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# RECENT DEVELOPMENTS AND FUTURE DIRECTIONS FOR STABLE ISOTOPE APPLICATIONS IN NUTRITION RESEARCH

Report of a Consultants Meeting

Vienna, Austria , 4 – 8 December 2000

INTERNATIONAL ATOMIC ENERGY AGENCY

*EDITORIAL NOTE*

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# RECENT DEVELOPMENTS AND FUTURE DIRECTIONS FOR STABLE ISOTOPE APPLICATIONS IN NUTRITION RESEARCH

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## SUMMARY

The International Atomic Energy Agency (IAEA) at its Headquarters in Vienna convened a consultants meeting to provide the Agency with an overview of the current status of isotopic techniques in nutritional science with respect to both methodology and applications. The main objectives were:

- To assess the practice of stable isotope methodologies in human nutrition research
- To explore high quality stable isotope spikes for use in humans
- To standardise the mathematical approaches to evaluate mass spectrometric data when using stable isotope labels within metabolic studies
- To identify new strategies for improving sensitivity of nutrition monitoring techniques for use in projects in nutrition.

This exercise was conducted to also identify strengths and weaknesses of methodologies currently used in IAEA funded research (CRPs and Technical Cooperation Projects) and to see how they can be improved for the general user, and to provide a basis for the assessment of outcomes delivered by collaborating laboratories in IAEA funded studies. The consultants who participated in the meeting were: Dr. William A. Coward, Dr. Rosalind S. Gibson, Dr. Anura V. Kurpad, Dr. Philip Taylor, Dr. Thomas R. Walczyk and Dr. Clive West.

The consultants reviewed the methods relating to the measurement of energy expenditure and noted that the analytical methodologies had changed substantially and that there was further refinement to data fitting and the calculation of uncertainties. They also felt that a repeat of a comparison of laboratory performances with a dilution series similar to the one carried out earlier should be performed for quality control. Other methods using labelled isotopes $^{13}C$ and $^{2}H$ were also discussed. The meeting noted that it was IAEA's intention to support the development of compound specific reference materials for $^{2}H$, $^{13}C$, $^{15}N$ and $^{18}O$.

Contrary to light isotope techniques where attempts have been made in the past towards standardization, in particular by IAEA and Stable Isotopes in Gastroenterology and Nutrition (SIGN) harmonization of techniques between laboratories is unsatisfactory for the minerals and trace elements. It was decided that considering the future expansion of stable isotope techniques in mineral and trace element nutrition research, ensuring comparability of results and improving data quality should be given high priority along with the still existent need to develop, evaluate and validate new techniques. This refers, in particular, to the IAEA which plays a leading role in the dissemination of these techniques, especially in the developing world. As multiple actions are required, emerging issues are briefly discussed in order of priority. Interactions between dietary components and/or multiple micronutrients and trace elements in deficiency states and their impact on human health have been discussed in some detail.

The risk of osteoporosis in the elderly in countries is becoming a significant problem, associated in part with changes in dietary patterns, declines in physical activity, and increases in life expectancy. It was agreed that efforts to identify country specific etiological factors associated with the development of osteoporosis should continue. Finally, the role of isotopic techniques in the development of biomarkers to validate intakes of protein, fatty acids, carotenoids and vitamin A, certain inorganic nutrients, as well as energy intakes was also briefly discussed.

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## 1. INTRODUCTION

As experts we have been asked to provide an overview of the current status of isotopic techniques in nutritional science with respect to both methodology (technology) and applications (need). The concepts of technology push or needs drive can be a contentious issue but nutrition has never been a battle ground between the two, indeed there exists in a large number of scientists who have already recognised what can be gained when nuclear technologies are linked to the basic nutritional needs of the developing countries, but herein lies the problem. The pace of the work and the variety of applications developed in the last few years has meant that elements of each discipline have not developed in an organised way and at the same pace.

This document examines the current areas in nutrition where the application of nuclear technology is needed. We have also addressed some of the shortcomings of the technology as it is routinely applied and how it could be improved.

To ensure comparability of data in research and application of isotopic methods, the problems that have to be addressed are much more complex than the essential underpinning of the work with quality control by the use of reference materials. This is illustrated in Fig. 1.

The quality of the analytical data determines both protocols and outcomes but before a meaningful value can be produced there may be many steps that can add random error or bias. The origin of these method-specific problems needs to be understood and quantified. Each measurement activity in which the IAEA is engaged should therefore have associated with it a set of well-defined methodologies for every stage.

Proper definition of uncertainties also has implications for the costs of a study on a single subject, number of subjects required for a study (leaving aside the question of population variances) and total costs. The aim should be to achieve success at the lowest possible cost and to avoid expensive failures.

An example of the importance of these questions is illustrated by the hypothetical investigation provided in Fig. 2. We will suppose that a UN organization wishes to support iron fortification in the form of a commonly used sauce, but its effectiveness is not known. It may be that constituents of the foods with which the sauce is commonly consumed interact with the iron to make it much less available than expected. This question is amenable to testing at the small study or even community level but if an erroneous result is produced in a trial:

- An effective product could be rejected, or
- An ineffective product could be recommended for nation-wide use.

The first error will cost money and the second will cost lives.

The current situation in respect to these considerations ranges from adequate to generally poor. There is rather better organized for reference materials than for methodology and data translation.

This paper will attempt to identify strengths and weaknesses of methodologies currently used in IAEA funded research (CRPs and Technical Cooperation Projects) and to see:

- How they can be improved for the general user, and
- To provide a basis for the assessment of outcomes delivered by collaborating laboratories in IAEA funded studies.

## 2. LIGHT ISOTOPE METHODOLOGIES

### 2.1 Measurement of energy expenditure with $^2H_2{}^{18}O$ (doubly labelled water, DLW)

An IAEA report (NAHRES-4) provides an organized and comprehensive guide to this methodology that has served the user community well. Relevant features of the report are:

- how to analyse the samples
- how to use IAEA reference materials for instrument calibration
- how to best fit data to the metabolic model used
- how to calculate overall measurement uncertainty, including the contributions of analytical and physiological variance
- the impact of these on dose requirements
- how to calculate the sizes of biases consequent on model imperfections
- how data should be reported in the scientific literature

Since this publication appeared the analytical methodologies have changed substantially. For $^2H$ preparation from water, hot uranium or zinc are now rarely used; the methods being superceded by Pt catalysts or the injection of the sample onto hot Chromium. Additional papers provide refinement to the methodology, especially in relation to data fitting and the calculation of uncertainties (Cole and Coward 1992; Ritz et al, 1996a; Jones et al, 2000). Alternate methods of curve fitting produce comparable results for overall error. The only substantial disagreement among users of the method relates to the question as to whether the covariant natural abundance variation between $^2H$ and $^{18}O$ that exists for the environment also exists as part for physiological variation in man and whether it can be used to produce an algorithm for optimum $^2H$ and $^{18}O$ doses (Schoeller 1983; Ritz et al, 1996b).

The results of a modest survey of the results produced by users of this methodology including reports on laboratory performances with dilution series from an actual experiment were also carried out (Roberts et al, 1995). This work was not, however, done with any long-term aim to improve laboratory performance and has not been repeated.

Since this methodology is a dilution methodology, there is no need for standardization with the use of reference materials, provided that the doses given are analyzed with the same procedures as those applied to samples. This is because the units customarily used to plot kinetic data in biological experiments (fraction of dose) are not dependent on absolute values. However, the use of standard materials provides assurance of linearity and the hydrology standard materials IAEA VSMOW, IAEA SLAP and IAEA GISP are reasonably well used as basic reference materials. However, they are a range of standards from natural abundance down wards and therefore inherently unsuitable. The enriched standards available from IAEA (IAEA 302A, IAEA 304A, IAEA 304B) are not widely used and in any case are not exactly appropriate to the methodology since they are separately enriched for $^2H$ and $^{18}O$. The range of $^2H$ enrichments (508.4 and 996 delta value vs. VSMOW) is appropriate but the $^{18}O$ enrichments (215.7 and 502.5 delta value vs. VSMOW) are much higher than those customarily used in the methodology. It is recommended that two standards enriched in both $^2H$ and $^{18}O$ be produced. Suggested values are about 500 and 1000 delta value vs. VSMOW for $^2H$ and 75 and 150 delta value vs. VSMOW for $^{18}O$. Consideration should be given to organizing a distribution of a dilution series similar to that used by Roberts et al (1995) so that current laboratory performance can be tested. A timely revision of NAHRES-4 to incorporate new developments would also be helpful for new users of the methods.

## 2.2 Measurement of body composition (lean body- and fat mass)

In contrast to the well-standardized but complex methodology of DLW it is less easy to define a formal consensus for the estimation of lean body- and fat mass by $^2H_2O$ dilution, although it is evident from published work that there is an underlying and informal consensus. It would be relatively easy for NAHRES staff to draw up a series of recommendations for dose size, which samples to take, post-dose equilibrium time, how to obtain uncertainty estimates, correction factors to convert $^2H$ dilution space to body water, and to distribute these to prominent users for agreement.

Recommendations for reference materials are the same as those for DLW.

## 2.3 Measurement of breast-milk intake

The dose-to-the-mother technique for measuring intake is fully described in NAHRES-44 but the necessary calculation of the infant's body water from body weight has been revised from new updates on the relationship between weight and total body water in infants (NAHRES-55). The isotopic doses currently used produce an initial maternal enrichment of about 150 ppm above base line for IRMS measurements and three times this value for Fourrier transform IR methods. The latter is the IR method of choice (Jennings et al. 1999) and works well with both Shimadzu and Genesis FTIR instruments. Data from these has been tested against IRMS procedures. The quality of data produced by conventional IR is less well documented. Translation of data to intake measurements in IAEA supported work is achieved by curve fitting using the "SOLVER" function in EXCEL. While this package is simple, universally useable and easily understood, it provides only a rough guide as to the quality of the data, and weighting of errors in the fitting can only be either absolute or proportional. It does not provide specific estimates of the uncertainties for the fitted parameters. Other modelling packages such as SAAM or Modelmaker can provide these and with these it is possible to weight data points according to the precision of individual data points defined by their standard deviation.

IAEA's current procedure provides no particular guidance for the use of reference materials but these can be the same as for DLW.

## 2.4 Breath tests

Several distinct techniques are subsumed under this heading and need to be treated separately from the analytical standpoint. A recent guide to the status of current methodology is provided by Harding and Coward (Harding and Coward 1998). This review describes work carried out by a European group. Standardization of $^{13}CO_2$ measurements relative to PDB is recommended and a ring test involving 15 laboratories was performed. This has not however led to the adoption of a common standard for routine use.

### *2.4.1. Test for Helicobacter Pylori infection*

This test identifies *Helicobacter Pylori* infection by providing an oral dose of sufficient $^{13}C$-urea to saturate the urease enzyme. A fixed value for $^{13}CO_2$, delta PDB above baseline, is usually used to define cut-off points for the test and data for specificity and sensitivity have been provided by some authors. Provided that sufficient $^{13}C$-urea is given, the test is not dose dependant but the response will depend on methodological procedures determining the length of time the substrate remains in the stomach and the accuracy with which values for enrichment relative to PDB are produced (i.e. instrumental calibration). IAEA nutrition activities are likely to focus on the use of this test in children and NAHRES-

56 provides comprehensive guidelines. These are however a little different from those described in Harding and Coward previously and used on a large scale in Africa (Harding and Coward 1998). It is, however, unlikely that these differences will affect study outcomes.

*2.4.2 Measurement of gastric emptying*

This clinical test may find its way into IAEA use at some future stage but it is not currently used for nutrition studies. While percent dose recovered (PDR) in breath $CO_2$ from $^{13}C$ octanoic acid dose is the response plotted against time, it is the shape of the curve that determines the relevant parameters such as half-emptying time. Standardization of the isotopic measurements against PDB is not therefore really necessary or as important as the standardization of the way in which the dose is given. A standard procedure for adults is described in web pages produced by a European network of clinical scientists (SIGN, http://www.med.rug.nl/sign/) but even in the European context there is variation in the way the test is done. The same is true for the "gold standard" scintigraphy methods. There is no standard test for children.

*2.4.3 Other breath tests (see also Section 4.2)*

Applications in children are developing and some are included in NAHRES-56. The end-point measurement is invariably % isotopic dose recovered in breath $CO_2$ with the assumption made that the quantity of isotope administered can be calculated from the un-validated information supplied by the manufacturer or supplier. Assuming that this information is correct, accuracy will only be achieved if the $^{13}CO_2$ measurements are properly measured relative to PDB but outside the SIGN collaboration, there is no organized or systematic way by which this is being achieved at present and so comparability of data between laboratories cannot be guaranteed. Additionally, in breath tests for macronutrient assimilation (e.g. the mixed trigyceride breath test) there are no universally accepted diagnostic cut-off points. Fortunately, this is likely to be a consequence of physiological rather than analytical uncertainty.

### 2.5 Other nutritional research with light isotopes

There are a huge variety of applications in metabolic work, including work with vitamins and these are too diverse with respect to substrates and techniques (GC/MS, LC/MS and GC/C/IRMS, to name three of the most important ones) to make any attempt at standardisation too complex and expensive, except in the case of GC/C/ IRMS. Although this technique is relatively new a substantial proportion of users are in metabolic research allied to nutrition. The meeting noted that it was IAEA's intention to support the development of compound specific reference materials for $^{2}H$, $^{13}C$, $^{15}N$, $^{18}O$. Of the mixtures suggested for use, alkanes, fatty-acid methyl ethers (FAMEs) and caffeine, FAMEs are most likely to be useful since current work features mainly $^{13}C$ and likely developments will also include $^{2}H$.

## 3. THE METHODOLOGICAL SITUATION FOR THE MINERALS/TRACE ELEMENTS

Over the past two decades, substantial progress has been made in developing and improving stable isotope techniques to study mineral and trace element metabolism in humans, establishing them as powerful tools in nutrition research. When compared to radio isotopic techniques, stable isotope techniques are preferable and their use in humans is absolutely safe. This is obvious from the constantly increasing number of laboratories worldwide using or intending to install these techniques in the nearby future.

Contrary to light isotope techniques where attempts have been made in the past towards standardization, in particular by IAEA and SIGN, harmonization of techniques between laboratories is unsatisfactory for the minerals and trace elements. Considering the future expansion of stable isotope techniques in mineral and trace element nutrition research, ensuring comparability of results and improving data quality have to be given high priority along with the still existent need to develop, evaluate and validate new techniques. This refers, in particular, to IAEA which plays a leading role in the dissemination of these techniques, especially in the developing world. As multiple actions are required, emerging issues are briefly discussed in order of priority.

### 3.1 Translation of mass spectrometric data into nutritional information

The terminology 'stable isotope tracers' implies that stable isotopes can be followed and quantified in the human body or in its excreta directly, similar to radiotracers. However, this is not the case, as the stable isotopes in a stable isotope tracer are physically indistinguishable from the natural element except that they differ from the natural element in their isotopic abundance. This means that quantification of stable isotope tracers in any material can only be done indirectly via the induced alteration of the natural isotopic abundances of the element. This principle is known as the 'isotope dilution concept' and requires more or less complex calculations to obtain the amount of tracer present in a sample/tracer mixture.

Calculations can be simplified substantially by assuming that the stable isotope tracer is monoisotopic, i. e. that the respective stable isotope is enriched to 100%. However, by making the calculation simpler, a bias is introduced that will be substantial when looking at the final nutritional conclusions to be drawn. To date, evaluation techniques range from completely neglecting all other isotopes in a stable isotope tracer to concepts where corrections are made to compensate finally for these inaccuracies (van Dokum et al, 1996; Barrett et al, 1992). Evaluation techniques which consider the specific isotopic composition of the stable isotope tracer from the beginning and which would also allow proper error statements at the end are usually not applied (Walczyk et al, 1997).

To exclude data evaluation as a bias source and to ensure comparability of results obtained within different studies the 'isotope dilution concept' should be propagated within the community. A consensus on the algorithms for data evaluation and the underlying mathematics should be achieved for the established and routinely used stable isotope techniques in mineral and trace element nutrition research. Dissemination could be accomplished by the development of a suitable software program with modules for each technique that can be directly referred to when transformed data are published. Regular updates would be required to address new developments in the field.

### 3.2. Consideration of the uncertainty in translated mass spectrometric data

Due to the complexity of the mathematical evaluations which are required to obtain nutritionally relevant information from stable isotope work, estimations of the combined uncertainty based on error propagation concepts is technically challenging. Because of the demanding mathematics involved, nutritionally relevant data as obtained using stable isotope techniques are generally reported without an error/uncertainty statement and users are commonly not aware of the uncertainty in the results they have generated. Attempts have been made to calculate the uncertainty in stable isotope derived iron absorption values (Barrett et al, 1992). However, this approach is unsatisfactory as it is based on a simplified calculation algorithm which itself induces a systematic bias.

The current situation has to be considered dangerous as over-interpretation of data becomes possible and wrong conclusions both on the scientific and the governmental level could be drawn regarding planned or on-going intervention programs. Suitable strategies have to be developed to make users aware of their risks. This could be accomplished by designing easy to use, method specific software programs that allow to calculate the combined uncertainty in the translated mass spectrometric data, e.g. in determined iron absorption values. As this program should be used as a standard in the field, it should be inexpensive to make it also affordable to laboratories in the developing world.

### 3.3. Common criteria for calculation of dose requirements

The combined uncertainty in a numeric value of nutritional significance (e. g. fractional absorption of a trace element from a given test meal) is largely determined by the isotopic enrichment that is finally present in the biological sample to be analyzed. This enrichment itself is proportional to the dose of stable isotope tracer that has been administered. Isotopic doses are usually kept as low as possible for physiological reasons and cost considerations.

To date, there is no consensus about the criteria for deciding about the minimum dose that results in an appropriate isotopic enrichment over baseline values in the biological samples to be analyzed. Common practice for accepting mass spectrometric data of isotopically enriched samples range from minimum isotopic shifts of 3 times above baseline values up to 10 times, the relative standard deviation as obtained for the respective isotopic ratio within a series of independent runs of an unenriched sample. Isotope doses for a study to be conducted are commonly calculated based on this arbitrary concept. This practice is questionable, as decisions on isotopic doses are not based on the combined uncertainty in the finally derived nutritional value as the primary criterion for rejecting data for interpretation. The aforementioned software program should not only be designed to calculate absorption values with combined uncertainties, it should also allow to model absorption studies for estimating the minimum dose that is required to reach a target combined uncertainty in the final absorption value. Such software would reduce the risk of subject underdosing substantially and would represent a common basis for deciding about dose requirements in this area of research.

### 3.4. Development and validation of novel stable isotope techniques

Bioavailability is defined as the fraction of a nutrient that is absorbed by the human body and utilized for normal physiological function. For the mineral and trace elements, iron is the only element for which it is currently possible to determine bioavailability directly using stable isotope techniques. This routinely used method is based on the erythrocyte incorporation of the isotopic label 14 days after isotope administration (Kastenmayer et al, 1994). For all other minerals and trace elements, bioavailability can only be estimated based on the true/apparent absorption or retention of the isotopic label. These estimates differ substantially in quality. Retention is a better estimate for bioavailabilty than absorption while true absorption is a better measure than apparent absorption. Furthermore, techniques differ significantly in invasiveness and in difficulties in conducting the experiments. As an example, a 24-hour urine sample is sufficient to determine Ca absorption by the urinary monitoring technique while complete feces and urine collections up to 10 days are required when the isotopic balance technique is used (Eastell et al, 1989). To extend the possibilities of using stable isotope techniques in mineral and trace element nutrition research and to allow their use more routinely, new techniques have to be developed and those existent improved:

- *urinary monitoring technique for Zn and Mg:* the urinary monitoring technique based on the simultaneous excretion of an oral and an intravenously given label has been developed and validated for determination of true Ca absorption (Eastell et al, 1989). In principle, the same technique could be used to determine true absorption of Zn and Mg, respectively. Attempts have been made to validate this technique in humans for Zn but data are conflicting (Friel et al, 1992; Rauscher and Fairweather Tait 1997). For Mg, no conclusions can be drawn based on the published preliminary data (Benech et al, 1998).
- *compartmental analysis for Mg and Zn based on kinetic tests:* compartment sizes and transfer rates between different compartments can be determined by following the appearance/disappearance of an oral and an intravenously administered isotopic label in plasma and excreta. For Ca, models for compartmental analysis are established (Weaver, 1998) while the situation remains unclear for Zn (Krebs et al, 1995). To date, Mg kinetic tests have been performed only once in a human study (Abrams and Ellis, 1998).
- *use of novel isotopic techniques in nutrition research:* recent investigations have shown that Ca in the skeleton can be labeled with the virtually stable, long-living radionuclide $^{41}$Ca (Freeman et al, 2000). This offers the unique opportunity to look at Ca losses from bone and Ca balance in bone directly via urinary excretion of the isotopic label. The use of this technique in nutrition research needs to be evaluated.
- *other elements:* stable isotope techniques are used routinely to look at the absorption of Fe, Cu, Zn, Se, Ca and Mg from test meals. Basically, it is possible to extend the range of application to other elements that are identified or discussed to be essential, e. g. Mo, Ni, V, Sn and B. Semi-stable, very long-living radionuclides ($^{26}$Al, $^{53}$Mn and $^{129}$I) can be used for monoisotopic elements, i. e. elements for which no stable isotope tracer is available. For the mentioned elements, stable/semi-stable isotopic absorption studies have been conducted to date only for Mo (Turnlund et al, 1995) and for Al using $^{26}$Al (Vogel et al, 1997).
- *heavy metals:* the use of stable isotope techniques for absorption studies is not limited to those elements essential to the human body. Techniques can also be used to study the absorption of elements of toxicological relevance, e. g. Pb, Cd, Hg and Cr. With the currently available mass spectrometric techniques (multicollector inductively coupled plasma mass spectrometry, MC-ICP-MS) it is possible to keep isotope doses below the usual dietary intake, making the techniques safe to use in humans. For the heavy metals, stable isotope techniques have been used in humans only once to study Cd absorption (Crews et al, 2000).

### 3.5. Strategies to reduce dose requirements

The main disadvantage of using stable isotope techniques to look at mineral and trace element absorption, in vivo, is the relatively large dose requirement. The reduction of dose requirements has to be considered a primary target in the improvement of existing methodologies. This would reduce the substantial costs for stable isotope labels and allow work to be done at physiologically more meaningful levels. This can be approached in three different ways:

- improving the mass spectrometric techniques for the relevant elements. As an example, it has been possible to reduce dose requirements for iron absorption studies by a factor of ten only by improving the reproducibility of isotopic analysis by thermal ionization mass spectrometry (TIMS) (Walczyk, 1997). With the advent of MC-ICP-MS, reproducibility of isotopic analysis has been improved substantially for Ca, Cu and Fe when compared to the commonly used isotopic analysis by TIMS

(Halicz et al, 1999; Marechal et al, 1999; Zhu et al, 2000, Belshaw et al, 2000). This opens up the possibility for dose reductions in the near future when these techniques become more accessible to human nutrition research.

- using gravimetrically prepared isotopic labels that allow internal normalization of mass spectrometric data to correct for mass dependent fractionation effects in the ion source. This approach would result in a higher reproducibility of isotopic analysis which would translate directly into reduced isotopic doses.
- developing suitable methodologies for obtaining higher isotopically enriched samples. For iron, this could be achieved by looking at the isotopic enrichment of newly formed erythrocytes instead of whole blood when using the erythrocyte incorporation technique (Heuvel et al. 1997).

### 3.6. Quality of available stable isotope labels

Worldwide, there are currently about 20 stable isotope suppliers for the minerals and trace elements. Most of the material currently distributed by the suppliers has been produced in the former Soviet Union and details on the production process (source material, enrichment techniques, location of enrichment facilities) are difficult to obtain. In the past, it has been observed on several occasions that stable isotope material imported from the former Soviet Union was contaminated with radioactive material. To ensure that subjects are not exposed to any health risk by ingestion of such radioactive contaminants, radioactivity tests should be mandatory before any stable isotope material is used on humans.

The risk of administering unsuitable isotopic labels to human subjects could be minimized by offering stable isotope material specified for use in humans only. This material should be certified for elemental and isotopic composition with a specific statement of the absence of radioactive contaminants. The preparation of standardized gravimetrically prepared isotopic labels as outlined above could consider these aspects.

### 3.7. Quality control

When using stable isotope techniques in mineral and trace element research, the results are often of relevance for whole population groups, e. g. when stable isotope techniques are used for the planning or the evaluation of national food fortification programs, in particular in the developing world. Currently, there is a strong discrepancy between public relevance and quality control for outcomes in this field of research. This is partly related to current publication policies of nutritional/medical orientated journals which do not request the description of analytical and mass spectrometric aspects in enough detail for independent evaluation of published data for quality. In particular, this poses a serious problem when looking at the use of ICP-MS for isotopic analysis. MC-ICP-MS has the potential to become the preferred tool in this field of research due to the high reproducibility of isotopic analysis and sample throughput that can be achieved. However, it is technically extremely challenging to measure isotopic ratios by MC-ICP-MS reliably at high reproducibility and high relative accuracy. Analytical artifacts can be easily generated which are impossible to identify by the reviewer or the reader of the publication without detailed information on the technique used and quality control observed. For this purpose, it would be useful to establish technical protocols within the community that consider all relevant aspects of quality control that should be addressed when results are published. This information should be reviewed independently within the regular publication process by a mass spectrometrist/analytical chemist.

### 3.8. Information to IAEA on planned activities

At the consultants meeting, Dr. Philip Taylor from the Joint Research Center of the European Commission, Institute of Reference Materials and Measurements (IRMM, Geel, Belgium) recognized the need for harmonization/standardization in the field of stable isotope applications in mineral and trace element research. The IRMM is willing to contribute to this undertaking as follows:

- It is the intention to prepare gravimetric iron isotopic labels for use in human nutrition research ($^{57}$Fe and $^{58}$Fe) within a Ph.D. program at the IRMM. Isotopic labels with defined $^{56}$Fe/$^{54}$Fe isotopic ratios will be produced and certified for elemental and isotopic composition. The produced material will be used for an iron absorption study at the Swiss Federal Institute of Technology (ETH) Zurich, Laboratory of Human Nutrition. The Ph.D. program is intended to develop the techniques to produce sufficient gravimetrically prepared and characterized iron isotopic labels for covering the needs in human nutrition research for about 5 years. As relatively large amounts of isotopic label are finally required, IAEA could contribute to this substantial step by pre-financing the isotopes to be transformed.
- A software program has been developed at the IRMM to calculate the combined uncertainty in a result even for the most complex analytical techniques. This software can be easily modified and modules can be written for each specific stable isotope technique in mineral and trace element nutrition research. This software would be easy to use and could serve as the future standard for translating mass spectrometric data into nutritional information including uncertainty statements. Using this software program, isotope doses can also be calculated based on a previously defined target uncertainty in the absorption value. Dr. Thomas Walczyk at ETH Zurich and Dr. Jack Dainty at the Institute of Food Research, Norwich, UK will provide the algorithms and help to design the programs specifically for the needs of users. It is intended to provide this software package at low costs to the nutritional community, specifically in the developing world. IAEA could contribute here by additional funding.
- To date there is no forum for stable isotope users in mineral and trace element nutrition research to discuss and critically evaluate new methodological developments in the field and to come up with commonly agreed standards for conducting experiments. Theoretically, the European Union could provide the funding for annual meetings of about 10 methodological specialists from European laboratories for this purpose. IAEA could contribute here with travel grants for specialists outside the European Union for making it a truly international forum.

## 4. FUNCTIONAL INDICATORS

### 4.1. Body composition

The characterization of functional changes in the body, in response to altered nutritional status, must be seen as an important goal of the stable isotope programme. One functional outcome of an altered nutrition state is a change in body composition. This change, if measurable, is useful in monitoring growth by partitioning the deposited tissues into fat and non-fat compartments. For example, the growth of a child in the first years of life can be probed more extensively by serial measurements of the fat content of the body and the calculations of "fat" and "lean" body mass indices (Wells, 2000). Characterizing the body fat in this manner may be useful in charting and predicting later childhood and adult obesity. A similar approach may be taken with apparently thin babies born in developing countries such as India. These babies have been shown, by examination of their sub-scapular skin folds, to

have as much fat as bigger as well nourished infants from developed countries. These "thin but fatty" babies may be at risk for later adult chronic diseases such as diabetes. The fat content of adults at different BMI (Body Mass Index, $Kg/M^2$) can also be investigated in transitioning societies, to investigate the appearance of glucose intolerance in relatively thin individuals who do not present with classical "at risk" BMI patterns. The important point is to ensure that the expanding group of deuterium users worldwide now recognizes the need for carrying out their experiments in a rational and standardized manner. This includes protocols for dosing, sample collection, sample analysis as well as the calculation of the final outcomes. It should be possible to carry out this exercise successfully (refer to Section 2).

### 4.2 Metaprobes

Another type of functional characterization involves the use of isotopic probes to dynamically measure the activity of selected enzyme pathways. Breath tests (Section 2.4) are a sub-set of these. The use of the stable isotope $^{13}C$ in selected substrates can encourage the development of non-invasive procedures to determine functional consequences of altered nutrition, both at an individual as well as at a population level. This strategy is already in use in several clinical applications, such as the determination of the gastric emptying rate, hepatic function and intestinal mal-absorption (Section 2.4). In these tests, a $^{13}C$ labelled substrate (selected according to the test being performed) is given either intravenously or orally, followed by serial breath sample collection for the determination of the $^{13}CO_2$ enrichment in the breath. At present, these tests are limited in their applicability, since their use is confined to clinical situations. In addition, these tests need to be developed more to provide dynamic information about the kinetics of the substrate. The shape of the breath tracer curve over time can be subjected to non-compartmental analysis at the very least, as well as, compartmental analyses. In addition, the rules for administering these tests needs to be set down in an accessible user guide, with rational standards for dosing, sample collection and analysis, as well as calculations.

Isotopic probes (in this case, also called metabolic probes or metaprobes) can also be developed to measure functional parameters in chronic disease of nutritional etiology (Young and Ajami 1999). Homocystinuric patients have a propensity for arteriosclerosis, leading to myocardial infarction and stroke. While transitions in lifestyle and nutrition in developing countries have led to an epidemic of cardiovascular disease in these populations (Yusuf and Reddy, 1999), epidemiological studies also suggest that an elevated plasma homocysteine is a risk factor for cardiovascular disease (Yusuf and Reddy, 1999). Therefore, it is worthwhile to measure homocysteine status of the human body. Homocysteine is cleared from body pools due to the activity of two enzyme systems: the re-methylating and the trans-sulphurating enzymes. The former are vitamin ($B_{12}$ and folate) dependent, while the latter is pyridoxine dependent. Intervention trials with these vitamins have shown that homocysteinemia can be reduced by up to 25%, suggesting that a vitamin deficiency may be partly responsible for the elevated plasma homocysteine levels.

However, the genes coding for these enzymes that clear homocysteine may also be subject to mutations (Zittoun et al, 1998; D'Angelo et al, 2000), and the ultimate phenotypic expression of hyper-homocystenemia may be due to a combination of environmental and genomic effects. It is also likely that this phenotype of a high homocysteine level is a late manifestation of variations in the clearance kinetics of homocysteine. It would therefore be useful to have a dynamic assessment of the homocysteine clearance system so that 1) earlier diagnosis and risk assessment is possible, and 2) the effect of interventions such as vitamin supplementation can be studied effectively. At present, the measurement of homocysteine clearance by the isotope labeled metaprobe method has only been performed in animals and is not available for use in humans, although this is anticipated. The metaprobe used is L-2-

oxo-tetrahydro,1,3 thiazine-4-carboxilic (1-$^{13}$C) acid which releases labelled homocysteine intra-cellularly, under the influence of oxoprolinase. The labelled homocysteine is then transsulphurated to cysteine and $^{13}$C-aminobutyrate. The latter, on oxidation, would release the label into the $CO_2$ pool, and this would then be detected in the breath. Therefore, a breath pattern of isotopic label release can be used to assess homocysteine dynamics, under different metabolic conditions, such as fasting or after ingesting a methionine load. Preliminary studies in rabbits support these projections (Young and Ajami 1999). However, at the present time, it cannot be assumed that this technique is easy to perform on an epidemiological basis.

Other potential functional applications using this approach include the measurement of Nitric Oxide production by the use of $^{15}$N-homoarginine or $^{18}$O inhalation (Young and Ajami 1999; Sakinis, Jungersten et al, 1999.

## 5. INTERACTIONS BETWEEN DIETARY COMPONENTS AND/OR MULTIPLE MICRONUTRIENTS; THEIR SIGNIFICANCE IN NUTRITION INTERVENTION PROGRAMMES

### 5.1. Introduction

Requirement estimates for all nutrients depend on age, sex, stage of growth, pregnancy, and lactation. In addition, for many of the inorganic nutrients, however, these requirements estimates must then be adjusted to take into account their bioavailability in the diet. Both physiological and dietary factors may influence the bioavailability of these inorganic nutrients. Physiological factors include the mineral or trace element status of the individual, their development and health status, as well as the existence of certain adaptive mechanisms that may occur such as increased absorption and utilization of the inorganic nutrient. The dietary factors affecting bioavailability, and thus in turn requirements, include the physicochemical properties of the inorganic nutrient in the food or diet (e.g., pH, solubility, charge density, state of oxidation), its level in the diet, the presence of certain dietary modifiers which may inhibit or enhance absorption, as well as the existence of competitive antagonism between ions (e.g., Cu-Zn; Cu-Fe; Fe-Zn. Mn-Fe, Cd-Zn) during digestion and absorption. In most cases, the content of trace elements in indigenous diets is not high enough to induce such competitive interactions but they could become important if high doses of micronutrient supplements are consumed or if staple foods or diets are fortified with multi-micronutrients. In addition, for certain emerging countries, where environmental pollution is a problem, antagonistic interactions between some essential trace elements and heavy metals may become of increasing health significance, especially in diets which have been manipulated to reduce their phytic acid content.

### 5.2. Interactions between dietary components and inorganic nutrients; their impact on bioavailability

In developing countries, there is an urgent need to quantify the impact of dietary factors on nutrient bioavailability in indigenous diets in developing countries using isotopic techniques, so that valid estimates of nutrient requirements can be made. In such countries, staple diets are generally plant-based, and intakes of flesh foods are often low. As a result, they often contain high levels of certain antinutrients (e.g., phytate, polyphenols) known to inhibit the bioavailability of calcium, iron, and/or zinc by forming insoluble complexes in the gastrointestinal tract. The extent to which these antinutrients influence the bioavailability of other essential inorganic nutrients such as copper, manganese, selenium, chromium, iodine, boron, and magnesium is not well established and requires further study (Fairweather Tait and Hurrell, 1996). As well, the extent to which certain adaptive mechanisms such as increased absorption and/or decreased endogenous losses via the intestine (e.g. for zinc) may

occur to compensate for the poor bioavailability of inorganic nutrients in the diets of persons consuming habitual plant-based diets, and thus modify requirement estimates, is poorly defined, and warrants further investigation using isotope techniques (Sian et al, 1996).

### 5.3. Interactions between co-existing multiple micronutrient deficiencies; their impact on pre-existing deficiency states

Recently, nutritionists have emphasized that consumption of predominantly plant-based diets leads to multiple micronutrient deficiencies, notably iron, zinc, vitamin A, riboflavin, vitamin B-12, and possibly selenium and iodine (depending on the soil concentrations of these two trace elements), especially among groups at risk such as infants, preschool children, pregnant and lactating women with high nutrient requirements (Rosado, 1999; Murphy et al, 1992). Such co-existing micronutrient deficiencies can induce interactions (e.g., vitamin A-Zn; vitamin A-Fe; Se-I; I-Fe, Se-Zn) which may exacerbate pre-existing deficiency states. Stable isotope techniques can play a critical role in studying the metabolic interactions of these micronutrients in humans, so that nutrient requirements can be defined more precisely (Udomkesmallee et al, 1992; Bloem, 1995; Contempre et al, 1992; Hetzel, 1998).

### 5.4. Interactions in multiple micronutrient supplements; their impact on bioavailability

As a result of the increasing recognition of the co-existence of multiple micronutrient deficiencies in developing countries, United Nations Agencies have urged that supplementation and fortification programmes are designed to combat multiple micronutrient deficiencies simultaneously. Care must be taken to ensure that the micronutrient levels used in these programmes will not induce competitive antagonistic nutrient interactions.

Possible antagonistic interactions that may interfere with the utilization of trace elements in multi-micronutrient supplements *per se*, and/or with the utilization of elements intrinsic to the food or the meal include those between Cu-Zn, Cu-Fe, Fe-Zn and Mn-Fe (Solomons, 1986; Yadrick et al, 1989; Fischer et al, 1984). Uncertainties still exist about: (i) the relative efficiency of absorption of different doses, chemical forms; and combinations of multiple micronutrient supplements; (ii) whether they should be given in the fasting or fed state; (iii) optimal dosing schedules to use (i.e., daily or intermittent); and (iv) the optimal levels required for apparently healthy individuals, and those with severe malnutrition.

### 5.5. Interactions in multiple micronutrient fortificants; their impact on bioavailability

When micronutrient deficiencies are endemic, fortification with multiple micronutrients is being increasingly advocated as a cost-effective and sustainable method for improving micronutrient status. Such programmes can be undertaken at a national level or targeted at specific regions and/or high-risk groups. For example, a recent publication (WHO, 1998) on complementary feeding has highlighted the need to establish fortificant levels for complementary foods for developing countries. Stable isotope techniques can play a crucial role in quantifying the optimal micronutrient fortificant levels and their bioavailability in a range of both complementary foods and indigenous meals containing varied levels of phytate. Efforts should be made to develop and quantify the bioavailability of a range of 'protected' micronutrient fortificants that are readily absorbed and utilized and resistant to any dietary inhibitors, as has been developed for iron (e.g., NaFeEDTA).

Fortificant levels must also be carefully selected to minimize risk of antagonistic interactions as has been discussed for supplements. They must also take into account the known toxic threshold levels for normal individuals. This requires knowledge of the lowest-observed-adverse effect level (LOAEL) for each essential micronutrient, which in the US, are used to set daily reference doses (RfD). The latter represents an estimate of daily exposure that is likely to be without appreciable risk or deleterious effects during a lifetime (US EPA, 1992). For certain inorganic nutrients (e.g., selenium), the RfD is known to be very close to the requirement estimate, whereas for others, RfD values are very poorly defined. Isotopic techniques can assist in defining LOAEL values with more certainty.

### 5.6. Interactions between dietary components and micronutrients in food-based programmes at the food production level; their impact on bioavailability

Dietary modification/diversification is becoming increasingly used as a more sustainable, economically feasible, equitable and culturally acceptable strategy to alleviate several micronutrient deficiencies simultaneously without risk of antagonistic interactions. The approach involves changes in food production practices, food selection patterns, and traditional household methods for preparing and processing indigenous foods. Strategies at the food production level include the use of traditional plant breeding or genetic engineering to enhance the micronutrient content and/or alter the level of micronutrient absorption modifiers in plant-based staples. Examples include the production of low-phytate strains of cereals or those with an enhanced content of absorption promoters of iron and zinc such as methionine or cysteine. Stable isotope techniques, used in two recent pilot studies investigating the impact of low phytic acid maize (LPM) hybrids on the bioavailability of iron and zinc in humans, have reported promising results. For example, when the phytic acid content of the maize was reduced by approximately one third, iron absorption from tortillas was 49% greater compared to that from the wild-type strain (Mendoza et al, 1998). Similarly, Adams et al. (2000) reported a 78% increase in zinc absorption of a maize-based diet of polenta, when the phytic acid content of the maize was reduced by 55-63%. Hence, transfer of the low phytic acid trait to tropical maize lines has the potential to have a major impact on the micronutrient status, specifically iron, zinc, and possibly calcium, of population groups who derive at least 50% of their dietary energy from maize, and in whom, as a consequence, the major causative factor of zinc and probably nutritional iron deficiency is poor absorption rather than low intakes of zinc and iron *per se*.

Alternatively, or in addition, new cereal varieties with higher concentrations of zinc and iron can be promoted. Stable isotope studies to assess the impact of these new cereal varieties with a low phytic acid content, enhanced content of absorption promoters, and/or higher trace element content, on the bioavailability of iron and zinc of populations groups consuming habitual plant-based diets high in phytic acid are urgently required.

### 5.7. Interactions between dietary components and micronutrients in food-based programmes at the household level; their impact on bioavailability

At the household level, certain food preparation and processing methods can also be promoted to reduce the level of absorption inhibitors, notably phytic acid or increase the content of absorption enhancers and thus improve the bioavailability of several micronutrients simultaneously in diets in developing countries. The methods for zinc and iron have been reviewed in detail by Gibson and Ferguson (1998). They are based mainly on enzymatic or non-enzymatic hydrolysis of phytic acid induced by germination, fermentation and soaking. To date, in vivo stable isotope studies quantifying the bioavailability of iron and zinc in total diets prepared using these household processing methods are not available, and are urgently required.

### 5.8. Interactions between heavy metal contaminants and miconutrients in food-based programmes designed to reduce phytic acid content

Although the micronutrient content of most indigenous diets in developing countries is generally not high enough to induce competitive antagonistic interactions, nevertheless, there has been some concern that a reduction in the phytic acid content of cereals has the potential to enhance the absorption of certain heavy metals such as cadmium and lead. This could be a problem, especially in emerging nations where air pollution is a concern and where controls for land-fills in mining operations are often inadequate. Antagonistic interactions are known to exist between Zn-Cd, Fe-Pb, Cr-Zn, Cd-Cu, As-I (Fairweather Tait and Hurrell, 1996).

## 6. RISK OF OSTEOPOROSIS IN THE ELDERLY IN EMERGING COUNTRIES

In both industrialized and in the emerging countries, osteoporosis in the elderly is becoming a significant problem, associated in part with changes in dietary patterns, declines in physical activity, and increases in life expectancy. Efforts to identify country specific etiological factors associated with the development of osteoporosis should continue, and the extent to which physiological adaptation can compensate for differences in calcium bioavailability in plant-based diets evaluated so that effective intervention strategies can be implemented. Diagnosis of osteoporosis and the impact of these strategies can be evaluated using isotopic techniques such as single photon absorptiometry, dual photon absorptiometry, and dual energy X-ray absorptiometry (DEXA).

## 7. FUTURE DEVELOPMENTS FOR ISOTOPIC TECHNIQUES IN NUTRITION RESEARCH: BIOMAKERS

Recognition of the uncertainties in the collection of food intake data has led to the increased use of external variables such as biomarkers to establish the validity of nutrient intake data (Hunter, 1990). When using such an approach all the factors that may confound the biomarker, including both technical and biological factors, must be identified, measured, or controlled. Isotopic techniques have the potential to play a role in the development of biomarkers to validate intakes of protein, fatty acids, carotenoids and vitamin A, certain inorganic nutrients, as well as energy intakes.

## 8. SUMMARY OF RESEARCH APPLICATIONS UTILIZING ISOTOPIC TECHNIQUES

- Support the development of compound specific reference materials for $^{2}H$, $^{13}C$, $^{15}N$, $^{18}O$ such as alkanes, fatty-acid methyl ethers (FAMEs) and caffeine.
- Standardise the mathematical approaches to evaluate mass spectrometric data when using stable isotope labels.
- Consensus to be achieved on the algorithms for data evaluation and the underlying mathematics for the established and routinely used stable isotope techniques in mineral and trace element nutrition research.
- Design a software program to calculate absorption values with combined uncertainties and to estimate the minimum dose that is required to reach a target combined uncertainty in the final absorption value.
- Use of the stable isotope $^{13}C$ in selected substrates for the development of non-invasive procedures to determine functional consequences of altered nutrition.

- Investigate etiology of osteoporosis and impact of intervention strategies in the elderly in emerging countries.
- Develop biomarkers based on isotopic techniques for validating nutrient intakes from food intake data.
- Quantify the impact of dietary factors on bioavailability of essential inorganic nutrients such as copper, manganese, selenium, chromium, iodine, boron, and magnesium.
- Quantify the extent to which adaptive mechanisms may occur to compensate for poor bioavailability in persons consuming habitual plant-based diets containing varied levels of phytate.
- Study absorption and metabolism of micronutrients in persons with co-existing micronutrient deficiency states.
- Compare the relative absorption efficiency of different chemical forms, doses, dosing schedules, and combinations of micronutrients for supplements and fortificants in food vehicles with a varied phytic acid content.
- Establish the optimal multi-micronutrient fortificant levels for complementary foods for infant and young child feeding.
- Investigate and compare the relative bioavailability of protected fortificants which are resistant to dietary absorption inhibitors.
- Use isotopic techniques to assist in defining with more certainty LOAEL levels for micronutrients which are used in multi-micronutrient supplements and fortificants.
- Conduct *in vivo* assessments utilising stable isotope techniques to quantify the bioavailability of micronutrients in new cereal varieties with a low phytic acid content, enhanced content of absorption promoters, and/or higher micronutrient content.
- Conduct *in vivo* assessments utilizing stable isotope techniques to quantify the bioavailability of iron and zinc in total diets prepared using household processing and preparation methods designed to reduce the level of phytic acid and/or increase the content of absorption enhancers.
- Carry out efficacy trials of the impact of multiple micronutrient supplementation, fortification, and dietary diversification/modification interventions on micronutrient status and other functional outcomes.
- Establish impact of phytic acid reduction in food staples on heavy metal absorption.

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## CONSULTANTS' MEETING

## RECENT DEVELOPMENTS AND FUTURE DIRECTIONS FOR STABLE ISOTOPE APPLICATIONS IN NUTRITION RESEARCH

## INFORMATION SHEET

### 1. Background

The International Atomic Energy Agency (IAEA) has been supporting activities in the field of nutrition since the 1970s. In 1987, it established a sub-programme on nutrition related research, with a focus on applications of isotopic techniques for measuring nutrients in foods and in the human body. In 1992, the IAEA increased its efforts to bridge the gap between industrialized and developing countries' access to isotopic techniques for monitoring the nutritional status, thereby arousing interest among the UN, national and bilateral organizations in the use of isotopic techniques in food and nutrition. In recent years, new strategies are being sought to address micronutrient malnutrition that is in epidemic proportions in developing societies today. Women and children representing the largest segment of society are in need of protection because of their increased vulnerability to malnutrition. Multi-factorial interventions to prevent malnutrition, infectious disease and environmental pollution are priority issues that need to be addressed as urgent public health problems. The IAEA is contributing to these efforts by facilitating the development of a variety of isotope based techniques to improve monitoring techniques for both nutrients and pollutants and to identify effective strategies in nutrition intervention schemes particularly among vulnerable groups in developing regions around the world.
Recently, the Nutritional and Health Related Environmental Studies Section (NAHRES), Division of Human Health of the IAEA has been very active in supporting activities related to the measurement of both organic and inorganic nutrients in foods and in the human body in several developing countries. In preparation to a systematic approach to help the needy developing countries, a new initiative under "Nutritional Metrology in Practice" has been initiated for the budget cycle 2002-2003. As a first step, it is planned to assemble a small group of consultants to seek their advice on the current methodological status related to nutrient measurements. Of particular interest to the IAEA is the use of isotopic methods since several nutritional monitoring programmes are likely to seek this technology for future projects supported by the IAEA.

### 2. IAEA's involvement with stable isotopes in nutrition

In recent years, NAHRES has turned more toward the use of stable isotopes. Work with stable isotopes involves nuclear techniques which are being increasingly used by the joint FAO/IAEA Division and the Section of Isotope Hydrology and Geochemistry. For the light isotopes ($^{2}H$, $^{13}C$, $^{15}N$, $^{18}0$), largely as a consequence of the activities of physiologists and nutritionists using IRMS, this type of analysis is now available with a high degree of automation with no significant reduction in performance. GC/MS (gas chromatography/mass spectrometry) and LC/MS (liquid chromatography/mass spectrometry) are never likely to be tools of choice for stable isotope ratio measurements but GC/C/IRMS (gas chromatography/combustion/IRMS) developments will be significant and will reduce isotope costs in many applications. For the heavy elements, lack of automation inhibits the development of good models for absorption, storage and turnover but magnetic sector ICPMS with multiple collectors is probably the way forward for future studies.

For several reasons cited above, the IAEA is involved in a range of nutritional topics covering quantitative measurements of vitamins in foods and biological fluids, assessment of

absorption of nutrients, nutrient supplementation, measurement of body nutrient status and energy metabolism, among others. For these and other reasons, the IAEA closely follows the progress in application of stable isotope methods in human nutrition research and recognises the need to periodically assess the state of the practice, by bringing together experts for consultants' meetings.

## 3. Objectives

- To assess the practice of stable isotope methodologies in human nutrition research
- To identify new strategies for improving sensitivity of nutrition monitoring techniques for use in Technical Co-operation (TC) Projects in nutrition
- To explore high quality stable isotope spikes for use in humans
- To standardise the mathematical approaches to evaluate mass spectrometric data when using stable isotope labels within metabolic studies
- To formulate an effective scientific programme for the IAEA symposia sessions that are part of the International Congress of Nutrition (ICN-17) and for the workshop on stable isotope users' group in human nutrition (also at ICN-17).

## 4. Expected outcomes

- Working material addressing the above-mentioned issues, describing on-going or recent work carried out by each participating scientist, to be presented during the meeting and made available to the IAEA.
- Recommendations on the use of emerging improved isotopic technique methodologies for IAEA Co-ordinated Research Projects and field applications in TC projects.
- Strategy to approach analytical quality control (AQC) issues of stable isotope measurements in support of the "Nutritional Metrology" activities foreseen for Programme and Budget 2002-2003.
- Technical guidance for initiating stable isotope incorporated natural matrix reference materials.
- A well-designed scientific and technical programme for the Stable Isotopes in Nutrition Research Symposium in August 2001 (ICN-17).
- Short report (not more than 20 pages) with recommendations, to be drafted and adopted by participants during the meeting. It is anticipated that the discussions during this consultants' meeting will shed light on the problems outlined above and facilitate formulating future projects to resolve the issues using nuclear and isotope based techniques.

# AGENDA

**MONDAY, 4 DECEMBER 2000: JOINT SESSION**

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|---|---|
| **09.00 – 09.30** | **Registration/Opening Session** |
| 09.30 - 10 00 | Gathering at the Meeting Room/coffee |
| 10:00 - 10.15 | Welcome and Introduction of the participants |
| 10.15 - 10.45 | NAHU perspectives (Dr. S. Groth, DIR-NAHU) |
| 10.45 - 11.00 | Aim of the meeting (s) (Dr. G.V. Iyengar, SH, NAHRES) |
| 11.00 - 11.20 | The world of Stable Isotope Measurements (Dr. W. A. Coward, U.K.) |
| 11.20 - 11.40 | The world of Organic Nutrient Measurements (Dr. C. West, Netherlands) |
| 11.40 - 12.00 | Stable Isotope Activities at the IAEA (Dr. M. Groening, NAAL-NAPC) |
| 12.00 - 12.30 | Discussions/selection of general chairpersons |
| 12:30 - 14:30 | Lunch/Administrative matters |
| **14:30 – 17:30** | **SESSION 1, Presentations by the consultants and discussions** |
| 14:30 - 15.15 | Dr. P. Taylor, Belgium |
| 15.15 - 16.00 | Dr. A. Kurpad, India |
| 16.00 - 16.15 | Coffee break |
| 16:15 - 17:00 | Dr. R. Gibson, New Zealand |
| 17.00 - 17.30 | General discussion/Administrative matters |

**TUESDAY, 5 DECEMBER 2000**

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|---|---|
| **09:00 – 12:30** | **SESSION 2: Presentations by the consultants and discussions** |
| 09.00 - 09.45 | Dr. T. Walczyk, Switzerland |
| 09.45 - 10.30 | Dr. W.A. Coward, U.K. |
| 10.30 - 11.00 | Coffee Break |
| 11.00 - 12.30 | Discussion to identify areas needing attention |
| 12.30 - 14:30 | Lunch and administrative arrangements, if any |
| **14:30 – 15:30** | **SESSION 3, Selected topics for in-depth discussions** |
| | Moderator: Dr. W.A. Coward<br>Input by Dr. P. Taylor<br>Comments by all participants |

**Suggested topics:**

- State of the practice of stable isotope methodologies in human nutrition research
- New strategies for improving sensitivity of nutrition monitoring techniques for use in Technical Co-operation (TC) Projects in nutrition
- Explore high quality stable isotope spikes for use in humans
- To standardise the mathematical approaches to evaluate mass spectrometric data when using stable isotope labels within metabolic studies).

15:30 – 16:00 Coffee break

**16.00 – 17.30 Framework for contributions to ICN-17 in Vienna:** JOINT SESSION

- To formulate an effective scientific programme for the workshop on stable isotope users' group in human nutrition for ICN-17, August 2001 (moderator Dr. Walczyck)
- Review IAEA scientific session topics for ICN-17 (moderator Dr. Iyengar)

Introduction by Dr. G.V. Iyengar
Input by all participants to develop the framework

**WEDNESDAY, 6 DECEMBER 2000**

**09:00 – 12:30 SESSION 4, Selected topics for in-depth discussions (continued)**
Moderator: Dr. Coward
Contributions by all participants

**Proposed topics:**

- State of the practice of stable isotope methodologies in human nutrition research
- New strategies for improving sensitivity of nutrition monitoring techniques for use in Technical Co-operation (TC) Projects in nutrition
- Explore high quality stable isotope spikes for use in humans
- To standardise the mathematical approaches to evaluate mass spectrometric data when using stable isotope labels within metabolic studies)

- **Other topics to be brought for discussion:**
  Proposed actions and identifying authors for writing specific segments
  Coffee break as needed
  Lunch

**14.30 - 17.00 SESSION 5: Combined session with both CS groups:** JOINT SESSION
Short reports by both groups by respective chairpersons
Comparative assessment: isotopic vs non-isotopic methods
Approaches for certification of organic nutrients in natural matrix materials: Specific examples
Other topics to be brought for discussion

**THURSDAY, 7 DECEMBER 2000**

**09:00 – 12:30 SESSION 6, Preparation of draft report**
Completion of individual contributions as assigned
Lunch
**14.30 - 17.00 SESSION 7, Preparation of draft report (continued)** Coffee break as needed

**FRIDAY, 8 DECEMBER 2000**

**09:00 – 12.00 SESSION 8, Finalising the Report**
Timetable for further action needed
Conclusions of the meeting
Closing of the meeting

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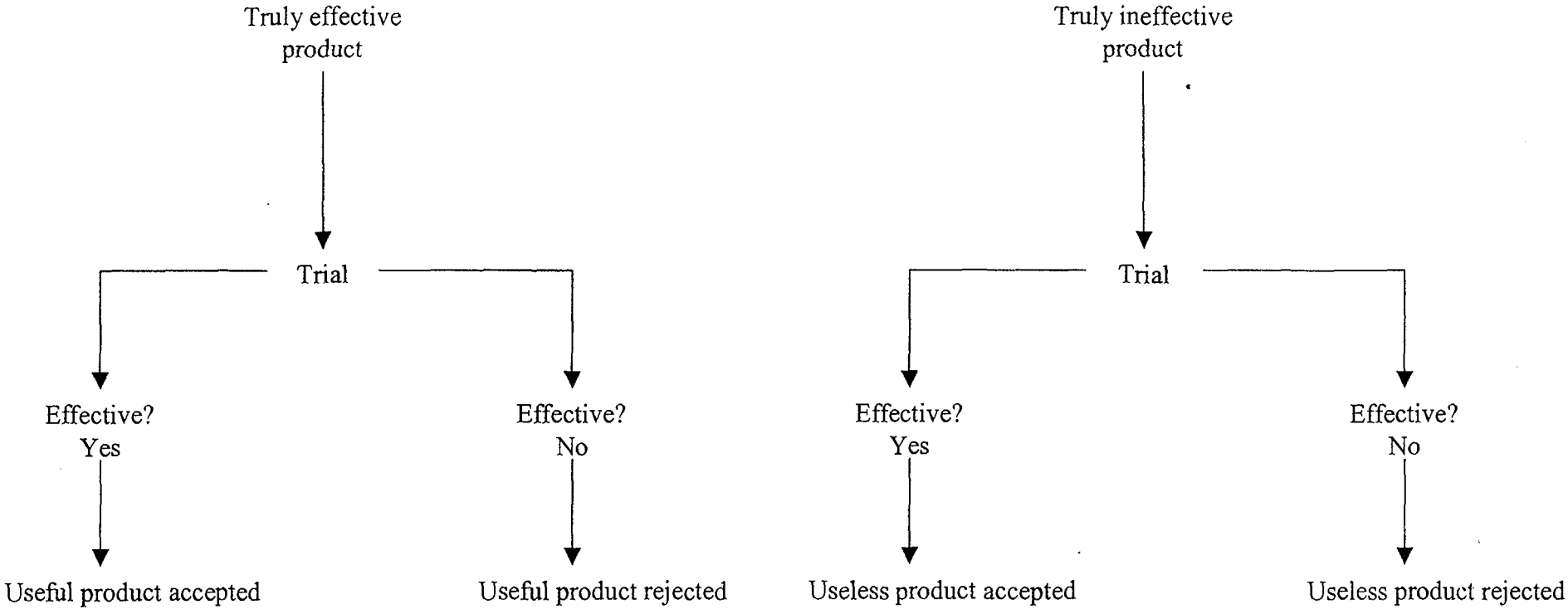
Truly effective product
Trial
Effective? Yes
Effective? No
Useful product accepted
Useful product rejected
Truly ineffective product
Trial
Effective? Yes
Effective? No
Useless product accepted
Useless product rejected

**Figure 1.**

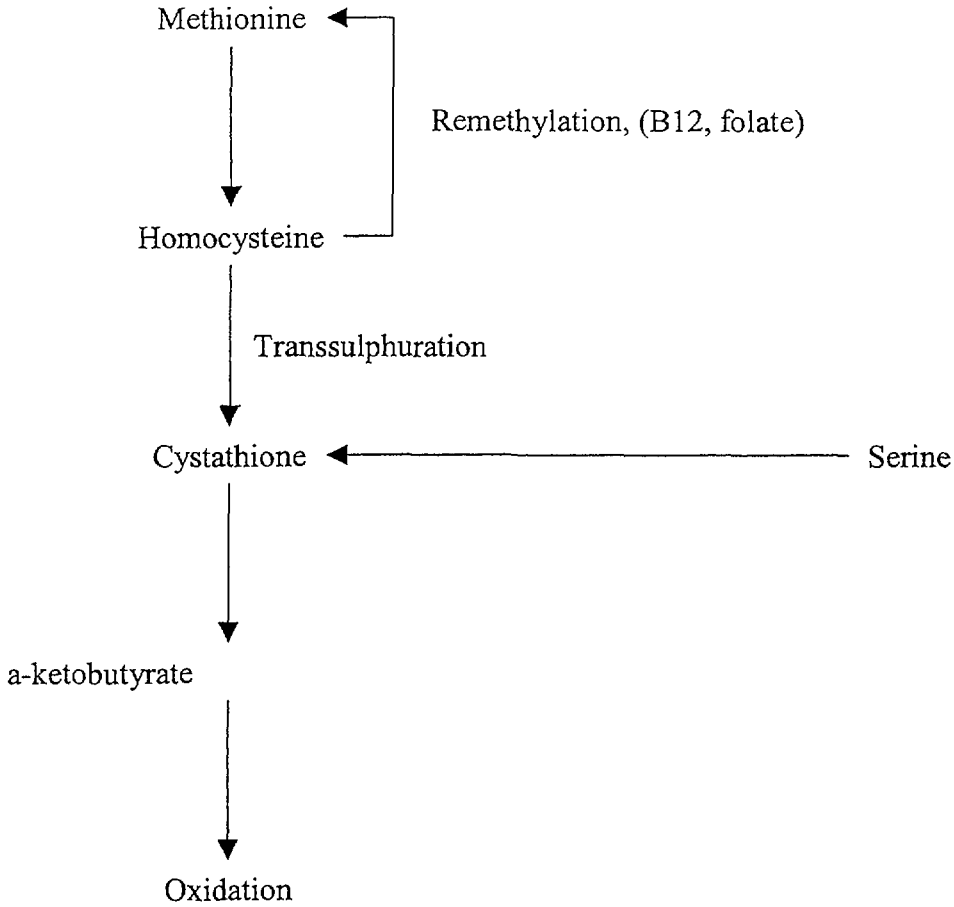
Methionine
Remethylation, (B12, folate)
Homocysteine
Transsulphuration
Cystathione
Serine
a-ketobutyrate
Oxidation

**Figure 2.**