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STABLE ISOTOPES USED IN NUTRITION RESEARCH: SOME ASPECTS OF SAFETY CONSIDERATIONS

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ABSTRACT: One of the major advantages of using stable isotopes in nutrition research is the effectiveness of using them as tracers for *in vivo* studies in human subjects. In particular, they are suitable for application during the entire human life cycle, namely embryo to elderly stages. Considering that there is a link between dosage and isotopic ratio measurements (precision, accuracy and adequate sensitivity of the technique to safeguard the required detection limits), this factor may be expected to influence the level of tracer administered. This becomes an important issue in carrying out nutritional studies in sensitive population groups such as pregnant women, infants and children. Although for most mineral stable isotopes the dose used as tracers is generally recognized to be safe, no specific safety guidelines seems to be currently available. Hence selected literature sources are examined to formulate a possible basis for evaluating the safety aspects of stable isotope usage. The discussion has been extended to include a brief comparison with radioactive isotopes usage to get an overall perspective of the increasing awareness to the use of stable isotopes in biomedical research studies.

Introduction

The first stable isotopic tracer used in metabolic research was deuterium, the heavy isotope of hydrogen, as reported by Schoenheimer and Rittenberg in 1935. At that time, it was predicted that "The isotopes of those elements which present in natural organic compounds, presented to the biochemist by physical chemist will certainly furnish a better insight into the details of the intricate mechanism" (Schoenheimer, Rittenberg, 1935). The use of stable mineral isotopes as research tools began in 1963 with Lowman and Krivit, who used radioactive ^{59}Fe combined with stable ^{58}Fe to determine plasma iron clearance (Jones, 1990). Recently, awareness about safety aspects (advantages and disadvantages) of using radioactive and stable isotopes has increased. Specially when carrying out an in-vivo nutritional bioavailability study in high-risk populations such as pregnant women and children, stable isotopes remain as the choice over radioactive isotopes (Turnlund, 1989; Jones, Leatherdale, 1991; Santiago, Bargbosa, 1995; de Meer et al., 1999; Abrams, Wong, 2003). With the development of new techniques like new applications of mass spectrometry, stable isotopic tracers have been used more and more frequently in biomedical research, especially for carrying out nutrient bioavailability (Bier, 1997; Walczyk, 2001).

The purpose of this report is to review briefly available information concerning the safe use of stable isotopes. The stable isotopes of light elements will be addressed briefly, while the emphasis will be placed on the safety limits of heavy (mineral) stable isotopes. Further, for the purpose of comparison, safety issues related to radioisotopes will also be included.

General consideration in using stable isotopes in human nutrition research

Care has to be taken that stable isotopes are chemically pure, and in the case of intravenous (iv) applications, they are sterile and pyrogen free (Thompson et al., 1989; Koletzko et al., 1997). An additional concern is raised when the labels, for deuterium water for example, includes a warning stating that "not for human use". The producer provides standard information on isotopic enrichment and the chemical impurities, if any, of the stable isotopes. They can also make special analysis on request, to determine for example the pyrogenicity. But the producer does not assume responsibility for using in human studies because they have not tested it by themselves. In the scientific circle, it is presumed to be of good quality for human use and is being used in such human studies, despite concerns about questionable labeling practices. Given this situation some researchers are confused and the concern for safety is still a lingering issue on this account.

Concerning mineral isotopes, it is important to recognize that the administrated isotope dosage does not exceed the recommended dietary intake for the mineral in question. It is also important to ensure that

the amount of isotope administered is sufficient to yield an average change in isotope ratio of at least 10 times the standard deviation of the measurements. An optimum precision is achievable when the isotope ratio(s) (R) does not deviate from the unity (0.1<R<1) (Crews et al., 1994).

Stable isotopes are administered directly as tracers or through labeled foods. Extrinsic labeling of food is achieved by adding the label to the foodstuff prior to feeding while intrinsic mode is through incorporating the label during food formation. Most bioavailability studies with stable mineral isotopes use extrinsic labels because it is the simplest and cheapest method. Some studies have shown similar absorption factors for Zn and Cu, irrespective of the mode of labeling (Aggett, 1997). However, some differences in absorption have been noted for the labels (extrinsic vs. intrinsic) for example for Se (selenite or selenomethionine) due to differences in the metabolism (Crews et al., 1994). In his recent publication, Dainty (2001) stated that out of the four minerals studied (Ca, Zn, Cu and Se), only Ca the oral and intravenous (iv) administered tracers were handled the same way by the body. Therefore the use of iv with other minerals should be viewed with caution. Table 1 shows some minerals essential to humans, and the use of their stable isotopes in nutritional research. The trace elements of interest in nutrition research that could make use of isotopic labels are iron, zinc, calcium, magnesium, copper and selenium.

Concerning costs, in general the lower the natural abundance of an isotope, higher is the requirement for enrichment, thus offering to be a good spike. Of course prices depends on enrichment and quantities. This can be seen on its price (Table 2). The price for deuterium oxide (99.9% enriched) is US $ 0.5–1/g or US$ 80/l. The price of $^{18}O_2$-Water (10% enriched) is US $ 9–10/g and for $^{18}O_2$-Water (95% enriched) is US $140–150/g. Currently, the former Soviet Union is a good source of stable isotopes sold through several companies in US and Europe. The Oak Ridge National laboratories (ORNL) used to be the main supplier for the mineral stable isotopes. It seems they are not operating now but still can offer isotopes produced prior to 1990 (Abrams, Wong, 2003).

Definitions and contradictions

Isotopes

In biological research, the term stable isotope is used to describe a chemical form of an element, which is non-radioactive and less abundant (Abrams, 1999; Junqueira-Franco et al., 1999; Young, Ajami, 1999) than the predominant and naturally occurring chemical form of an isotope. Chemically speaking, all forms of an element, except the radioactive ones, are stable.

The main (in terms of abundance) stable isotope of an element has the same number of protons and neutrons in its nucleus. Sometimes more than one stable combination is possible, in which case the number of neutrons may vary, giving the element a different mass. In fact, most chemical elements occur naturally as a mixture of stable isotopes. For example, calcium has the following six stable isotopes forms ^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca and ^{48}Ca, with natural abundances (atom %) of 96.9%, 0.6%, 0.1%, 2.1%, 0.004% and 0.2%, respectively. In contrast, radioisotopes are not naturally present (Mellon, Fairweather-Tait, 1997; Patterson, Veillon, 2001), except for a few long living radionuclides (Walczyk, pers.comm.). Enriched stable isotopes are man-made combinations in which the ratio of different isotopes differs from the naturally existing ratio (Mellon, Fairweather-Tait, 1997). For example, one could have a sample with 83.9% enrichment of

Table 1: Minerals essential to humans and the use of their stable isotopes in nutritional research (King et al., 1978; Mellon, Sandstrom, 1996; Abrams, 1999).

	Total body content (mg/kg)	Plasma/serum concentration	Absorption (%)	Tracer amounts	
				Children	Adults
Ca	14,000	2.2–2.5 mmol/l	30–60	^{42}Ca 1–2 mg iv*; ^{44}Ca 3–16mg iv (10–15mg po); ^{46}Ca 15–20 µg po**	^{42}Ca 4 mg po; ^{48}Ca 10mg po;
Mg	270	0.8–1.2mmol/l	25–45	^{25}Mg 0.3–0.5 mg/kg iv; ^{26}Mg 0.2–0.4 mg/kg iv	
Fe	60	14–32 µmol/l	0–40	^{57}Fe^3 5–15 mg po;	^{54}Fe 5–25 mg po;
				^{58}Fe^3 1–3 mg po (0.2–0.4 mg iv)	^{58}Fe 2.7 mg po
Zn	33	9–22 µmol/l	15–40	^{67}Zn 1–3 mg po; ^{70}Zn 0.2–0.5mg iv	^{70}Zn 3.6–13.6 mg po (iv)
Cu	1	13–22 µmol/l	30–40		^{65}Cu 3.6 mg po
Se	0.05	0.7–1.5 µmol/l	60–80		^{74}Se 100–200 µg po

* iv — intravenously; ** po — orally.

Table 2. Isotopic composition, natural abundance, enrichment and cost of some minerals used in nutritional studies (*).

Elements	Natural abundance %	Enrichment (Atom %)	Enrichment Avail. From Trace (atom%)	Cost 2003 dollar/mg ($/mg)
Fe-54	5.8	98.4	99+	15.00
Fe-56	91.7	99.9	99.5	0.75
Fe-57	2.2	93.5	95+	8.00
Fe-58	0.3	82.5	90	75.00
Zn-64	48.6	99.7	same	3.50
Zn-66	27.9	99.3	96+	4.50
Zn-67	4.1	94.6	88.6	20.50
Zn-68	18.8	99.7	95+	1.90
Zn-70	0.6	88.2	95+	80.00
Se-74	0.9	77.7	98.5	17.50
Se-76	9.0	97.1	90	14.25
Se-77	7.6	94.4	65+	10.00
Se-78	23.5	98.8	97+	8.50
Se-80	49.6	99.5	99.2	3.50
Se-82	9.4	97.4	90+	20.00
Ca-40	96.9	99.9	same	0.55
Ca-42	0.6	94.4	87.8	35.00
Ca-43	0.1	83.9	52	100.00
Ca-44	2.1	98.8	95.9	15.00
Ca-46	0.004	30.9	7.5	135.00
Ca-48	0.187	97.8	89.5	125.00
Cu-63	69.17	99.88	98.5	1.65
Cu-65	30.83	99.69	99.5	3.50
Mg-24	78.99	99.92	same	1.20
Mg-25	10.00	98.81	99.0	7.00
Mg-26	11.01	99.56	99.6	6.15

*Courtesy: Trace Sciences International Corp., 40 Vogell Road, Suite 42, Richmond Hill, ON. Canada L4B 3N6; Internet: www.isotopetrace.com; E-mail: websales@isotopetrace.com

^{43}Ca; this would change the normal predominance of ^{40}Ca in the sample.

Non-metal elements used in biomedical research such as ^{13}C, ^{15}N, ^{18}O and ^{2}H (deuterium) are referred to as light isotopes. The human body contains substantial quantities of stable isotopes. A person who weights 50 kg, for example, has 225 g of "light" isotopes ^{2}H, ^{15}N, ^{17}O, and ^{18}O. In contrast, metal elements used in biomedical research, such as iron, calcium, chromium, copper, zinc, magnesium, selenium, and molybdenum, are called heavy isotopes.

Tracer

There seems to be some difference of opinion concerning identifying stable isotopes as true tracers. This arises from the fact that a tracer is defined as "a marked form of a substance that is used to determine certain properties of the substance in a biological system such as pathways through chemical reactions, transfer rates, its localization, exchangeable mass or volume" (Young, Ajami, 1999). A true tracer, therefore, is one that does not disturb the system investigated (Dainty, 2001). It is quite possible that when certain stable isotopes are administered in mg quantities, (e.g. Fe and Zn), the term tracer comes into question. Therefore, according to another opinion the term "label" might be more appropriate for stable isotopes (Dainty, 2001). For stable isotopes to be useful as labels they must be determinable above the natural abundance of endogenous isotopes in the samples.

Radioactive vs. stable isotopes in human nutrition research

There is evidence that low ionizing radiation doses of low linear energy transfer have no harmful effects (Viteri, Warren, 2002). Yet, a certain stigma exists that at any concentration level radioisotope usage is harmful for human subjects. There are some beneficial effects of radioisotopes, e.g. cancer treatment and this is recognized. However, for metabolic studies of healthy subjects, they are generally unwelcome while in animal studies and in *in-vitro* studies related to human health they are used. In bioavailability studies for nutrients usually a combination of both radioisotopes and stable isotopes is used. While radioisotopes find their application in duplicating physiological conditions under laboratory conditions (simulation), stable isotopes find direct use in *in vivo* investigations. With modern instrumentation reaching near perfection in their detection capabilities and permitting use of extremely small quantities of radioactivity, the safe usage of radioisotope in human nutritional studies should be re-explored (Viteri, Warren, 2002).

The following qualities make stable isotopes ideal tools for research: (i) the chemical properties of an isotopic label and those of the native element are identical (Walczyk, pers.comm.), except for mass; (ii) Even very small amounts of the isotopic element can be quantified (Walczyk, pers.comm.) due to the precision and sensitivity of the instrumentation. Therefore, stable isotopes can easily be followed *in vivo*; and (iii) since stable isotope labels are the same ones as those already existing in nature, only the changes in abundance ratio will make an indirect quantification possible. Walczyk (2001) notes "stable isotope techniques and radioisotope methods are the only reliable tools available for determination of the absorption, retention or utilization of a nutrient by the human body i.e. its bioavailability". Moreover, the safety of stable isotopes makes them especially important tools for drug metabolism studies, particularly in high-risk groups (Abramson, 2001).

The advantages and disadvantages of using radioactive and stable isotopes are summarized in the Table 3.

Table 3. Advantages and disadvantages of radioactive and stable isotopes.

Isotopes / Utility	Radioisotopes	Enriched Stable Isotopes
Advantages	• authentic tracers[1] • easily detectable[2] • generally inexpensive[1] • sample preparation minimal[4] • whole body measurement[4], retention can be determined	• minimal health risk, can be used in infants, pregnant and lactating women[3] • multi-elements procedure[2] • tracers may be followed for longer periods[1] • samples can be stored without loss of tracers[1] • reanalysis possible[3]
Disadvantages	• safety concerns, some risk through exposure to radiation[5] • unsuitable for infants and children[2], pregnant and lactating women[3] • decay time[2] • only one radioactive element can be studied[2] • sample analysis must be timed based on half-life[2] • expensive waste problem[5]	• not true tracers, larger amount needed[1] • expensive[2] • extensive sample preparation[1] • still complex and costly analysis[2] • direct determination of retention not possible[6]

[1] Mellon, Fairweather-Tait, 1997; [2] Mellon, Sandstrom, 1994; [3] Viteri, Warren, 2002; [4] Turnlund, 1989; [5] Abrams, 1999; [6] Abramson, 2001.

Differences in physical, chemical and biochemical behavior of isotopes

Due to their mass variations, stable isotopes can demonstrate differences in their physical, chemical and biochemical behavior, resulting in kinetic and thermodynamic effects (King et al., 1978). Adverse effects have been observed (Koletzko et al., 1997) with low atomic weight elements such as deuterium (double mass of the hydrogen), which at high doses that can even be lethal. However, the doses applied as tracers are much below such lethal levels, ranging from 1-80 mg deuterium/kg body weight, while the threshold associated with relevant side effects lies in the range of 200–400 mg deuterium/kg body weight (Koletzko et al., 1997, 1998).

The amount of 20 mg of deuterium/kg of body weight for neonatal in energy studies or enrichment studies in animals and humans up to 60% for ^{13}C and ^{18}O rarely exceeds 25 mg/kg body weight and had not shown adverse effects (Koletzko et al., 1998). The natural abundance of stable isotopes in carbon containing molecules in the human body, for example, is in the order of 2,000 mg of 13 C /kg body weight (Koletzko et al., 1997, 1998).

Minerals have higher atomic weights than light elements and consequently the relative mass differences between different isotopes are very small. As expected, since the physicochemical characteristic of these isotopes does not change to any relevant or detectable degree, the probability of side effects in total body enrichment is small. Isotope dilution studies with stable minerals are therefore very unlikely to have *in vivo* effects large enough to be detectable (Koletzko et al., 1997). Therefore, it seems the margin of safety in the application of stable mineral isotopes is larger. An exception to this seems to be the alkali metal lithium (Lieberman et al., 1986). It has two stable isotopes: ^{7}Li (92.58%) and ^{6}Li (7.42%). Different biochemical, pharmacological, behavioral and toxicological effects have been demonstrated for ^{6}Li. In rat studies, the LD-50 for ^{6}Li was 13meq/kg and the LD-50 for ^{7}Li was 16meq/kg. Mortality, death and decrease of spontaneous motility occurred earlier for ^{6}Li animals. *In vitro* uptake studies in human erythrocyte membrane have also shown a greater accumulation of ^{6}Li than ^{7}Li.

Walczyk (2001) has reported in investigations using NTI-MS (negative thermal ionization), that "iron circulating in human body is depleted of heavier isotopes, and that the extent of this mass-dependent isotopic fractionation effect differs slightly between healthy males". These findings can have the following implications: a. Open new possibilities of using Fe isotope effects to trace back the origin of a food, and b. Such an effect might indicate future problems in using stable isotopes.

Certainly further investigation is needed to evaluate these effects and whether there are dosage limits for safe use of mineral stable isotopes in human research.

Analytical methods used for mineral stable isotopes

The first studies with mineral stable isotopes used Neutron Activation Analysis as the analytical method, but this methodology lacks sensitivity. It was not until the late 1970's in combination with mass spectrometry (MS), that the use of stable isotopes (King et al., 1978) was well adopted as a tool for nutrition research. Since several types of MS exist, the appropriate one has to be chosen according to the study, required sensitivity, time and cost factors (Jones, Leatherdale, 1991). There are several mass spectrometry methods available. The most widely used are Gas Chromatography Mass Spec-

Table 4. Methods for stable isotope analysis of the most common minerals in nutrition research (Turnlund, 1983; Crews et al., 1994; Aggett, 1997; Bier, 1997; Walczyk, 2001).

Method	Minerals in nutrition	Precision	Tracer / Tracee ratio (%)	Analytical level required / preparation (↑↓)	Matrix
Neutron Activation Analysis	Zn, Fe, Ca, Mg, Cu	1–10%			plasma
Thermal ionization (TIMS) Magnetic Quadrupole	Ca, Cu, Fe, Mg, Zn	 <0.1% 0.1–1%		<µg (↑)	solid samples?; blood, plasma, urine, faeces
Inductively coupled plasma MS (ICP-MS) quadrupole	Mg, Ca, Fe, Cu, Zn, Se	0.3–1%		(↓)	blood, plasma, urine, faeces tissue
Fast atom bombardment MS		0.3–2%		100ng–1µg (↓)	plasma. urine
Isotope ratio GC/ MS		0.001	0.0005–0.01	µg	breath, urine
Gas chromatography MS (GC-MS)	Ca, Cu, Fe, Mg, Zn, Se	0.1–10%	0.1–100	picogram (10^{-3}µg)	plasma, urine

↑ preparation demanding ;↓ preparation simple.

trometry (GC-MS), Thermal Ionization Mass Spectrometry (TIMS), Fast Atom Bombardment Mass Spectrometry (FABMS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). These various methods are compared in Table 4.

Monoisotopic elements

Only elements that have two or more naturally occurring isotopes can be used in stable mineral enrichment studies (Turnlund, 1983). Monoisotopic elements (only one naturally isotope) cannot be studied by methods based on mass difference and isotope ratios. The important monoisotopic elements in nutritional studies (Walczyk, 2001) are: F, I, Na, Mn, and P.

However, monoisotopic elements, as well as multiisotopic elements can be studied by themselves using the following analytical techniques: Ion Chromatography (IC), Flame-Atomic Absorption Spectrophotometry (F-AAS), Graphite furnace-AAS (GF-AAS), Atomic Emission Spectrophotometry/Flame emission spectrophotometry (AES/FES), Inductively coupled plasma AES (ICP-AES), Instrumental Neutron Activation Analysis (INAA), Radiochemical Neutron Activation Analysis (RNAA), Photon Neutron Activation Analysis (PAA), Epithermal Neutron Activation Analysis (ENAA), Inductively coupled plasma mass spectrometry (ICP-MS), Prompt Gamma Activation Analysis (PGAA) and Proton Induced X-ray fluorescence (PIXE). These are summarized (WHO, 1996) well in Table 5.

Conclusions

1. Despite the wide commercial availability of stable isotopes, there are currently no official guidelines, national or international, on their use in nutritional research studies (Thompson et al., 1989). Generally, individual ethical committees assume responsibility for the approval of stable isotopes' usage for the proposed research projects (Thompson et al., 1989). Ambiguous circumstances are faced concerning warning for deuterium water labeling that should be clarified. Consultations by an expert group can help to resolve the safety concerns and develop guidelines for future use.

2. The increased sensitivity of new mass spectrophotometers is a great advancement for nutritional and metabolic studies. It allows a greater precision, accuracy, and the use of smaller samples and lower risks to the subjects.

3. Stable isotopes have been used for about 50 years for metabolic research. To our knowledge, no adverse effects from light or heavy stable isotopes have been reported at the levels used in clinical research. The safety margin in the use of light stable isotopes is quite large, as described by Jones and Leatherdale (1991).

4. There seems to be a general agreement that biological effects of labeled compounds of higher elements (minerals) are minimal because of smaller mass differences, and almost identical physical and chemical properties between stable isotopes and their predominant form (Jones, Leatherdale, 1991). Also, the threshold of toxicity for these elements is far in excess of the concentrations used in human studies.

5. There is also concern on standardization of new techniques and methods for "heavy" isotopes. The "light" isotopes users have been successful in accomplishing substantial standardization of procedures (Walczyk et al., 2002) and it should also be possible for the heavy isotopes group to accomplish similar success.

6. The IAEA has been working to strengthen the application of nuclear and isotopic techniques in applied human nutrition research. The IAEA through its Coordinated Research projects- and Technical Cooperation (TC) projects- has been able to achieve some

Table 5. Analytical Techniques for the Determination of Minor and Trace Elements in Foods and Related Biomaterials, (slightly modified and updated from WHO, 1996).

Analyte	Simple and Practical	High Costs, Greater Skills	Limited Use
Frequently Determined Minor Elements			
Calcium	F-AAS, AES/FEES	ICP-AES, INAA	PIXE, X-RAY
Chlorine	SP	IC, INAA	PGAA, PIXE, X-RAY
Magnesium	F-AAS	ICP-AES	INAA
Phosphorus	SP	ICP-AES	PGAA, PIXE, X-RAY
Potassium	F-AAS, AES	ICP-AES, INAA	PGAA, PIXE, X-RAY
Sodium	F-AAS, AES	ICP-AES, INAA	PGAA, PIXE, X-RAY
Sulfur	SP	IC	PGAA, PIXE, X-RAY
Frequently Determined Trace Elements			
Cadmium	ASV	GF-AAS, RNAA	ICP-AES
Copper	F-AAS, ASV, SP	ICP-AES, RNAA, ICP-MS	PIXE, X-RAY
Iodine	POT (CAT, ISE)	RNAA, ICP-MS	EPNAA, PAA, X-RAY
Iron	F-AAS, ASV, SP	GF-AAS, INAA, ICP-AES, ICP-MS	PIXE, X-RAY
Lead	ASV	GF-AAS, ICP-AES, ICP-MS	IDMS, PIXE, X-RAY
Mercury		CV-AAS, INAA	GC
Selenium	SP (FLU)	HG-AAS, INAA, ICP-MS	GC-MS
Zinc	F-AAS, ASV, SP	GF-AAS, INAA, ICP-AES, ICP-MS	PIXE, X-RAY
Less Frequently Determined Trace Elements			
Aluminium		GF-AAS, ICP-AES	INAA
Arsenic	SP	HG-AAS, RNAA	X-RAY
Barium	F-AAS, AES	INAA, ICP-AES	X-RAY
Boron	SP, AES	ICP-AES	NA-MS, PGAA
Bromine		INAA	X-RAY, PIXE
Cesium	F-AAS, AES	INAA, ICP-AES	
Chromium		GF-AAS, INAA, RNAA	GC-MS
Cobalt	SP	GF-AAS, INAA, ICP-AES	
Fluorine	POT (ISE), SP	IC	INAA
Lithium	AES	F-AAS/GF-AAS, ICP-AES	IDMS, NA-MS
Manganese	F-AAS	GF-AAS, ICP-AES, INAA	PIXE, X-RAY
Molybdenum	SP	GF-AAS, INAA/RNAA	ICP-AES
Nickel	ASV, SP	GF-AAS, ICP-AES	NAA, X-RAY
Rubidium	F-AAS, AES	GF-AAS, INAA, ICP-AES	X-RAY
Silicon	SP	ICP-AES	NAA, PIKE, X-RAY
Strontium	F-AAS	GF-AAS, INAA, ICP-AES	X-RAY
Tin		HG-AAS, INAA/RNAA	ICP-AES
Vanadium		GF-AAS, RNAA	ICP-AES

Methods: AAS — Atomic Absorption Spectrophotometry, CV-AAS — Cold Vapor AAS; F-AAS — Flame AAS; GF-AAS — Graphite Furnace AAS; HG-AAS — Hydride Generation AAS; AES — Atomic Emission Spectrophotometry, FES — Flame Emission Spectrophotometry; ICP-AES — Inductively Coupled Plasma AES; ASV — Anodic Stripping Voltammetry; POT — potentiometry (specific versions); CAT — Catalytic Techniques; ISE — Ion Selective Electrode; IC — Ion Chromatography; MS — Mass Spectrometry; GC-MS — Gas ChromotographyMS, ICP-MS — Inductively Coupled Plasma MS; IDMS — Isotope Dilution MS; NA-MS — Neutron ActivationMS; NAA — Neutron Activation Analysis; ENAA — Epithermal NAA; INAA — Instrumental NAA; PAA — Photon Activation Analysis; PGAA — Prompt Gamma Activation Analysis; RNAA — Radiochemical NAA; SP — Spectrophotometry (specific version for Se — Fluorometry); XRF — X-Ray Fluorescence; PIXE — Proton Induced X-Ray Fluorescence.

sound results. The TC project in Latin America has helped the joint FAO/WHO/UNU experts committee to establish new energy recommendations, for the first time using data from developing countries. The same TC project has shown on its evaluation in Chile that the National Nutrition program was delivering more energy than necessary for the children leading to obesity, and thus changes were necessary (Velasquez et al., 2002). In Mexico, the evaluation has shown that the iron form used to fortify the corn tortillas was not bioavailable due to its high phytate content, therefore another form of iron had to be introduced.

7. Finally, although the effects of stable isotopes, especially minerals, are considered negligible, it is in the interest of everyone to minimize the amounts used — yet without sacrificing the accuracy and precision of measurements.

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