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► **To cite this version:**

Anne-Laure Nivesse, Nicolas Baglan, Gilles Montavon, G. Granger, Olivier Peron. Non-intrusive and reliable speciation of organically bound tritium in environmental matrices. *Talanta*, 2021, 224, pp.121803. <10.1016/j.talanta.2020.121803>. <hal-03137938>

HAL Id: hal-03137938

<https://hal.science/hal-03137938v1>

Submitted on 19 Sep 2024

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Non-intrusive and reliable speciation of organically bound tritium in environmental matrices

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Abstract:

Measurements of tritium in its various forms within the environment and especially in organic matter are keys to improving the current understanding of its environmental behavior and distribution. Validated or standardized analytical procedures for tritium determination methods have now been developed for several forms of tritium in environmental samples, yet an analytical lack remains regarding the quantifications of exchangeable and non-exchangeable forms of organically bound tritium (OBT) fractions. The present work therefore aims to provide a means of developing a standardized method for OBT fraction determination by evaluating the robustness and relevance of two methods (intrusive and non-intrusive methods) developed for non-exchangeable OBT quantification on a broad panel of pertinent environmental matrices. The validity and reliability of a non-intrusive method has thus been confirmed through a robust comparative study. Moreover, its relevance for standardization purposes is discussed, while the fundamental weakness of the conventional and most widespread method is highlighted and directly quantified for the first time in relying on many demonstrated biases.

Keywords: Organically Bound Tritium, tritium speciation, tritium analysis, standard method.

1. Introduction

In many countries, the fate of tritium (^3H) in the environment has become a growing concern since the release rates of this radionuclide are expected to rise significantly over the upcoming decades. Changes in fuel management methods along with new tritium-emitting facilities, such as the International Thermonuclear Experimental Reactor (ITER) and Evolutionary Power Reactor (EPR), are thus expected to generate increasing discharges of tritium into the environment [1,2]. Tritium can be found in several forms within the environment, e.g. gaseous, liquid or integrated into living organisms as Tissue Free Water Tritium (TFWT) or Organically Bound Tritium (OBT) [[3], [4], [5]]. OBT is typically subdivided into exchangeable OBT (E-OBT), which rapidly equilibrates with the surrounding atmosphere, and non-exchangeable OBT (NE-OBT) that remains in the organic matter until its degradation [6,7]. At the present time, only TFWT and OBT quantifications have yielded validated or standardized analytical procedures [8,9] serving as tritium determination methods in environmental samples, yet without considering the exchangeable and non-exchangeable fractions. However, the NE-OBT fraction is directly representative of the amount of tritium released into the environment during growth of the biological organism and, consequently, considered to be a reliable environmental marker [[6], [10], [11], [12]]. The validation of a reliable and robust determination method for the various forms of OBT should therefore be of great benefit in the context of retrospective studies of tritium releases, as well as a necessity in assessing tritium distribution and behavior in the environment.

The conventional and most widespread method for quantifying exchangeable and non-exchangeable OBT fractions in an environmental matrix is carried out in the aqueous liquid phase [[5], [11], [13], [14]]. The E-OBT fraction is then eliminated from the sample by means of isotopic exchange, and the remaining tritium activity measured in the sample after treatment is directly attributed to the NE-OBT fraction. Some possible drawbacks of this intrusive method have nonetheless been pointed out, including the potential solubilization effect and a partial degradation of organic matter occurring during sample immersion in water. Investigations have made it possible to identify families of solubilized molecules on specific environmental matrices [15], yet several questions still persist on the resulting loss of information and potential induced bias on the quantification of the various OBT fractions. To overcome these complications, initial tests were previously conducted under the vapor phase by steam percolation, though sample degradation remained an ongoing issue [13]. Most recently, a new method has been developed from a custom designed system with controlled and stable temperature ($20.0 \pm 0.1^\circ\text{C}$) and relative

humidity ($85\% < RH < 88\%$) parameters [16]. This non-intrusive method has since been introduced in other works to study the exchangeability parameter of targeted environmental matrices [16,17]. The exchangeability parameter is thus able to quantify the distribution of exchangeable / non-exchangeable hydrogen positions, in offering access to a calculated value of the NE-OBT fraction in a sample [18,19]. Therefore, this non-intrusive method allows for not only direct NE-OBT measurement in a sample, after E-OBT removal by depletion, without any demonstrated bias, but also a means of confirming the validity and reliability of the obtained value through an exchangeable capacity study on the targeted matrix.

In order to provide a means of developing a standardized method for OBT sub fractions determination, this work proposes assessing the robustness of both intrusive and non-intrusive methods for NE-OBT fraction determination on a large panel of previously studied environmental matrices [20]. The two main objectives of the present study are: (i) to confirm the validity of the non-intrusive direct measurement method by means of verification with calculated results, and (ii) to evaluate the bias impact on the reliability of the intrusive method by comparing results as regards their correlation with the non-intrusive method.

2. Materials and methods

2.1. Materials

2.1.1. Sample preparation

Samples of various matrices were collected around areas of known tritium releases into the environment. For each studied environmental matrix, the major constituent was extracted according to Nivresse *et al.* [20] and subsequently added to the present study to explore several degrees of sample solubility. In order to be free from any sample composition bias, the comparative study was carried out on a set of environmental matrices and the comparison step was executed through the different results obtained from the intrusive or the non-intrusive method for a same initial matrix.

Wheat grains (matrix A-1) were sampled as part of the Organically Bound Tritium (OBT) working group activities [21], and their starch (matrix A-2) was extracted according to a procedure adapted from Liu and Ng [22], Verwimp *et al.* [23] and Xie *et al.* [24]. Water milfoil (matrix B-1) was sampled from the Loire River (47°45'18.7"N 2°28'25.3"E) 2 km downstream of the Dampierre Nuclear Power Plant (NPP) (France), and their cellulosic wall (matrix B-2) was recovered by extraction and elimination of the cytoplasmic fraction according to a procedure adapted from Mochochoko *et al.* [25] and Sun *et al.* [26]. Apples (matrix C-1) were sampled 2 km from the Cernavodă Nuclear Power Plant (NPP) (Romania) (44°20'10.0"N 28°02'13.9"E), and their simple carbohydrates (matrix C-2) were extracted according to the procedure described in Besle and Pitiot [27].

All samples analyzed were dehydrated by freeze-drying and then stored under vacuum until testing. The organic matter compositions of all studied matrices and their initial OBT activity are presented in Table 1 (*insert Table 1 here*).

2.1.2. Sample post-treatment and ^3H activity determination

In order to determine OBT (E-OBT + NE-OBT) and NE-OBT levels in solid samples before and after the isotopic exchange procedure, the solid samples were dehydrated by freeze-drying and then heat-treated in a tubular furnace (Eraly, France). The organic matter was thus transformed into carbon dioxide and combustion water containing the tritium fraction of the sample. The sample mass m_{sample} needed to obtain a sufficient mass of combustion water m_{comb} (both of which expressed in kilograms) for an optimal measurement by liquid scintillation has been

estimated from the combustion efficiency ε_{comb} , expressed in %, according to the following equation (Eq. 1):

$$\varepsilon_{comb} = \frac{m_{comb} \times \%H_{water}}{m_{sample} \times \%H_{sample}} \quad (1)$$

where $\%H_{water}$ and $\%H_{sample}$ denote the % by weight of hydrogen respectively in water and in the sample.

Combustion water was purified by a single distillation under atmospheric pressure after pH adjustment with sodium peroxide (Na_2O_2). The isotopic exchange water aliquots were also purified by one or more distillations under atmospheric pressure in order to eliminate organic residues or remaining salt. The tritium activities of purified liquid samples (combustion water and isotopic exchange water aliquots) were measured by liquid scintillation counting using an Ultima Gold LLT cocktail and two types of equipment, i.e.: a Tri-Carb 3170 TR/SL model (PerkinElmer) with a detection limit estimated at 3 Bq.kg^{-1} of water for a counting time of 180 minutes and a blank value of 2 counts per minute, and a Wallac Quantulus 1220 model (PerkinElmer) with a detection limit of approx. 1 Bq.kg^{-1} of water for a counting time of 24 hours and a blank value of 0.7 counts per minute. A quench consideration was applied for each measurement according to a quenching curve calculated after calibration using laboratory-prepared quenched standards.

The tritium activity concentrations (A^{HTO}) of the combustion water (A_{comb}^{HTO}) and isotopic exchange water aliquots ($A_{iso-liq}^{HTO}$), expressed in Bq.kg^{-1} of water, were determined using the formula presented in Equation 2:

$$A^{HTO} = \frac{(N - N_0)}{t \times \eta} \times \frac{1}{m} = \frac{(n - n_0)}{m \times \eta} \quad (2)$$

where N and N_0 denote the sample count number and blank number, n and n_0 the sample and blank counting rates expressed in counts per second, t the counting time expressed in seconds, η the counting efficiency of the counter (dimensionless number between 0 and 1), and m the mass of water expressed in kilograms.

To improve the accuracy of results on each sample, n aliquots of the same sample (combustion water and exchange water) were typically measured ($n \geq 3$), and the statistical uncertainty of the

experimental mean value U_{AHTO} was determined with a coverage factor k equal to 2, according to Equation 3:

$$U_{AHTO} = 2 \times \sqrt{\frac{s^2}{n} + \left(\frac{u_m}{m}\right)^2 + \left(\frac{u_\eta}{\eta}\right)^2 + \left(\frac{u_{\varepsilon_{comb}}}{\varepsilon_{comb}}\right)^2 + \left(\frac{u_{\%H_{water}}}{\%H_{water}}\right)^2} \quad (3)$$

where s is the standard deviation between the n values, and u_m , u_η , $u_{\varepsilon_{comb}}$ and $u_{\%H_{water}}$ are the uncertainties on the mass m, counting efficiency η , combustion efficiency ε_{comb} and % by weight of hydrogen in water $\%H_{water}$, respectively.

2.1.3. Elemental analysis (C, H, N, S)

Elemental analyses were conducted on each studied matrix using a FlashEA[®] 1112 Scientific apparatus (ThermoFisher) in order to determine the % content by weight of carbon, hydrogen, nitrogen and sulfur elements. The result obtained on the proportion of an element x, expressed in %, was calculated from a calibration line according to Equation 4:

$$x = \frac{m_x}{m_{sample}} = \left(\frac{A_x - b_{0,x}}{b_{1,x} \times m_{sample}} \right) \quad (4)$$

where m_x and m_{sample} denote respectively the mass of element x and the mass of the introduced sample, and A_x the area of the peak identified on the chromatogram; moreover, $b_{0,x}$ and $b_{1,x}$ relate to the calibration line parameters.

To improve the accuracy of results on each sample, n aliquots of the same sample were typically measured ($n \geq 5$), and the statistical uncertainty of the experimental mean value $U_{\bar{x}}$ was determined with a coverage factor k equal to 2, according to Equation 5:

$$U_{\bar{x}} = 2 \times \sqrt{\frac{s^2}{n} + \left(\frac{u_{cal}}{m_x}\right)^2 + \left(\frac{u_{m_{sample}}}{m_{sample}}\right)^2} \quad (5)$$

where s is the standard deviation between the n values, u_{cal} the calibration uncertainty according to Neuilly and CETAMA [28], and $u_{m_{sample}}$ the uncertainty on the mass of the introduced sample.

The elemental compositions of each matrix, both before and after isotopic exchange in non-tritiated water, are reported in Table 2 (*insert Table 2 here*).

2.1.4. Conversion of the tritium activity concentration

The % content by weight of hydrogen was then used to convert the tritium activity concentration from Bq.kg^{-1} of water (A_{comb}^{HTO}) to Bq.kg^{-1} of dry matter (A_{ms}^{HTO}) using the formula presented in Equation 6:

$$A_{ms}^{HTO} = A_{comb}^{HTO} \times \frac{\%H_{sample}}{\%H_{water}} \quad (6)$$

To improve the accuracy of results on each sample, n aliquots of the same sample were typically measured ($n \geq 3$), and the statistical uncertainty of the experimental mean value $U_{A_{ms}^{HTO}}$ was determined with a coverage factor k equal to 2, according to Equation 7:

$$U_{A_{ms}^{HTO}} = 2 \times \sqrt{\left(\frac{u_{A_{comb}^{HTO}}}{A_{comb}^{HTO}}\right)^2 + \left(\frac{u_{\%H_{sample}}}{\%H_{sample}}\right)^2 + \left(\frac{u_{\%H_{water}}}{\%H_{water}}\right)^2} \quad (7)$$

where $u_{A_{comb}^{HTO}}$ is the uncertainty of the tritium activity concentration (A_{comb}^{HTO}), $u_{\%H_{sample}}$ the uncertainty of $\%H_{sample}$, and $u_{\%H_{water}}$ the uncertainty of $\%H_{water}$.

2.1.5. Total Organic Carbon (TOC)

Total organic carbon (TOC) analyses were carried out using a Multi N/C 2100S model TOC-meter apparatus (Analytik Jena). The TOC content was determined in the isotopic exchange waters from the intrusive method in order to evaluate the partial sample solubilization phenomenon occurring during the isotopic exchange procedure. Since the NE-OBT is widely assumed to be principally involved in carbon binding, the loss in carbon atoms during the intrusive method is also information of great utility in grasping method bias and weaknesses. The TOC content was calculated from the difference between the total carbon (TC) and inorganic carbon (IC) measurements in the liquid sample. The concentration of solubilized carbon from the dry sample during the isotopic exchange was derived in milligrams of carbon per gram of solution $[TOC]_{mgC/g_s}$, yielding relation 8 below:

$$[TOC]_{mgC/g_{sample}} = \frac{[TOC]_{mgC/g_s} \times m_s}{m_{sample}} = \frac{m_{C\ solub}}{m_{sample}} \quad (8)$$

where $[TOC]_{mgC/gsample}$ expresses the TOC concentration in milligrams of carbon per gram of solid sample, while m_s , m_{sample} and $m_{C\ solub}$ represent the masses of analyzed solution, solid sample and solubilized carbon respectively in the exchange water solution analyzed.

The solubilized fraction of carbon from the sample could thus be determined according to the initial amount of carbon in the sample, as obtained by elemental analysis using the formula presented below:

$$\% C_{solub} = \frac{m_{C\ solub}}{m_{C\ sample}} \quad (9)$$

where $\% C_{solub}$ is the proportion of the solubilized fraction of carbon from the sample, and $m_{C\ sample}$ the initial mass of carbon in the solid sample.

To improve the accuracy of results on each sample, n aliquots of the same sample (analyzed solutions) were typically measured ($n \geq 5$), and the statistical uncertainty of the experimental mean value $U_{\%C_{solub}}$ was determined with a coverage factor k equal to 2, according to Equation 10:

$$U_{\%C_{solub}} = 2 \times \sqrt{\frac{s^2}{n} + \left(\frac{u_{m_{sample}}}{m_{sample}}\right)^2 + \left(\frac{u_{m_s}}{m_s}\right)^2 + (\Delta TOC)^2} \quad (10)$$

where s is the standard deviation between the n values, $u_{m_{sample}}$ the uncertainty of the solid sample mass m_{sample} , u_{m_s} the uncertainty of the analyzed solution mass m_s , and ΔTOC the uncertainty related to the TOC-meter efficiency determined from experimental calibrations.

2.2. Methods

2.2.1 Intrusive isotopic exchange method

i) *Depletion procedure to access the (NE-OBT) fraction*

The intrusive isotopic exchange procedure is a technique performed in a closed system, where the isotopic equilibrium reaction is carried out under the aqueous liquid phase [5,29]. Briefly described, freeze-dried samples are immersed in a large volume (around factor 10) of very low-level tritium drilling water from the CEA Bruyères-le-Châtel site ($HTO < 0.01 \text{ Bq.kg}^{-1}$ of water) [30] and kept under magnetic stirring for 2 days at room temperature. Each sample is then placed in an individual container closed with a special waterproof plastic film in order to avoid atmospheric tritium contamination, as recommended in Pointurier *et al.* [5]. Isotopic exchanges

occur between the exchangeable organically bound tritium (E-OBT) fraction in the sample and the excess of stable hydrogen atoms in the exchange water solution. At the end of the solid-liquid contact time, it is assumed that an isotopic equilibrium has been reached between the tritium content of the exchangeable part of the organically bound hydrogen in the sample and that of the water bath. Next, the exchangeable organically bound tritium (E-OBT) fraction is completely removed from the sample, and the remaining activity measured in the sample is directly attributed to the non-exchangeable organically bound tritium (NE-OBT) fraction.

For each matrix, this procedure was applied to three to five aliquots in order to confirm reproducibility. The samples were then recovered by means of filtration and centrifugation, freeze-dried and kept under vacuum until subsequent use. The isotopic exchange water aliquots were also sampled at equilibrium and held at a temperature below 5°C prior to distillation.

2.2.2 Non-intrusive isotopic exchange method

i) Depletion procedure to access the (NE-OBT) fraction

The non-intrusive isotopic exchange procedure is a technique carried out under an aqueous vapor phase with controlled and stable parameters: temperature ($20^\circ \pm 0.1^\circ\text{C}$) and relative humidity ($85\% < \text{RH} < 88\%$), as ensured by a KCl-saturated solution in a closed system [16]. Briefly described, a specific atmosphere with a defined T/H ratio is set up in a glove box (Plas-Labs 890-THC) from the KCl-saturated solution prepared with a very low-level tritium commercial water, i.e. “Eau des Abatilles” ($\text{HTO} < 0.2 \text{ Bq.kg}^{-1}$ of water). An initial isotopic equilibrium is established between the liquid and vapor phases of the saline solution. Next, freeze-dried samples are exposed to the specific atmosphere, and a second isotopic equilibrium is reached with both the exchangeable organically bound hydrogen positions of the matrix and the water condensed at the sample surface, which can be described by the following equation:

$$\left(\frac{T}{H}\right)_{l, \text{ bath}} = \left(\frac{T}{H}\right)_{g, \text{ vapor}} = \left(\frac{T}{H}\right)_{l, \text{ cond}} = \left(\frac{T}{H}\right)_{s, \text{ E-OBT}} \quad (11)$$

where $\left(\frac{T}{H}\right)_{l, \text{ bath}}$ is the T/H ratio in the KCl-saturated bath, $\left(\frac{T}{H}\right)_{g, \text{ vapor}}$ the T/H ratio in the vapor phase, $\left(\frac{T}{H}\right)_{l, \text{ cond}}$ the T/H ratio in the free water fraction of the sample, and $\left(\frac{T}{H}\right)_{s, \text{ E-OBT}}$ the T/H ratio of the exchangeable organically bound hydrogen fraction of the sample.

When the isotopic equilibrium described by Equation 11 is reached, the exchangeable organically bound tritium (E-OBT) fraction is then completely removed from the sample, and all remaining activity measured in the sample is directly attributed to the non-exchangeable organically bound tritium (NE-OBT) fraction.

For each matrix, this procedure was applied to three aliquots with three different contact times (2, 3 and 4 days) to confirm both the reproducibility and effective achievement of equilibrium within the system. The transfer airlock of the glove box was then used to ensure sample removal without disrupting the atmospheric system set-up. For this purpose, a process of purging with dried air and a (T/H) vapor pressure ratio reset from a custom “module” filled with the same KCl-saturated solution used in the glove box was employed (see Péron *et al.* [16] for further details).

The samples were then recovered outside the glove box, immediately immersed in liquid nitrogen, freeze-dried and kept under vacuum until subsequent use. The isotopic exchange bath aliquots were also sampled at equilibrium and held at a temperature below 5°C prior to distillation.

ii) *Calculation of the non-exchangeable organically bound tritium (NE-OBT)*

In previous studies [20], the non-intrusive isotopic exchange procedure was conducted on each matrix of our present study using different (T/H) ratios in the vapor phase for the enrichment experiments (tritium baths with HTO activities = 120, 300 and 500 Bq.kg⁻¹ of water). From these experiments along with the present depletion experiment, the isotopic exchangeable (α_{iso}) parameter was determined for each studied matrix; the details of the set-up procedure and results obtained are presented in Nivesse *et al.* [20]. As stated in Péron *et al.* [16], the (α_{iso}) parameter describes the isotopic exchangeable hydrogen pool versus total hydrogen atoms in a specific matrix and moreover may quantify the distribution of exchangeable to non-exchangeable OBT according to the following equation:

$$\left(\frac{T}{H}\right)_{s, OBT} = (\alpha_{iso}) \times \left(\frac{T}{H}\right)_{s, E-OBT} + (1 - (\alpha_{iso})) \times \left(\frac{T}{H}\right)_{s, NE-OBT} \quad (12)$$

where $\left(\frac{T}{H}\right)_{s, OBT}$ is the T/H ratio of the organically bound hydrogen fraction of the sample and $\left(\frac{T}{H}\right)_{s, NE-OBT}$ the T/H ratio its non-exchangeable organically bound hydrogen fraction.

The non-exchangeable organically bound tritium (NE-OBT) fraction of the sample has been calculated from Equations 11 and 12, in addition to Equation 13 below:

$$\left(\frac{T}{H}\right)_{s, \text{ NE-OBT}} = \frac{\left[\left(\frac{T}{H}\right)_{s, \text{ OBT}} - \alpha_{\text{iso}} \times \left(\frac{T}{H}\right)_{\text{l,bath}}\right]}{(1 - \alpha_{\text{iso}})} \quad (13)$$

This value was automatically calculated from each of the four steady-state results, and the statistical uncertainty of the experimental mean value $U_{\overline{ANE-OBT}}$ was determined with a coverage factor k equal to 2, according to Equation 14:

$$U_{\overline{ANE-OBT}} = 2 \times \sqrt{\frac{s^2}{n} + \left(U_{(\alpha_{\text{iso}})} \times (1 - \alpha_{\text{iso}}) \times \left(\frac{T}{H}\right)_{s, \text{ NE-OBT}}\right)^2} \quad (14)$$

where s is the standard deviation between the n values and $U_{(\alpha_{\text{iso}})}$ the uncertainty on the isotopic exchangeable (α_{iso}) parameter.

3. Results and discussion

3.1. Evaluation of solubilization events during application of the intrusive method

Solubilization phenomena occurring during application of the intrusive method procedure were monitored by sampling and an exchange water analysis. Slight variations in elemental compositions before and after the intrusive method procedure could be observed on samples depending on their matrix type (see Table 2). However, these relative values are insufficient to invalidate the occurrence of partial solubilization events since the absence of variation could simply be related to a solubilization that respects the stoichiometric distributions of the initial sample. Effective solubilization events of partial samples during the immersion step of the intrusive procedure were demonstrated by increasing TOC levels in exchange water before and after isotopic exchange. The proportion of the solubilized carbon fraction in the exchange water for each sample, $\% C_{\text{solubilized}}$, is reported in Table 2. The extent of the solubilization phenomenon was therefore observed in proportion to the rate of water-soluble compounds in each studied matrix, which remains completely dependent on the preparation step for the extracted major constituent matrices (A-2), (B-2) and (C-2). From those results, the largest impact was recorded for the simple carbohydrate matrix types (C-1) and (C-2). For the latter, a complete solubilization of the solid sample was observed, thus resulting in an infeasible use of the matrix for quantifying the NE-OBT via the intrusive method. To a lesser but still noticeable extent, results on water milfoil (B-1) and starch (A-2) showed significant solubilization phenomena during the intrusive method procedure, while expected minor impacts were observed on the cellulosic wall (B-2). In the case of wheat grains (A-1), a minor solubilization seems to have taken place despite the presence of water-soluble compounds in the matrix, leading to the assumption that they were mostly preserved from external water contact interaction by the grain hull.

3.2. Validation of the non-intrusive method reliability

The tritium activity concentrations measured in the samples after contact times of 2 days, 3 days and 4 days of depletion experiment by the non-intrusive method were observed to be repeatable results for each matrix, thus confirming that isotopic equilibrium had been reached within the system with at least 2 days of contact time, exactly as previously suggested in Péron *et al.* [16] and Nivresse *et al.* [17]. A comparison of results between the NE-OBT fractions of studied matrices obtained by measurement and calculation from the non-intrusive method is presented in Figure 1 (*insert Figure 1 here*). For each matrix, the results on NE-OBT quantifications from both sources

are very similar, and the statistical differences between each value has been invalidated by a comparative statistical test (Student t-test). From these assessments, very small deviations have been observed on each of the six studied matrices, with a maximum of $0.17 \pm 0.04\%$ for simple carbohydrates (matrix C-2). As previously described in Section 2.2.2, the calculated NE-OBT is obtained from a series of isotopic exchanges via the non-intrusive method, which then yields access to a reliable value of the non-exchangeable fraction in a sample. As such, the robustness of the non-intrusive isotopic exchange method for an effective tritium depletion and reliable quantification of NE-OBT by means of direct measurement after sample treatment could be agreed. When considering the associated relative uncertainties, only the measured value of each of the studied matrices has been introduced in the following comparative study.

3.3. Comparative study: Shortcomings of the intrusive method

Results on the NE-OBT fractions of studied matrices obtained by measurement from both the intrusive and non-intrusive method procedures are described in Table 3 (*insert Table 3 here*). A complete depletion process of the exchangeable fraction of tritium (E-OBT) in samples and the absence of external contamination during procedures were both verified by measuring the tritium activities in exchange water. The quantification of tritium activity concentration in a solid sample was carried out by measurements in the combustion water of the treated solid sample. The NE-OBT results obtained after both isotopic exchange methods could thus be directly expressed in terms of Bq.kg^{-1} of combustion water. Among all matrices studied, a significant deviation was observed between the intrusive and non-intrusive method results on NE-OBT values after direct measurement, i.e. from $6.9 \pm 0.9\%$ up to $49.8 \pm 6.2\%$ deviation. The statistical difference between each value was verified and validated by means of a comparative statistical test (Student t-test). The solubilization phenomena of the sample during the intrusive method procedure are undoubtedly at the origin of a bias on the measurement of the NE-OBT fraction in a given matrix.

Nevertheless, the concentration activities measured in combustion water are entirely dependent on the hydrogen yield of the combustion (Equation 1) and thus directly associated with the hydrogen content of the sample. Yet, the solubilization caused by the intrusive method procedure could lead to a hydrogen content modification of the initial sample, while the non-intrusive method procedure is intended to preserve the elemental composition.

To allow for a comparison of values in taking into account the evolution of hydrogen contents in each sample, results expressed in Bq.kg^{-1} of combustion water were converted into Bq.kg^{-1} of dry matter (Table 3). Reductions in deviations between both quantified values of NE-OBT from the

two methods were observed in the case of cellulosic wall (B-2) matrix. However, significant deviations of up to $52.4 \pm 6.7\%$ in the case of apple (matrix C-1) still remained for the majority of matrices studied, thereby suggesting that the % by weight of hydrogen information is not a satisfactory correction to the intrusive method bias. Along with the other results, the statistical difference between each value was verified and validated by means of a comparative statistical test (Student t-test). This situation is likely to occur whenever the NE-OBT is not distributed evenly across the matrix constituents. According to Nivesse *et al.* [20], an imbalance between exchangeable capacities of hydrogen from one biomolecule to another in the same matrix could be at the origin of an NE-OBT distribution that fails to respect the mass distribution of the biomolecules themselves. Deviations on NE-OBT quantifications were observed in closed relation with the extent of solubilization events of the sample during the intrusive method procedure. Furthermore, although a trend was obvious, the extent of the solubilization phenomenon did not seem to cause a proportional loss of sample NE-OBT in the exchange water. Hence, the starch matrix (A-2) showed a deviation in NE-OBT results between the intrusive and non-intrusive method procedures of the same order of magnitude as that of water milfoil (B-1) (i.e. approx. 25% deviation); whereas the extent of its solubilization events was much lower ($5.4 \pm 0.8\%$ for starch vs. $17.3 \pm 1.4\%$ for water milfoil). In addition to solubilization, prolonged contact with water is assumed to alter molecular compound evolution by the partial degradation of the organic matter of environmental matrices. During these processes, the biochemical reactions of organic matter maturation could lead to the simultaneous consumption and renewal of hydrogen atoms. As such, the loss in non-exchangeable OBT originally found in the sample is likely to undergo significant expansion during the intrusive method procedure, without any evolution in either solubilization events or hydrogen content.

After disturbing the initial nature of a sample, no further correction based on simple parameters from solubilization events, such as carbon loss or hydrogen content evolution, can guarantee a viable quantification of the NE-OBT fraction in the final sample. Consequently, the intrusive method fails to offer a reliable solution for accurate quantification of the NE-OBT fraction in environmental matrices.

Nevertheless, measured OBT concentration activities from combustion waters of samples from environmental monitoring purpose present commonly relative uncertainties of about 10%. In the case of matrix (A-1), the deviation between NE-OBT results from the intrusive and the non-intrusive method was obtained at $8.5 \pm 1.0\%$, without any results correction with the hydrogen content of the sample. On this sample type, the deviation between the two methods results is then

found to be of the same order of magnitude as the commonly encountered uncertainty on OBT value measurement for environmental monitoring. Hence, the NE-OBT value quantified by the intrusive method on this matrix can be considered acceptable for NE-OBT quantification to the environmental monitoring purpose. In the case of matrices (B-1) and (C-1) however, larger deviations between NE-OBT results obtained around 20 to 50% remains problematic aspect to approve and allow the validation of the intrusive method results within the monitoring context. As part of environmental monitoring, the intrusive method is therefore relevant and acceptable for NE-OBT quantifications in samples with a very low content in water soluble compounds or a very effective protective structure like cereals samples. Yet, the non-intrusive method still can't be recommended for others matrices with medium to high content in water soluble compounds such as proteins and more especially simple carbohydrates in fruit or vegetables samples.

4. Conclusion

A comparative study has been carried out on NE-OBT quantification results obtained by means of intrusive and non-intrusive method procedures on a large panel of environmental matrices. The impact of the intrusive method drawbacks has been highlighted, and the lack of a possible correction to the observed biases has been confirmed. If the intrusive method reliability to strongly accurate NE-OBT quantification has been refuted, its relevance for NE-OBT measurements as part of environmental monitoring was yet evaluated in environmental samples according to their content in water soluble compounds and validated for cereals matrices types. In stark contrast, the non-intrusive method robustness has been demonstrated by a strong validation method over the wide range of studied matrices. Despite requiring a larger set-up than that of the intrusive protocol, this method still offers a similar depletion time (equilibrium reached after 2 days) and moreover provides a reliable NE-OBT measurement in environmental matrices.

Acknowledgments

This work was financed by the CEA and Subatech institutes, as well as by France's Loire Valley Regional Council (under the aegis of the POLLUSOLS OSUNA Project) and by the EDF energy company. The authors would like to thank the members of the Environmental Laboratory at the Cernavodă Nuclear Power Plant for contributing the apple matrix as part of this work program.

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Tables

	(A-1)	(A-2)	(B-1)	(B-2)	(C-1)	(C-2)
Starch (%)	75.3 (± 3.7)	85.1 (± 2.1)	18.1 (± 1.9)	7.6 (± 0.9)	0.8 (± 0.1)	-
Maltose (%)	-	14.9 (± 2.1)	-	-	-	-
Glucose (%)	-	0.8 (± 0.2)	-	-	17.3 (± 0.9)	19.0 (± 2.3)
Fructose (%)	-	-	-	-	51.0 (± 1.5)	58.5 (± 7.1)
Sucrose (%)	3.6 (± 0.4)	-	0.8 (± 0.8)	0.1 (± 0.6)	19.9 (± 0.6)	22.5 (± 2.7)
Cellulose (%)	4.2 (± 0.9)	-	16.5 (± 1.5)	21.7 (± 2.3)	3.7 (± 1.6)	-
Lignin (%)	-	-	13.7 (± 1.8)	26.2 (± 3.0)	2.6 (± 0.4)	-
Hemicellulose (%)	-	-	10.3 (± 2.5)	19 (± 7)	1.6 (± 4.0)	-
Protein⁽ⁱ⁾ (%)	13.9 (± 1.1)	-	-	-	-	-
Protein⁽ⁱⁱ⁾ (%)	-	-	37.3 (± 2.7)	25.1 (± 2.7)	-	-
Fat (%)	3.0 (± 0.9)	-	3.2 (± 1.3)	0.2 (± 0.9)	0.9 (± 0.9)	-
OBT (reference date: July 1, 2020)	31.3 (± 2.1)	18.4 (± 2.0)	18.6 (± 3.8)	11.8 (± 1.6)	36.2 (± 2.8)	8.9 (± 1.2)

Table 1: Analytical compositions in % by weight and initial OBT activity in Bq.kg^{-1} of dry matter for: wheat grains (A-1), starch (A-2), water milfoil (B-1), cellulosic wall (B-2), apples (C-1), and simple carbohydrates (C-2). ⁽ⁱ⁾ and ⁽ⁱⁱ⁾ denote wheat grain protein type and water milfoil protein type, respectively.

	C (%)		H (%)		N (%)		% C _{solubilized}
	(1)	(2)	(1)	(2)	(1)	(2)	
(A-1)	45.1	44.8	6.6	6.4	2.3	2.9	1.1 (± 0.1)%
(A-2)	42.6	43.6	6.3	6.3	0.0	0.0	5.4 (± 0.8)%
(B-1)	37.4	35.2	4.7	4.5	3.3	4.1	17.3 (± 1.4)%
(B-2)	40.5	40.1	5.1	5.1	3.7	3.7	1.0 (± 0.4)%
(C-1)	40.4	42.9	6.6	6.2	0.4	0.7	77.1 (± 4.3)%
(C-2)	40.2	-	6.9	-	0.3	-	100.3 (± 17.1)%

Table 2: Elemental composition in % by weight on (1) before and (2) after intrusive isotopic exchange procedure and resulting solubilized carbon from samples on: wheat grains (A-1), starch (A-2), water milfoil (B-1), cellulosic wall (B-2), apples (C-1), and simple carbohydrates (C-2). The relative uncertainties of these elemental compositions were all calculated at less than 0.4%.

	(A-1)	(A-2)	(B-1)	(B-2)	(C-1)	(C-2)
NE-OBT ^(a) (Bq.kg ⁻¹)*	30.3 (±2.6)	24.3 (±2.1)	26.5 (±2.3)	26.4 (±2.2)	13.3 (±1.1)	-
NE-OBT ^(b) (Bq.kg ⁻¹)*	33.1 (±2.6)	32.7 (±2.5)	33.7 (±3.1)	24.6 (±2.3)	26.4 (±2.4)	13.3 (±2.8)
NE-OBT deviation ^(a) (Bq.kg ⁻¹)* / NE-OBT ^(b) (Bq.kg ⁻¹)* in %	8.5 (±1.0)	25.8 (±2.9)	21.3 (±2.7)	6.9 (±0.9)	49.8 (±6.2)	-
NE-OBT ^(a) (Bq.kg ⁻¹)**	17.8 (±1.6)	13.8 (±1.2)	10.8 (±1.0)	12.2 (±1.1)	7.5 (±0.7)	-
NE-OBT ^(b) (Bq.kg ⁻¹)**	19.7 (±1.6)	18.6 (±1.4)	14.4 (±1.4)	11.4 (±1.1)	15.8 (±1.5)	8.4 (±1.8)
NE-OBT deviation ^(a) (Bq.kg ⁻¹)** / NE-OBT ^(b) (Bq.kg ⁻¹)** in %	9.9 (±1.4)	25.8 (±3.0)	25.1 (±3.2)	6.4 (±0.8)	52.4 (±6.7)	-
% C _{solubilized}	1.1 (± 0.1)%	5.4 (± 0.8)%	17.3 (± 1.4)%	1.0 (± 0.4)%	77.1 (± 4.3)%	100.3 (± 17.1)%

Table 3: Isotopic exchanges in NE-OBT quantification results and deviation from (a) the intrusive method and (b) the non-intrusive method, as expressed in Bq.kg⁻¹ of combustion water* and Bq.kg⁻¹ of dry matter**, along with the resulting solubilized carbon from samples of: wheat grains (A-1), starch (A-2), water milfoil (B-1), cellulosic wall (B-2), apples (C-1), and simple carbohydrates (C-2)

Figure

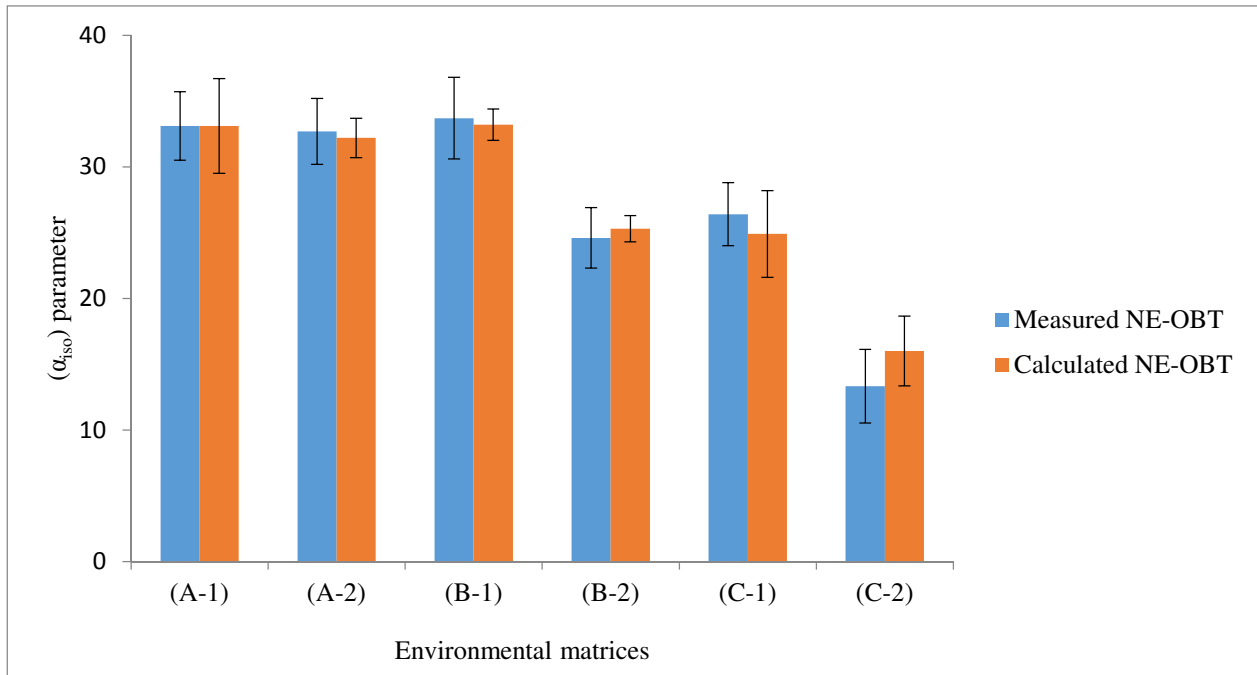


Figure 1: Comparison of non-intrusive method results on NE-OBT quantifications, as expressed in Bq.kg^{-1} of combustion water, by (a) measurements after depletion experiment and (b) calculation from the exchangeable parameter (α_{iso}) results presented in Nivesse *et al.* [20] on: wheat grains (A-1), starch (A-2), water milfoil (B-1), cellulosic wall (B-2), apples (C-1), and simple carbohydrates (C-2)