	0.085	**0.904**	**0.876**	**0.855**
Vegetables	-0.255	0.346	0.102	0.216	0.352	0.659	0.564	**0.882**	**0.748**	0.047	0.469	0.455	0.333
Fruits & Berries	0.516	-0.679	-0.564	-0.549	-0.424	-0.597	-0.494	-0.264	-0.394	-0.262	-0.328	-0.345	-0.334
Meat & Prods.	0.508	-0.054	-0.183	0.239	-0.075	-0.093	-0.147	-0.040	-0.195	0.444	-0.063	-0.194	0.307
Fish & Seafood	-0.110	-0.128	-0.331	0.599	-0.230	0.122	-0.060	0.599	0.389	0.541	-0.271	-0.214	-0.174
Milk Prods.	-0.504	**0.798**	**0.900**	-0.073	**0.875**	0.662	**0.833**	0.373	-0.045	-0.287	**0.780**	**0.818**	0.580
Cheese	-0.162	-0.584	-0.327	-0.606	-0.529	-0.561	-0.654	**-0.848**	-0.088	-0.560	-0.483	-0.606	-0.562
Food Fats	-0.390	**0.803**	**0.797**	0.282	**0.715**	0.590	**0.766**	0.405	-0.006	0.033	0.590	**0.775**	0.390
Fluids (no juice)	0.245	-0.673	-0.693	0.172	**-0.849**	-0.662	**-0.840**	-0.580	-0.026	0.283	**-0.848**	**-0.844**	-0.677
Other Foods	-0.339	-0.247	-0.075	0.065	-0.392	-0.344	-0.498	-0.642	-0.122	0.005	-0.522	-0.577	-0.425
Tea/Coffee	**0.711**	-0.487	-0.548	0.060	-0.599	-0.644	-0.639	-0.600	-0.333	0.304	-0.550	-0.523	-0.331
Soft Drinks	-0.271	-0.523	-0.254	-0.584	-0.466	-0.484	-0.600	**-0.804**	-0.053	-0.568	-0.439	-0.585	-0.511
Water	-0.141	-0.315	-0.438	0.540	-0.519	-0.169	-0.392	0.096	0.308	0.474	-0.579	-0.517	-0.478
Wine													

Table 4.18. Correlation coefficients of elemental concentrations and mass fractions of food groups for men

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
%Dry Mass	0.061	0.680	-0.149	0.356	0.546	0.636	0.684	**0.747**	0.668	0.288	0.523	0.284	-0.267
%Moisture	-0.350	**-0.791**	0.095	-0.021	-0.378	-0.545	-0.620	-0.656	-0.647	0.033	-0.500	-0.166	0.253
Cereals/Nuts	-0.176	0.280	**0.699**	0.517	0.167	-0.286	0.517	**0.897**	**0.750**	0.466	0.590	0.655	0.023
Vegetables	-0.011	0.622	0.655	-0.342	-0.173	-0.558	**0.722**	0.516	**0.794**	-0.401	**0.833**	0.345	0.583
Fruits & Berries	0.312	0.601	0.172	-0.184	0.185	0.468	0.097	0.287	0.227	-0.209	0.056	0.179	-0.451
Meat & Prods.	**-0.741**	-0.623	0.215	0.517	0.126	-0.375	-0.252	-0.102	-0.30	0.522	-0.174	0.339	0.216
Fish & Seafood	0.140	0.291	0.184	0.083	-0.047	0.446	-0.063	0.123	0.116	0.068	-0.131	0.024	-0.550
Milk Prods.	-0.149	0.657	-0.123	-0.561	0.165	-0.020	0.681	0.057	0.400	-0.625	0.644	0.044	0.653
Cheese	0.060	-0.579	-0.121	-0.049	0.094	-0.335	-0.555	-0.299	-0.591	0.014	-0.430	0.025	0.072
Food Fats	-0.216	0.350	0.448	-0.048	-0.304	-0.478	**0.702**	0.359	**0.725**	-0.105	**0.737**	0.121	0.572
Fluids (no juice)	0.019	**-0.974**	-0.414	0.339	-0.231	-0.115	**-0.737**	-0.561	**-0.741**	0.423	**-0.729**	-0.430	-0.197
Other Foods	0.579	-0.490	**-0.732**	0.185	-0.170	0.347	-0.428	-0.316	-0.417	0.258	-0.536	-0.671	-0.462
Tea/Coffee	0.680	0.049	**-0.699**	0.063	0.085	0.612	-0.057	0.018	-0.063	0.096	-0.210	-0.485	-0.505
Soft Drinks	0.526	-0.393	0.168	-0.144	**-0.743**	-0.073	-0.695	-0.458	-0.390	-0.071	-0.650	-0.514	-0.530
Water	-0.622	-0.684	-0.334	0.252	0.158	-0.180	-0.323	-0.477	-0.572	0.273	-0.316	-0.031	0.328
Wine	-0.218	0.417	0.631	0.207	0.264	-0.446	0.675	**0.839**	**0.766**	0.147	**0.781**	0.698	0.388

Correlations for the whole group (Table 4.16) are discussed first. Dry mass, cereals and milk products appear to be mutually correlated with Br, iodine, K, Rb, Se, and possibly Ca. These elements were also negatively correlated with fluids (perhaps due to the milk). The only strong correlation involving Fe was with dry mass although its pattern of weak correlations appeared to follow that of the percent dry mass/cereals/milk group. The only strong correlations for Mg and Mn were with wine. There were no noteworthy correlations for Cl, Na or Zn. Aluminum was correlated with tea/coffee.

The women's group revealed similar correlations to those discussed for the men and women. Bromine, Ca, Fe, K, Rb, Se and possibly iodine and Zn were mutually correlated with dry mass, cereals, milk and fats, and negatively correlated with fluids. Magnesium and Mn were strongly correlated with vegetables. Magnesium was negatively correlated with soft drinks (probably due to competition with fruit juices as beverages) and cheese. Sodium and Cl had no strong correlations. Aluminum was strongly correlated with tea/coffee.

Correlations between measured elements and food group mass fractions were somewhat different for the men. Ca, K, Mg, Mn and Rb were positively correlated with cereals vegetables, fats and wine, and negatively correlated with fluids. Ca was negatively correlated with other foods and tea/coffee. The only strong correlation for Fe was a negative correlation with soft drinks. Se was moderately correlated with cereals and wine and negatively correlated with other foods. Aluminum was negatively correlated with meat and positively correlated with tea/coffee.

4.2.7 Correlation Between Elements and Calculated Nutrients: Daily Intakes

The daily intakes of several nutrients were calculated by Chatt and Pegg (27) from the food records, using the Nutrient Analysis System of Mount Saint Vincent University. The daily intakes were calculated for protein, energy, phosphorus, vitamins D, A, C, B-6 and B-12, Thiamin, Riboflavin, Niacin, Folacin, and percent (with respect to energy intake) protein, fat and carbohydrate. The results may be found in Appendix A. In order to study associations between these nutrients and the measured elements, and to determine common food sources, correlation calculations were performed. The correlation coefficients for all subjects, the women and the men are presented in Tables 4.19, 4.20 and 4.21, respectively.

Overall, the elements Br, Ca, Mg, Mn, K, Rb, Se and Zn appeared to be mutually correlated with protein, energy, phosphorus, vitamin D, vitamin A, Riboflavin, Niacin, vitamin B-6, Folacin and vitamin B-12. These elements were strongly correlated with cereals and vegetables, so these nutrients likely come from these sources as well. Correlation calculations between nutrients and food groups support this conclusion (data not shown). Iron was correlated similarly to the elements listed above, but was more weakly correlated with vitamin A, Niacin, vitamin B-6 and vitamin B-12, and more strongly correlated with thiamin. Chlorine, iodine and Na were not strongly correlated with any of the calculated nutrients, suggesting that they are not concentrated in the foods containing these nutrients, but are either dispersed more widely throughout the diet (as is likely for Cl, iodine and Na) or are highly associated with foods that contain little of these nutrients.

Table 4.19. Correlation coefficients of the intakes of measured elements and some calculated nutrients for men and women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Protein	-0.073	**0.838**	**0.781**	0.526	0.694	0.417	**0.925**	**0.789**	**0.776**	0.441	**0.920**	**0.819**	**0.912**
Energy	0.033	**0.831**	0.692	0.497	0.582	0.319	**0.961**	**0.832**	**0.893**	0.395	**0.916**	**0.761**	**0.713**
Phosphorus	-0.072	**0.830**	**0.790**	0.578	**0.725**	0.332	**0.956**	**0.865**	**0.864**	0.485	**0.934**	**0.824**	**0.783**
Vitamin D	-0.238	0.610	0.495	0.092	0.263	0.345	0.680	0.420	0.563	-0.022	**0.708**	0.480	0.601
Vitamin A	-0.202	**0.703**	**0.780**	0.324	0.586	0.272	**0.800**	0.580	0.606	0.227	**0.829**	**0.782**	**0.893**
Vitamin C	0.335	0.139	0.299	0.584	0.509	-0.025	0.318	0.604	0.366	0.633	0.303	0.553	0.108
Thiamin	0.061	0.439	0.436	0.472	**0.784**	0.085	0.496	0.516	0.248	0.437	0.422	0.676	0.404
Riboflavin	-0.065	**0.781**	**0.782**	0.358	**0.704**	0.309	**0.868**	0.684	0.690	0.279	**0.905**	**0.836**	**0.806**
Niacin	0.035	**0.811**	0.665	0.524	0.651	0.357	**0.953**	**0.827**	**0.844**	0.440	**0.924**	**0.776**	**0.857**
Vitamin B-6	-0.136	0.694	0.559	0.484	0.611	0.260	**0.893**	**0.844**	**0.855**	0.378	**0.848**	**0.705**	0.644
Folacin	0.033	0.536	0.483	0.626	**0.796**	0.325	**0.721**	**0.869**	0.661	0.579	**0.712**	**0.819**	0.499
Vitamin B-12	-0.110	**0.784**	0.697	0.435	0.509	0.269	**0.871**	**0.705**	**0.807**	0.339	**0.881**	**0.705**	**0.739**
%Protein	-0.214	0.293	0.411	0.232	0.411	0.301	0.233	0.177	0.047	0.234	0.299	0.360	0.629
%Fat	-0.366	0.236	0.226	-0.012	-0.113	0.172	0.295	0.100	0.251	-0.094	0.316	0.17	0.402
%Carbohydr.	0.378	-0.328	-0.342	-0.070	-0.022	-0.235	-0.378	-0.176	-0.296	0.007	-0.413	-0.242	-0.574

Table 4.20. Correlation coefficients of the intakes of measured elements and some calculated nutrients for women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Protein	-0.025	**0.747**	0.593	0.296	**0.802**	0.686	**0.842**	0.648	0.048	0.257	**0.742**	0.590	**0.941**
Energy	-0.009	0.621	0.412	-0.113	0.695	0.562	**0.851**	0.556	-0.208	-0.160	**0.770**	**0.879**	0.507
Phosphorus	-0.155	**0.703**	**0.800**	0.104	**0.861**	0.500	**0.792**	0.422	-0.492	0.070	0.671	0.636	**0.724**
Vitamin D	-0.324	0.697	0.486	-0.094	0.613	0.617	**0.746**	0.383	-0.312	-0.252	0.590	**0.722**	0.277
Vitamin A	-0.297	**0.917**	**0.845**	-0.088	**0.883**	0.658	**0.864**	0.175	-0.535	-0.209	**0.789**	**0.786**	0.639
Vitamin C	0.481	-0.436	-0.224	-0.056	-0.090	-0.408	-0.111	0.174	-0.043	0.153	-0.039	0.034	0.007
Thiamin	0.388	0.254	0.380	0.097	0.325	-0.167	0.368	0.032	-0.550	0.163	0.251	0.615	0.104
Riboflavin	0.014	0.643	**0.715**	-0.146	**0.796**	0.359	**0.748**	0.165	-0.598	-0.136	**0.737**	**0.785**	0.610
Niacin	0.168	0.551	0.282	0.261	0.623	0.603	**0.773**	**0.781**	0.295	0.264	0.699	0.608	**0.824**
Vitamin B-6	-0.295	0.094	0.070	-0.253	0.331	0.363	0.378	0.556	-0.100	-0.283	0.335	0.281	0.140
Folacin	0.123	0.030	-0.066	-0.098	0.301	0.260	0.418	0.680	0.147	-0.030	0.412	0.386	0.330
Vitamin B-12	-0.008	**0.855**	**0.793**	0.066	**0.869**	0.545	**0.868**	0.256	-0.414	0.021	**0.803**	**0.816**	**0.778**
%Protein	-0.002	0.550	0.506	0.473	0.569	0.476	0.524	0.463	0.150	0.455	0.429	0.206	**0.841**
%Fat	-0.407	0.590	0.328	0.279	0.385	0.608	0.558	0.553	0.010	0.066	0.263	0.379	0.152
%Carbohydr.	0.370	**-0.756**	-0.499	-0.451	-0.576	**-0.744**	**-0.717**	-0.694	-0.076	-0.250	-0.409	-0.425	-0.476

Table 4.21. Correlation coefficients of the intakes of measured elements and some calculated nutrients for men

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Protein	-0.156	**0.859**	**0.888**	0.505	0.582	0.350	**0.935**	**0.792**	**0.867**	0.419	**0.965**	**0.863**	**0.898**
Energy	0.072	**0.930**	**0.844**	0.512	0.446	0.483	**0.970**	**0.819**	**0.966**	0.428	**0.969**	**0.726**	**0.756**
Phosphorus	-0.062	**0.897**	**0.834**	0.626	0.640	0.458	**0.981**	**0.897**	**0.953**	0.543	**0.992**	**0.843**	**0.788**
Vitamin D	-0.172	0.625	0.518	0.169	0.17	-0.001	**0.776**	0.498	**0.769**	0.084	**0.811**	0.416	**0.791**
Vitamin A	-0.240	0.639	**0.804**	0.338	0.467	0.117	**0.774**	0.575	0.646	0.269	**0.826**	**0.761**	**0.972**
Vitamin C	0.199	0.484	0.651	**0.901**	**0.788**	0.696	0.463	**0.780**	0.459	**0.904**	0.436	**0.774**	0.122
Thiamin	-0.137	0.291	0.394	0.425	**0.852**	0.384	0.301	0.422	0.115	0.414	0.310	0.659	0.365
Riboflavin	-0.166	**0.874**	**0.820**	0.500	0.661	0.385	**0.951**	**0.810**	**0.871**	0.411	**0.975**	**0.853**	**0.881**
Niacin	-0.054	**0.903**	**0.847**	0.483	0.555	0.411	**0.978**	**0.803**	**0.916**	0.396	**0.993**	**0.795**	**0.881**
Vitamin B-6	-0.117	**0.875**	**0.753**	0.563	0.563	0.378	**0.966**	**0.861**	**0.964**	0.474	0.981	**0.771**	**0.760**
Folacin	-0.043	**0.737**	**0.738**	**0.836**	**0.939**	0.620	**0.780**	**0.930**	**0.728**	**0.787**	**0.777**	**0.932**	0.510
Vitamin B-12	-0.245	**0.737**	0.658	0.440	0.302	0.129	**0.848**	**0.723**	**0.909**	0.350	**0.885**	0.633	0.695
%Protein	-0.682	0.145	0.394	0.161	0.515	-0.240	0.256	0.232	0.108	0.119	0.348	0.637	0.635
%Fat	-0.380	0.130	0.222	-0.081	-0.262	-0.507	0.352	0.093	0.381	-0.133	0.427	0.106	0.607
%Carbohydr.	0.459	-0.198	-0.311	0.027	0.123	0.473	-0.419	-0.167	-0.417	0.086	-0.502	-0.242	**-0.703**

Regarding the female diets, Br, Ca, Fe, K, Rb and Se appear to be mutually correlated with protein, energy, phosphorus, vitamin D, vitamin A, Niacin, Riboflavin and vitamin B-12, and negatively correlated with % carbohydrates. Zinc shares many of these correlations but was weaker in energy and vitamin A, and had a stronger association with percent protein. The only strong correlation for Mg was with Niacin. Chlorine, Mn and Na had no strong correlations.

The results for the male diets were similar to the females. Bromine, Ca, K, Mg, Mn, Rb, Se and Zn appeared to be mutually correlated with protein, energy, phosphorus, vitamin A, Riboflavin, Niacin, vitamin B-6, Folacin, vitamin B-12 and possibly vitamin C. Zinc differed slightly in being uncorrelated with vitamin C and strongly negatively correlated with percent carbohydrates. Iron might also be included but the correlations were generally weaker, except for vitamin C and Folacin. This large mutual correlation differs from the females by the inclusion of Mg, Mn and Zn, folacin and vitamin C in a large mutually correlated group. Sodium and Cl were correlated with vitamin C and with Folacin. The only noteworthy correlation with aluminum was a negative correlation with percent protein. Iodine had no noteworthy correlations.

4.2.8 Correlation Between Elements and Calculated Nutrients: Concentrations and Mass Fractions

Correction of the daily intakes for total mass of the diet should give more of an indication of the balance of nutrients in the diet. The correlations between

elemental concentration and nutrient intake divided by wet mass of the diet are presented in Tables 4.22, 4.23 and 4.24.

The overall correlations are shown in Table 4.22. Bromine, K, Rb, Se, Zn and possibly Fe appear to be mutually correlated with protein, energy, phosphorus, vitamin D, Riboflavin, Niacin and vitamin B-12. Zinc is similar, but more weakly correlated with energy and vitamin D and more strongly correlated with percent protein. Magnesium was correlated strongly with Folacin. Aluminum, Ca, Cl, Mn and Na had no strong correlations.

The women had several strong correlations and again a large group of mutual correlations involving Br, Ca, Fe, iodine, K, Rb, Se, protein, energy, phosphorus, vitamin D, vitamin A, Riboflavin, Niacin and vitamin B-12. Magnesium was similar, but more weakly correlated to some of the aforementioned nutrients, more strongly correlated to Folacin and percent fat. Zinc was also similar, but had weaker correlations with energy, vitamin D, vitamin A, Riboflavin and was strongly correlated with percent protein. There were no strong correlations for aluminum, Cl, Mn or Na.

The correlations in the male group were slightly different from the females. K, Rb and perhaps Se were mutually correlated with protein, energy, phosphorus, Riboflavin, Niacin, vitamin B-6, Folacin, and vitamin B-12. Magnesium and Mn appeared to have similar correlations. Aluminum was negatively correlated to protein, Riboflavin, and percent protein. Fe was positively correlated with Thiamin and Folacin. Iodine was negatively correlated with vitamin A and positively correlated with percent carbohydrate. Zinc was positively correlated with vitamin A, Riboflavin, percent protein

Table 4.22. Correlation coefficients of elemental concentrations and mass nutrient intake per gram of wet diet for men and women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Protein	-0.470	0.736	0.640	0.224	**0.716**	0.681	**0.834**	0.516	0.332	0.116	0.829	0.693	**0.881**
Energy	-0.416	0.667	0.514	0.126	0.571	0.573	**0.852**	0.521	0.496	-0.030	0.789	0.671	0.457
Phosphorus	-0.448	0.743	0.667	0.200	**0.738**	0.496	**0.906**	0.656	0.501	0.075	0.857	**0.748**	0.685
Vitamin D	-0.368	0.586	0.596	0.095	0.431	0.513	**0.736**	0.312	0.305	-0.040	0.682	0.644	0.502
Vitamin A	-0.410	0.602	0.672	-0.099	0.482	0.317	0.673	0.278	0.243	-0.213	0.715	**0.720**	**0.763**
Vitamin C	0.201	-0.220	-0.098	-0.031	0.105	-0.090	-0.088	0.316	0.027	0.063	0.029	0.158	-0.144
Thiamin	-0.281	0.170	0.123	0.300	0.662	0.121	0.269	0.083	-0.312	0.246	0.154	0.387	0.169
Riboflavin	-0.348	**0.709**	**0.746**	-0.044	**0.718**	0.522	**0.804**	0.345	0.242	-0.146	0.841	**0.816**	0.695
Niacin	-0.345	0.623	0.355	0.196	0.649	0.519	**0.844**	0.639	0.482	0.098	0.798	0.584	**0.783**
Vitamin B-6	-0.478	0.334	0.166	0.187	0.516	0.278	0.679	0.643	0.493	0.069	0.579	0.458	0.333
Folacin	-0.194	0.234	0.114	0.117	0.583	0.319	0.512	**0.708**	0.346	0.085	0.531	0.552	0.344
Vitamin B-12	-0.333	0.711	0.640	0.286	0.554	0.410	**0.829**	0.526	0.489	0.171	0.778	0.675	0.624
%Protein	-0.236	0.280	0.339	0.186	0.386	0.248	0.219	0.171	-0.063	0.202	0.284	0.297	**0.709**
%Fat	-0.373	0.291	0.347	0.182	0.019	0.244	0.457	0.220	0.318	0.084	0.391	0.299	0.455
%Carbohydr.	0.395	-0.348	-0.397	-0.203	-0.113	-0.267	-0.493	-0.271	-0.318	-0.118	-0.451	-0.346	-0.618

Table 4.23. Correlation coefficients of elemental concentrations and mass nutrient intake per gram of wet diet for women

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Protein	-0.321	**0.863**	**0.751**	0.260	**0.913**	**0.903**	**0.895**	**0.726**	0.238	0.121	0.872	**0.724**	**0.974**
Energy	-0.393	**0.758**	**0.698**	-0.119	**0.823**	**0.795**	**0.913**	0.609	0.396	-0.351	0.888	**0.913**	0.596
Phosphorus	-0.395	**0.853**	**0.883**	0.103	**0.947**	**0.758**	**0.898**	0.585	-0.063	-0.057	0.836	**0.824**	**0.781**
Vitamin D	-0.347	**0.773**	**0.713**	0.157	**0.737**	0.672	0.843	0.566	0.193	-0.089	0.702	**0.870**	0.427
Vitamin A	-0.360	**0.942**	**0.930**	0.17	**0.912**	**0.746**	**0.914**	0.409	0.041	-0.091	0.839	**0.905**	0.689
Vitamin C	0.230	-0.331	-0.217	-0.482	0.002	-0.175	-0.076	0.162	-0.291	-0.280	0.053	-0.022	0.044
Thiamin	-0.065	0.626	0.679	-0.081	0.655	0.355	0.671	0.213	-0.250	-0.204	0.605	**0.824**	0.326
Riboflavin	-0.240	**0.802**	**0.848**	-0.094	**0.897**	0.636	**0.858**	0.389	-0.113	-0.232	0.848	**0.890**	0.688
Niacin	-0.185	**0.706**	0.536	0.181	**0.796**	**0.853**	**0.832**	**0.828**	0.365	0.087	0.834	0.684	**0.905**
Vitamin B-6	-0.427	0.311	0.330	-0.220	0.516	0.538	0.581	0.681	0.311	-0.357	0.553	0.528	0.318
Folacin	-0.091	0.166	0.125	-0.186	0.426	0.425	0.464	**0.728**	0.182	-0.174	0.490	0.411	0.418
Vitamin B-12	-0.205	**0.928**	**0.895**	0.166	**0.926**	**0.724**	**0.894**	0.426	-0.064	0.015	0.851	**0.876**	**0.808**
%Protein	-0.023	0.422	0.339	0.496	0.416	0.401	0.295	0.349	-0.143	0.545	0.275	0.074	**0.708**
%Fat	-0.392	0.614	0.462	0.610	0.464	0.605	0.614	0.702	0.303	0.362	0.353	0.514	0.265
%Carbohydr.	0.364	**-0.726**	-0.552	-0.760	-0.585	**-0.711**	-0.674	**-0.781**	-0.219	-0.555	-0.428	-0.492	-0.524

Table 4.24. Correlation coefficients of elemental concentrations and mass nutrient intake per gram of wet diet for men

	Al	Br	Ca	Cl	Fe	I	K	Mg	Mn	Na	Rb	Se	Zn
Protein	**-0.862**	0.333	0.370	0.197	0.384	-0.277	**0.706**	0.421	0.523	0.107	0.728	0.635	0.674
Energy	-0.455	0.465	0.159	0.308	0.166	0.153	**0.730**	0.467	0.667	0.224	0.629	0.261	0.213
Phosphorus	-0.559	0.558	0.340	0.261	0.454	-0.166	**0.931**	**0.756**	**0.818**	0.164	0.927	0.650	0.547
Vitamin D	-0.493	0.188	0.108	0.112	0.062	-0.343	0.688	0.281	0.519	0.051	0.682	0.197	0.689
Vitamin A	-0.533	0.147	0.583	-0.263	0.066	**-0.842**	0.430	0.207	0.321	-0.306	0.617	0.547	**0.917**
Vitamin C	0.113	0.109	0.385	0.450	0.334	0.154	-0.051	0.598	0.203	0.445	-0.012	0.509	-0.516
Thiamin	-0.554	-0.342	-0.586	0.394	**0.714**	0.309	-0.102	-0.128	-0.425	0.389	-0.182	0.196	0.101
Riboflavin	**-0.717**	0.511	0.242	0.063	0.551	-0.221	**0.849**	0.531	0.605	-0.033	**0.865**	0.667	**0.747**
Niacin	-0.673	0.471	0.186	0.139	0.398	-0.161	**0.864**	0.492	0.649	0.045	**0.841**	0.504	0.674
Vitamin B-6	-0.623	0.378	0.078	0.418	0.451	0.034	**0.817**	0.585	0.651	0.330	0.743	0.464	0.425
Folacin	-0.417	0.415	0.195	0.399	**0.829**	0.053	0.606	**0.815**	0.516	0.330	0.616	**0.823**	0.206
Vitamin B-12	-0.559	0.366	0.467	0.337	0.083	-0.220	**0.743**	0.569	**0.743**	0.255	**0.735**	0.445	0.402
%Protein	**-0.773**	-0.059	0.481	-0.065	0.411	-0.641	0.130	0.111	-0.033	-0.100	0.313	0.747	**0.724**
%Fat	-0.453	-0.051	0.384	-0.008	-0.310	-0.685	0.416	0.067	0.363	-0.041	0.500	0.110	**0.724**
%Carbohydr.	0.546	0.008	-0.463	0.037	0.207	**0.750**	-0.449	-0.116	-0.375	0.078	-0.560	-0.253	**-0.811**

and percent fat, but negatively correlated with percent carbohydrate. There were no strong correlations involving Br, Ca Cl and Na.

4.3 HIERARCHICAL CLUSTER ANALYSIS (HCA)

Hieracrchical cluster analysis was performed on both concentrations and daily intakes. Since the results were similar, results for concentrations only will be discussed. Cluster analyses for the elements themselves, and for the elements and mass fractions of the food groups were performed.

Dendrograms were calculated using different clustering techniques. The dendrogram for the elemental concentrations, percent moisture and percent dry mass, shown in Fig. 4.1, was calculated using the "complete link" technique. The most similar distinct pairs were Na and Cl, and Rb and K. There appeared to be two large clusters, two clustered pairs, and two single points (marked by the cursor at a similarity of 0.44). The first cluster involved elements associated with dry mass. They were Br, Ca, iodine, Fe, K, Rb, Se and Zn. The clusters pairs were Mn and Mg, and Na and Cl. The individual points, which are actually quite similar, are aluminum and percent moisture. In order to link the elements more closely with their sources in the diet, mass fractions were subsequently included with the elements in the cluster analysis.

Figure 4.2 shows the dendrogram also calculated using the "complete link" technique for the cluster analysis of elements and food groups. For the most part, there were not great distinctions between clusters. However, there appeared to be two main clusters. The first, from wine to fruits and berries, appeared to be elements and foods

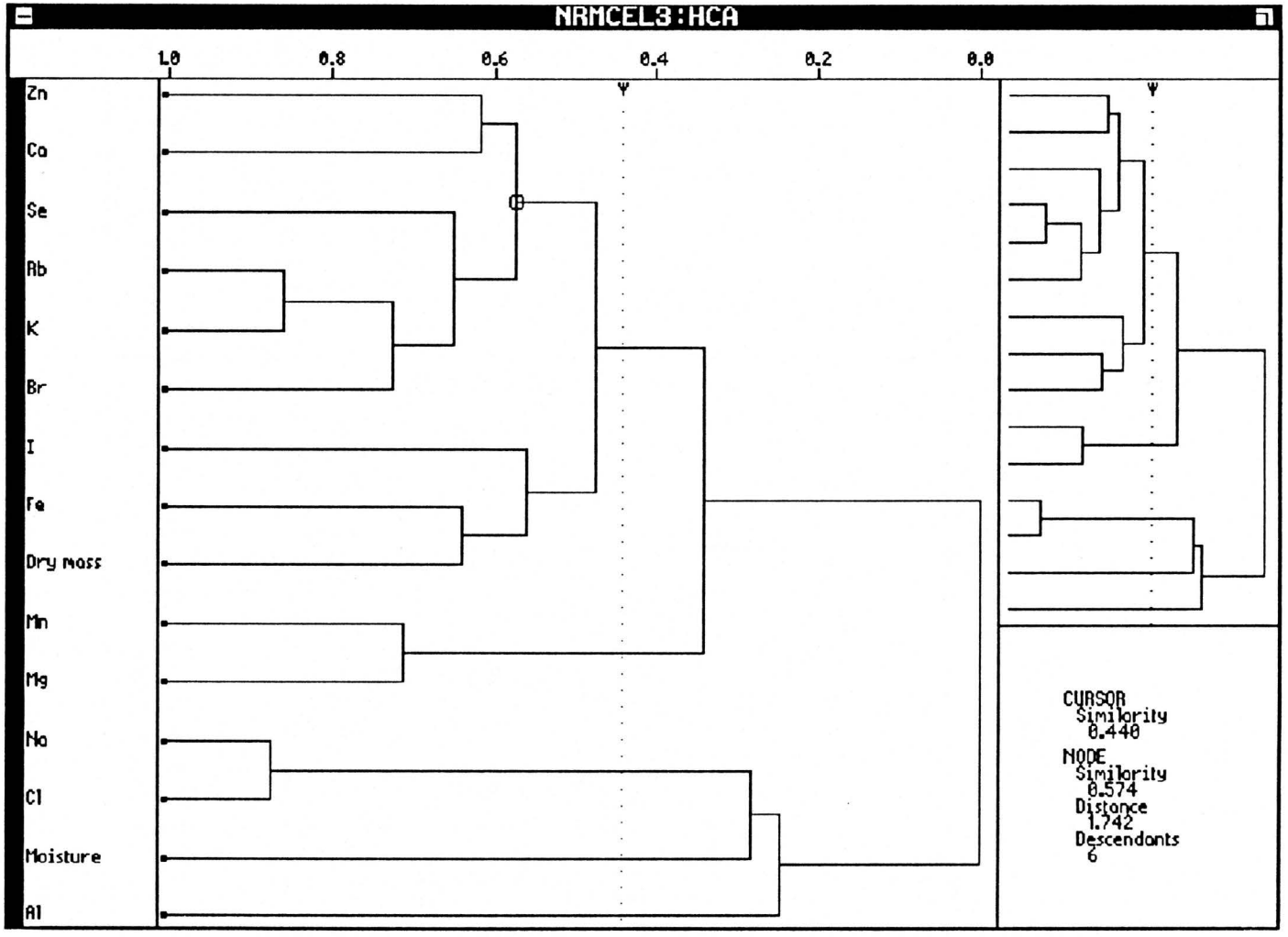


Fig. 4.1. Dendrogram for the cluster analysis of elemental concentrations of duplicate diet composites and food group mass fractions

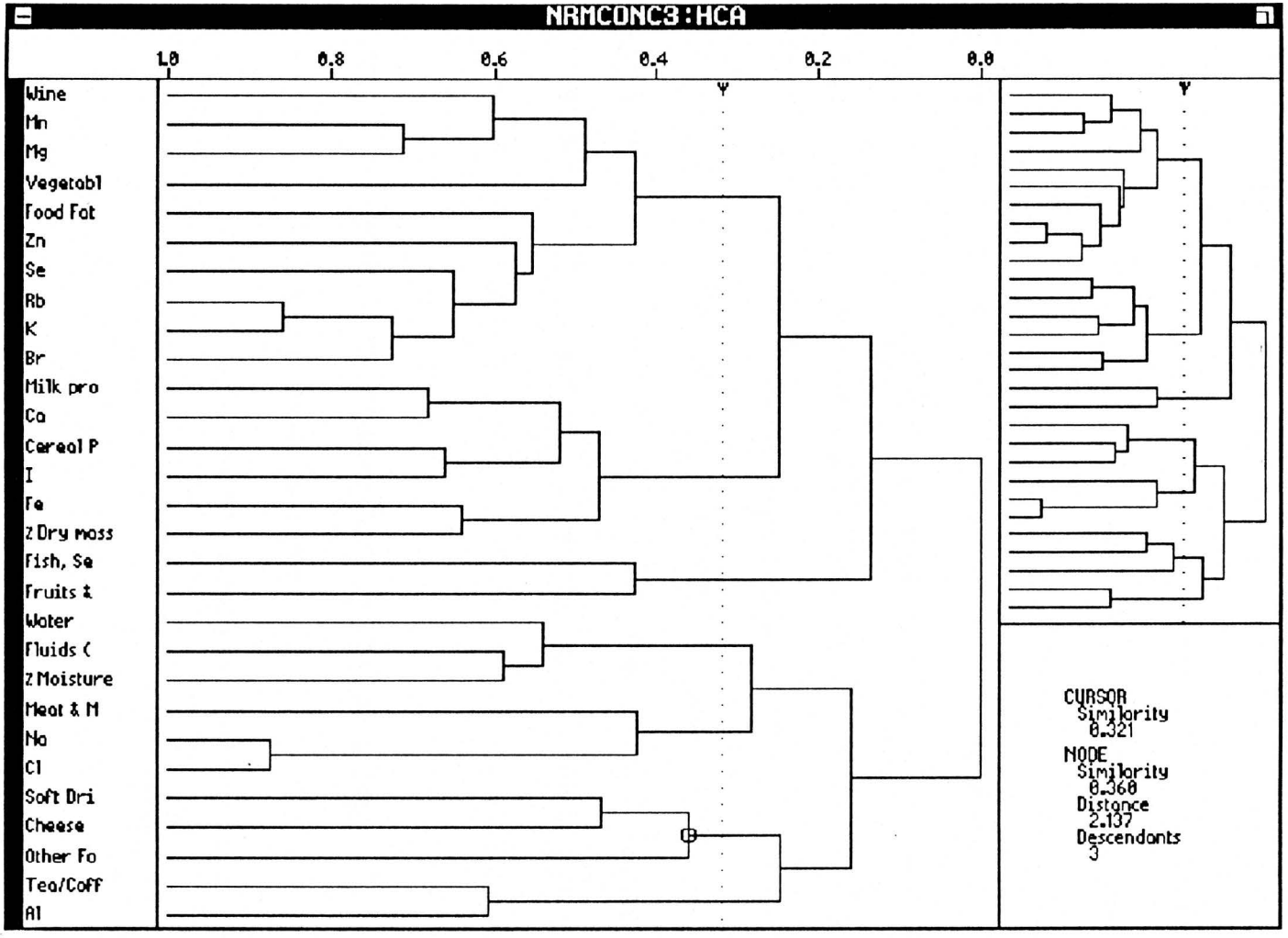


Fig. 4.2.. Dendrogram for the cluster analysis of elemental concentrations of duplicate diet composites and food group mass fractions

mainly associated with dry mass. The second cluster appeared to be primarily liquids, beverages and snack foods. The dry mass cluster appeared to be subdivided into three main subclusters: the clusters were all foods and elements shown in Fig. 4.2 from wine to Br, milk products to I, and fish and seafoods to fruits and berries. Within the first of these, Rb and K had a high degree of similarity. Within the beverages/liquids group, the most distinct links were between sodium and chlorine, and Al and tea/coffee.

These results appeared to indicate that most of the trace elements (Br, Ca, iodine, Fe, Mg, Mg, K, Rb, Se and Zn) were associated with the dry mass (plus milk and wine), including cereals, vegetables and food fats. Sodium and Cl and meat were not linked to particular food groups within the dry mass cluster. Neither fish and seafood nor fruits and berries were associated with mineral intake. Fluids did not appear to be major contributors to the elemental intake of the study subjects. The association of aluminum with tea/coffee was previously demonstrated by the correlation calculations.

4.4 PRINCIPAL COMPONENT ANALYSIS (PCA)

4.4.1 PCA of Elemental Concentrations

As for HCA, PCA of the elements alone was performed before including the food group mass fraction data. The daily intake results are similar to the concentration results, so only concentrations will be discussed.

The variance calculated for each principal component is shown in Fig. 4.3 and Table 4.25. As these results show, the first principal component accounts for 55% of the variance. The first four principal components comprise 88% of the variance.

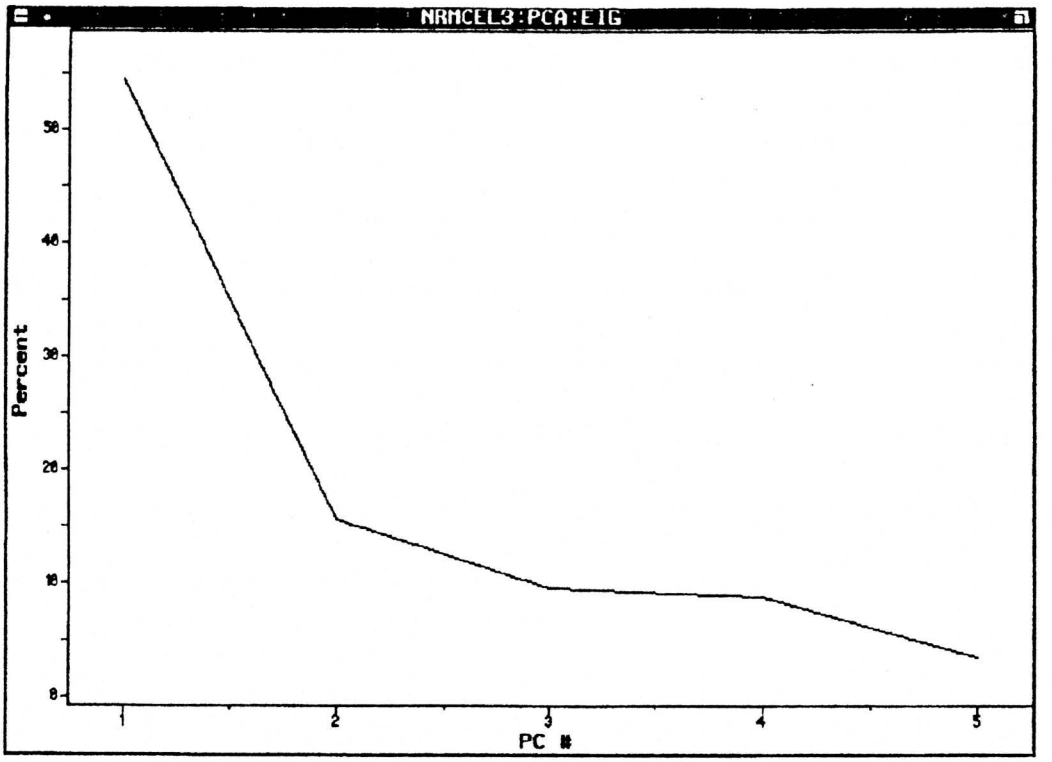


Figure 4.3 Plot of the percent variance calculated for the principal components

Table 4.25. Variance for each of the principal components calculated for elemental concentrations

PC	Variance	Percent	Cumulative
1	106.5	54.6	54.6
2	30.4	15.6	70.2
3	18.7	9.6	79.8
4	17.0	8.74	88.5
5	6.7	3.5	92.0

Figure 4.4 shows a plot of the scores of the first three principal components. Distinct clusters can be seen. Aluminum, Na and Cl, and Mg and Mn are well separated from the other elements. There is a large cluster consisting of Br, percent dry mass, K, Rb, Se and Zn. Calcium and iodine were quite close although it is not entirely clear whether these two elements should be considered separate from the large cluster. The inclusion of Fe with iodine and Ca is somewhat arbitrary and may not be meaningful. In Fig. 4.5, the first two principal components are plotted. The separation between clusters can be seen clearly, except for Ca, iodine and Fe. In order to determine whether the clusters can be associated with particular food groups, the food group mass fractions were included in the data set and PCA performed.

4.4.2 PCA of Elemental Concentrations and Food Group Mass Fractions

The variances calculated for the first principal components are shown in Table 4.26. The first principal component accounts for 41% of the variance. The first five principal components account for only 78.5% of the variance. The data thus does not appear to be highly correlated.

The data plotted against the first three principal components are shown in Fig. 4.6. The elements have been grouped with the foods with which they are most closely associated (except for Na and Cl which are not closely associated with any particular group). Bromine, Fe, K, Rb and Se were most closely associated with percent dry mass, cereals and fats. Magnesium and Mn were most closely associated with wine and vegetables (the two diets containing alcohol also contained large proportions of

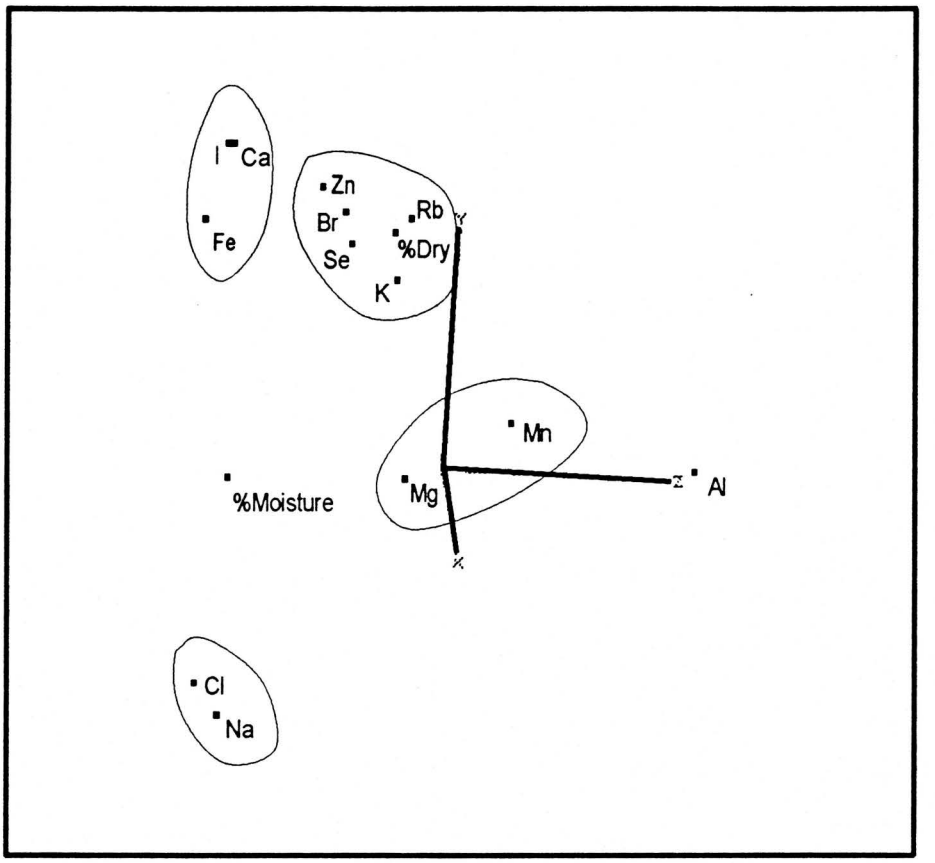


Fig. 4.4. Plot of the principal component scores for the first three principal components

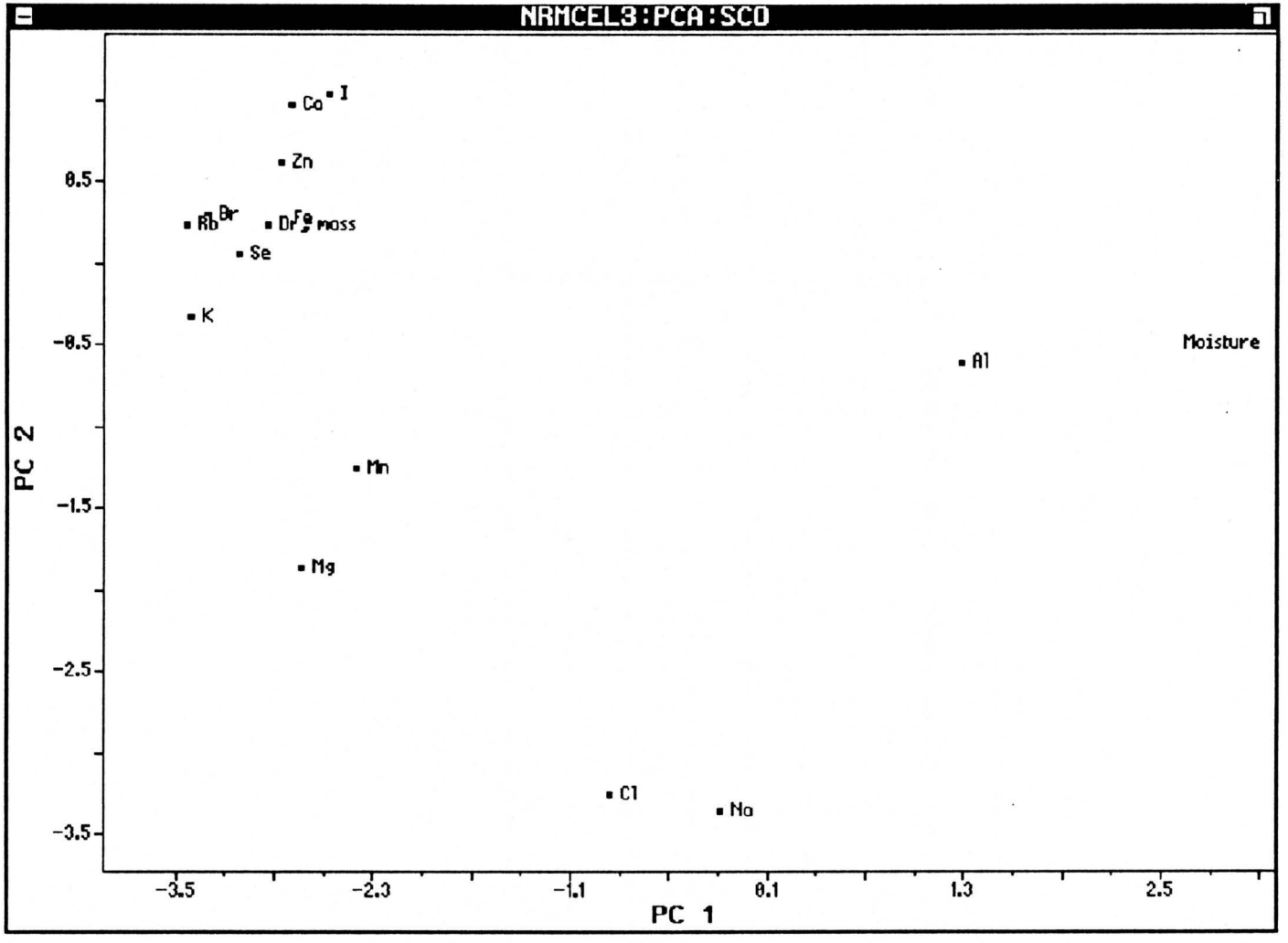


Fig. 4.5. Scores plot of the first and second principal components

Table 4.26. Variance for each of the principal components calculated for elemental concentrations

PC	Variance	Percent	Cumulative
1	155.5	41.2	41.2
2	46.7	12.4	53.3
3	38.5	10.2	63.8
4	28.4	7.5	71.4
5	26.7	7.1	78.5
6	19.4	5.1	83.6
7	16.5	4.4	88.0

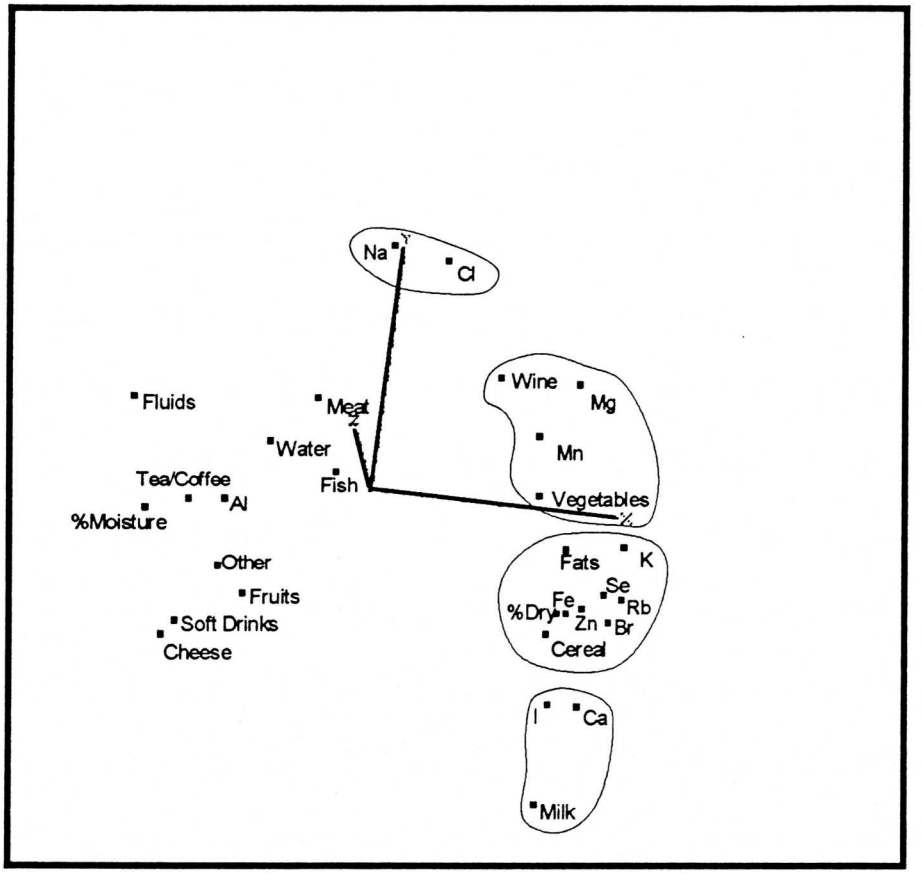


Fig. 4.6 Plot of the principal component scores for the first three principal components, elemental concentration, percent dry mass, percent moisture, and food group mass fractions.

cereals and vegetables). Calcium and iodine were located between cereals and milk. The association drawn with milk may not be meaningful, and they may just as well belong to the cereals group. Aluminum was relatively closely associated with tea/coffee. Most of the fluids were located to the left of the plot, whereas the dry foods were located on the right. This is shown clearly in Fig. 4.7, a two-dimensional plot of principal component 2 versus principal component 1. The scores for PC 1 are highest for elements associated with vegetables, cereals, milk and food fats, and most negative for fluids, percent moisture, tea/coffee, cheese soft drinks and water. Principal component 1 therefore seems to be a factor relating to fluid versus dry mass intake. The dominating score of PC 2 appears to be Na chloride, with small scores relating to fluid and beverage intake. Figure 4.8 shows the second and third principal components plotted with each other. Principal component 3 has the highest scores for fruits and berries and aluminum, and the most negative scores for water, milk and percent moisture. The third principal component therefore appears to represent competition between beverages. Interestingly, in this plot the beverages are located in the corners, while dry foods are located in the center. This plot therefore shows the competition between beverages. As in the correlation results and HCA, aluminum appears to be most closely associated with tea/coffee and fruits and berries.

The loadings of the first two principal components are shown in Fig. 4.9. The diet composites do not appear to be highly clustered. There may be loose clusters in the bottom left and right of the plot, and a pair at top left. The foods and elements

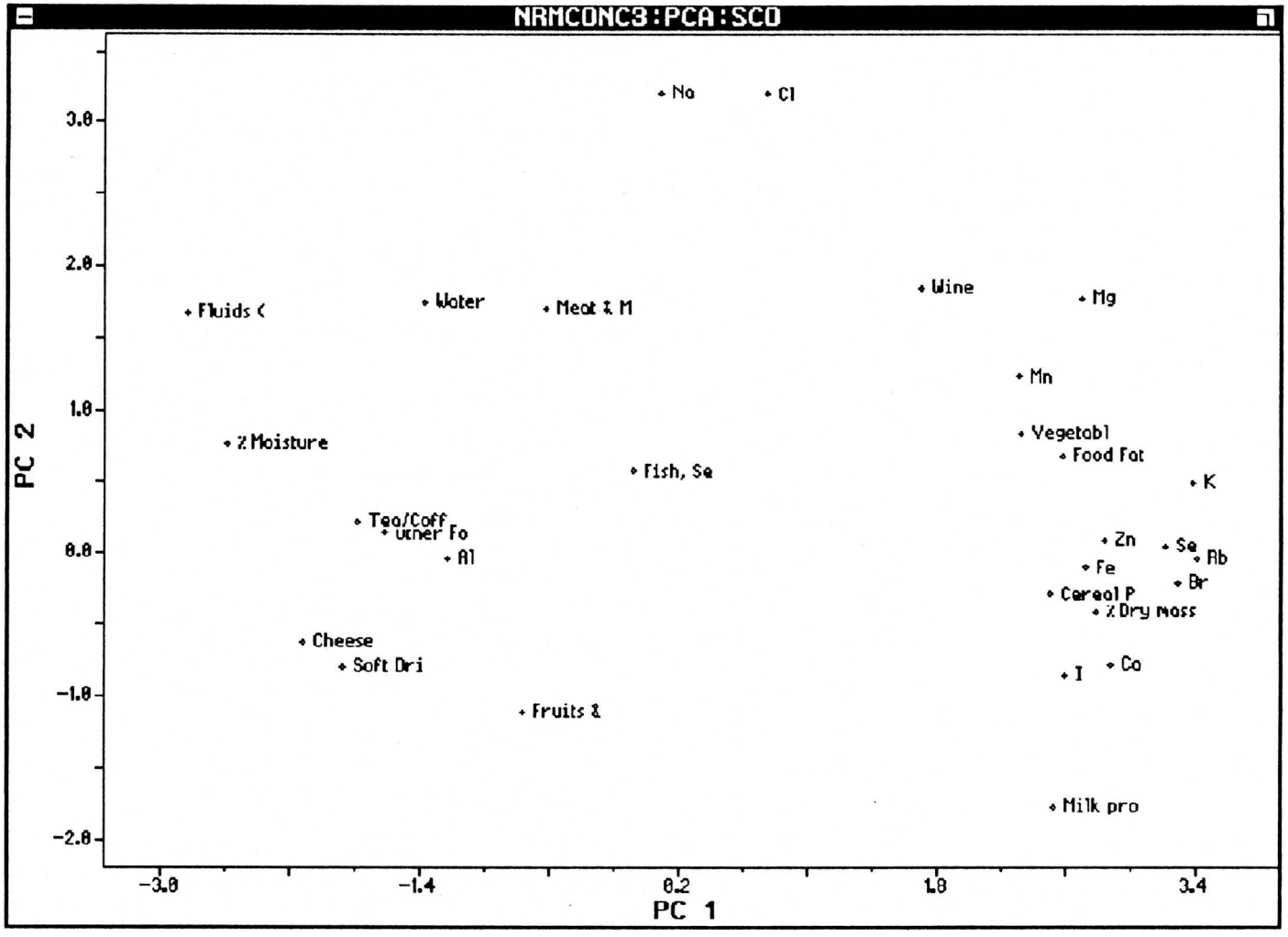


Fig. 4.7 Plot of scores of the first two principal components

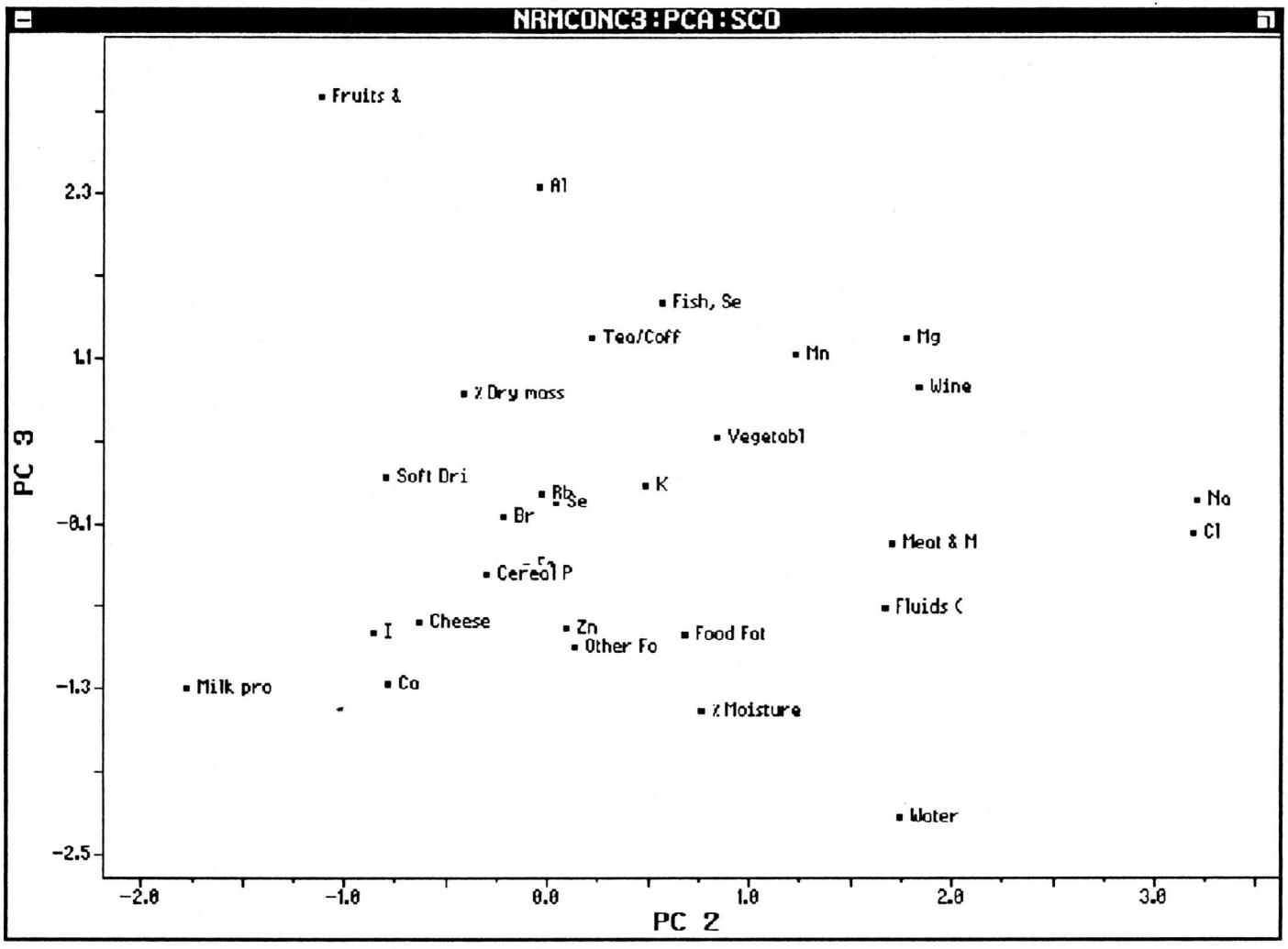


Fig. 4.8 Plot of scores of the second and third principal components .

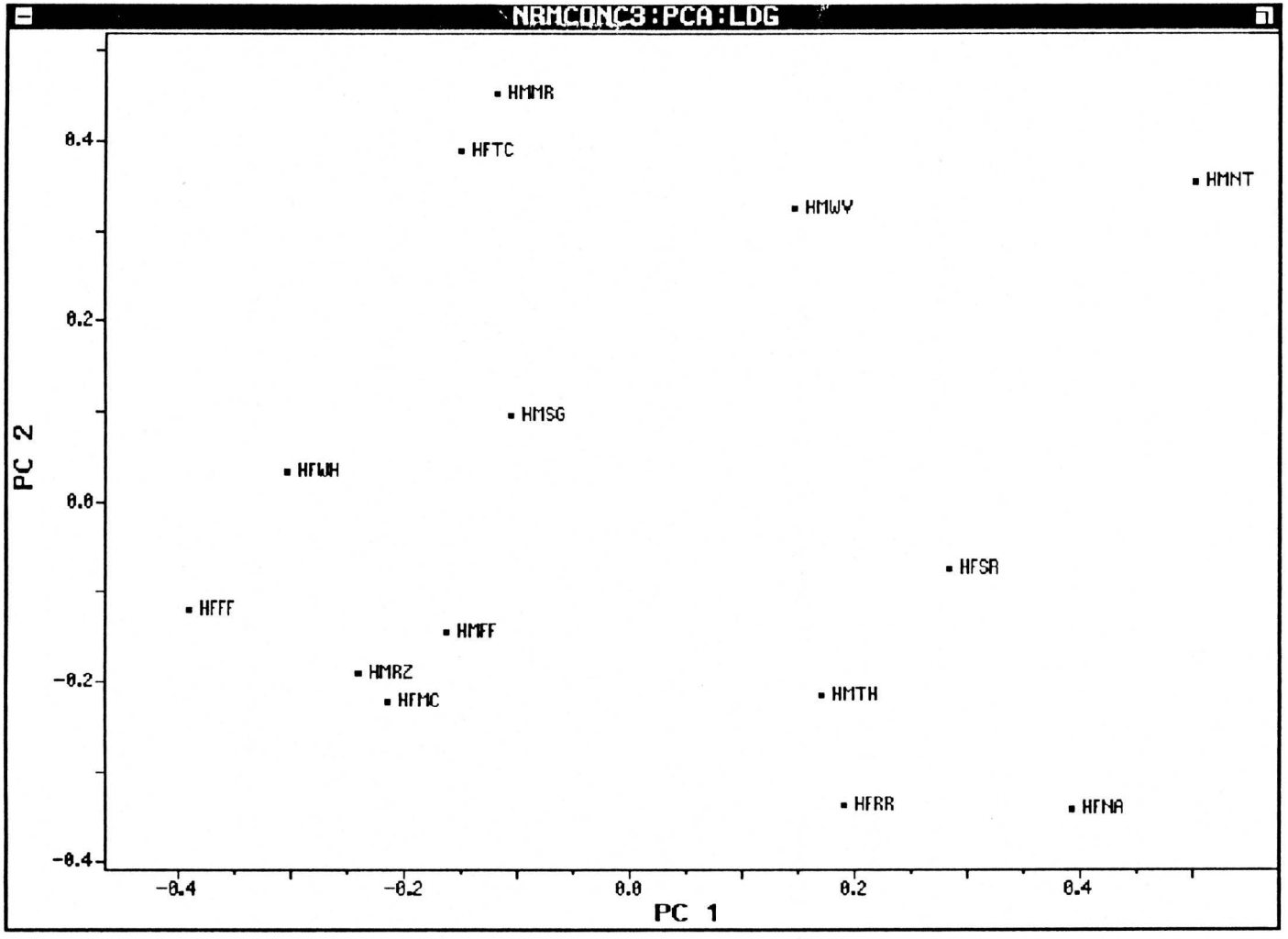


Fig. 4.9. Plot of loadings of principal component 2 versus principal component 1.

most strongly associated with these diets can be estimated by comparing the loadings plot (Fig. 4.9) with the scores plot (Fig. 4.8) and then verifying these results by reference to the actual concentrations and mass fractions in Appendix A. The two diets in the top right of Fig. 4.9, HMWV and HMNT, are the only diets which contained alcoholic beverages and were also high in cereals and vegetables. The diets in the bottom right of Fig. 4.9, HFRR, HFSR, HFNA and HMTH had high mass ratios of cereals, vegetables and particularly milk. Diet composites in the bottom left of the plot, HFFF, HFMC, HFWH, HMFF, HMRZ and HMSG tended to be higher in snack foods like cheese, soft drinks and tea/coffee. The two diets in the top left of the plot, HFTC and HMMR had higher levels of fluids and water in their composites and were highest in Na and Cl.

4.5 EXPLORATORY DATA ANALYSIS: CONCLUSIONS

Exploratory data analysis has proven to be useful in identifying associations between elements and associations between elements and foods sources. Associations which were not observed, or only hinted at were much more clearly revealed. Most of the elements measured to date not surprisingly were associated with the dry mass. Only Al, which was strongly correlated with tea/coffee was associated with fluids. Pennington has reviewed the content of several foods and diets (84), including tea and coffee. Tea leaves and tea powder have quite high levels of Al (67.0-140.0 mg/100g), although the concentration in brewed tea is relatively small (0.02-0.446 mg/100g). Brewed coffee has roughly the same concentration as tea, but Al coffee pots were reported to increase the concentrations significantly.

The elements Br, Fe, K, Rb, Se and Zn were most closely associated with the cereals/nuts group. Health Canada has determined various elements in "representative diets" (71, 74, 77, 246, 259). In these studies, they reported the contribution of various food groups to the elemental intake. Meranger and Smith (74), and Kirkpatrick and Coffin (77) reported that most of the Fe and Zn were contributed by the meat, fish and poultry group. Cereals and potatoes were also significant contributors. A significant portion of the zinc content was from the milk group, while the sugars group contributed significantly to the Fe content. In a later study by Shah *et al.*, cereals were the major contributor of Fe, followed by meat, and meat was the principal source of Zn. Shah *et al.* (71), found K to have a fairly wide distribution among the food groups, with 23% contributed by milk, 16% from potatoes, 15% from fruit, 14% from cereals, 10% from cereals, and 9% from vegetables. In contrast to the work of Shah *et al.*, the statistical analysis of the NAA data of this thesis suggests that K was quite strongly associated with the cereals food group.

In this thesis, Mg and Mn were most closely associated with vegetables. In the Kirkpatrick and Coffin, and Meranger *et al.* studies, Mg was not determined. The principal source of Mn was found to be cereals. Vegetables contributed only 10% of the DI of the Shah *et al.* study (259), the major contributors being dairy products and cereals.

The data analysis suggested that Ca and I could be associated with cereals or milk. Shah *et al.* (259) found that 72% of the Ca intake of the representative diet was from milk. Only 9% of the content was from cereals. In the work of Fischer and Giroux

(246), table salt was the major source (65%) of I, followed by dairy products (23%). Cereals were a relatively minor contributor, *viz.* 8%.

In this thesis, Na and Cl were not strongly associated with any particular food group, suggesting a wide distribution through the diet. This agrees with the work of Shah *et al.* (71), who found that about 70% of the sodium content derived from three major food groups, namely milk, meat products, and cereals.

Examination of the correlation coefficients suggested differences between the men's and women's diets. The women appeared to have a higher correlation of total mass with intake of fruits and berries, meat and meat products and tea/coffee whereas the men had a higher correlation of total diet mass with cereals and vegetables. However, when mass fractions were considered, cereals were strongly correlated with the women's percent dry mass whereas there was no such correlation for the men. The men had a higher correlation of the mass fraction of meats and meat products with percent moisture. The loadings plot calculated from PCA did not reveal clear differences in dietary habits or dietary intake

between the men and women, although it did aid differentiation of the diet composites according to food group and trace element content.

5. CONCLUSIONS AND RECOMMENDATIONS

During the course of this thesis, INAA methods were developed for the determination of thirteen elements, namely Al, Br, Ca, Cl, Fe, I, K, Mg, Mn, Na, Rb, Se and Zn in diet samples. Fifteen duplicate diet samples were prepared and subjected to analysis by these INAA methods. Quality assurance/quality control procedures included the analysis of replicates, analysis of reference materials, preparation of QA charts, and the development of a laboratory information management system (LIMS) to aid data management, archiving and traceability. A dialysis method was used to determine the fractions of the diets which were insoluble or bound to macromolecules, and to reduce the levels of interfering elements Na and Cl, allowing previously undetected elements to be measured. Finally, exploratory data analysis was performed on the concentrations and daily intakes (DIs) determined by INAA. It should be noted here that the number of diet samples collected in this study was fairly small for several reasons such as the difficulty in selecting volunteers who met our criteria, in securing their cooperation for collecting the diets, and for the lack of funds for more extensive studies.

Five INAA methods, including INAA using ^{75}Se, as well as INAA, CINAA, PCINAA, and INAA with anti-coincidence gamma-ray spectrometry using ^{77m}Se, were evaluated for their suitability to the analysis of diet composites for Se. This was done by comparing sensitivities and detection limits, and by evaluating precision and accuracy of analysis of reference materials (RMs) and Standard Reference Materials (SRMs). Because of its superior sensitivity, detection limits, precision and accuracy, the

PCINAA method was found to be the best for the analysis of diet composites. The duplicate diets were analyzed by INAA and PCINAA using ^{77m}Se. The median Se concentrations and DIs determined in the duplicate diets were 260 ppb and 130 μg d^{-1}, respectively. All diets met or exceeded the Recommended Nutrient Intake (RNI) for Se. The residue from the dialysis of the duplicate diets was analyzed for Se. The percent of Se retained by the dialysis tubing varied between 8 and 107%, the median being 73%. Five of the diet samples subject to the enzymolysis procedure were analyzed for Se, the percent potentially available being 9-64% for the freeze-dried diets, and 5- 79% for the wet slurry.

Methods evaluated for iodine analysis included EINAA using conventional and anti-coincidence (AC) gamma-ray spectrometry, and EINAA with boron carbide shielding in combination with AC spectrometry. The best method in terms of sensitivity and signal-to-background ratio was the EINAA-AC spectrometry combination, although further optimization of a method using extra boron shielding may be worth pursuing. The median iodine content and DI of the duplicate diets were 590 ppb and 280 μg d^{-1}, respectively. This DI is much lower than the Canadian DI estimated by Fischer and Giroux (246) of 1 046 μg d^{-1}.

Magnesium was determined by INAA. The relatively low Mg levels and insensitivity of the 1014.4-keV gamma-ray of ^{27}Mg led to somewhat decreased precision. The median Mg concentration and DI determined in the duplicate diets were 760 ppm and 350 mg d^{-1}. Calculations based on food composition tables (FCT) showed the general

trends, but significantly underestimated the DI. The percent Mg retained in the dialyzed diet samples varied from 20-70%, the median being 37%.

Although the 1810.7 keV gamma-ray of ^{56}Mn was interference-free under the conditions employed, its sensitivity was considered to be too low for the analysis of the duplicate diets. Therefore, the 846.8-keV gamma-ray of ^{56}Mn was used, after allowing sufficient time for the decay of the interfering 843.8-keV gamma-ray of ^{27}Mg. Precision and accuracy of INAA was considered to be very good. The median Mn concentration and DI were 8.4 ppm and 3.6 mg d^{-1}, respectively. Two DI values were below and one was above the SADDI of 2.0-5.0 mg d^{-1}. From Mn analysis of the dialyzed diet samples, the percent retained by the dialysis tubing was found to be 28-81%, with a median of 56%. The percent retained Mn was weakly correlated with the proportion of vegetables. The uncertainty in percent potentially available, calculated from Mn determined in the dialysis residue of the enzymolyzed sample, was relatively high. Percent Mn potentially available varied from near 0 to 59%, the median being 32% for freeze-dried diet, and 29% for wet slurry.

Analysis for Al involved INAA and an INAA-EINAA method used to correct for interference from the reaction $^{31}P(n,\alpha)^{28}Al$. Significant and variable levels of Al in the irradiation vials used led to decreased precision of analysis. Therefore the corrections for ^{31}P were too low to be significant. Values of 9.4 ppm and 4.8 mg d^{-1} were obtained for the median concentration and DI of Al, respectively, in the diets. The highest DI reported was 32 mg d^{-1}. There do not appear to be any previous Canadian values for Al intake reported in the literature. The percent Al dialyzed in the diet samples

were very low. Due to high blank values, Al in enzymolyzed samples were reported only for the diets with the highest Al contents. Potentially available Al in these diets varied from 7-89%. Future work on Al could involve the development of a method with a lower blank, in order to improve the precision of analysis.

The median concentration and DI of calcium, determined by INAA, were 2060 ppm and 1080 mg d^{-1}, respectively. Calcium DIs of four of the diets were below the RNI, although the overall median was larger than most other reported Canadian values. Calcium DIs calculated from FCT agreed reasonably well with those from INAA. The percentage retained by the dialysis tubing was 20-70%, the median being 46%. The uncertainties in calculating percent potentially available from the enzymolysis experiments only allowed a qualitative interpretation that the percent available was very low.

Sodium, K and Cl were analyzed by INAA. The concentrations and DIs for Na were 7180 ppm and 3230 mg d^{-1}, for K were 8500 ppm and 3800 mg d^{-1}, and for Cl were 9780 ppm and 4750 mg d^{-1}. There is little Canadian data the DI of these elements in the literature. Calculations based on FCT agreed well with DIs determined using INAA. Dialysis of the duplicate diets reduced the content of these elements by >95%.

Low count rates and high detection limits during INAA for Fe led to poor precision as assessed by the relative standard deviations (RSDs) of replicate calculations. The median Fe concentration and DI were 52 ppm and 21 mg d^{-1}. The FCT-based calculations of the Fe intakes agreed well with DIs determined from INAA. The percent potentially available was calculated by difference from the Fe concentrations determined

in undigested, and digested diets. Because the differences were small, and the RSD of the concentrations were large, the uncertainties in the percent potentially available were quite large. The calculated availability for all diets, except HMFF, were low. Future work could include the development of a method for Fe analysis with improved precision. Longer counting times could be used, but not on a routine basis, so a preconcentration method may be required.

The overall median Zn concentration and DI, determined by INAA, were 26 ppm and 11 mg d^{-1}, respectively. Intakes calculated using FCT significantly underestimated the Zn intakes. As for Fe, the calculated percent available was low and the uncertainties in the calculated values were high.

The overall median concentrations and DIs determined by INAA were 11.0 ppm and 5.07 mg d^{-1} for Br, and 6.4 ppm and 3.0 mg d^{-1} for Rb. There is little data in the literature on DI of Rb and Br.

Three elements were detected in the dialyzed diets, which were not previously detected before dialysis. One of these, Cu, was most likely a result of contamination presumably during the washing and freeze-drying steps. Titanium was detected in some diets, although the content was possibly from the Ti blade of the blender used for homogenization of the diets. Vanadium was detected in all of the dialyzed diets. A potential interference was contamination from the ink of the markers used to identify the sample vials, although this was not considered to be a significant interference. The V concentrations in dialyzed NIST 1515 Apple Leaves, and 1577b Bovine Liver were 51 and 67%, respectively, of their certified values. For the diets, the median V concentration

and DI were 0.065 ppm, and 0.024 mg d^{-1}, respectively. There appear to be no previous values of V intake by Canadians reported in the literature. It would be useful to determine the V and Ti concentration in the original undialyzed diets in order to calculate the percent dialyzable. The development of a preconcentration method for this purpose could be the subject of future work.

A few other elements were detected in some diets, including long-lived nuclides of Au, Ba, Co, Cr, Cs, Sb, and Sc. Accuracy and precision of determination of these elements were not considered to be satisfactory. Cobalt was also subject to interference from Co contamination contributed by the markers used to identify the sample vials. Some of these elements are of nutritional interest, particularly Cr. Future work could involve the determination of these through the use of longer counting times than were used during the course of this thesis work. There are other elements of nutritional or toxicological interest, such as As, Cd, Co, Cu, and Ni which could not be analyzed by the instrumental methods. The determination of these elements through preconcentration or radiochemical methods would be a valuable addition to the data reported in this thesis.

Quality assessment by the analysis of RMs and SRMs, and replicate analysis of selected duplicate diets, indicated that the accuracy and precision of INAA was good to excellent, with the exception of Fe and Al, where low ^{59}Fe count rates and relatively high detection limits, and high Al blanks led to poor precision of analysis. Future work could involve the development and application to the diets of methods using lower Al blanks, to improve the precision of analysis. Agreement between measured and

certified values of RMs and SRMs was generally good. A notable exception is Se in NIST SRM 1548 Total Diet, where the measured value was higher than the certified value, which has an unusually narrow confidence interval. This discrepancy could be the subject of future work. Detection limits (ppm) were calculated to be 0.026-0.074 for Se, 0.090-0.370 for I, 50-550 for Mg, 0.09-0.54 for Mn, 0.31-4.5 for Al, 33-310 for Ca, 10-120 for Na, 240-1160 for K, 18-120 for Cl, 18-54 for Fe, 3.3-4.9 for Zn, 0.096-0.12 for Br, and 0.36-0.44 for Rb. The QA charts indicated that the INAA methods were in statistical control. The LIMS database was successfully developed and proved to be invaluable in quality management and control.

Exploratory data analysis of the NAA results yielded some interesting relationships. The elements Br, Fe, K, Rb, Se, and Zn were associated with the cereals food group, which appears to be the major source of these elements. Calcium and I were associated with the cereals and also with milk. This may be due to the consumption of milk with cereal products, for example in the case of ready-to-eat breakfast cereals. Magnesium and Mn were associated partly with cereals, but mainly with the vegetables food group. Sodium and Cl were not associated with any particular food group, but were mutually correlated, suggesting NaCl to be their major source in the diet. Aluminum was associated strongly with the tea/coffee food group. The major factors distinguishing the diets, as determined from the loadings plot of PCA, were cereals and vegetables, milk, snack foods, and fluids and water.

The dialysis experiments demonstrated that the fraction of a diet sample insoluble or bound to macromolecules could be relatively easily determined. In future

work, the effect of various inhibitors and enhancers on dialyzability of various elements could be studied using this method.

In this thesis, only the residues of the enzymolyzed diets were analyzed, requiring the percent potentially available to be calculated by difference. The uncertainties of these calculated values were very high, highlighting the need to analyze the dialysates directly. Future work could involve the development of chemical separation and preconcentration techniques to achieve this objective.

APPENDIX 1

CONTENTS OF DUPLICATE DIET COMPOSITES AND FORMS USED BY PARTICIPANTS

A1.1 INTRODUCTION

This appendix lists the food items contained in each duplicate diet composite. Although the collection of the food items, with the exception of the trial diet HMFS collected by this author, was performed by other members of Prof. Chatt's group (27), the contents of the composites may be relevant to the reader and therefore are included here. Also, the forms used in the participant selection process are found in section A1.2 of this appendix.

The food items and amounts have been copied from the unpublished food records (27). Based on an IAEA system, the food items were classified by this author into ten categories, namely cereal products/nuts, vegetables, fruits and berries, meat and meat products, fish, seafoods and products, milk products (liquids), cheese, food fats, fluids (excluding fruit and vegetable juices) and other foods. The "other foods" category included items which could not be put in any of the above food groups, and is primarily comprised of candies and sugar added to food items such as tea and coffee. Fluids were subdivided into four categories: tea/coffee, soft drinks, water, and wine. Wine included all alcoholic drinks such as beer, sherry, *etc.*

There are obvious problems with categorization into such broad groups. A given food may contain several items from different food groups. For example, pizza

is composed of crust (cereals), tomato sauce (vegetables), and cheese, and could contain meats and other vegetables. In this case, it would be grouped under cereals because the crust comprises the main component, although it is recognized that the remaining ingredients of the pizza could fall into other categories. Furthermore, some foods like toast and butter were returned to the lab in combination. Although all foods were weighed, it was not always possible to weigh components separately, so that the masses of each component in a combined food item were estimated where possible. This problem occurred frequently with the food fats (since butter or margarine were not usually separated from the food items they were served with, such as toast, potatoes, other vegetables, *etc.*) and the other foods category (since tea and coffee additives such as sugar were included in this category). Because of their small quantities, spices including salt and pepper were generally not weighed separately either. Despite these limitations, the categories do help to give some structure and aid understanding the diets.

The contents of the fifteen duplicate diets analyzed in this work are given in Tables A1.1 through A1.15. Following these, the author has composed Tables A1.16 (masses of foods by category) and Table A1.17 (mass fractions of food groups, *i.e.* wet mass of individual food groups divided by the total wet mass of the composite).

Table A.1. A list of contents of the HMFS duplicate diet.

(1) Cereal Products & Nuts
1 tbsp. Squirrel brand peanut butter (in sandwiches)

Wheat Products
2 Slices Grainhouse brand Oatmeal Brown bread (in sandwiches)
1 Ben's brand honey bran muffin with raisins
Macaroni (in casserole)
2 slices pizza crust (in pizza)

(2) Vegetables
Sobey's brand frozen peas (in casserole)
Four Star brand canned mushrooms (in casserole)
1 bag (55g) Hostess brand All-Dressed flavor potato chips
Tomato sauce (in pizza)

(3) Fruits & Berries
1 tsp. homemade raspberry jam (in sandwich)
1 cup Five Alive brand fruit drink
1/4 cup strawberries

Canned Products

(4) Meat & Meat Products
browned ground beef (in casserole)
ground beef (in pizza)

(5) Fish, Seafoods & Products

(6) Milk Products (as liquids)
1 cup milk
1 1/2 cup Dairy Queen chocolate milk shake

(7) Cheese
Mozzarella cheese (in pizza)

(8) Food Fats

(9) Fluids (excl. fruit & vegetable juices)

Tea or Coffee

Soft Drinks
1 can Coca-Cola
Mineral Water
1 1/2 cups tap water
Wine

(10) Other Foods
1 tsp. Smeltzer's brand honey (in sandwich)

Table A.2. A list of contents of the HFFF duplicate diet.

(1) Cereal Products & Nuts
2 cups Kellogg's brand corn flakes
Wheat Products
1 Honey Maid brand graham wafer
2 slices Ben's brand 100% whole wheat bread (in sandwich)

(2) Vegetables
1 small fresh tomato (in sandwich)

(3) Fruits & Berries
1/2 small fresh cantaloupe
Canned Products
2 cups Sobey's brand apple juice

(4) Meat & Meat Products
1 slice IGA brand smoked ham (in sandwich)
1 large soft-boiled egg

(5) Fish, Seafoods & Products

(6) Milk Products (as liquids)
1/2 cup Farmer's brand skim milk
142 g Laura Secord brand "Light Touch" chocolate pudding
175 g Crescent brand strawberry yogurt

(7) Cheese
1 tsp Kraft grated parmesan cheese
1 slice Kraft brand processed cheese (in sandwich)
2":x1" block M°Cain brand old cheddar cheese

(8) Food Fats
20 mL Kraft brand "Miracle Whip" light salad dressing (in sandwich)

(9) Fluids (excl. fruit & vegetable juices)
1 cup Lipton "Cup-a-soup" chicken noodle supreme
Tea or Coffee
1 1/2 cup Lipton tangy orange herbal tea
Soft Drinks
355 mL Schweppes brand diet ginger ale
Mineral Water
1 cup tap water
Wine

(10) Other Foods
14 lozenges Vick's brand cherry cough drops

Table A.3. A list of contents of the HFMC duplicate diet.

(1) Cereal Products & Nuts
2 homemade oatcakes

Wheat Products
1 Stone Hearth brand bagel, with sesame seeds
2 Peak Frean's brand chocolate-coated digestive biscuits
6 Christie brand Premium Plus soda crackers - salted
1 Tbsp. Shake and Bake brand Italian coating (with Pork Chop)

(2) Vegetables
250 mL Popcorn popped in corn oil with salt

Potatoes
2 Baked potatoes (baked with onions, onions removed after cooking)

Canned Products

(3) Fruits & Berries
250 mL Tang brand grape juice drink

Canned Products

(4) Meat & Meat Products
1 Pork Chop

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
125 mL Milk

(7) Cheese
2 tbsp Cream Cheese

(8) Food Fats
2 tsp. Imperial brand margarine

(9) Fluids (excl. fruit & vegetable juices)

Tea or Coffee
1 cup Red Rose brand tea
1 cup perked coffee

Soft Drinks
1 glass pepsi cola

Mineral Water
1 glass water

Wine

(10) Other Foods
2 tsp. Ross brand coffee whitener
2 tsp. white sugar
6 pieces Nib-its brand red licorice

Table A.4. A list of contents of the HFNA duplicate diet.

(1) Cereal Products & Nuts
1 cup Kellogg's brand raisin brand
Wheat Products
4 slices French pastry enriched white bread
1 cup Lancia brand boiled spaghetti
1 piece homemade chocolate-zucchinni

(2) Vegetables
1 small homegrown tomato
1 cup homemade spaghetti sauce

Potatoes

Canned Products

(3) Fruits & Berries
3/4 cup McCain's brand frozen reconstituted orange juice

Canned Products

(4) Meat & Meat Products

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
4 cups Farmer's brand 2% milk

(7) Cheese

(8) Food Fats
4 tbsp. Hellman's brand mayonnaise
2 tbsp. Fleischmann's brand margarine

(9) Fluids (excl. fruit & vegetable juices)

Tea or Coffee

Soft Drinks

Mineral Water

Wine

(10) Other Foods
2 tbsp. Lantic brand white granulated sugar
salt and pepper

Table A.5. A list of contents of the HFRR duplicate diet.

(1) Cereal Products & Nuts
1 1/2 cups Quaker brand corn bran
1 granola bar

Wheat Products
2 slices honey brown bread (in sandwich)
1 whole wheat donut

(2) Vegetables
1/6 cup frozen peas (in sandwich)
1 green green onion (in sandwich)
1 baby carrot (in sandwich)
1 small stalk celery (in sandwich)
1 cob corn
3/4 cup steamed broccoli w/pepper
1/2 microwaved onion

Potatoes
1 medium baked potato

Canned Products

(3) Fruits & Berries
1 1/2 cups Niagara brand frozen reconstituted orange juice
1/2 banana
1/2 nectarine
1 plum
1 orange
1/6 Granny Smith apple (in sandwich)

Canned Products

(4) Meat & Meat Products
80 g microwaved inside round beef steak

Canned Products

(5) Fish, Seafoods & Products

Canned Products
1/6 can tuna (in sandwich)

(6) Milk Products (as liquids)
2 1/2 cup milk

(7) Cheese
2 tbsp. Kraft brand grated parmesan cheese

(8) Food Fats
1/12 cup Kraft brand Miracle Whip light salad dressing (in sandwich)
2 tsp. Fleishmann's brand light margarine

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee

Soft Drinks

Mineral Water

Wine

(10) Other Foods
1/12 tsp. curry powder (in sandwich)

Table A.6. A list of contents of the HFSR duplicate diet.

(1) Cereal Products & Nuts

Wheat Products
3/4 cup Cream of Wheat cereal w/milk, sugar & unsalted butter
1 slice Grainhouse Mills brand oatmeal brown bread w/butter
2 slices Grainhouse Mills brand oatmeal brown bread (in sandwich)
3 Nabisco brand arrowroot cookies
1 Hamburger roll & bread crumbs (in hamburger)

(2) Vegetables
6 slices lightly salted carrot
onions (in hamburger)
garlic (in hamburger)
2 slices tomato
1 stalk broccoli

Potatoes
30-50 French-fried potato slices, fried in Crisco vegetable oil

Canned Products

(3) Fruits & Berries
1/2 cup Ocean Spray brand cranberry juice

Canned Products

(4) Meat & Meat Products
3 slices pastrami (in sandwich)
2 patties lean ground beef (in hamburger)
egg (in hamburger)

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
3 cups & 2 tbsp. Farmer's brand 2% milk

(7) Cheese

(8) Food Fats
butter (in hamburger)
1 tbsp. butter (with broccoli)

(9) Fluids (excluding fruit & vegetable juices)
Worcestershire sauce, tabasco sauce (in hamburger)

Tea or Coffee
1 cup Tetley brand tea
1 cup Red Rose brand decaffeinated tea

Soft Drinks

Mineral Water

Wine

(10) Other Foods
1 tsp. Dijon mustard (in sandwich)
tarragon, salt, pepper (in hamburger)
Mustard & barbecue sauce (in hamburger)
1 1/2 tbsp. brown sugar

Table A.7. A list of contents of the HFTC duplicate diet.

(1) Cereal Products & Nuts
1 tbsp. Kraft brand crunchy peanut butter
4 oz. Libby's brand brown beans with pork

Wheat Products
1 slice Ben's brand 60% whole wheat bread
2 slices Ben's brand 60% whole wheat toasted bread
1/2 jelly-filled donut
1 piece"snowball" dessert containing coconut, rice Krispies, walnuts, peanut butter, icing sugar, butter, margarine & water

(2) Vegetables
2 tbsp. Heinz brand ketchup
1 1/2 tbsp. homemade mustard pickles

Potatoes
1 1/2 small potatoes

Canned Products
5 oz. Graves brand canned peas & carrots

(3) Fruits & Berries
1 small apple

Canned Products

(4) Meat & Meat Products
2 Sunrise brand boiled weiners

Canned Products

(5) Fish, Seafoods & Products
2 1/2 baked fish sticks w / salt & pepper

Canned Products

(6) Milk Products (as liquids)
1/2 cup & 1 tbsp. whole milk

(7) Cheese

(8) Food Fats
3 tbsp. Farmer's brand 20/80 margarine

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee
2 cups Melitta brand coffee

Soft Drinks

Mineral Water
4 cups tap water

Wine

(10) Other Foods
1 tsp. granulated sugar
1 small Laura Secord brand chocolate
1 tbsp. Schwartz brand prepared mustard

Table A.8. A list of contents of the HFWH duplicate diet.

(1) Cereal Products & Nuts

Wheat Products
1 1/2 walnut crunch donut
2 slices whole wheat bread (in sandwich)

(2) Vegetables
lettuce (in sandwich)
parsley, onion, celery (in omelet)
4 tbsp. ketchup

Potatoes

Canned Products

(3) Fruits & Berries
300 mL Everfresh brand grapefruit juice
1 apple
2 cups Oceanspray brand cranapple juice

Canned Products

(4) Meat & Meat Products
ham (in sandwich)
3 eggs (in omelet)

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
6 tsp. evaporated milk
1 tsp. milk

(7) Cheese
mozzarella cheese (in sandwich)

(8) Food Fats
1 tsp. butter (in sandwich) butter (in omelette)

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee
2 cups Melita brand coffee
1 cup Maxwell House brand coffee
1 1/2 cups Tetley brand tea

Soft Drinks

Mineral Water

Wine

Other Foods
mustard, butter, salt & pepper (in sandwich)
3 tsp. sugar (in tea)

Table A.9. A list of contents of the HMFF duplicate diet.

(1) Cereal Products & Nuts

Wheat Products
1 bowl Kellog's brand corn flakes
2 slices Cabin brand white toasted bread
4 Christie brand premium plus unsalted crackers
1 large molasses cookie

(2) Vegetables
1/2 stalk celery (in beef stew)
1 oz. carrots (in beef stew)
1/7 green pepper (in beef stew)
1/14 Spanish onion (in beef stew)
1 tomato
1/4 cucumber
1 carrot

Potatoes
60 g potatoes (in beef stew)

Canned Products

(3) Fruits & Berries
2 tsp. Sultana seedless raisins
1/2 cup Niagara brand reconstituted orange juice
10 mL President's Choice brand grape jelly
1 nectarine

Canned Products

(4) Meat & Meat Products
1 kg round steak (in beef stew)
350 g pork chop, fried in Mazola brand corn oil

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
1 1/2 cup Baxter's brand 2% milk

(7) Cheese
4 pieces Kraft brand mild cheddar

(8) Food Fats
1 tsp. Parkay brand margarine

(9) Fluids (excluding fruit & vegetable juices)
3 mL Worcestershire sauce (in beef stew)
5 mL Worcestershire sauce (on pork chip)

Tea or Coffee

Soft Drinks
355 mL Orange Crush brand orange soda w / 10% real juice

Mineral Water
50 mL water (in beef stew)
4 cups tap water

Wine
2 mL Canadian sherry (in beef stew)

(10) Other Foods
peppercorn, corn starch (in beef stew)

Table A.10. A list of contents of the HMMR duplicate diet.

(1) Cereal Products & Nuts
2 cups Kellogg's brand corn flakes
Wheat Products
2 slices brown bread (in sandwich)
1 slice white bread

(2) Vegetables
1 leaf of lettuce (in sandwich)
Potatoes
40g O'Ryan's brand sour cream & onion potato chips
2 tbsp. Cavendish brand frozen hash browns fried in corn oil
Canned Products

(3) Fruits & Berries

Canned Products

(4) Meat & Meat Products
1 slice ham (in sandwich)
1 medium egg, fried
2 slices Schneider's brand cooked ham
Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
1 cup Baxter's brand 2% milk

(7) Cheese
1 slice mozzarella cheese (in sandwich)
3 cm x 1 1/2 cm x 1 cm block of Capitol brand mild cheddar

(8) Food Fats
1 tsp. butter (in sandwich)
1 tbsp. Elmsdale brand 100% vegetable margarine

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee
1 cup tea
Soft Drinks

Mineral Water
5 cups tap water
Wine

(10) Other Foods
1 tbsp. granulated white sugar
3 Wine gums candy, pinch of salt

Table A.11. A list of contents of the HMNT duplicate diet.

(1) Cereal Products & Nuts
1 tbsp. peanut butter
1/2 cup Planter's brand salted peanuts
Wheat Products
2 1/2 homemade bran muffins
11 Christie's brand Premium low-salt crackers
1/2 piece Grainhouse Mills brand 100% whole wheat toasted bread
2 homemade peanut butter cookies

(2) Vegetables
1 cup Campbell's brand vegetable soup
1 1/2 cups salad (lettuce green peppers, tomatoes, zucchini)
6 Heinz brand bread and butter pickles

Potatoes
1 1/2 baked potatoes w / pepper & salt

Canned Products

(3) Fruits & Berries
1 cup Dole brand unsweetened pineapple juice
1 Gravenstein apple
Canned Products

(4) Meat & Meat Products
3 tbsp. Sobey's brand liverwurst
9 oz. roasted sirloin tip beef w / salt & pepper

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)
2 cups Brookfield brand 1% milk
1 tbsp. whole milk (in coffee)
2 cups Brookfield brand 2% milk

(7) Cheese

(8) Food Fats
4 tbsp. 20/80 blend margarine
4 tbsp. homemade salad dressing (egg, oil, vinegar, mayonnaise, garlic, pepper, salt)
1 tbsp. 20/80 blend margarine
6 oz. gravy

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee
1 1/2 cups coffee
Soft Drinks

Mineral Water

Wine
2 cups home-brewed beer

(10) Other Foods

Table A.12. A list of contents of the HMRZ duplicate diet.

(1) Cereal Products & Nuts

Wheat Products

17 Christie brand Premium Plus salted crackers
1/2 bowl raisin bran cereal
2 slices enriched white bread (in sandwich)l

(2) Vegetables

1 tomato
2 cobs microwaved corn-on-the-cob w / salt
1/2 cucumber w / salt

Potatoes

35 g Humpty-Dumpty brand barbecue flavor potato chips

Canned Products

(3) Fruits & Berries

1 plum
2/3 cup orange drink

Canned Products

(4) Meat & Meat Products

30 g roasted white chicken meat (in sandwich)

Canned Products

(5) Fish, Seafoods & Products

Canned Products

(6) Milk Products (as liquids)

7/12 cup & 3 1/2 tsp. Farmer's brand 2% milk

(7) Cheese

20 g Kraft brand Mozzarella
1 wedge Kraft brand gruyere

(8) Food Fats

3/4 tsp. hard Parkay margarine (in sandwich)
1 tsp. Kraft Miracle Whip salad dressing (in sandwich)
1 tsp. Parkay brand margarine

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee

4 cups Maxwell House brand automatic drip coffee

Soft Drinks

1/3 cup Pepsi cola
355 mL Coca cola

Mineral Water

Wine

(10) Other Foods

7 1/4 tsp. Lantic brand granulated white sugar
46 g Neilson brand "Mr. Big" Chocolate bar
2 tsp. Carnation brand Coffeemate coffee whitener
salt & pepper (in sandwich)
1 pkg. Sunkist brand grape "Fun Fruits"

Table A.13. A list of contents of the HMSG duplicate diet.

(1) Cereal Products & Nuts

Wheat Products

2/3 cup Aunt Jemima brand pancake mix (in 2 pancakes)

2 Sobey's brand hot dog rolls (in 2 hot dogs)

(2) Vegetables

2 tsp. relish (in 2 hot dogs)

35 g zucchini, fried in butter from garden

30g boiled leek from garden

Potatoes

225 g potatoes w / margarine, mashed w / peel

Canned Products

(3) Fruits & Berries

1/3 cup strawberries (in pancakes)

2 Paula Red variety apple

2 cups Five Alive brand citrus beverage

Canned Products

(4) Meat & Meat Products

2/3 egg (in 2 pancakes)

2 Schneider's brand barbecue wieners (in 2 hot dogs)

Canned Products

(5) Fish, Seafoods & Products

130 g haddock, coated w / eggs & cracker crumbs, fried in butter

Canned Products

(6) Milk Products (as liquids)

2/3 cup 2% milk (in 2 pancakes)

(7) Cheese

(8) Food Fats

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee

1 cup drip coffee

Soft Drinks

2 1/2 cups Fountain Fresh brand black cherry pop

Mineral Water

Wine

(10) Other Foods

45 mL Table syrup

2 tsp. mustard (in 2 hot dogs)

Table A.14. A list of contents of the HMTH duplicate diet.

(1) Cereal Products & Nuts

Wheat Products
250 mL Kellogg's brand bran flakes cereal
1 Kaiser bun w / salt & pepper (in sandwich)
1/3 tbsp. wheat flour (in sauce)

(2) Vegetables
1 leaf lettuce (in sandwich)
75 mL frozen peas, boiled
Potatoes
250 mL mashed potatoes
Canned Products

(3) Fruits & Berries
2 tbsp. Sultana raisins
100 mL fresh-squeezed orange juice
2 Christie brand fig newtons
2 date squares
350 mL lemonade
Canned Products

(4) Meat & Meat Products
48 g Chicken salad (in sandwich)
Canned Products

(5) Fish, Seafoods & Products
6 cm x 6cm x 1.5 cm haddock, poached in milk
Canned Products

(6) Milk Products (as liquids)
350 mL 2% milk
75 mL milk (in sauce)
30 mL milk (in mashed potatoes)
3 scoops homemade ice cream (milk, cream, sugar, eggs, corn starch, artificial vanilla extract)

(7) Cheese

(8) Food Fats
1 tsp. butter (in sandwich)
2/3 tbsp. margarine (in sauce)

(9) Fluids (excluding fruit & vegetable juices)
Tea or Coffee
500 mL coffee
Soft Drinks

Mineral Water

Wine

(10) Other Foods
1 tsp. granulated white sugar
3 tsp. coffee creamer
1/3 tsp. tarragon (in sauce)

Table A.15. A list of contents of the HMWV duplicate diet.

(1) Cereal Products & Nuts

Wheat Products

1 bowl Kellogg's brand bran flakes
1 slice cracked wheat bread, toasted w / margarine
1 donut
1 hot dog roll (in hot dog)
2 slices Butternut brand oatmeal brown bread (in sandwich)
8 Ritz brand cheese crackers
4 slices Butternut brand oatmeal brown bread, toasted

(2) Vegetables

1 fried green tomato
1 bowl tomato soup
1 carrot
1 leaf lettuce (in sandwich)
1 stalk celery
1 Heinz brand pickle
1 slice cabbage

Potatoes

Canned Products

(3) Fruits & Berries

1 fresh, peeled orange
1 glass Niagara brand reconstituted orange juice
1/2 handful Thompson brand raisins
1 plum
1" x 4" piece watermelon

Canned Products

(4) Meat & Meat Products

2 slices fried Sobey's brand bacon
1 wiener, boiled (in hot dog) w / relish & mustard
2 boiled eggs

Canned Products

(5) Fish, Seafoods & Products

Canned Products

44g tuna salad (Clover leaf brand flaked tuna, Kraft brand Miracle Whip salad dressing, green onion, pepper)

(6) Milk Products (as liquids)

1 1/2 cups Brookfield brand 2% milk
2 tbsp. Farmer's brand 10% cream
1/2 tsp. cream from commercial creamer

(7) Cheese

1 slice Kraft brand processed cheese (in sandwich)
3 tbsp. Brookfield brand 2% cottage cheese
Cheddar cheese

(8) Food Fats

(9) Fluids (excluding fruit & vegetable juices)

Tea or Coffee

3 cups Melitta brand coffee

Soft Drinks

Mineral Water

Wine

2 bottles Keith's brand beer

(10) Other Foods

2 tbsp. Kraft brand honey
1/2 tsp. whitener

Table A.16. Masses of Foods by Category

Food Category	*HFFF*	*HFMC*	*HFNA*	*HFRR*	*HFSR*	*HFTC*	*HFWH*	*HMFF*	*HMMR*	*HMNT*	*HMRZ*	*HMSG*	*HMTH*	*HMWV*
Total Mass	2455	1717	1703	1871	1931	2132	2594	2768	1277	3244	2891	2215	2189	3491
Cereal Products/Nuts	90	153	351	267	436	253	247	164	96	374	179	224	138	467
Vegetables	20	153	195	322	196	341	148	365	119	654	368	265	2796	518
Fruits & Berries	542	250	41	569	101	78	868	269	0	283	408	604	713	765
Meat & Meat Products	121.28	38.09	0	61	209.58	78.84	248.93	250.79	138.19	157.46	32.4	171.02	48.12	224.2
Fish, Seafoods & Products	0	0	0	26	0	81.7	0	0	0	0	0	131.83	82.56	34.59
Milk products (liquids)	412	125	1052	608	504	108	14	401	140	866	375	0	557	264
Cheese	52	49	0	1	0	0	10	42	9	0	36	0	0	46
Food Fats	15	4	54	17	19	23	4	10	12	142	6	17	4	10
Fluids (excl. fr. & veg. juices)*	1172	921	0	0	453	1149	1045	1265	739	767	1397	802	364	1140
Other Foods	31	20	10	0	12	19	8	0	23	0	89	0	2	21

**Fluids*	*HFFF*	*HFMC*	*HFNA*	*HFRR*	*HFSR*	*HFTC*	*HFWH*	*HMFF*	*HMMR*	*HMNT*	*HMRZ*	*HMSG*	*HMTH*	*HMWV*
Tea/Coffee	573	356	0	0	453	287	1045	0	186	262	890	94	370	522
Soft Drinks	349	328	0	0	0	0	0	373	0	0	525	708	0	0
Mineral Water	250	238	0	0	0	862	0	811	552	80	0	0	0	0
Wine	0	0	0	0	0	0	0	30	0	504	0	0	0	618

Table A.17. Mass Fractions (wet) of Foods by Category

Food Category	*HFFF*	*HFMC*	*HFNA*	*HFRR*	*HFSR*	*HFTC*	*HFWH*	*HMFF*	*HMMR*	*HMNT*	*HMRZ*	*HMSG*	*HMTH*	*HMWV*
Cereal Products/Nuts	0.0365	0.0891	0.2063	0.1427	0.2258	0.1189	0.0954	0.0594	0.0754	0.1153	0.0618	0.1013	0.0631	0.1337
Vegetables	0.0081	0.0896	0.1146	0.1722	0.1014	0.1598	0.057	0.1318	0.0935	0.2017	0.1272	0.1194	0.1275	0.1484
Fruits & Berries	0.2208	0.1459	0.0239	0.3041	0.0523	0.0366	0.3348	0.0973	0.	0.0874	0.141	0.2729	0.326	0.2192
Meat & Meat Products	0.0494	0.0222	0.	0.0326	0.1085	0.037	0.096	0.0906	0.1082	0.0485	0.0112	0.0772	0.022	0.0642
Fish, Seafoods & Products	0.	0.	0.	0.0139	0.	0.0383	0.	0.	0.	0.	0.	0.0595	0.0377	0.0099
Milk products (liquids)	0.1679	0.0729	0.6176	0.3249	0.2608	0.0507	0.0054	0.1447	0.1096	0.267	0.1298	0.	0.2544	0.0758
Cheese	0.0211	0.0288	0.	0.0005	0.	0.	0.0039	0.0153	0.0074	0.	0.0126	0.	0.	0.0133
Food Fats	0.0061	0.0023	0.0317	0.0091	0.0098	0.0108	0.0015	0.0036	0.0094	0.0438	0.0021	0.0077	0.0018	0.0029
Fluids (excl. fr. & veg. juices)*	0.4774	0.5379	0.	0.	0.2347	0.5389	0.4029	0.4571	0.5783	0.2363	0.4834	0.3621	0.1664	0.3266
Other Foods	0.0126	0.0114	0.0059	0.	0.0062	0.0089	0.0031	0.	0.0182	0.	0.0309	0.	0.0009	0.006

*Fluids	*HFFF*	*HFMC*	*HFNA*	*HFRR*	*HFSR*	*HFTC*	*HFWH*	*HMFF*	*HMMR*	*HMNT*	*HMRZ*	*HMSG*	*HMTH*	*HMWV*
Tea/Coffee	0.2335	0.2077	0.	0.	0.2346	0.176	0.4029	0.	0.1459	0.0809	0.3079	0.0424	0.169	0.1496
Soft Drinks	0.1421	0.1915	0.	0.	0.	0.	0.	0.177	0.	0.	0.1816	0.3198	0.	0.
Mineral Water	0.1018	0.1387	0.	0.	0.	0.4044	0.	0.293	0.4324	0.0247	0.	0.	0.	0.
Wine	0.	0.	0.	0.	0.	0.	0.	0.0108	0.	0.1554	0.	0.	0.	0.1769

A1.2 Forms Used in the Selection of Study Paricipants

DETERMINATION OF DAILY DIETARY INTAKES OF TRACE ELEMENTS BY CANADIAN ADULTS

Consent Form

This research project is being carried out by the Trace Analysis Research Centre, Dalhousie University, Halifax, Nova Scotia.

This research project involves:

(a) completing a questionnaire
(b) keeping a one-day food record
(c) collecting a one-day food composite according to instructions provided by the researchers.

Participation in the project is voluntary and I am free to withdraw at any time, I will not be paid the $10.00 to defray the cost of food if I do not provide the items (a) through (c).

Results of the food record and diet sample analyses will be kept strictly confidential and are for the exclusive use of the research project. My name will not appear on any report of the work.

I understand the above and am willing to participate in this project.

_____
signature

_____
name (printed)

_____
date

Telephone Number - home (___)_____

work (___)_____

DETERMINATION OF DAILY DIETARY INTAKES OF TRACE ELEMENTS BY CANADIAN ADULTS

Questionnaire for Prospective Participants

PLEASE PRINT ALL ANSWERS

1. Name:_____
   last name first name

2. Home Address: _____
   street apt.

   _____
   city province

   _____
   postal code

3. Telephone: Home (___)_____
   Work (___)_____

4. Birthdate: _____
   year month date

5. Sex: Male_____ Female_____

   Are you pregnant? yes___ no___

   Are you nursing? yes___ no___

6. Height (without shoes): _____ cm / inches

7. Weight (without clothes): _____ kg / Ibs

8. Drinking water:

   (a) Do you drink water from the metropolitan water supply? yes__ no__

   (b) Does your drinking water contain added fluoride? yes__ no__

   (c) Do you get your drinking water from a private well? yes__ no__

   (d) Do you drink bottled/mineral water? yes__ no__

   brand_____

   (c) Do you use bottled/mineral water for cooking? yes__ no__

   brand_____

9. Household:

(a) Do you live alone? yes_____ no_____

(b) If no, how many other people on your household are in the following age groups:

0-9 years _____

10-19 years _____

20-39 years _____

40-64 years _____

65 years or older _____

(c) Do you have facilities for refrigerating food? yes_____ no_____

(d) Do you have facilities for freezing food? yes_____ no_____

10. Health and Diet:

(a) Are you presently in good health? yes_____ no_____

(b) Are you presently following a special diet? yes_____ no_____

If yes, please specify:

diabetic _____

vegetarian _____

weight reduction _____

allergy, specify _____

other, specify _____

(c) Do you take a vitamin and/or mineral supplement? yes_____ no_____

If yes, please specify:

brand_____

type and amount of each nutrient as specified on label:

frequency of use_____

(d) During a typical one-week (7-day) period how many times do you eat the following meals (please specify the number of days):

breakfast _____

lunch _____

supper _____

(e) During a typical one-week (7-day) period how many times do you eat the following meals at home (please specify the number of days):

breakfast _____ (include lunch carried

lunch _____ using food supplied from

supper _____ home)

(f) During a typical one-week (7-day) period how many times do you eat the following meals away from home (please specify the number of days):

breakfast _____

lunch _____

supper _____

11. Occupation: _____

12. Date this questionnaire was completed _____

year month day

13. Return questionnaire to:

Dr, A. Chatt
Trace Analysis Research Centre
Department of Chemistry
Dalhousie University
Halifax, Nova Scotia
B3H 451

TO BE COMPLETED BY THE RESEARCHER:

Participant Code _____

Sex Code _____

Day Code _____

City Code _____

APPENDIX 2

NUTRIENT ANALYSIS USING CANADIAN NUTRIENT FILE

Table A.18 contains calculated intakes of some nutrients in the duplicate diets collected by the participants of this study. The calculations were based on the Canadian Nutrient File and performed using the Nutrient Analysis System of Mount Saint Vincent University in 1987. These previously unpublished values were calculated by Chatt and Pegg (27) and are reproduced here by permission.

Table A.18. Intake of various nutrients calculated from diet records and weighed masses.

Nutrient	*HFFF*	*HFMC*	*HFNA*	*HFRR*	*HFSR*	*HFTC*	*HFWH*	*HMFF*	*HMMR*	*HMNT*	*HMRZ*	*HMSG*	*HMTH*	*HMWV*
Protein (g)	55.21	34.06	65.86	71.68	94.49	60.37	63.29	92.2	47.65	17.28	58.04	67.85	75.88	115.24
Energy (cal.)	1606.8	1553.7	2317.2	2264.8	1972.1	1799.7	1945.3	2278.2	1493.5	4200.7	2228.7	2470.2	2532.6	3285.4
Phosphorus (mg)	1255.9	542.0	1560.7	1402.1	1442.1	962.8	1110.8	1359.8	880.6	3030.3	1127.3	1072.7	1483.7	2657.7
Vit. D (µg)	1.9	2.64	14.53	8.28	5.47	6.95	2.98	5.42	5.24	19.15	3.44	1.38	4.02	6.28
Vit. A (RE)	661.7	400.50	1761.0	856.2	1307.3	566.0	608.7	2473.0	445.6	3142.6	533.4	237.2	534.6	2285.3
Vit. C (mg)	184.18	69.19	74.51	254.59	89.33	67.34	245.53	114.58	50.34	114.68	142.87	186.22	121.4	384.92
Thiamin (mg)	1.65	0.97	2.13	1.55	1.32	1.14	1.98	3.19	2.29	1.98	2.34	1.19	2.67	4.08
Riboflavin (mg)	1.52	0.78	2.39	1.88	1.9	0.8	1.71	1.92	1.01	3.25	1.17	1	1.8	2.81
Niacin (NE)	19.66	15.23	25.09	34.25	38.05	26.6	30.18	39.95	23.2	69.82	31.59	27.95	38.01	55.4
Vit. B-6 (mg)	0.87	0.73	1.21	1.8	0.75	1.1	0.8	1.36	1.17	3.18	1.18	1.24	1.67	2.54
Folacin (µg)	119.63	69.36	158.75	398.56	164.59	167.76	222.02	191.57	127.77	346.94	162.6	114.3	246.65	621.02
Vit. B-12 (µg)	2.09	0.85	4.07	2.45	4.02	1.41	2.65	2.81	2.38	8.74	1.27	3.95	3.16	5.13
%Protein*	13.49	8.71	11.19	12.34	19.04	13.11	12.95	16.05	12.5	12.89	9.35	10.8	11.77	14.49
%Fat*	29.75	29.15	44.42	35.66	34.13	44.4	27.77	36.9	38.28	50.92	26.63	29.88	23.19	27.19
%Carbo.*	56.76	62.13	44.38	51.99	46.82	42.29	59.28	47.06	49.22	33.2	64.02	59.32	65.04	58.32

*By energy (cal.)

REFERENCES

1. Health and Welfare Canada. Nutrition Recommendations. The Report of the Scientific Review Committee. 1990. Canadian Government Publishing Centre, Ottawa, 1990.

2. M. Abdulla, A. Behbehani and H. Dashti. Biol. Tr. Element Res. 21, 173 (1989).

3. S. Hattangadi. Industrial Chemist 8, 10, (1987).

4. E.J. Underwood. *Trace Elements in Human and Animal Nutrition*. Academic Press, New York, p. 442, 1977.

5. K. Heydorn. *Neutron Activation Analysis for Clinical Trace Element Research*, Vol. I. C.R.C. Press Inc., Boca Raton, Florida, 1984.

6. T. Hazell. World Rev. Nutr. Diet 46, 1 (1985).

7. M.F. Robinson and C.D. Thomson. Nutr. Abstr. Rev. 53, 3 (1983).

8. B.L. O'Dell. Nutr. Rev. 42, 301 (1984).

9. J.M. McKenzie. "Bioavailability of Trace Elements in Foodstuffs and Beverages" in *Changing Metal Cycles and Human Health*, Springer-Verlag, New York, 1984.

10. B.A. Chowdry and R.K. Chandra. Progr. Food Nutr. Sci. 11, 55 (1987).

11. F.H. Nielsen. "Ultratrace elements of possible importance for human health: an update." in *Essential and Toxic Tace Elements in Human Health and Disease: An Update*, Wiley-Liss Inc., pp. 355-376, 1993.

12. R.H. Garrison Jr. and E. Somer. *The Nutrition Desk Reference*. Keats Publishing Inc., New Canaan, Connecticut, 1985.

13. Health and Welfare Canada. *Recommended Nutrient Intakes for Canadians*, Canadian Government Publishing Centre, Ottawa, 1983.

14. J.N. Hathcock. J. Nutr. 126 Supplement, 2386S (1996).

15. J.R. Coughlin. "Essentiality vs. Toxicity of Essential Trace Elements: A Nutritional Toxicologist Looks at the Upper Safe Level" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, 1997, pp. 399-400, 1997.

16. R.L. Pike and M.L. Brown. *Nutrition: an Integrated Approach 3rd Edition.* John Wiley and Sons, New York, 1984.

17. National Research Council. *Recommended Dietary Allowances 10th edition,* National Academic Press, Washington, D.C., 1989.

18. E.R. Monsen. J. Am. Diet. Assn. 96, 754 (1996).

19. J.C. King. "Considerations Regarding Future Trace Element Dietary Reference Intakes" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 388-390, 1997.

20. National Academy of Sciences. Nutr. Rev. 55, 319 (1997).

21. National Academy of Sciences. Nutr. Rev. 55, 327 (1997).

22. S.W. Gunner and O.C. Kirkpatrick. Can. Inst. Food Sci. Technol. J..12, 27 (1979).

23. G. Block. Am. J. Epidemiol. 115, 492 (1982).

24. M. Pekkarinen. World Rev. Nutr. Dietetics 12, 145 (1970).

25. C.M. Young. "Dietary Methodology" in *Assessing Changing Food Consumption Patterns.* Committee on Food Consumption Patterns, Food and Nutrition Board, National Research Council, National Academic Press, Washington D.C., pp. 89-147, 1981.

26. M. Abdulla, R.M. Parr, and G.V. Iyengar. "Trace Element Requirements, Intake and Recommendations," *Essential and Toxic Trace Elements in Human Health and Disease: An Update,* Wiley-Liss Inc., pp. 311-328, 1993.

27. A. Chatt and D. Pegg. Unpublished.

28. S.G. Capar. "Analytical Method Aspects of Assessing dietary Intake of Trace Elements." in *ACS 445. Biological Trace Element Research. Multidisciplinary Perspectives*, American Chemical Society, 1991, p. 181.

29. A.-S. Sandberg. "Food Processing Influencing iron and Zinc Bioavailability" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 10-13, 1997.

30. J.C. Sherlock. "Aluminum in Foods and the Diet" in *Special Publication No.73. Aluminium in Food and the Environment*, R. Massey and D. Taylor eds., Royal Society of Chemistry, p.64, 198.

31. M.J. Baxter, J.A. Burrell, H.M. Crews and R.C. Massey. "Aluminum in Infant Formulae and Tea and Leaching During Cooking" in *Special Publication No.73. Aluminium in Food and the Environment*, R. Massey and D. Taylor eds., Royal Society of Chemistry, p. 77, 1989..

32. L. Mei and T. Yao. Intern. J. Environ. Anal. Chem. 50, 1 (1993).

33. H.C. Brittin and C.E. Nossaman. J. Am. Diet. Assoc. 86, 897 (1986).

34. G. Yang and R. Zhou. J. Tr. Elements Electrolytes Health Dis. 8, 159 (1994).

35. J. Park, and H.C. Brittin. J. Am. Diet. Assn. 97, 659 (1997).

36. V. Iyengar, J. Kumpulainen, K. Okamoto, M. Morita, S. Hirai and S. Nomoto. "Recent Trends in Analytical Approaches for Trace Element Determinations in Biomedical Investigations" in *Essential and Toxic Trace Elements in Human Health and Disease: An Update*, Wiley-Liss Inc., p. 329, 1993.

37. N.T. Crosby. Analyst 102, 225 (1977).

38. A.H. Khan, S.A. Tarafdar, M. Ali, S.K. Biswas, S. Akhter, D.K. Saha, A. Islam, M. Billah, D.A. Hadi, F.B.A. Maroof. J. Radioanal. Nucl. Chem. Articles 17, 367 (1989).

39. L. Wang, X. Zhang, G. Cheng. Anal. Lett. 23, 2233 (1990).

40. L. Singer and R.H. Ophaug. J. Agr. Food Chem. 34, 510 (1986).

41. *A.O.A.C. Official Methods of Analysis 14th edition.* Association of Official Analytical Chemists, Washington, D.C., 1984.

42. A. Chatt, H.S. Dang, B.B. Fong, C.K. Jayawickreme, L.S. McDowell, D.L. Pegg. J. Radioanal. Nucl. Chem. Articles 124, 65 (1988).

43. S.M. Liu, C. Chung, J.T. Chuang, C.F. Wang and N.K. Aras. J. Radioanal. Nucl. Chem. Articles 150, 397 (1991).

44. S.A. Kerr and N.M. Spyron. J. Radioanal. Chem. 44, 159 (1978).

45. R.R. Rao, L.S. McDowell, C.K. Jayawickreme and A. Chatt. J. Radioanal. Nucl. Chem. Articles 151, 167 (1991).

46. R.R. Rao, J. Holzbecher and A. Chatt. Fresenius' J. Anal. Chem. 352, 53 (1995).

47. A. Chatt. Trans. Am. Nucl. Soc. 55, 176 (1987).

48. N. Lavi and Z.B. Alfassi. Analyst 115, 817 (1990).

49. R.R. Rao and A. Chatt. Anal. Chem. 63, 1298 (1991).

50. D.A. Skoog. *Principles of Instrumental Analysis*, Saunders College Publishing, Philadelphia, pp. 511-520, 1985.

51. D. De Soete, R. Gijbels and J. Hoste. *Neutron Activation Analysis*, Wiley-Interscience, Birkenhead, Cheshire, England, 1972.

52. W.D. Ehmann and D.E. Vance. *Radiochemistry and Nuclear Methods of Analysis*, John Wiley & Sons, New York, pp.253-306, 1991.

53. S.J. Parry. *Activation Spectrometry in Chemical Analysis*, John Wiley & Sons, New York, 1991.

54. G. Hevesy and S. Levi. "The Action of Neutrons on the Rare Earth Elements." in K. Dan. Vidensk. Selsk., Mat.-Fys. Medd. XIV, 5 , pp. 3-34, 1936; as cited in *Aspects of Precision and Accuracy in Neutron Activation Analysis*, K. Heydorn, Roskilde, Denmark, p. 1, 1978.

55. D.E. Ryan, A. Chatt and J. Holzbecher. Anal. Chim. Acta 200, 89 (1987).

56. D.E. Ryan, D.C. Stuart and A. Chattopadhyay. Anal. Chim. Acta 100, 87 (1978).

57. J. Holzbecher, A. Chatt and D.E. Ryan. Can. J. Spectrosc. 30, 67 (1985).

58. P. Beazley. M.Sc. Thesis, Dalhousie University, 1993.

59. S. Landsberger. J. Radioanal. Nucl. Chem. Articles 179, 67 (1994).

60. S. Landsberger and S. Peshev. J. Radioanal. Nucl. Chem. Articles 202, 201 (1996).

61. W. Zhang. Ph.D. Thesis, Dalhousie University, 1997.

62. J.K. Taylor. *Quality Assurance of Chemical Measurements*, Lewis Publishers, Inc., Chelsea, Michigan, 1987.

63. Analytical Methods Committee of the Royal Society of Chemistry. Analyst 120, 29 (1995).

64. D. Mullins. Analyst 119, 369 (1994).

65. *Nutrition Canada. The Nova Scotia Survey Report.* the Bureau of Nutritional Sciences, Department of National Health and Welfare, 1975.

66. L.S. McDowell. *Determination of Total and Bioavailable Selenium in Food by Neutron Activation.* M.Sc. Thesis, Dalhousie University, 1987.

67. L.S. McDowell, P.R. Giffen, A. Chatt, J. Radioanal. Nucl. Chem. Articles 110, 519 (1987).

68. D. Arthur. Can. Inst. Food Sci. Technol. J. 5, 165 (1972).

69. J.N. Thompson, P. Erdody, D.C. Smith. J. Nutr. 105, 274 (1975).

70. R.S. Gibson, A.C. MacDonald and O.B. Martinez. "Dietary trace element intakes of a selected sample of Canadian elderly women" in *Proceedigs of the 5th International Conference on Nuclear Methods of Environmental Energy Research*, pp. 844-851, 1984.

71. B.G. Shah, A. Giroux and B. Belonje. Nutr. Res. 2, 669 (1982).

72. D. O'Connor, R.S. Gibson and O.B. Martinez. J. Can. Diet. Assn. 46, 45 (1985).

73. R.S. Gibson, J.K. Friel and C.A. Scythes. J. Can. Diet. Assoc. 46, 182 (1985).

74. J.C. Meranger and D.C. Smith. Can. J. Public Health 63, 53 (1972).

75. D.C. Kirkpatrick and D.E. Coffin, J. Inst. Can. Sci. Technol. Aliment. 7, 56 (1974).

76. R.S. Gibson and C.A. Scythes. Biol. Tr. Element Res. 6, 105 (1984).

77. D.C. Kirkpatrick and D. E. Coffin. Can. J. Public Health 68, 162 (1977).

78. J.T. Tanner and M.H. Friedman. J. Radioanal. Chem. 37, 529 (1977).

79. J.A.T. Pennington. J. Am. Diet. Assn. 82, 166 (1983).

80. R.D. Johnson, D.D. Manske, D.H. New and D.S. Podrebarac. J. Assn. Official Anal. Chems. 67, 154 (1984).

81. J.A.T. Pennington, D.B. Wilson, R.F. Newell, B.F. Harland, R.D. Johnson and J.E. Vanderveen. J. Am. Diet. Assoc. 84, 771 (1984).

82. D.S. Podrebarac. J. Assn. Official Anal. Chems. 67, 176 (1984).

83. J.A.T. Pennington, B.E. Young, D.B. Wilson, R.D. Johnson and J.E. Vanderveen. J. Am. Diet. Assoc. 86, 876 (1986).

84. J.A.T. Pennington. J. Am. Diet. Assoc. 87, 1644 (1987).

85. J.A.T. Pennington, B.E. Young and D.B. Wilson. J. Am. Diet. Assoc. 89, 659 (1989).

86. J.A.T. Pennington and B.E. Young. J. Am. Dietetic Assoc. 91, 179 (1991).

87. J.A.T. Pennington and S.A. Schoen. Food Additives and Contaminants 12, 119 (1995).

88. M. Allegrini, K.W. Boyer and J.T. Tanner. J.A.O.A.C. 64, 1111 (1981).

89. M. Allegrini, J.A.T. Pennington and J.T. Tanner. J. Am. Diet. Assoc. 83, 18 (1983).

90. M. Abdulla, I. Andersson, P. Belfrage, I. Dencker, M. Jagerstad, A. Melander, A. Norden, B. Schersten, T. Thulin and B. Akesson. Scand, J. Gastroent. 14, Suppl. 52, 28 (1979).

91. M. Abdulla, M.D.I. Andersson, N.-G. Asp, K. Berthelsen, D. Birkhed, I. Dencker, C.-G. Johansson, M. Jagerstad, K. Kolar, B.M. Nair, P. Nilsson-Ehle, A. Norden, S. Rassner, B. Akesson and P.-A. Ockerman. Am. J. Clin. Nutr. 34, 2464 (1981).

92. M. Abdulla. *Nutritional adequacy, nutrient availability and needs.* Ed. J. Mauron. Birkhauser Verlag Basel, Boston, Stuttgart, p. 338.

93. M. Abdulla, M.D.I. Andersson, N.-G. Asp, K. Berthelsen, D. Birkhed, I. Dencker, C.-G. Johansson, M. Jagerstad, K. Kolar, B.M. Nair, P. Nilsson-Ehle, A. Norden, S. Rassner, B. Akesson and P.-A. Ockerman. Am. J. Clin. Nutr. 34, 2464 (1981). Am. J. Clin. Nutr. 40, 325 (1984).

94. M. Abdulla. *Inorganic Chemical Elements in Prepared Meals in Sweden,* Departement of Clinical Chemistry, University of Lund, Lund, Sweden, 1986.

95. O.A. Levander, G. Alfthan, H. Arvilommi, C.G. Gref, J.K. Huttunen, M. Kataja, P. Koivistoinen and J. Pikkarainen. Am. J. Clin. Nutr. 37, 887 (1983).

96. P. Koivisotoinen and J.K. Huttunen. Annals. Clin. Res. 18, 13 (1986).

97. M. Sinisalo, J. Kumpulainen, M. Paakki and R. Tahvonen. J. Human Nutr. Diet. 2, 43 (1989).

98. W. Becker and J. Kumpulainen. Br. J. Nutr. 66, 151 (1991).

99. M.H. Eurola, P.I. Ekholm, M.E. Ylinen, P.E. Koivistoinen and P.T. Varo. J. Sci. Food Agric. 56, 57 (1991).

100. A. Aro, G. Alfthan and P. Varo. Analyst 120, 841 (1995).

101. R. Schelenz. J. Radioanal. Chem. 37, 539 (1977).

102. R. Schelenz. "Intake of Zn, Mn, and Se by adult females- a total diet study." *Biological Trace Element Analytical Chemistry in Medicine and Biology, 3rd International Workshop,* p. 73, 1984.

103. O.A. Levander. Federation Proc. 42, 1721 (1983).

104. W. van Dokkum. "The Significance of Speciation for Predicting Mineral Bioavailability" in *Special Publication Number 72. Nutrient Availability: Chemical and Biological Aspects,* p. 89, 1988.

105. E.R. Monsen, L. Hallber, M. Layrisse, D.M. Hegsted, J.D. Cook, W. Mertz and C.A. Finch. Am. J. Clin. Nutr. 31, 17 (1978).

106. M. Brune, L. Rossander-Hulten, L. Hallberg, A. Gleerup and A.-S. Sandberg. J. Nutr. 122, 442 (1992).

107. D.L. Kirk. *Biology Today* 3rd Edition. Random House, New York 1980.

108. J.G. Reinhold, B. Faradji, P. Abadi and F. Ismail- Beigi. J. Nutr. 106, 493 (1976).

109. M. Torre, A.R. Rodriguez and F. Saura-Calixto. Crit. Rev. Food Nutr. 1, 1 (1991).

110. M.B. Reddy and J.D. Cook. Am. J. Clin. Nutr. 54, 723 (1991).

111. D. Siegenberg, R.D. Baynes, T.H. Bothwell, B.J. Macfarlane, R.D. Lamparelli, N.G. Car, P. MacPhail, U. Schmidt, A. Tal and F. Mayet. Am. J. Clin. Nutr. 53, 537 (1991).

112. F.M. Clydesdale, Chi-Tang Ho, C.Y. Lee, N.I. Mondy and R.L. Shewfelt. Crit. Rev. Food Sci. Nutr. 30, 599 (1991).

113. B.S. Narasinga Rao and T. Prabhavathi. Am. J. Clin. Nutr. 31, 169 (1978).

114. B.S. Narasinga Rao. J. Food Sci. Technol. 31, 353 (1994).

115. J. Luten, H. Crews, A. Flynn, P. Van Dael, P. Kastenmayer, R. Hurrell, H. Deelstra, L.-H. Shen, S. Fairweather-Tait, K. Hickson, R. Farre, U. Schlemmer and W. Frohlich. J. Sci. Food Agric. 72, 415 (1996).

116. D.D. Miller, B.R. Schricker, R.R. Rasmussen and D. Van Campen. Am. J. Clin. Nutr. 34, 2248 (1981).

117. I. Kaldor. Australasian Ann. Med. 6, 244 (1957).

118. R. Sanford. Nature 185, 533 (1960).

119. A. Jacobs and D.A. Greenman. Br. Med. J. 1, 673 (1969).

120. T. Hazell, D.A. Ledward and R.J. Neale. Br. J. Nutr. 39, 631 (1978).

121. K.B. Nolan and P.A. Duffin. J. Sci. Food Agric. 40, 79 (1987).

122. S.R. Gifford and F. Clydesdale. J. Food Sci. 55, 1720 (1990).

123. R.C. Massey, J.A. Burrell, D.J. McWeeny and H. Crews. Toxicological Env. Chem. 13, 85 (1986).

124. D.B. Nadeau and F.M. Clydesdale. J. Food Sci. 56, 146 (1991).

125. M.G.E. Wolters, H.A.W. Schreuder, G. van den Heuvel, H.J. van Lonkhuijsen, R.J.J. Hermus and A.G.J. Voragen. Br. J. Nutr. 69, 849 (1993).

126. L. Savoie. Can. J. Physiol. Pharmacol. 72, 407 (1994).

127. S.F. Gauthier, C. Vachon, J.D. Jones and L. Savoie. J. Nutr. 112, 1718 (1982).

128. L. Savoie and S.F. Gauthier. J. Food Sci. 51, 494 (1986).

129. H.M. Crews, J.A. Burrell and D.J. McWeeny. J. Sci. Food Agric. 34, 997 (1983).

130. H.M. Crews, J.A. Burrell and D.J. McWeeny. Z. Lebensmit. Unters. Forsch. 180, 405 (1985).

131. l.-H. Shen, J. Luten, H. Robberecht, J. Bindels and H. Deelstra. Zeitschr. fur Lebensm. Z. Lebensm. Unters. Forsch. 199, 442 (1994).

132. A. Chatt, D. Pegg and E. Sullivan. Unpublished.

133. A. Chatt. Submission to the International Atomic Energy Agency. To be published, 1992.

134. World Health Organization. *Trace elements in human nutrition and health.* World Heath Organization, Belgium, 1996.

135. G. Yang, K. Ge, J. Chen and X. Chen. World Rev. Nutr. Diet. 55, 98 (1988).

136. M.F. Robinson. Nutr. Rev. 47, 99 (1989).

137. G. Yang, S. Wang, R. Zhou and S. Sun. Am. J. Clin. Nutr. 37, 872 (1983).

138. J.D. Cross, R.M. Raie, H. Smith and L.B. Smith. Radiochem. Radioanal. Lett. 35, 281 (1978).

139. H.A. Schroeder, D.V. Frost and J.J. Balassa. J. Chron. Dis. 23, 227 (1970).

140. S.N. Ganapathy and R. Dhanda. The Ind. J. Nutr. Dietet. 17, 53 (1980).

141. C.D. Thomson and M.F. Robinson. Am. J. Clin. Nutr. 33, 303 (1980).

142. O.A. Levander. Ann. N.Y. Acad. Sci. 393, 70 (1982).

143. O.A. Levander. Ann. Rev. Nutr. 7, 227 (1987).

144. O.A. Levander. J. Am. Diet. Assoc. 91, 1572 (1991).

145. P. Van Dael and H. Deelstra. Int. J. Vitamin Nutr. Res. 63, 312 (1993).

146. J.R. Arthur and G.J. Beckett. Proc. Nutr. Soc. 53, 615 (1994).

147. O.A. Levander and P.D. Whanger. J. Nutr. 126 Supplement, 2427S (1996).

148. L.H. Foster and S. Sumar. Crit. Rev. Food Sci. Nutr. 37, 211 (1997).

149. K. Schwartz and C.M. Foltz. J. Am. Chem. Soc. 79, 3292 (1957).

150. T.C. Stadtman. Ann. Rev. Biochem. 49, 93 (1980).

151. R.A. Ahrens. J. Nutr. 127 Supplement, S1052 (1997).

152. J.R. Arthur. "Selenium Biochemistry and Function" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 1-4, 1997.

153. J.R. Arthur, F. Nicol and G.J. Beckett. Biochem. J. 272, 537 (1990).

154. J.R. Arthur, F. Nicol and G.J. Beckett. Am. J. Clin. Nutr. Suppl. 57, 236S (1993).

155. H.P.C.K. Jayawickreme. *Studies on Selenoproteins and Protein-Bound Trace Elements in Bovine Kidneys.* Ph.D. thesis, Dalhousie University, 1987.

156. R.F. Burk and K.E. Hill. Annu. Rev. Nutr. 13, 65 (1993).

157. B.J. Lysenko. *Selenoproteins and selected protein-bound trace elements in bovine kdney cytosol fraction.* Ph.D. thesis, Dalhousie University, 1995.

158. D. Behne, C. Weiss-Novak, M. Kalcklosch, C. Westphal, H. Gessner and A. Kyriakopoulos. Analyst, 120, 823 (1995).

159. R.A. Johnson, S.S. Baker, J.T. Fallen, E.P. Maynard, J.N. Ruskin, Z. Wen, K. Ge, H.J. Cohen. New Engl. J. Med. 304, 1210 (1981).

160. M.R. Brown, H.J. Cohen, J.M. Lyons, T.W. Curtis, B. Thumberg, W.J. Cochran and W.J. Klish. Am. J. Clin. Nutr. 43, 549 (1986).

161. D.A. Frankel. Nutr. Res. 13, 583 (1993).

162. Keshan Disease Research Group of the Chinese Academy of Medical Sciences, Beijing. Chinese Med. J. 92, 477 (1979).

163. X.S. Chen. Ann. N.Y. Acad. Sci. 393, 224 (1982).

164. G. Yang, J. Chen, Z. Wen, K. Ge, L. Zhu, X. Chen and X. Chen. Adv. Nutr. Res. 6, 203 (1984).

165. K. Ge and G. Yang. Am. J. Clin. Nutr. Suppl. 57, 259S (1993).

166. Keshan Disease Research Group of the Chinese Academy of Medical Sciences, Beijing. Chinese Med. J. 92, 471 (1979).

167. P.J. Collipp and S.Y. Chen. The Lancet 2, 1304 (1984).

168. B. Corvilain, B. Contempre, A.O. Longombe, P. Goyens, C. Gervy-Decoster, F. Lamy, J.B. Vanderpas and J.E. Dumont. Am. J. Clin. Nutr. Suppl. 57, 244S (1993).

169. T. Ma, J. Guo and F. Wang. Am. J. Clin. Nutr. Suppl. 57, 264S (1993).

170. C.-H. Thilly, B. Swennen, P. Bourdoux, K. Ntambue, R. Moreno-Reyes, J. Gillies and J.B. Vanderpas. Am. J. Clin. Nutr. Suppl. 57, 267S (1993).

171. J.T. Salonen, G. Alfthan, J.K. Huttunen, J. Pikkarainen and P. Puska. The Lancet 2, 175 (1982).

172. M. Vinceti, S. Rovesti, C. Marchesi, M. Bergomi and G. Vivoli. Biol. Tr. Element Res. 40, 267 (1994).

173. J. Ringstad, B.K. Jacobsen and Y. Thomassen. J. Tr. Elem. Electrolytes Health Dis. 1, 27 (1987).

174. O.A. Levander, A. Ager and M.A. Beck. Proc. Nutr. Soc. 54, 475 (1995).

175. E. Sun, H. Xu, Q. Liu, J. Zhou, P. Zuo and J. Wang. Biol. Tr. Element Res. 48, 231 (1995).

176. E. Sun, H. Xu, Q. Liu, J. Zhou, P. Zuo and J. Wang. Biol. Tr. Element Res. 48, 239 (1995).

177. R.A. Goyer. Am. J. Clin. Nutr. 61(suppl.), 646S (1995).

178. B. Wlodarczyk, B. Biernacki, M. Minta, W. Kozaczynski and T. Juszkiewicz. Bull. Environ. Contam. Toxicol. 54, 907 (1995).

179. M.C. Mondragon and W.G. Jaffe. Arch. Latinoam. Nutr. 26, 341 (1976).

180. M.P. Longnecker, P.R. Taylor, O.A. Levander, M. Howe, C. Veillon, P.A. McAdam, K.Y. Patterson, J.M. Holden, M.J. Stampfer, J.S. Morris and W.C. Willett. Am. J. Clin. Nutr. 53, 1288 (1991).

181. National Research Council. *Recommended Dietary Allowances 9th edition*, National Academic Press, Washington, D.C. (1980).

182. D.J. Higgs, V.C. Morris and O.A. Levander. J. Agr. Food Chem. 20, 678 (1972).

183. G. Tolg. "Selenium Analysis in Biological Materials" in *Trace Element Analytical Chemistry in Medicine and Biology Vol. 3.*, pp.95-125.

184. G. Alftman, A. Aro, H. Arvilommi and J.K. Huttunen. Am. J. Clin. Nutr. 53, 120 (1991).

185. D.M. McKown and J.S. Morris. J. Radioanal. Chem. 43, 411 (1978).

186. W. Zhang and A. Chatt. A quality assurance program for the determination of selenium in foods by instrumental neutron activation analysis. International Symposium on Harmonization of Health-Related Environmental Measurements using Nuclear and Isotopic Techniques, International Atomic Energy Agency Proceedings, Vienna, Austria. IAEA -SM-344/35. In Press (1997).

187. R.L. Heath. *Gamma-Ray Spectrum Catalogs.* Idaho National Engineering & Environmental Laboratory, Idaho Falls, ID, USA, β Relaease (1997 March).

188. *Table of Isotopes.* Eds.: C.M. Lederer and V.S. Shirley, 7th Edn., Wiley Interscience, New York, NY, USA, 1978.

189. G. Erdtmann. "Neutron Activation Tables." Verlag Chemie, Weinheim, 1976.

190. M. Makarewicz and R. Zeisler. Biol. Tr. Element Res. 43, 95 (1994).

191. L.A. Currie. Anal. Chem. 40, 586 (1968).

192. M.J. Gartrell, J.C. Craun, D.S. Podrebarac and E.L. Gunderson. J. Assoc. Off. Anal. Chem. 68, 862 (1985).

193. K.P. McConnell, J.C. Smith, P.J. Higgins and A.J. Blotcky. Nutr. Res. 1, 235 (1981).

194. S.O. Welsh, J.M. Holden, W.R. Wolf and O.A. Levander. J. Am. Diet. Assn. 79, 277 (1981).

195. G.V. Iyengar, J.T. Tanner, W.R. Wolf and R. Zeisler. Sci. Total Env. 61, 235 (1987).

196. O.A. Levander, P.B. Moser and V.C. Morris. Am. J. Clin. 46, 694 (1987).

197. C.A. Swanson, M.P. Longnecker, C. Veillon, M. Howe, O.A. Levander, P.R. Taylor, P.A. McAdam, C.C. Brown, M.J. Stampfer and W.C. Willett. Am. J. Clin. Nutr. 52, 858 (1990).

198. J.J. Fardy, G.D. McOrist and Y.J. Farrar. J. Radioanal. Nucl. Chem. Articles 133, 397 (1989).

199. H.J. Robberecht and H.A. Deeltra. Z. Lebensm. Unters. Forsch. 178, 266 (1984).

200. E.J. Rockens, H.J. Robberecht and H.A. Deelstra. Z. Lebensm. Unters. Forsch. 182, 8 (1986).

201. H.J. Robberecht, P. Hendrix, R. Van Cauwenbergh and H.A. Deelstra. Z. Lebensm. Unters. Forsch. 199, 251 (1994).

202. M. Cresta, M. Allegrini, E. Casadei, M. Gallorini, E. Lanzola and G.B. Panatta. Food and Nutr. 2, 8 (1976).

203. D.I.T. Favaro, V.A. Maihara, M.J.A. Armelin, M.B.A. Vasconcellos and S.M. Cozzolino. J. Radioanal. Nucl. Chem. Articles 181, 385 (1994).

204. H. Benemariya, H. Robberecht and H. Deelstra. Sci. Total Env. 136, 49 (1993).

205. X. Luo, H. Wei, C. Yang, J. Xing, C. Qiao, Y. Feng, J. Liu, Z. Liu, Q. Wu, Y. Liu, B.J. Stoecker, J.E.. Spallholz and S.P. Yang. Am. J. Clin. Nutr. 42, 31 (1985).

206. S. Laiyan, L. Fengying, S. Rongwei and Z. Houxi. J. Radioanal. Nucl. Chem. Articles 151, 277 (1991).

207. S. Bro, B. Sandstrom and K. Heydorn. J. Tr. Elements Electrolytes Health Dis. 4, 147 (1990).

208. P. Varo and P. Koivistoinen. Int. J. Vit. Nutr. Res. 51, 79 (1981).

209. M. Mutanen, J. Kumpulainen, J. Lehto, and P. Koivistoinen. Nutr. Res. 5, 693 (1985).

210. M. Lamand, J.C. Tressol and J. Bellanger. J. Tr. Elements Electrolytes Health Dis. 8, 195 (1994).

211. J. Thorn, J. Robertson and D.H. Buss. Br. J. Nutr. 39, 391 (1978).

212. M.S.. Bratakos, T.F. Zafiropoulos, P.A. Siskos and P.V. Ioannou. J. Food Sci. 52, 817 (1987).

213. M.S. Bratakos and P.V. Ioannou. Sci. Total Env. 105, 101 (1991).

214. L.C. Rossi, G.F. Clemente and G. Santaroni. Arch. Env. Health 31, 160 (1976).

215. G.F. Clemente, L. Cigna Rossi and P. Santaroni. J. Radioanal. Chem. 37, 549, (1977).

216. L.C. Rossi, G.F. Clemente and G. Santaroni. Rev. Env. Health 1, 19 (1978).

217. A. Stacchini, E. Coni, M. Baldini, E. Beccaloni and S. Caroli. J. Tr. Elem. Electrolytes Health Dis. 3, 193 (1989).

218. V. Singh and A.N. Garg. J. Radioanal. Nucl. Chem. 217, 139 (1997).

219. K. Yasumoto, K. Iwami, M. Yoshida and H. Mitsuda. J. Jap. Soc. Food Nutr. 29, 511 (1976).

220. C.J. Wyatt, J.M. Melendez, N. Acuna and A. Rascon. Nutr. Res. 16, 949 (1996).

221. J. Yoshinaga, T. Suzuke, R. Ohtsuka, T. Kawabe, T. Hongo, H. Imai and T. Inaoka. Ecol. Food Nutr. 26, 27 (1991).

222. M.F. Robinson, J.M. McKenzie, C.D. Thomson and A.L. van Rij. Br. J. Nutr. 30, 195 (1973).

223. N.M. Griffiths. Proc. Univ. Otago Med. Sch. 51, 8 (1973).

224. J.H. Watkinson. New Zealand Med. J. 80, 202 (1974).

225. R.D.H. Stewart, N.M. Griffiths, C.D. Thomson and M.F. Robinson. Br. J. Nutr. 40, 45 (1978).

226. J.H. Watkinson. Am. J. Clin. Nutr. 34, 936 (1981).

227. K. Bibow, H.M. Meltzer, H.H. Mundal, I.T. Paulsen and H. Holm J. Tr. Elements Electrolytes Health Dis. 7, 171 (1993).

228. I.H. Qureshi, A. Mannan, J.H. Zaidi, M. Arif and N. Khalid. Int. J. Env. Anal. Chem. 38, 565 (1990).

229. A. Mannan, S. Waheed, S. Ahmad and I.H. Qureshi. J. Radioanal. Nucl. Chem. Articles 162, 111 (1992).

230. M.F. Reis, J. Holzbecher, E. Martinho, and A. Chatt. Biol. Tr. Element Res. 26-7, 629 (1990).

231. R.H. de Vos, W. van Dokkum, P.D.A. Olthof, J.K. Quirijns, T. Muys and J.M. van der Poll. Food Chem. Toxicol. 22, 11 (1984).

232. W. van Dokkum, R.H., de Vos, TH. Muys and J.A. Wesstra. Br. J. Nutr. 61, 7 (1989).

233. T. Mumcu, I. Gokmen, A. Gokmen, R.M. Parr and N.K. Aras. J. Radioanal. Nucl. Chem. Art. 124, 289 (1988).

234. U. Ari, M. Volkan and N.K. Aras. J. Agric. Food Chem. 39, 2180 (1991).

235. P. Bratter, V.E. Negretti, and S.U. Rosick. Tr. Elem. Anal. Chem. Med. Biol. Proc. 3rd Int. Workshop, 29 (1984).

236. O. Oster and W. Prellwitz. Biol. Tr. Element Res. 20, 1 (1989).

237. F. Chen, P. Cole, L. Wen, Z. Mi and E.J. Trapido. J. Nutr. 124, 196 (1994).

238. P. Hakala, J. Marniemi, L.-R. Knuts, J. Kumpulainen, R. Tahvonen and S. Plaami. Food Chem. 57, 71 (1996).

239. C. Drobner, B. Rohrig, M. Anke, and G. Thomas. "Selenium Intake of Adults in Germany Depending on Sex, Time, Living Area and Type of Diet" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 158-159, 1997.

240. E.I. Hamilton and M.J. Minski. Sci. Total Env. 1, 375 (1972/1973).

241. M.C. Linder "Chapter 7. Nutrition and Metabolism of the Trace Elements" in *Nutritional Biochemistry and Metabolism with Clinical Applications.* ed. by M.C. Linder, Elsevier Science Publishing Co., Inc., New York, N.Y., p. 151, 1985.

242. H.D. Belitz and W. Grosch. *Food Chemistry*, Springer- Verlag, Berlin, Heidelberg, 1987, pg. 320.

243. B.S. Hetzel, B.J. Potter and E.M. Dulberg. World Rev. Nutr. Diet. 62, 59 (1990).

244. P.O.D. Pharoah. Am. J. Clin. Nutr. Suppl. 57, 276S (1993).

245. A.T. Diplock, B. Contempre, J. Dumont, N. Bebe and J. Vanderpas. "Interaction of Selenium and Iodine Deficiency in the Development of Human Iodine Deficiency Diseases" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 63-67, 1997.

246. P.W.F. Fischer and A. Giroux. J. Can. Diet. Assoc. 48, 24 (1987)

247. M. Dermelj, Z. Slekovec, A.R. Byrne, P. Stegnar, V. Stibilj and M. Rossbach. Fresenius' J. Anal. Chem. 338, 559 (1990).

248. M. Dermelj, V. Stibilj, J. Stekar and A.R. Byrne. Fresenius' J. Anal. Chem. 340, 258 (1991).

249. X. Hou, C. Chai, Q. Qian, C. Li, and K. Wang. Fresenius J. Anal. Chem. 357, 1106 (1997).

250. M. Anke, B. Groppel, U. Krause, W. Arnhold and M. Langer. J. Tr. Elements Electrolytes Health Dis. 5, 69 (1991).

251. P.O. Wester. Am. J. Clin. Nutr. 45, 1305 (1987).

252. P.E. Johnson and F.H. Nielsen. Adv. Meat Res. 6, 275 (1990).

253. E.J. Brink, P.R. Dekker, E.C.H. Van Beresteijn and A.C. Beynen. J. Nutr. 121, 1374 (1991).

254. J.L. Kelsay and E.S. Prather. Am. J. Clin. Nutr. 38, 12 (1983).

255. H.S. White. J. Am. Diet. Assn. 55, 38 (1969).

256. M.A. Walker and L. Page. J. Am. Diet. Assoc. 70, 260 (1977).

257. K. Shiraishi, H. Kawamura and G.-I. Tanaka. J. Radiat. Res. 27, 121 (1986).

258. P. Hendrix, R. Van Cauwenbergh, H.J. Robberecht and H.A. Deelstra. Zeitschr. fur Lebensm. Unters. Forsch. 201, 213 (1995).

259. B.G. Shah, A. Giroux, B. Belonje and P.W.F. Fischer. Nutr. Res. 11, 265 (1991).

260. M. Glei and M. Anke. Magnesium-Bulletin 17, 1 (1995).

261. M. Glei, M. Anke, and B. Rohrig. "Magnesium Intake and Magnesium Balance of Adults Eating Mixed or Vegetarian Diets" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 181-182, 1997.

262. S.D. Soman, V.K. Panday, K.T. Joseph and S.J. Raut. Health Phys. 17, 35 (1969).

263. O. Moreiras, C. Cuadrado, M. Lamand and J.C. Tressol. Nutr. Res. 13, 851 (1993).

264. T. Thulin, M. Abdulla, I. Dencker, M. Jagerstad, A. Melander, A. Norden, B. Schersten and B. Akesson. Acta Med. Scand. 208, 367 (1980).

265. A. Gormican. J. Am. Diet. Assn. 56, 397 (1970).

266. H.S. White and T.N. Gynne. J. Am. Diet. Assn. 59, 27 (1971).

267. M.W. Marshall, J.M. Iacono, C.W. Young, V.A. Washington, H.T. Slover and P.M. Leapley. J. Am. Diet. Assn. 66, 470 (1975).

268. R. Ellis, E.R. Morris, A.D. Hill and J.C. Smith Jr.. J. Am. Diet. Assn. 81, 26 (1982).

269. J.L. Greger and M.J. Baier. Food Chem. Toxicol. 21, 473 (1983).

270. J.T. Holbrook, J.C. Smith Jr. and S. Reiser. Am. J. Clin. Nutr. 49, 1290 (1989).

271. P.D. Saltman and L.G. Strause. J. Am. College Nutr. 12, 384 (1993).

272. P. Saltman. "More Precious than Gold: Trace Elements in Osteoporosis" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 517-519, 1997.

273. R.S. Gibson, A.C. Macdonald and O.B. Martinez. Hum. Nutr.: Appl. Nutr. 39A, 43 (1985).

274. L. Davidsson, A. Cederblad, B. Lonnerdal and B. Sandstrom. Am. J. Clin. Nutr. 54, 1065 (1991).

275. P.E. Johnson, G.I. Lykken and E.D. Korynta. J. Nutr. 121, 711 (1991).

276. R.S. Gibson and C.A. Scythes. Br. J. Nutr. 48,241 (1982).

277. J. Ross, R.S. Gibson and J.H. Sabry. Tropical Geogr. Med. 38, 246 (1986).

278. R.W. Wenlock and D.H. Buss. Br. J. Nutr. 41, 253 (1979).

279. J.D. Cross, I.M. Dale, R.M. Rate, H. Smith and L.B. Smith. Radiochem. Radioanal. Lett. 37, 365 (1979).

280. J.J. Fardy G.D. McOrist and Y.J. Farrar. J. Radioanal. Nucl. Chem. Articles 163, 195 (1992).

281. J.P. Buchet, R. Lauwerys, A. Vandevoorde and J.M. Pycke. Food Chem. Toxicol. 21, 19 (1983).

282. H.J. Robberecht, P. Hendrix, R. Van Cauwenbergh and H.A. Deelstra. Z. Lebensm. Unters. Forsch. 199, 446 (1994).

283. G. Ellen, E. Egmond, J.W. van Loon, E.T. Sahertian and K. Tolsma. Food Additives and Contaminants 7, 207 (1990).

284. B.E. McLeod and M.F. Robinson. Br. J. Nutr. 27, 15 (1972).

285. B.E. Guthrie and M.F. Robinson. Br. J. Nutr. 38, 55 (1977).

286. R. Barbera, R. Farre and A. Lozano. J. Micronutrient Anal. 6, 47 (1989).

287. R. Barbera, R. Farre and D. Mesado. Die Nahrung 3, 241 (1993).

288. M. Spencer, C.R. Asmussen, R.B. Holtzman and B. Kramer. Am. J. Clin. Nutr. 32, 1867 (1979).

289. D.B. Milne, D.D. Schnakenberg, H.L. Johnson and G.L. Kuhl. J.. Am. Diet. Assoc. 76, 41 (1980).

290. G. Chazot and E. Brousolle. "Alterations in Trace Elements During Brain Aging and in Alzheimer's Dementia" in *Essential and Toxic Trace Elements in Human Health and Disease: An Update*, p. 269, 1993.

291. N.D. Priest. Proc. Nutr. Soc. 52, 231 (1993).

292. J.L. Greger. Annu. Rev. Nutr. 13, 43 (1993).

293. J.J. Powell and R.P.H. Thompson. Proc. Nutr. Soc. 52, 241 (1993).

294. Y. Wang, C. Lu, Z. Xiao, G. Wang, S.S. Kuan, E.J. Rigsby. J. Agric. Food Chem. 39, 724 (1991).

295. Anonymous. Nutr. Rev. 49, 287 (1991).

296. H.J.M. Bowen and A. Peggs. J. Sci. Food Agric. 35, 1225 (1984).

297. N.W. Solomons, *Absorption and Malabsorption of Mineral Nutrients*, Alan R. Liss Inc., 1984, p. 269.

298. A. Chatt and J. Holzbecher, "Determination of Aluminum in Biological Materials by Neutron Activation Analysis." The 1989 Congress of Pacific Basin Societies, Honolulu, Hawaii, December 17-22.

299. Shahla Shakerinia. *Determination of Aluminum in Biological Materials by Instrumental and Preconcentration Neutron Activation Analysis.* Bachelor of Science Honours Report, Dalhousie University, 1991.

300. I.H. Tipton, P.L. Stewart and J. Dickson. Health Phys. 16, 455 (1969).

301. M. Nabrzyski and R. Gajewska. Z. Lebensm. Unters. Forsch. 201, 307 (1995).

302. K. Shiraishi, K. Yoshimizu, G. Tananka and H. Kawamura. Health Phys. 57, 551 (1989).

303. M. Muller, M. Anke, and H. Illing-Gunther. " *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 177-178, 1997.

304. M.J. Barger-Lux and R.P. Heaney. J. Nutr. 124, 1406S (1994).

305. Osteoporosis Society of Canada. Nutr. Quarterly 18, 62 (1994).

306. G.D. Miller and C.M. Weaver. J. Nutr. 124, 1404S (1994).

307. G. Ewan. Nutr. Quarterly 18, 58 (1994).

308. R.P. Heaney. Annu. Rev. Nutr. 13, 287 (1993).

309. A.A. Licata. World Rev. Nutr. Diet. 73, 27 (1993).

310. L. Nilas. World Rev. Nutr. 73, 1 (1993).

311. D.I. Levenson and R.S. Bockman. Nutr. Rev. 52, 221 (1994).

312. C. Hasling, K. Sondergaard, P. Charles and L. Mosekilde. J. Nutr. 122, 1119 (1992).

313. K.H. Fleming and J.T. Heimbach. J. Nutr. 124, 1426S (1994).

314. K.M. Chapman, M.W. Chan and C.D. Clark. J. Am. College Nutr. 14, 336 (1995).

315. F. Bronner. J. Nutr. 122, 641 (1992).

316. Osteoporosis Society of Canada. Nutr. Quarterly 18, 70 (1994).

317. U.S. Srivastava, A.K. Rakashit and I. Khare. Nutr. Rep. Int. 18, 313 (1978).

318. A.J. Speek, S. Speek-Saichua and W.H.P. Schreurs. Food Chem. 40, 251 (1991).

319. B. Borgstrom, A. Norden, B. Akesson and M. Jagerstad. Scand. J. Social Med. Suppl. 10, 14 (1975).

320. S.M. Walberg and W.S. Adams. J. Am. Diet. Assoc. 47,37 (1965).

321. R. Manalo and J.E. Jones. Am. J. Clin. Nutr. 18, 339 (1966).

322. J.M. Krebs, V.S. Schneider, A.D. Leblanc, M.C. Kuo, E. Spector and H.W. Lane. Am. J. Clin. Nutr. 58, 897 (1993).

323. B.G. Shah and B. Belonje. Nutr. Res. 3, 629 (1983).

324. N.L. Bull and D.H. Buss. Proc. Nutr. Soc. 39, 30A-31A (1980).

325. M.K. Sweeten, G.C. Smith and H.R. Cross. J. Food Qual. 9, 263 (1986).

326. S. Fairweather-Tait. Proc. Nutr. Soc. 54, 465 (1995).

327. World Health Organization. *Diet, nutrition and the prevention of chronic disease.* World Health Organization, Switzerland 1990.

328. S. Fairweather-Tait. Int. J. Vit. Nutr. Res. 63, 296 (1993).

329. Y.-D. Zhou and H.C. Brittin. J. Am. Diet. Assoc. 94, 1153 (1994).

330. R.J. Neale. "Meat Iron Bioavailability: Chemical and Nutritional Considerations" in *The Chemistry of Muscle-based Foods.* ed. D.E. Johnson, M.K. Knight and D.A. Ledward, Royal Society of Chemistry, p. 183, 1992.

331. E.R. Monsen, I.N. Kuhn and C.A. Finch. Am. J. Clin. Nutr. 20, 842 (1967).

332. C.T. Walsh, H.H. Sandstead, A.S. Prasad, P.M. Newberne and P.J. Fraker. Env. Health Perspect. 102 (Suppl. 2), 5 (1994).

333. U.S. Srivastava, M.H. Nadeau and N. Carbonneau. J. Can. Diet. Assn. 38, 302 (1977).

334. P.B. Moser and R.D. Reynolds. Am. J. Clin. Nutr. 38, 101 (1983).

335. L. Hussein and J. Bruggeman. Food Chem. 58, 391 (1997).

336. T.D.B. Lyon, H. Smith and L.B. Smith. Br. J. Nutr. 42, 413 (1979).

337. M. Anke, M. Muller, M. Glei, H. Illing-Gunther, A. Trupschuch, M. Seifert, O. Seeber, and B. Rohrig. "Zinc Intake and Zinc Balance of Adults in Central Europe - Depending on Time, Sex, Living Area, Eating Habits, Age and Live Weight" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 160-164, 1997.

338. D. Osis, E. Wiatrowski and H. Spencer. Am. J. Clin. Nutr. 25, 582 (1972).

339. E.D. Brown, M.A. McGuckin, M. Wilson and J.C. Smith. J. Am. Diet. Assn. 69, 632 (1976).

340. L.M. Klevay, S.J. Reck and D.F. Barcome. J. Am. Med. Assoc. 241, 1916 (1979).

341. J.M. Holden, W.R. Wolf and W. Mertz. J. Am. Diet. Assoc. 75, 23 (1979).

342. J.D. Cross, R.M. Raie, H. Smith and L.B. Smith. Radiochem. Radioanal. Lett. 35, 291 (1978).

343. F.H. Nielsen. Annu. Rev. Nutr. 4, 21 (1984).

344. K. Yokoi, M. Kimura and Y. Itokawa. Biol. Tr. Element Res. 51, 199 (1996).

345. M. Anke, H. Gurtler, L. Angleow, J. Gottschalk, C. Drobner, S. Anke, H. Illing-Gunther, M. Muller, W. Arnhold and U. Schafer. "Rubidium- An Essential Element for Animals and Humans" in *Trace Elements in Man and Animals - 9. Proceedings of the Ninth International Symposium on Trace Elements in Man and Animals.* P.W.F. Fischer, M.R. L'Abbe, K.A. Cockell and R.S. Gibson eds., NRC Research Press, Ottawa, pp. 189-191, 1997.

346. E.O. Uthus and C.D. Seaborn. J. Nutr. 126 Supplement, 2452S (1996).

347. E. Sabbioni, E. Marafante, R. Pietra, L. Goetz, F.Girardi and E. Orvini. "The Association of Vanadium with the Iron Transport System in Human Blood as Determined by Gel Filtration and Neutron Activation Analysis" in *Nuclear Activation Techniques in the Life Sciences 1978. Proceedings of an International Symposium on Nuclear Activation Techniques in the Life Sciences held by the International Atomic Energy Agency in Vienna, 22-26 May 1978.* International Atomic Energy Agency, Vienna, pp. 179, 1979.

348. K.E. Hill, Y. Xia, B. Akesson, M.E. Boeglin and R.F. Burk. J. Nutr. 126, 138 (1996).

349. B.F. Harland and B.A. Harden-Williams. J. Am. Diet. Assn. 94, 891 (1994).

350. D.R. Myron, T.J. Zimmerman, T.R. Shuler, L.M. Klevay, D.E. Lee and F.H. Nielsen. Am. J. Clin. Nutr. 31, 527 (1978).

351. H. Illing-Gunther, M. Anke, M. Muller, A. Trupschuch and S. Anke. "Biological Importance, Analysis and Supply of Vanadium" in *Nuclear Activation Techniques in the Life Sciences 1978. Proceedings of an International Symposium on Nuclear Activation Techniques in the Life Sciences held by the International Atomic Energy Agency in Vienna, 22-26 May 1978.* International Atomic Energy Agency, Vienna, pp. 179-180, 1997.

352. A. Krushevska and R.M. Barnes. Analyst 119, 131 (1994).

353. T. Berg, O. Royset and E. Steinnes. Elemental Monitoring and Assessment 31, 259 (1994).

354. M. Krieg and J. Einax. Fresenius J. Anal. Chem. 348, 490 (1994).

355. M.J. Latorre, C. Garcia-Jares, B. Medina and C. Herrero. J. Agric. Food Chem. 42, 1451 (1994).

356. F. Rincon, R. Moreno, G. Zurera and M. Amaro. J. Dairy Res. 61, 262 (1994).

357. L. Currie. "The Importance of Chemometrics in Biomedical Measurements" in *ACS 445. Biological Trace Element Research. Multidisciplinary Perspectives,* American Chemical Society, Washington, DC, p. 75, 1991.

358. M.A. Sharaf, D.L. Illman and B.R. Kowalski. *Chemometrics.* John Wiley and Sons Inc., New York, 1986.

359. D.L. Massart, B.G.M. Vandeginste, S.N. Deming, Y. Michotte and L. Kaufman. *Chemometrics: a Textbook.* Elsevier Science Publishers, Amsterdam, 1988.

360. S.J. Haswell. *Practical Guide to Chemometrics.* Marcel Dekker Inc., New York, 1992.

361. J.G. Lease. Nutr. 93, 523 (1967).

362. B.R. Schricker, D.D. Miller, R.R. Rasmussen and D. Van Campen. Am. J. Clin. Nutr. 34, 2257 (1981).

363. S. Reiser, J.C. Smith Jr., W. Mertz, J.T. Holbrook, D.J. Scholfield, A.S. Powell, W.K. Canfield and J.J. Canary. Am. J. Clin. Nutr. 42, 242 (1985).

364. G.V. Iyengar, Report on the First Research Coordination Meeting, Co-ordinated Research Programme on Human Daily Dietary Intakes of Nutritionally Important Trace Elements as Measured by Nuclear and Other Techniques, Gaithersburg, Beltsville and Washington, D.C., U.S.A., 19-22 March 1985, Annex 14).

365. M.L.G. Gardner. Ann. Rev. Nutr. 8, 329 (1988).

366. E J. Brink, A.C. Beynen, P.R. Dekker, E.C.H. van Bereseijn and R. van der Meer J. Nutr. 122, 580 (1992).