NOTE: The information given below is a short compilation of discussions and presentations of the Advisory Group Meeting. The opinions expressed in the recommendations are those of the invited experts and do not imply that the IAEA will directly implement them. A prioritization of recommended actions will be necessary in view of the IAEA’s mandate, the available limited resources and keeping in mind related ongoing activities (e.g. establishing whole-air standards for isotopic studies on trace gases in air; re-calibration of existing carbonate reference materials). First activities have already started concerning the production of suitable organic stable isotope reference materials. Some other activities like those proposed for labelled compounds could be only started after careful check of the financial implications and the associated workload.

An IAEA TECDOC is in preparation including the complete working group reports and all submitted and presented papers.

Some of the recommendations and conclusions of this meeting certainly will be discussed again in the forthcoming 8th Advisory Group Meeting on stable isotope reference materials planned for 11-15 September 2000 in Vienna.

CONTENTS OF THIS DOCUMENT

- Summary
- Table: List of recommended possible new reference materials
- Recommendations of the Working Group Reports:
  - Biogeochemistry, Food and Ecology
  - Hydrology and Oceanography
  - Medicine and Biology (labelled compounds)
  - Atmosphere
- List of submitted background papers
SUMMARY

During the past decade, the applications requiring measurements of light element stable isotopes (H,C,N,O,S) has expanded dramatically. Previously, geochemists were the dominant developers and users of isotope techniques, especially at natural abundance levels. However, the situation has changed with the broader range of disciplines now adapting and incorporating isotopes to, for example, characterize physiochemical or biological pathways, fingerprint substances and trace material fluxes. As a consequence, the requirements of the instruments and infrastructure to support them have also changed. Coupled with this wider recognition of isotope applications, there have also been significant developments in instrumentation. This document reports several of the instrumental improvements made during this decade. The two most important developments are (1) Continuous Flow-Isotope Ratio Mass Spectrometers (CF-IRMS), and (2) optical techniques for stable isotope determinations. However, with these new instruments and the increase in isotope user base, there are new needs placed on the isotope community.

CF-IRMS is a generic term for IRMS instruments that are coupled on-line to preparation and partitioning lines or instruments. This includes Elemental Analyser-IRMS (EA-IRMS) that has greatly simplified the isotope measurements of bulk constituents. It also refers to the coupling of Gas Chromatographs to IRMS systems, either with or without intervening combustion or reduction interfaces (e.g., GC-IRMS, GC-C-IRMS, GC-Py-IRMS). The key features of CF-IRMS include sample size and analytical time reductions, but perhaps most significant is the possibility of making isotope measurements on individual compounds separated on-line from complex mixtures. With the availability of GC-C-IRMS, we are now able to merge the molecular and stable isotope information contained in complex samples, such as oils, fatty or amino acids, flavours and biomarkers.

Optical spectroscopic techniques for stable isotope ratio determinations (OIRS), typically use the absorbance of different spectral lines for the various isotope species, e.g., $^{12}\text{C}^{16}\text{O}^{16}\text{O}$ vs. $^{13}\text{C}^{16}\text{O}^{18}\text{O}$, in the infrared region. Detection arrangements vary, e.g., from direct absorbance measurements (correlation or non-dispersive IR spectroscopy), phase sensitive detection to optogalvanic configurations, and the light sources can be spectrally narrow (laser) or broad (e.g. glow bars). Currently, large developments are being realised in OIRS techniques, and they ultimately offer some significant operational advantages in the near and medium future. These include simplified instrumentation, multiple measurements, non-destructive determinations, and reduction in contaminations, e.g. isobaric problems.

Despite the analytical improvements, the measurement of stable isotopes is still reported as ratios relative to standards of known isotope ratio. This is necessitated by the continuing difficulties in making precise and accurate direct determinations of individual isotope abundances, e.g., true counts of $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$. Fortunately, the instrumentation configurations are such that several of the errors are common for the different isotope species, and cancel if the results are presented as a ratio. Again because the measurements are not based on absolute determinations, the measured isotope ratio has to be calibrated to an internationally recognized isotope ratio. The creation of reliable isotope standards or reference materials is critical to compare results between runs on a single machine, but also to relate them to other machines and laboratories. The requirement for isotope standards has increased dramatically with the new technologies, but also due to the wider range of applications. As a result, there are new challenges for the isotope community to extend the offering of standard materials.
The Advisory Group Meeting resulting from the considerations as outlined above took place from 20 to 23 September 1999 at IAEA Headquarters in Vienna, Austria. It was attended by eight invited experts and by nine observers from other institutions. The meeting was structured in a way to present the state-of-the-art in various scientific fields and – if possible – to find solutions to urgent problems for the different user communities from the perspective of IAEA and its international role in providing reference materials for the standardization of stable isotope measurements.

The meeting started with experts’ reports on various techniques, instruments and stable isotope applications, reviewing the “state-of-the-art” in their respective fields. In almost all cases, the measurement or application requirements drive the technique and instrument development, which in turn drives the requirements for calibration protocols and reference materials. The great advances in GC techniques with various separation columns and post conversion for IRMS analysis have allowed more sophisticated studies with smaller samples while still maintaining adequate precision to draw valid scientific conclusions. The major current limitations to accuracy and precision in many measurements with complex molecules and bulk samples is fractionation in the conversion of components to the gas analyzed and overlap in the column separation- combustion cycle.

A diversity of applications applying the continuous flow technologies toward increasing the understanding and documenting the origins of organic materials in a wide variety of problems was presented during the meeting. The applications to food analysis, biogeochemical studies, ecological investigations, etc. indicated a number of limitations on utilizing the technologies to their fullest. These limitations included the need for derivatization of non-volatile components, co-elution in complex mixtures and especially the lack of pure reference materials, which would be available for each type of analysis. It was apparent that each technology presented specific requirements, but in many cases for both bulk isotope analysis and compound specific isotope analysis, more universal standards should be available with prospects for multi-elemental compositions.

Atmospheric studies, in contrast to food, flavour and other hydrocarbon studies, require the highest precision in the order of 0.02 ‰ for $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ in the study of a few simple molecules e.g. water, carbon dioxide, methane, N$_2$. The situation was reviewed on labelled species measurements in terms of highly variable concentrations and change of isotopic ratios over long time periods (weeks to decades) with problems on dilution effects, scale stability and interlaboratory comparisons. In particular, the problem of traceability to a primary isotopic abundance scale is significant.

The state-of-the-art in other than mass spectrometric isotope ratio measurement was reviewed with specific details on compound specific devices for water and carbon dioxide. The advantage of the new optical techniques is their molecular specificity and their potential for wide field deployment. GC-laser devices should be considered, especially using nitrogen as the carrier gas for CO$_2$ studies with the new LARA device. Resolution limitations and traceability of results to primary standards was also considered.

A considerable part of the discussion was devoted to the calibration of measurements and the availability of suitable international reference materials. The assessment started with the discussion of the existing IAEA stable isotope reference materials (RMs) and their suitability for the new analytical techniques. Most of these RMs were prepared in the seventies and therefore they quite clearly do not comply with the requirements in terms of homogeneity at tiny sample amounts – at that time only offline preparation techniques were available using samples sizes about two orders of magnitude.
larger than possible today. Further on those measurement techniques limited considerably the number of determinations used for calibration of the “old” materials. Together with a lack of documentation of those measurements it causes a gap in the traceability of those materials.

The assessment of needs and wishes of the different user communities resulted in the preparation of a list of candidate materials (table 1), which could serve as reference materials for a variety of different applications and would have to be complemented by specialized materials to be prepared and maintained by interested groups within the relevant scientific communities themselves. Serious problems were identified to find dedicated institutions offering their services in initial testing and certification of possible new candidates for reference materials. Fortunately several of the scientists present at the meeting committed themselves to provide such assistance for implementing the ambitious plans. The IAEA Isotope Hydrology Laboratory will try to assist within its mandate to coordinate some of these efforts. It is envisaged within the next twelve months to further screen available materials on their suitability and their isotopic composition and to initiate the testing of possible materials for bulk analysis and of one mixture of compounds for GC separation techniques. It is planned to report first findings and results to the experts at the next regular IAEA Advisory Group Meeting on Reference Materials during 11-15 September 2000 in Vienna, where the next steps and further implementation of the programme on organic stable isotope reference materials should be reviewed.

The synergism of discussions among experts from a variety of scientific fields and disciplines, but having both a common vocabulary and common interest in advancing the measurement science of stable isotope ratios was apparent from the discussions. In particular, both common and unique problems, solutions and calibration needs were discussed. Finally the instrument development experts were able to determine the limitations of devices and the potential to advance the applications science through the new instrumentation techniques and technologies.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Isotope</th>
<th>Technique</th>
<th>Reference material</th>
<th>Precision required for RM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>$^{13}$C, $^{18}$O</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;0.2 ‰</td>
</tr>
<tr>
<td></td>
<td>$^{2}$H</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;2 ‰</td>
</tr>
<tr>
<td>Thiourea</td>
<td>$^{13}$C, $^{15}$N, $^{18}$O, $^{34}$S</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;0.2 ‰</td>
</tr>
<tr>
<td></td>
<td>$^{2}$H</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;2 ‰</td>
</tr>
<tr>
<td>Methionine</td>
<td>$^{13}$C, $^{15}$N, $^{18}$O, $^{34}$S</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;0.2 ‰</td>
</tr>
<tr>
<td></td>
<td>$^{2}$H</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;2 ‰</td>
</tr>
<tr>
<td>N-Methylanthranilic acid ester</td>
<td>$^{13}$C, $^{15}$N, $^{18}$O, $^{34}$S</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;0.2 ‰</td>
</tr>
<tr>
<td></td>
<td>$^{2}$H</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;2 ‰</td>
</tr>
<tr>
<td>Fatty acid methyl ester (C12, C18)</td>
<td>$^{13}$C, $^{18}$O</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;0.2 ‰</td>
</tr>
<tr>
<td></td>
<td>$^{2}$H</td>
<td>CF-IRMS</td>
<td></td>
<td>&lt;2 ‰</td>
</tr>
<tr>
<td>GROB-Test (mixture)</td>
<td>$^{13}$C, $^{18}$O</td>
<td>GC-IRMS</td>
<td></td>
<td>&lt;0.2 ‰</td>
</tr>
<tr>
<td>Enriched dilution series</td>
<td>$^{13}$C, $^{15}$N, $^{18}$O</td>
<td>CF-IRMS, optical techniques</td>
<td>H$_2$O, C-containing material (CO$_2$, carbonate, other), N-containing material</td>
<td>0.1 ‰ 1 ‰ of δ</td>
</tr>
<tr>
<td></td>
<td>$^{2}$H</td>
<td>CF-IRMS, optical techniques</td>
<td></td>
<td>0.8 ‰ 1 ‰ of δ</td>
</tr>
<tr>
<td>CH$_4$</td>
<td>$^{2}$H</td>
<td>CF-IRMS, TD-LAS</td>
<td>Whole air samples</td>
<td>&lt;0.5 ‰</td>
</tr>
<tr>
<td></td>
<td>$^{13}$C</td>
<td>CF-IRMS, TD-LAS</td>
<td>Whole air samples</td>
<td>&lt;0.05 ‰</td>
</tr>
<tr>
<td>CO</td>
<td>$^{13}$C, $^{18}$O</td>
<td>CF-IRMS</td>
<td>Whole air samples</td>
<td>&lt;0.1 ‰</td>
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<td>N$_2$O</td>
<td>$^{15}$N (bulk), $^{15}$N (center), $^{18}$O</td>
<td>CF-IRMS, TD-LAS, FT-IR</td>
<td>Whole air samples</td>
<td>&lt;0.05 ‰</td>
</tr>
<tr>
<td>H$_2$, CH$_4$, CO, N$_2$O</td>
<td>$^{2}$H, $^{13}$C, $^{14}$C, $^{15}$N, $^{18}$O</td>
<td>irmMS</td>
<td>Trace gas concentrate cocktail</td>
<td>as above</td>
</tr>
<tr>
<td>CH$_4$, CO, N$_2$O, O$_3$ in stratosphere</td>
<td>$^{2}$H, $^{13}$C, $^{15}$N, $^{17}$O, $^{18}$O</td>
<td>irmMS, TD-LAS, FT-IR</td>
<td>Enriched (pure) gases ($^{2}$H: 0 ‰, $^{13}$C: 0 ‰, $^{15}$N: 150 ‰, $^{18}$O: 100 ‰)</td>
<td>as above</td>
</tr>
<tr>
<td>NMHC</td>
<td>$^{13}$C</td>
<td>CF-IRMS</td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>CH$_4$</td>
<td>$^{2}$H</td>
<td>CF-IRMS, TD-LAS</td>
<td>Whole air samples</td>
<td>&lt;0.5 ‰</td>
</tr>
</tbody>
</table>
Working Group Recommendations- BIOGEOCHEMISTRY, FOOD and ECOLOGY

- All primary standard materials must be certified according to the ISO 9000 guidelines. For $^{18}$O the VSMOW-SLAP and the VPDB scale have to be connected for organic and inorganic substances.
- The cellulose standard (IAEA-CH-3) calibration for carbon, oxygen and hydrogen isotope determinations needs to be accomplished as a reference material for bulk analysis versus the primary calibrated standards.
- At present there is no appropriate organic standard material for bulk analysis which contains nitrogen. Such a material could be developed, and should contain all five light isotope elements, an example of which could be thiourea or methionine.
- For compound specific standards, there is a need for a calibrated material that contains carbon, oxygen, nitrogen and hydrogen. This standard could be used for assessing multielement isotope determinations by a variety of techniques. We suggest the methyl and a longer carbon chain (perhaps octyl) derivatives of the N-methyl anthranilic acid esters.
- An additional need exists in GC-Pyrolysis for calibration with a material which does not contain nitrogen as an alternative to the multielement standard recommended. Such a material could be two compounds, such as, C$_{12}$ and C$_{18}$ fatty acids methyl esters.
- A need for rigorous testing for chemical and isotope stability is required for all these new standards.
- The compound specific reference materials should be available as either a single component or in a mixture.
- The members of the working group recommend that the distribution of these materials should be undertaken by the IAEA.

Working Group Recommendations - HYDROLOGY and OCEANOGRAPHY

- All laboratories handling water samples should follow a routine for calibration and normalisation on a daily basis. This calibration and normalisation should be based on 2 local water standards, that are distinctly different (preferably one similar to VSMOW, the other to SLAP). The local water standards should be checked for stability, and should be periodically calibrated against the primary standards VSMOW and SLAP.
- It should be realised that the value of the “light” local water standard w.r.t VSMOW (that is, calibrated, but not yet normalised) forms a valuable record, with information about stability of the IRMS system and the water preparation line.
- All laboratories co-operating in maintaining the IAEA GNIP network (Global Network of Isotopes in Precipitation) should recognise that careful and reliable calibration & normalisation is crucial for the quality and usefulness of the GNIP database. Only in that way the total GNIP network, consisting of measurements of tens of individual laboratories, can be merged successfully. To test the consistency, regular intercomparisons are necessary.
The IAEA should make available a so-called dilution series of reference materials for water (both $^{18}$O and $^2$H), a C-containing material (either pure CO$_2$ gas, carbonates or some other material), and N-containing material. This IAEA material is to be used to calibrate a local dilution series which each laboratory will need to prepare individually, or to obtain it from a commercial supplier (as in the case of medical applications is to be foreseen). The local dilution series RM should be used for daily calibration of the instrument.

Commercial suppliers offering isotopically substituted materials should be put to the test regularly, as the quality (isotopic signature) of such materials are critical for the derived local dilution series (as well as for medical diagnostics). This can be done by preparing a dilution series based on the manufacturer’s product certificate, and by checking the resulting standards against a well-characterized dilution series.

For new techniques like clinical applications of isotope measurements and laser spectrometry techniques to be accepted by the isotope community they should be adequately compared with existing state-of-the art equipment and checked against isotope reference materials that are back-traceable to (IAEA) primary standards. Publications should distinguish between precision, accuracy, as well as instrument stability. In addition they should clearly state the procedure followed for calibration (defining the scale zero) and normalization (scale multiplication factor). In practice this means, e.g. for the case of water, that VSMOW, SLAP, GISP and a number of local standards should be measured.

Set up techniques to trace back working standards to primary IAEA calibration standards VSMOW, NBS19 and atmospheric nitrogen.

Use whole air samples as intercomparison materials to exchange between labs making these measurements. This has the advantage that the same preparation and analysis path as unknown air samples is followed. With dual inlet IRMS this is difficult because samples are huge and require very expensive shipment of high pressure cylinders of air as dangerous goods. CF-IRMS will bring in the great advantage that much smaller air samples can be used. We recommend that low pressure air samples in glass flasks are used. These can be sent by regular parcel post very cheaply.

For the studies in the stratospheric background, highly enriched standards are needed for CH$_4$, N$_2$O, O$_3$, and CO. We recommend to have standards for the enriched end member in D, $^{13}$C, $^{15}$N, and $^{18}$O in the range of around 0 for $\delta$D, 0 for $\delta^{13}$C, 150 for $\delta^{15}$N, and 100 for $\delta^{18}$O respectively.

Another possibility to avoid the use of high-pressure cylinders would be the preparation of a synthetic ”trace gas concentrate” mixed into pure N$_2$ at relative concentration levels as well as isotopic compositions close to the environmental values of the different components (e. g., CH$_4$, CO, N$_2$O and H$_2$). The ”trace gas concentrate” could be shipped in amounts of only 1 liter STP to laboratories around the world where the initial concentrations would then be mixed by ”zero air” depleted by the relevant component(s). The reconstitution could be achieved either dynamically using mass flow controllers or statically by mixture into a large volume. The required gases would be extracted from ambient air, wherever applicable. The isotopic and concentration integrality of the enriched material in the primary cylinder have to ascertained.

For other species like NMHC, isoprene and terpenes the situation is more difficult and will require further research. We recommend contacting an expert in this field (names stated).
A. Optical spectroscopic techniques

1. E.R.Th. Kerstel, R. van Trigt, N. Dam, J. Reuss, H. A. J. Meijer: Laser spectrometry applied to the simultaneous determination of the $\delta^2\text{H}$, $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ isotope abundances in water:

   **Abstract** - We demonstrate the first successful application of infrared laser spectrometry to the accurate, simultaneous determination of the relative $^2\text{H}/^1\text{H}$, $^{17}\text{O}/^{16}\text{O}$, and $^{18}\text{O}/^{16}\text{O}$ isotope abundance ratios in natural water. The method uses a narrow line width color center laser to record the direct absorption spectrum of low-pressure gas-phase water samples (presently 10 µl liquid) in the 3 µm spectral region. The precision of the spectroscopic technique is shown to be 0.7 ‰ for $\delta^2\text{H}$ and 0.5 ‰ for $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$, while the calibrated accuracy for natural waters amounts to about 3 ‰ and 1 ‰, respectively.

2. D.E. Murnick: Laser assisted ratio analysis - an alternative to GC/IRMS for CO$_2$:

   **Abstract** - A new technique for laser based analysis of carbon isotope ratios, with the acronym LARA, based on large isotope shifts in molecular spectra, the use of fixed frequency isotopic lasers, and sensitive detection via the laser optogalvanic effect is reviewed and compared with GC/IRMS for carbon dioxide in specific applications. The possibility for development of new classes of isotope ratio measurement systems with LARA is explored.

B. CF-techniques applied for stable isotopes in organic materials

3. H. Förstel, M. Boner, H. Prast: On-line measurement of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratio by elemental analyser - diluter mass spectrometer combination:

   **Abstract** - Food control necessitates rapid procedures to test the authenticity of a material not dependent on chemical adulterations. A foodstuff is produced in an individual „isotopic environment“ from where an specific isotopic composition like a fingerprint originates. The isotope ratio mass spectrometry IRMS can measure the stable isotopic composition of simple gases only. These gases are produced by an elemental analyser. After separating the gases their content is determined by an TCD. The outflow is partly sucked into the mass spectrometer via a capillary reducing the atmospheric pressure down to vacuum. For the portions of the biological important elements differ significantly, e.g. C : N as 25 : 1, the amount of gases produced from a single sample have to be adapted to a usable level by help of a diluter. The diluter configuration can be used to measure repeatedly the $^{13}\text{C}/^{12}\text{C}$ ratio of carbon dioxide in mineral waters, as well as to measure $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ ratios of biological and soil samples simultaneously within one run. In the first application different kinds of carbon dioxide, produced naturally (well) or technically (process), can be distinguished. The second application offers the possibility to trace the fate of fertilizer in vineyards measuring the natural isotopic variation of nitrogen and carbon in soil and vines.


   **Abstract** - A new method for the automated sample conversion and on-line determination of deuterium, carbon, nitrogen and oxygen isotopes for organic and inorganic substances is presented. The samples are pyrolytically decomposed in a high temperature pyrolysis system (HTP) at a temperature higher than 1400 °C in the presence of reactive carbon. The method has a great potential for the future analysis of hydrogen, carbon, nitrogen and oxygen isotopes. The instrumentation and application is very simple and cost effective. The reproducibility of the delta values for D/H is 3 ‰, for $^{18}\text{O}$ is 0.3 ‰, and for $^{13}\text{C}$ and $^{15}\text{N}$ 0.2 ‰ respectively. The system is suitable for solid, liquid and gaseous samples. The isotopic composition inter-laboratory and international reference materials show of the accuracy of the method.
5. C. Guillou, F. Reniero: Isotope methods for the control of food products and beverages:  
(no abstract included)

6. S. A. Macko, M. H. Engel: Methods in the use of GC/C/IRMS for the analysis of biochemical and pollutant isotope signatures of compounds:  
Abstract - The potential for application of GC/C/IRMS analysis to any multitude of environmental, ecological or biochemical research areas is only beginning to be realized. Extension of compound-specific isotope analytical data derived from modern organisms and settings to yield interpretations of ancient depositional environments certainly appears possible. Further application of GC/C/IRMS approaches to understand the cycling of carbon and nitrogen, the identification and alteration of pollutants, or resolve metabolic relationships between compounds in living or extinct organisms are all within the scope of future research.

7. A. Mosandl, U. Hener: Capillary gas chromatography, online coupled with isotope ratio mass spectrometry (GC-IRMS) - current developments:  
(no abstract included)

8. I. Tolosa, S. de Mora: Stable carbon isotope ratios of lipid biomarkers and their applications in the marine environment:  
(no abstract included)

9. M. J. Whiticar, M. Eek: Challenges of $^{13}$C/$^{12}$C measurements by CF-IRMS of biogeochemical samples at sub-nanomolar levels:  
(no abstract included)

C. CF-techniques applied to non-organic compounds

10. D. C. Lowe, D. J. Ferretti, R. J. Francey, C. E. Allison: Taking the atmosphere’s pulse: The application of GC-IRMS to stable isotopes in atmospheric trace gases:  
(no abstract included)

11. H. A. J. Meijer: Isotope ratio analysis on water: A critical look at developments:  
(no abstract included)

12. D. Yakir: On-line stable isotope measurements during plant and soil gas exchange:  
(no abstract included)

13. N. Yoshida: Continuous flow IRMS application to CH₄, NMHCs, and N₂O in the atmosphere and the oceans:  
(no abstract included)