REFERENCE SHEET FOR STABLE ISOTOPE REFERENCE MATERIALS

IAEA-604 (water, low level \(^{2}\)H enriched)
IAEA-605 (water, medium level \(^{2}\)H enriched)
IAEA-606 (water, high level \(^{2}\)H enriched)

Certified Reference values for \(\delta^{2}\)H and \(\delta^{18}\)O stable isotopic composition

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>Reference value (\delta^{2})H</th>
<th>Combined standard uncertainty for (\delta^{2})H</th>
<th>Reference value (\delta^{18})O</th>
<th>Combined standard uncertainty for (\delta^{18})O</th>
<th>Information value(^a) (\delta^{17})O</th>
<th>Estimated uncertainty for (\delta^{17})O</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA-604</td>
<td>799.9</td>
<td>0.4</td>
<td>-5.86</td>
<td>0.04</td>
<td>-3.2</td>
<td>0.4</td>
</tr>
<tr>
<td>IAEA-605</td>
<td>5997.9</td>
<td>0.5</td>
<td>-3.02</td>
<td>0.04</td>
<td>-2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>IAEA-606</td>
<td>15993.6</td>
<td>1.0</td>
<td>2.43</td>
<td>0.04</td>
<td>-0.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

\(^{a}\)The \(\delta^{17}\)O values in this table are derived from mass 45 data of CO\(_{2}\), and are therefore depending on additional factors like the \(^{13}\)C abundance. These \(\delta^{17}\)O values are therefore given for information purposes only. For the moment, the \(\delta^{17}\)O scale relies only on its zero point as defined by VSMOW and is not a normalized 2-point scale.
The reference values for δ²H and δ¹⁸O in these three water reference materials as given in Table 1 were determined from the isotopic compositions of two parent waters by gravimetric mixing: a demineralized natural water sample, and a highly ²H enriched water (specified as ²H fraction = 99.993 %, Sigma Aldrich, Zwijndrecht, The Netherlands). The isotopic composition of the natural water had been calculated from calibrated values submitted by five laboratories. The pure ²H water was also assessed for its oxygen isotopic composition. Independent validation measurements have been performed to ensure correctness of the applied mixing procedure. The full details are reported in [1].

The combined uncertainties of reference values were calculated from the isotopic assessment of raw materials including necessary isotopic corrections, and the weighing uncertainties including necessary corrections. A special spreadsheet was developed and validated to facilitate the calculation including isotopic abundances, and to derive uncertainties from the mixing process [2]. Derived reference values and uncertainties were checked by analyses in two different laboratories using three different isotopic measurement methods and found to be consistent, and as expected with the performed isotopic analyses showing a much higher level of uncertainty. The potential inhomogeneity of produced ampoules was checked by measurements under repeatability conditions and found to be analytically insignificant and was therefore not included as uncertainty component.

**Rationale for preparation of these reference materials**

Bio-medical research using water with enriched levels of the rare stable isotopes of hydrogen and/or oxygen requires well-characterized enriched reference waters [1]. The International Atomic Energy Agency (IAEA) did have such reference materials available (IAEA-302 and IAEA-304 sets) [3], but these are exhausted since several years. In an IAEA Reference Material expert meeting in 2010 the necessity for replacement materials was discussed, which resulted in an IAEA technical contract for the Centre for Isotope Research of the University of Groningen (CIO), Netherlands, providing seed funding for preparation of two sets of three new reference materials each in sufficient quantity as replacement. These three water reference materials enriched in δ²H constitute one out of two sets produced, with the other set containing three further water reference materials enriched in both δ²H and δ¹⁸O (reference materials IAEA-607 to IAEA-609) [1].

**Origin and preparation of the material**

The major constituent of the three materials was a natural local tap water sample, deionized in a large quantity to prepare all three samples (approximate δ²H = -42.7 ‰ and δ¹⁸O = -6.3 ‰). The second component was an isotopically highly ²H enriched water (specified ²H fraction = 99.993 %, Sigma-Aldrich, Zwijndrecht, The Netherlands). Note: This “highly ²H enriched water” should not be confused with the name used for IAEA-606 “water, high level ²H enriched”, which is a 1:360 dilution of the latter material.

For preparation and storage of these reference waters, 30-L stainless steel containers were used that are normally used for potable liquids such as wine. The lids of these containers were modified and equipped with a self-developed water extraction system excluding evaporation and contamination with atmospheric water vapour when drawing water [4]; for this purpose the containers are kept under slight overpressure of Argon gas.

Each of the three containers was initially filled with about 20 kg of this demineralized local tap water. The water amount was determined to a precision of better than 1 g.
The isotopically $^2$H enriched water was added in small open glass vials such that added quantities ranging from about 1.5 g to over 50 g could still be weighted to a precision of between 0.01 to 0.1 mg. Weighted masses are reported in Table 2. Care was taken to limit evaporative losses during weighing by covering the vials and working quickly. The filled vials with the $^2$H enriched water were then completely immersed into the containers for complete mixing, and the containers were tightly capped with the special lids. During the next three weeks of storage, the containers were regularly moved/rolled to ensure complete mixing.

The actual isotopic values obtained differed only marginally from the originally aimed values calculated by use of the spreadsheet due to the limits of the actual filling process. A buoyancy correction for the two waters differing in density due to different isotopic composition was applied.

### TABLE 2. MASSES OF THE PARENT WATERS USED FOR THE PREPARATION OF REFERENCE MATERIALS IAEA-604, IAEA-605 AND IAEA-606

<table>
<thead>
<tr>
<th>Name</th>
<th>Mass of natural water (g)</th>
<th>Mass of highly $^2$H enriched water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAEA-604</td>
<td>22000.2 ± 0.7</td>
<td>3.21228 ± 0.00002</td>
</tr>
<tr>
<td>IAEA-605</td>
<td>20041.4 ± 0.7</td>
<td>20.9764 ± 0.00010</td>
</tr>
<tr>
<td>IAEA-606</td>
<td>20219.0 ± 0.7</td>
<td>56.1803 ± 0.00010</td>
</tr>
</tbody>
</table>

### Characterization study

The $\delta^2$H and $\delta^{18}$O isotopic composition of the natural demineralized tap water was assessed in an interlaboratory comparison involving five different experienced laboratories versus VSMOW2/SLAP2 on the normalized scale. The $\delta^{17}$O is assumed to be coupled to the $\delta^{18}$O isotopic composition due to the natural origin of this water.

The isotopic values of the individual laboratories are provided in Table 3. The best estimate as weighted mean value for the natural water isotopic composition is calculated from the set of data (excluding one outlier for $\delta^{18}$O) and is provided at the last row of Table 3. The associated combined standard uncertainties of the two weighted mean values had been expanded by a factor of two as conservative estimate, and are both given at 1σ level.

### TABLE 3. STABLE ISOTOPIC COMPOSITIONS ($\delta^2$H, $\delta^{18}$O) OF USED NATURAL WATER AND ASSOCIATED COMBINED STANDARD UNCERTAINTIES (1σ LEVEL) AS DETERMINED BY FIVE LABORATORIES

<table>
<thead>
<tr>
<th>Lab number</th>
<th>Method</th>
<th>$\delta^{18}$O (‰) vs VSMOW2/SLAP2</th>
<th>$\delta^2$H (‰) vs VSMOW2/SLAP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Laser spectrometry</td>
<td>-6.22 ± 0.07</td>
<td>-42.1 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>Laser spectrometry</td>
<td>-6.34 ± 0.05</td>
<td>-42.7 ± 0.3</td>
</tr>
<tr>
<td>3</td>
<td>Mass spectrometry</td>
<td>-6.30 ± 0.03</td>
<td>-42.5 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>Mass spectrometry</td>
<td>-6.36 ± 0.03</td>
<td>-43.3 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>Mass spectrometry</td>
<td>-6.70 ± 0.04</td>
<td>-42.9 ± 0.4</td>
</tr>
<tr>
<td>Weighted mean</td>
<td></td>
<td>-6.32 ± 0.04</td>
<td>-42.7 ± 0.4</td>
</tr>
</tbody>
</table>
The highly $^2\text{H}$ enriched water used for preparation of the reference materials had a high $^2\text{H}$ purity of 99.993 %, certified by the supplier (Sigma-Aldrich) for that batch by $^1\text{H}$-NMR spectroscopy. Performed independent test measurements corroborated this value, however at lower precision, and a conservative uncertainty estimate of ± 0.005 % for the certified value was used in calculations.

Measurements of $\delta^{18}\text{O}$ and $\delta^{17}\text{O}$ were performed at CIIO on 1:17 dilutions of the highly enriched water by mass spectrometry using the water CO$_2$ equilibration method, in order to test potential co-enrichment of oxygen isotopes in the highly enriched $^2\text{H}$ water. Indeed, a co-enrichment was indicated in both $^{18}\text{O}$ and $^{17}\text{O}$ (isotopic abundances increased by a factor of 4 ($^{18}\text{O}$) resp. 2 ($^{17}\text{O}$) compared to natural samples). This effect is visible in the oxygen data of the set of three single labelled waters scaling with the $^2\text{H}$ enrichment (IAEA-604, IAEA-605, IAEA-606 in the sequence of increasing $\delta^{2}\text{H}$ values). The measurements resulted in a fit correction applied for the oxygen isotopic abundances in the highly $^2\text{H}$ enriched water, which were used in the calculation spreadsheet.

$\delta^{17}\text{O}$ data were calculated based on the CO$_2$ gas used by the conventional CO$_2$-H$_2$O equilibration technique, by calculating the $^{17}\text{O}$ abundance from the mass 45/44 signal ratio. As the $\delta^{17}\text{O}$ values calculated strongly depend on the $^{17}\text{O}$ and $^{13}\text{C}$ absolute abundances in use and are not expressed on a normalised scale like the VSMOW/SLAP scale, these $\delta^{17}\text{O}$ data should be considered as information values only. The achievable precision of this $\delta^{17}\text{O}$ value calculation was limited to about ± 0.4 ‰ in the best case.

For validation of the mixing process and the calculation of reference isotope values, independent measurements were performed on the produced materials both at CIIO and IAEA. At CIIO, for hydrogen analysis, pyrolysis coupled to mass spectrometry and a self-developed SARA laser system were used. For oxygen analysis, a CO$_2$ water equilibration coupled with mass spectrometry, a pyrolysis system, and the SARA laser system were used. At the IAEA, analyses of hydrogen and oxygen were performed using a Picarro laser system, with 9 individual measurement sets per material, either performing direct measurements of enriched materials or diluting samples to a natural isotope level. The memory and drift corrections were applied according the procedure described in [5]; these corrections were considered as one of the uncertainty components.

TABLE 4: BIAS (IN PERMIL) OF HYDROGEN ISOTOPE MEASUREMENTS PERFORMED WITH THREE INDEPENDENT METHODS VERSUS REFERENCE VALUES. THE STANDARD UNCERTAINTIES (1 σ) OF THE INDIVIDUAL METHODS ARE TABULATED

<table>
<thead>
<tr>
<th>$\delta^2\text{H}$</th>
<th>Bias IAEA-604 (%)</th>
<th>Uncertainty IAEA-604 (%)</th>
<th>Bias IAEA-605 (%)</th>
<th>Uncertainty IAEA-605 (%)</th>
<th>Bias IAEA-606 (%)</th>
<th>Uncertainty IAEA-606 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>n.a.</td>
<td>0.4</td>
<td>n.a.</td>
<td>0.5</td>
<td>n.a.</td>
<td>1.0</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>10</td>
<td>10</td>
<td>-13</td>
<td>50</td>
<td>270</td>
<td>120</td>
</tr>
<tr>
<td>Sara-Laser</td>
<td>4</td>
<td>25</td>
<td>22</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picarro-Laser</td>
<td>2.2</td>
<td>3.1</td>
<td>10</td>
<td>14</td>
<td>-14</td>
<td>60</td>
</tr>
</tbody>
</table>

Low bias values in Table 4 for $\delta^2\text{H}$ indicate good agreement of the three methods with the reference value (except for one value on pyrolysis). However, the achievable uncertainty for calibrated measurements is by a factor of 7 to 120 higher than that achievable by use of gravimetric mixing.
TABLE 5: BIAS (IN PERMILLE) OF OXYGEN ISOTOPE MEASUREMENTS PERFORMED WITH FOUR INDEPENDENT METHODS VERSUS REFERENCE VALUES. THE STANDARD UNCERTAINTIES (1 σ) OF THE INDIVIDUAL METHODS ARE TABULATED

<table>
<thead>
<tr>
<th>δ¹⁸O</th>
<th>Bias IAEA-604 (%)</th>
<th>Uncertainty IAEA-604 (%)</th>
<th>Bias IAEA-605 (%)</th>
<th>Uncertainty IAEA-605 (%)</th>
<th>Bias IAEA-606 (%)</th>
<th>Uncertainty IAEA-606 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>n.a.</td>
<td>0.04</td>
<td>n.a.</td>
<td>0.04</td>
<td>n.a.</td>
<td>0.04</td>
</tr>
<tr>
<td>Equilibration</td>
<td>-0.06</td>
<td>0.06</td>
<td>-0.06</td>
<td>0.06</td>
<td>-0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Pyrolysis</td>
<td>0.4</td>
<td>0.5</td>
<td>-0.5</td>
<td>0.5</td>
<td>-0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Sara-Laser</td>
<td>-0.02</td>
<td>0.15</td>
<td>-0.08</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picarro-Laser</td>
<td>-0.08</td>
<td>0.24</td>
<td>-0.2</td>
<td>2.1</td>
<td>-0.5</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Low bias values in Table 5 generally indicate good agreement of the four methods with the reference value within uncertainty limits. The uncertainty of δ¹⁸O for one laser method was severely affected by the use of various dilution approaches, and eventually related to the high enrichment of δ²H.

Homogeneity of the material

Homogeneity tests were performed after flame sealing of 500 ampoules of each reference material from the corresponding bulk storage container. For each material six randomly selected ampoules were split each into six 0.8 mL portions. The produced 36 individual sub-samples were then measured at IAEA under repeatability conditions by laser spectrometry (Picarro system) in one consecutive measurement run. Due to the high enrichment in δ²H, the 36 sample measurements were performed en block without any attempt to calibrate by normal abundance isotopic standards in order not to induce large memory effects. Despite of this effort, the standard deviations obtained within a measurement run were at least one order of magnitude higher than the calibration values obtained by the gravimetric. The average values from each of the six ampoules were tested statistically and no reason was found to believe that the averages differ, both for δ²H and for δ¹⁸O. Therefore the materials and produced ampoules can be considered as being homogeneous within the limits of analytical precision of usual isotope determinations at these levels (see uncertainty values in tables 4 and 5). No heterogeneity component was added to the uncertainty propagation of reference values.

Assignment of values – Certification procedure

The reference values were assigned by using of a specially developed spreadsheet [2] using the data produced by the isotopic assessment of the natural water, by the isotopic abundances provided by the provider of the highly δ²H enriched water, by the oxygen isotopic abundances for the enriched water calculated by back-fitting, and by the weighing proportions of mixing components. Both the isotopic abundances and delta-values of hydrogen and oxygen isotopes were calculated including their combined standard uncertainties. The reference values in Table 1 were calculated with all the information as reported in this reference sheet. More details concerning all reported results as well as the performed value assignment for certification can be found in [1]. Based on this information, the IAEA Reference Materials Certification Committee decided to accept these assigned values as presented in the Table 1 above.
Statement on metrological traceability and uncertainty of assigned values

The δ²H and δ¹⁸O property values assigned to the reference materials IAEA-604, IAEA-605 and IAEA-606 for hydrogen and oxygen isotopes are calculated from isotopic abundance values and from delta-values calibrated versus the international VSMOW-SLAP scale. The measurement uncertainty associated with the assigned value is expressed as absolute value in the respective delta-scale. δ¹⁷O values are given for indicative purposes only, as they depend on several parameters taken for the ¹⁷O-correction procedure [1]. Metrological traceability to the SI units is based on the isotope abundance values of the international measurement standards VSMOW and SLAP, which are linking the delta-values defined by VSMOW and SLAP to the absolute isotope abundance scale.

Intended use

These reference materials are intended to be used for calibration of isotopically enriched (above the natural isotopic abundance range) materials, and for the validation of isotopic abundance statements from commercially available isotopically enriched products.

Instructions for use

The reference materials IAEA-604, IAEA-605 and IAEA-606 are supplied in quantities of 5 g water in flame sealed glass ampoules. The ampoules should be stored under ambient temperature in the dark. The materials are intended for immediate use after opening an ampoule. Due to the high enrichment level for ²H, a contamination risk by water vapour evaporating from the opened ampoule to other open samples nearby should be considered. Storage of opened ampoules is discouraged due to the possible change of its isotopic composition due to dilution with atmospheric moisture by diffusion and isotopic exchange; any residual content is recommended to be discarded by flushing it away with lots of water in a sink.

Issue and expiry date

The issue date of IAEA-604, IAEA-605 and IAEA-606 is 14 December 2015. Based on experience with similar materials, the expiry date is 30 November 2025, provided the original ampoules are handled and stored in accordance with the instructions given in this reference sheet (see “Instructions for use”).

The IAEA is monitoring the long term stability of the material and customers will be informed in case of any observed change.

Limit of distribution

Each material is available for limited distribution to laboratories by allowing the purchase of up to four units of each material within one year (12 months). This limitation is set to keep these reference materials available to as many laboratories as possible for an extended time period, and is therefore a measure to maximize the reference material usability.
Absolute isotopic abundances

Absolute isotope abundance values for these materials are provided in [1]. They can also easily be calculated by entering the delta-values in a special freely available spreadsheet [2].

Legal disclaimer

The IAEA makes no warranties, expressed or implied, with respect to the data contained in this reference sheet and shall not be liable for any damage that may result from the use of such data.

Compliance with ISO Guide 31:2015

The content of this IAEA Reference Sheet is in compliance with the ISO Guide 31:2015, Reference materials – Contents of certificates, labels and accompanying documentation [6].

Citation of this reference sheet

It is suggested to cite this reference sheet according to the following example, as appropriate to the citation format used: INTERNATIONAL ATOMIC ENERGY AGENCY, Reference Sheet for IAEA-604/605/606. IAEA, Vienna, 8pp. and including the revision number and the download path if possible (The latest version published applies, see “Note” below).

Note

Certified values as stated in this reference sheet may be updated if more information becomes available. Users of this material should ensure that the reference sheet in their possession is current. The current version may be found in the IAEA’s Reference Materials online catalogue:

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