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Isotopic fractionation of tritium in biological systems

Pierre Le Goff, Michel Fromm, Laurent Vichot, Pierre-Marie Badot, Philippe Guétat

Abstract
Isotopic fractionation of tritium is a highly relevant issue in radiation protection and requires certain radioecological considerations. Sound evaluation of this factor is indeed necessary to determine whether environmental compartments are enriched/depleted in tritium or if tritium is, on the contrary, isotope-well-distributed in a given system. The ubiquity of tritium and the standard analytical methods used to assay it may induce biases in both the measurement and the signification that is accorded to the so-called fractionation: based on an exhaustive review of the literature, we show how, sometimes large deviations may appear. It is shown that when comparing the non-exchangeable fraction of organically bound tritium (neOBT) to another fraction of tritium (e.g. tritiated water) the preparation of samples and the measurement of neOBT reported frequently led to underestimation of the ratio of tritium to hydrogen (T/H) in the non-exchangeable compartment by a factor of 5% to 50%. In the present study, corrections are proposed for most of the biological matrices studied so far. Nevertheless, the values of isotopic fractionation reported in the literature remain difficult to compare with each other, especially since the physical quantities and units often vary between authors. Some improvements are proposed to better define what should encompass the concepts of exchangeable and non-exchangeable fractions.

Keywords: Tritium; Environment; Isotopic fractionation, OBT

1 Theoretical background

Tritium is the only natural radioactive isotope of hydrogen. As hydrogen is a major element in the biosphere (as a component of water, mineral or organic matter) and as tritium is one of the most released radionuclides by the nuclear industry in the environment, tritium is ubiquitous in every environmental compartment. Tritium transfer from release compartments to biological organisms (microorganisms, plants, animals) along the food chains is a key question in radioecology of tritium. Taking into account the methods of sample treatment and measurement of tritium specific activities, some cautions have to be taken when interpreting results. The aims of this article are to review works dealing with flows of tritium in the environment and to propose some improvements in the analysis and presentation of the results.

1.1 Sources of tritium in the environment and the pathways in which it is involved

Most of tritium is produced in the high atmosphere (from 10 to 30 km of altitude) by the action of cosmic particles (protons and neutrons) on $^{14}$N and $^{16}$O. Tritium produced in these conditions reacts mainly with atoms of $^1$H to form tritiated hydrogen (HT) (Belot and others 1996). In lower layers of the atmosphere, tritium is incorporated into water molecules and forms tritiated water, also called tritium oxide (HTO) (Balonov and others 1974). Natural
production of tritium is estimated at 200 g y\(^{-1}\) and the natural stock of tritium at 3.5 kg (Okada and Momoshima 1993).

Anthropic tritium is mainly released into the environment as HT or HTO by nuclear facilities (nuclear power stations, fuel reprocessing plants, military applications, tritium extraction plants) and as volatile tritiated compounds in minor quantities (essentially CH\(_3\)T). At the world scale, these sources are equivalent to about one third of natural emissions (UNSCEAR 2008).

As HT and CH\(_3\)T are taken up very slowly by the different compartments of the environment, it is assumed that their oxidation into HTO (in particular by soil microorganisms) is their main environmental fate (Galeriu and others 2008). On the other hand, HTO is ubiquitous in abiotic or biotic compartments. Its transport and transfer in the environment follows the general water cycle (IAEA 1981), with the exception of a few fractionation effects.

### 1.2 Biological incorporation

As HTO follows the water cycle, plants and animals incorporate it. The part of tritium in living organisms that remains as HTO is termed Tissue Free Water Tritium (TFWT) (Spencer 1984). TFWT in plants soon reaches equilibrium with water of its environment (water in soil and in the atmosphere) because plant, soil and atmosphere constitute a continuum (Boyer and others 2009b). For example, the half-life of TFWT is estimated at 20-30 minutes in sunflower (*Helianthus annuus*) (Koranda and Martin 1972). In animals, TFWT results from the drinking water, the water in food and the water that is produced by the catabolism of organic molecules. It has a short biological half-life (Pinson 1951): in humans, the average half-life of TFWT is about 10 days (Moghissi and others 1972; Moghissi and others 1971).

In plants, animals and in most environmental compartments, the hydrogen of water and labile hydrogen (hydrogen bonded with atoms of oxygen, nitrogen or sulphur, accounting for approximately a third of the hydrogen in organic molecules) reach a quasi-instantaneous equilibrium. This kind of tritiation is termed “exchangeable Organically Bound Tritium” (eOBT) (Diabaté and Strack 1993). Nevertheless, some labile hydrogen can stay “buried” in the core of macromolecules and thus remain inaccessible to isotopic exchange until the macromolecule “breathes” (following enzymatic action for example) (Baumgärtner and Donhaerl 2004). This latter situation can influence measures of tritium in biological matrices (see § 4.1).

Plants produce organic matter from HTO essentially through the photosynthesis cycle. When this leads to a tritium atom bound to an atom of carbon, the tritium is termed as “non-exchangeable OBT” (neOBT) because it is assumed to remain bound until the catabolism of the tritiated molecule (Diabaté and Strack 1993).

The sum of eOBT and neOBT is called total OBT (tOBT).

Animals can also integrate tritium into their tissues and fluids by consuming tritiated food. The integrated fraction of ingested OBT\(^1\) depends on the form in which it occurs (Taylor 2008). When animals take up tritiated organic compounds, the absorption rate and the

\(^1\) In this article OBT is only used when it can be termed tOBT, neOBT or eOBT
retention time depend on the organic molecules involved and their function in body: Diabaté indicates in (Diabaté and Strack 1993) that the retention time and the incorporation into the dry matter of tissues/organs are generally higher for constitutive and storage molecules than for water.

.1.3 Isotopic effects and isotopic fractionation: some theoretical considerations

The isotopic substitution affects translational, rotational and vibrational motions of a substrate relative to the transition state or product of a reaction. Two kinds of isotopic effects might arise from these modifications: kinetic isotopic effects and equilibrium isotopic effects (Klinman 2006).

First, the difference of mass between isotopes leads to variation of molecular speed, the heaviest isotope having a lower molecular speed than the lightest. It conduces to a slower diffusion for the molecule marked by the heavy isotope and a less frequent collision with other molecules. This latter point explains why molecules with light isotopes generally react faster (IAEA 2000).

Secondly, the energy of activation for a reaction is also dependant of the mass of the atoms involved in the reaction by the effect of the mass variation on the force constant of the vibrating bond. In the normal isotopic effect, the heaviest isotope has a higher binding energy and reacts less than the lightest isotope. The difference of vapour pressure between \(^1\text{H}_2\ ^{18}\text{O}\) or \(^1\text{H}_2\ ^{16}\text{O}\) and \(^1\text{H}_2\ ^{16}\text{O}\) illustrates well this case. Nevertheless, inverse isotope effects are also observed, especially in polyatomic molecules or in reactions involving hydrogen atoms (Bigeleisen and Wolfsberg, 1958).

As the equilibrium isotopic effects is reversible, it is generally significant only when chemical intermediates in a given reaction can reach equilibrium, as in some enzymatically catalyzed reactions. In nature, kinetic and equilibrium isotopic effects are combined.

These mechanisms might lead to modifications of the isotope ratio (R which is the number of atoms of one isotope to the number of atoms of another isotope of the same chemical element in the same system) between reactants and products along metabolisms. Differences of isotopic ratios may also appears by the transition of a compound from one state to another (liquid water to water vapour for example) or between two compounds in equilibrium or in physical equilibrium. These modifications of the isotopic ratios are termed as isotopic fractionation (IAEA 2000). As isotopic fractionation in nature is the results of both kinetic and equilibrium isotopic effect, it should be referred as non-equilibrium fractionation.

The changes in isotopic composition between reactant and product can be measured by the isotopic fractionation factor (\(\alpha\)) which is the ratio of R of products to R of reactants. The isotopic fractionation (\(\varepsilon\)) is defined as \(\alpha – 1\) and is useful when discussing distributions of isotopes between substances. It is also termed as isotopic enrichment factor, discrimination, isotopic discrimination or isotopic fractionation constant. The relative difference of isotope ratios (\(\delta\) or isotope delta) expresses the variation of isotope ratio from product to reactants compared to the isotope ratio of the product. Delta values may also be expressed relative to an international measurement standard (Coplen 2011).
When dealing with radioactive isotopes, the specific activity is also used to compare the isotopic composition of different substances. In tritium, this is particularly easy as specific activity is usually measured by liquid scintillation counting on water, whenever it is on extracted free water or on combustion water. Comparing the specific activity of two matrices by establishing a specific activity ratio (SAR) appears convenient in most of the studied matrices.

Tritium has another specificity: it is the natural isotope which has the most important difference of mass comparing to the main isotope, $^1$H. As isotopic effects depend on mass, this important relative difference of mass between hydrogen and tritium induces the most important variations in the energy activation for a given reaction.

### 1.4 Sample preparation and tritium assay

Two standard techniques are used to assay tritium in environmental samples:
- $^3$Helium ingrowth method and measurement by Accelerator Mass Spectrometry (AMS) (Jean-Baptiste and others 2010)
- Liquid Scintillation Counting (LSC) (Wood and others 1993).

For both techniques, three fractions of tritium have to be separated. Most laboratories usually use the same protocol (Baglan and others 2005; Pointurier and others 2004):
- extraction of the free water of the sample and measurement of TFWT, then
- measurement of tOBT,
- isotopic exchange of hydrogen isotopes by washing the dry fraction with non-tritiated water, then a second extraction of water to measure (if possible) eOBT and determine neOBT.

$\text{eOBT}$ is frequently not measured but simply deduced:

$$\text{eOBT} = \text{tOBT} - \text{neOBT}$$

The reliability of the measurement of the tritium fractions obviously depends on the reliability of each of the steps involved. An isotopic effect in one of these steps may induce an error in the measurement of the specific activity of extracted free water and of OBT (Baumgärtner and Kim 1990; Kim and Baumgärtner 1991; Le Goff and others 2013).

When using liquid scintillation counting (LSC), measurements of tOBT and neOBT are carried out on oxidised forms (Galeriu and Melintescu 2010). Generally, oxidation is performed in a catalytic furnace or in a Parr bomb where the dry sample is completely oxidised in pure $O_2$. Thus, the combustion water results exclusively from the oxidation of the hydrogen of the sample. It is precisely on this water that LSC is performed.

The tritium content of plants or animal products can be expressed in Bq L$^{-1}$ (for total sample, or for free water or for combustion water related to tOBT, or neOBT), in Bq kg$^{-1}$ of fresh mass in Bq kg$^{-1}$ of dry mass or in Bq kg$^{-1}$ of hydrogen. Each of these unities has its own advantages:
- Bq L$^{-1}$ (and Bq L$^{-1}$ of combustion water) and Bq kg$^{-1}$ of hydrogen allow easy comparison of the ratio T/H in the different fractions of the matrix or even in different compartments of the environment.
- Bq kg\(^{-1}\) fresh weight and Bq kg\(^{-1}\) of dry weight are more appropriate when assessing global transfers of tritium through the food chain to man and considering the relative contributions of water and organic molecules.

### .2 Isotopic fractionation between tritium in abiotic compartments and Tissue Free Water Tritium of living organisms

In most terrestrial plants free water represents about 70 % to 85 % of their non-lignified mass (Boyer 2009; Hopkins 1995).

Experiments have shown that there is little (< 10\(^{-6}\) %), if any, HT conversion in HTO in plants (Dunstall and others 1985). The only pathway for tritium from HT to plant seems to be the oxidation by microbial action in the first centimetres of the soil (Belot 1986). In most cases, it is thus relevant to consider only HTO when considering the exposure of an animal or a plant.

So, in plants TFWT integrates soil water and atmosphere water with different kinetics and relative contributions. To reveal any fractionation between water of the environment and free water of plants, measurements should ideally be systematically performed at the same time in the three compartments.

Kim and Baumgärtner have run experiments in steady state conditions with hydroponic solution and atmosphere in equilibrium (Kim and Baumgärtner 1994). The isotopic fractionation effect due to evaporation of water led to a depletion in HTO of the atmosphere by about 10% compared to the hydroponic solution. They showed that in these conditions, barley and maize have different levels of equilibrium with HTO of their environment: tissue water in barley was slightly enriched in tritium (+ 2% compared to hydroponic solution) while tissue water of maize was tritium depleted (- 8.6 % compared to hydroponic solution). The authors explain this by the difference in metabolism of the two species (respectively C3 and C4 plants) which leads to differences in the contribution from soil water and air vapour in tissue free water and to differences in transpiration rate (which lead to another isotopic fractionation effect).

Tritium concentration of tissues free water is not the same for all parts and organs of plants. Belot et al. (Belot and others 1979), Raney and Vaadia (Raney and Vaadia 1965), Amano et al. (Amano and others 1995) investigated the TFWT in different organs. They found that the specific tritium activity of tissue free water could match with that of soil water in some organs (stem, petiole or fruit) but not in leaves where it only reached 60% of the activity of their nutrient solution due to dilution with atmospheric water. A simple model was deduced from these experiments and optimized (IAEA 2009):

\[
C_{TFWT} = \left[ RH \cdot C_{atm} + (1 - RH) \cdot C_{soil} \right] / \gamma
\]  

(2)

Where \(C_{TFWT}\) is the TFWT in leaves, \(C_{soil}\) the concentration of HTO in soil water, \(C_{atm}\) the concentration of HTO in atmospheric vapour, RH the relative humidity of the atmosphere and \(\gamma\) (=0.909) is the ratio of the HTO vapour pressure to that of H\(_2\)O under the thermodynamic conditions (T, P) of their experiments.

This relationship does not fit with all plant species: “This was explained by assuming that water in leaf veins is not always entirely accessible to HTO exchange” (Belot 1986).
Nevertheless, this equation assumes that no significant isotopic fractionation (except that due to the differences of vapour pressures between HTO and H₂O) occurs during the incorporation into plants of water from soil or atmosphere.

In other experiments under controlled conditions, Strack et al. (Strack and others 1995) have shown that the more leaves were illuminated (with a photosynthetic photon flux density ranging from 0 to 900 µmol m⁻² s⁻¹), the more atmospheric water contributes to TFWT. As the relation between photosynthetic rate and temperature and/or illumination is not linear, this can be generalised by considering that the higher the photosynthetic rate in leaves (depending on illumination, temperature and growth stage of plant), the larger the relative contribution of atmospheric water to TFWT. This is in good agreement with results obtained in lettuce at different stages of growth and in different conditions of illumination (Boyer 2009).

In natural conditions, atmospheric water is generally sampled by bubbling atmospheric air through non-trititated water for a few days even though the TFWT results from the tritium activity of water in the soil but also in the atmosphere during the hours before sampling. This explains why data in such conditions are rarely able to describe mechanisms involved in plants or the equilibrium between TWFT in plants or their organs with atmosphere and soil water (Boyer and others 2009b). By comparison, soil water dynamics is quite slow (Guétat 2013) and is thus a fairly stable source of tritiated water on the scale of a week. It integrates dry deposition of water vapour in the air and rain deposition over weeks. For air-leaf transfer, strong variations occur on the scale of the day or less, because of the instability of wind direction and the changes in stomatal opening throughout the day that may induce fast exchanges between plant water and air, or not. As a result, fluctuations of TFWT in plants follows the variation of HTO in atmospheric water vapour which are moderated by soil water inputs (Guétat and others 2013).

Animals are also composed of a large proportion of water. In mammals, water represents about 60% of their mass (Richmond and others 1962). The major source of water for terrestrial animals is their drinking water. For man, drinking water represents about 50 % of the daily intakes while the rest comes from food (about 35%) and from water produced by metabolism (about 15 %). Absorption by skin and lungs is negligible (ANC 2001). The different sources of water make it difficult to estimate an isotopic fractionation between water intakes and animal free water in environmental conditions. Experiments have rather indicated that no isotopic effects occur after intravenous injection, ingestion or inhalation of HTO (Balonov and others 1974; Pinson and Anderson 1950).

Tritium as TFWT is well distributed in the fluids and organs of the whole body after an absorption of HTO (Trivedi and others 1995) or tritiated glucose (Arai and others 1985). This behaviour is shared by compounds that enter specifically into energy metabolism. Equilibrium in body fluids is reached 45 minutes after absorption of tritiated water (Pinson and Anderson 1950).

.3 Isotopic fractionation between tissue free water and organic matter (from TFWT to OBT)

Total OBT includes exchangeable OBT and non-exchangeable OBT. The fraction of eOBT is usually estimated as 22 % of the tOBT of a living organism (IAEA 2009). Nevertheless, the relative amount of eOBT in a matrix depends on the relative fraction of labile hydrogen,
which in turn depends on the exact chemical composition of that matrix. Table 1 gives values of different organic compounds. It appears that, in most cases, 22% is an underestimation of the fraction of labile hydrogen.

Table 1: Exchangeable hydrogen content of the main components of organic material as estimated from stoichiometric calculations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean hydrogen content (% dry weight)</th>
<th>Calculated hydrogen content (% dry weight)</th>
<th>Non-Exchangeable hydrogen (% weight of total hydrogen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose C_{12}H_{22}O_{11}</td>
<td>6.1</td>
<td>6.4(^1)</td>
<td>64 (55(^1))</td>
</tr>
<tr>
<td>Cellulose (C_{6}H_{10}O_{5})_n</td>
<td>6.2</td>
<td>6.2(^1)</td>
<td>70</td>
</tr>
<tr>
<td>Starch (C_{6}H_{10}O_{5})_n</td>
<td>6.8</td>
<td>6.2(^1)</td>
<td>72</td>
</tr>
<tr>
<td>Proteins</td>
<td>6.8</td>
<td>7.5(^2)</td>
<td>75 (73(^2))</td>
</tr>
<tr>
<td>Fats</td>
<td>12.0</td>
<td></td>
<td>95-100</td>
</tr>
</tbody>
</table>

\(^1\): based on the chemical formula  
\(^2\): estimated for typical mammal protein  
Other values are extracted from (Diabaté and Strack 1993)

Some of the variations in the relative amounts of eOBT also depend on the conditions of exposure to tritium (Kim and Korolevych 2013) and on the time between exposure, sampling and measurement (especially if not enough care is taken to avoid demarking or marking by atmospheric vapour). eOBT is assumed to quickly reach equilibrium with TFWT (Belot 1986) but a fraction of labile hydrogen – that is not measurable – “[is] exchanged very slowly because of [its] inaccessibility to cellular water” (Diabaté and Strack 1993). Kim and Baumgärtner have proceeded to an experiment where they kept a dry powdered sample of tritiated maize plant in outdoor conditions and measured the evolution of its tOBT. The results are presented in Fig. 1 (Kim and Baumgärtner 1991).
Fig. 1: Exchange velocity of eOBT in dried maize plants with ambient air at 30.1 % relative humidity. The level of eOBT is given by the value of OBT obtained after isotopic washing. The plot is based on data from (Kim and Baumgärtner 1991).

In this experiment, 8% of the eOBT is still in the ground maize plants after one day of exposure to the atmosphere. These results cannot be compared with environmental conditions: firstly, plant or animal metabolism may increase the rate of accessibility to buried hydrogen; secondly, as the matter is dry and pulverised, it is certainly more accessible to water vapour due to the increase in specific surface area.

Some authors (for example in (Belot 1986; Brudenell and others 1997; Koranda and Martin 1972)) have measured the evolution of TFWT in plants exposed to ambient air following exposure to HTO. Decrease of TFWT fits with a double sometimes even triple exponential decay. The first (about 99 % of HTO), with a half-time of 30 to 60 minutes, is in good agreement with common conclusions on the half-time of TFWT in plants. The second one (about 1 % of HTO) has a half-time of about 15-30 hours. The authors explain this second component by the loss of some less accessible tritium in plants (in stem or root tissue). As this second component is very small compared to the first one, it also seems likely that “breathing” of biomolecules and the progressive accessibility of labile hydrogen positions may explain such an observation. This hypothesis fits with the estimation of the half time of exchangeable OBT by Heling and Galeriu: about 0.5-1.0 d⁻¹ (Heling and Galeriu 2002). Belot explains the third component by the decontamination of the root and stem tissues which were labelled by phloem translocation. Since this third component has a period similar to neOBT (Belot 1986; Koranda and Martin 1972), it may also be due to the anabolism of tritiated organic molecules.

These examples of results concerning the half-life of eOBT stress the difficulties to measure the half-life and the magnitude of the buried fraction. It results that neOBT cannot be deduced from tOBT (as mentioned by others (Baglan and others 2011; Kim and others 2011; Pointurier and others 2004)) even when paired with measurements of TFWT, and data on fractionation between TFWT and tOBT are not reproducible. Experiments have shown that the ratio of neOBT to eOBT may vary over a very wide range of values depending on the conditions of exposure (Hill and Johnson 1993).
.4 Isotopic fractionation between Tissue Free Water and non-exchangeable hydrogen of organic matter

.4.1 Measured neOBT is not (only) neOBT

As reported above, neOBT is evaluated after “washing” the dry sample with non-tritiated water. Two groups of techniques are available. The first group establishes equilibrium between the exchangeable hydrogen of the dry matter with non-tritiated hot water vapour flowing through the sample. After exchange, the water vapour is recovered in a cold trap. The exchange is stopped when the specific activity of recovered water is lower than a fixed threshold (generally the decision threshold) (see for example (Guénot and Belot 1984; Sauer and others 2009; Schimmelmann 1991)). The other group of isotopic exchange techniques consist in mixing dry powdered samples with liquid non-tritiated water and maintaining the mixture under constant stirring for 24 h to 72 h. The water is then extracted from the sample. The first step can be repeated if necessary. The extracted water has to be under the decision threshold after exchange (see for example (Baglan and others 2008; Baumgärtner and others 2009; Kim and Korolevych 2013)). As techniques of the second group are less time consuming than those of the first (5 to 7 days (exchange + freeze drying) vs. many weeks of exchange), they are generally preferred (Kim and others 2008; Pointurier and others 2004).

Some biases in the quantification of neOBT may be caused by the isotopic exchange. First, when biological samples are pulverised and mixed with water for 24 h to 72 h, several types of event may occur in the samples: enzymatic reactions, growth of microorganisms, fermentation, etc. This may induce variations (increase or decrease) of the levels of HTO and neOBT.

Secondly, some organic matter (sugars, amino-acids, etc.) gets into solution during the exchange. When water and dry matter are separated by filtration, this soluble fraction may stay with the water and is thus not-quantified as neOBT. This can also modify the tritium/hydrogen ratio in the neOBT (Bacchetta and others 2012).

Finally, the most important question is the reliability of standard isotopic exchange for quantification of neOBT. Some authors, in particular in (Baumgärtner and Donhaerl 2004; Baumgärtner and others 2009), show that a significant fraction of the eOBT can remain in the dry matter, even after two isotopic exchanges with non-tritiated water. They consider that a fraction of exchangeable hydrogen (water or hydrogen bound with X-atoms (X = oxygen, sulphur or nitrogen) of biomolecules) is not accessible for isotopic exchange due to their inaccessibility for water (steric hindrance and/or inaccessibility due to the molecular folding). This is in good agreement with observations made in cellulose (see for example (Yamada and others 1992)). Baumgärtner and his co-authors also consider that this fraction may be enriched in tritium because of a cumulative transfer from water to hydrogen bonds of biomolecules. They term this fraction “buried tritium”. Other authors have investigated the effect of this buried tritium in quantification of neOBT (Kim and others 2008). They conclude that it could lead to a maximum overestimation of neOBT of between 5 % (in plants) and 20 % (in fish).

.4.2 Tritium concentration in combustion water is not neOBT.
neOBT is currently one of the main topics in tritium radioecology, radiotoxicology and radioprotection. The most intriguing and controversial issue deals with possible isotopic fractionation along metabolisms in an organism or an ecosystem that would amplify the non-exchangeable fraction. It is usual to compare the specific activity of HTO, in the environment or in an organism, to the specific activity of neOBT (measured on combustion water obtained from isotopically washed dry matter as described above). This allows the T/H ratio to be compared between compartments of differing chemical nature.

In the literature, the ratio of specific activity of neOBT to the specific activity of TFWT or to HTO is termed the relative specific activity (RSA), SAR, or simply R. This ratio appears as being a common way to evaluate isotopic fractionation. Nevertheless, an important correction is necessary before interpreting this ratio.

Even though the tritium measured in the combustion water is generally assumed to arise exclusively from neOBT, the hydrogen that constitutes the bulk of the combustion water finds its origin in both non-exchangeable hydrogen and exchangeable hydrogen (Gontier and Siclet 2011; Jean-Baptiste and others 2010). Rinsing the dry matter in fact causes an isotopic dilution of the neOBT. Let us, for example, consider a molecule of fully-tritiated glucose (C$_6$T$_{12}$O$_6$) as represented in Fig. 2a. This extreme case of tritiation gives a clear illustration of this isotopic dilution.

![Fig. 2: (a) All hydrogen positions in the molecule are replaced with tritium (T). (b) Labile positions (exchangeable tritium) have been replaced by hydrogen.](image)

To measure tOBT, combustion is performed following reaction (3):

$$C_6T_{12}O_6 + 6 O_2 \rightarrow 6 CO_2 + 6 T_2O$$

To reach neOBT, the molecule first has to be rinsed in an excess of tritium-depleted water:

$$C_6T_{12}O_6 + n H_2O \rightarrow C_6T_7H_5O_6 + 5 HTO + (n - 5) H_2O$$

Then, neOBT is determined after the product of (4) is subjected to complete combustion (5):

$$C_6T_7H_5O_6 + 6 O_2 \rightarrow 6 CO_2 + 5 HTO + T_2O$$

The combustion water of the neOBT is 7/12 less concentrated in tritium than the combustion water of tOBT while the non-labile hydrogen fraction is still saturated in tritium. If we compare this specific activity to the specific activity of pure T$_2$O, the RSA would be 7/12. This does not give an accurate description of the real fraction of tritium in non-exchangeable positions (100 %). The correction to apply is thus the division of the RSA by the fraction of non-exchangeable hydrogen in the studied organic matter.
This correction is in good agreement with results in (Weinberger and Porter 1953): the authors took care to double “rinse” the dry matter of *Chlorella pyrenoidosa* with distilled water but did not evaluate the equilibrium established between distilled water and the eOBT of the algae. They conclude that the experimental data was 47 % of the theoretical value without fractionation. If the experimental data are set right by considering the fraction of non-exchangeable hydrogen at 79 % of total hydrogen (Spoehr and Milner 1949), the experimental data give 62 % of the expected value without fractionation effect.

Table 2 summarises the results presented in the literature with and without the proposed correction. We have only presented results from papers in which the authors give enough details in materials and methods to be sure that it is neOBT that is compared to HTO. The fraction of non-exchangeable hydrogen (the correction factor) was estimated using data found in different references: the contribution of proteins, fats, sugar and fiber to dry matter has been evaluated for each matrix, then the weighted mean of non-exchangeable hydrogen calculated using the data presented in Table 1.
### Table 2: Examples of measured neOBT/HTO ratios with a proposed correction to evaluate the discrimination effect

<table>
<thead>
<tr>
<th>Plants</th>
<th>Environmental data</th>
<th>Water taken as reference</th>
<th>Nature of the analysed dry matter</th>
<th>Name of the ratio as termed in the reference</th>
<th>Ratio as in the text</th>
<th>Correction factor *</th>
<th>Corrected ratio</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree leaves</td>
<td>HTO in rain</td>
<td></td>
<td></td>
<td></td>
<td>1.6</td>
<td>0.70</td>
<td>2.29</td>
<td>(Pointurier and others 2004)</td>
</tr>
<tr>
<td>Plants</td>
<td>HTO atm</td>
<td>plant OBT/air HTO ratios</td>
<td>0.12-0.56</td>
<td>0.78&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.15-0.72</td>
<td>(Davis and others 2005)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetation</td>
<td>TFWT</td>
<td>OBT/TFWT ratio</td>
<td>0.6-1.0</td>
<td>0.78&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.77-1.28</td>
<td>(Kötzler and Workman 1999)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fucus serratus</td>
<td>TFWT</td>
<td>ratio OBT/HTO</td>
<td>1.3</td>
<td>0.66</td>
<td>1.97</td>
<td>(Lebaron-Jacobs and Renaud 2009)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fucus vesiculosus</td>
<td>TFWT</td>
<td>ratio OBT/HTO</td>
<td>1.3</td>
<td>0.66</td>
<td>1.97</td>
<td>(Lebaron-Jacobs and Renaud 2009)</td>
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<td>Tree leaves</td>
<td>TFWT</td>
<td>R</td>
<td>0.04-1.0</td>
<td>0.70</td>
<td>0.06-1.43</td>
<td>(Baglan and others 2011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits and tubers</td>
<td>HTO atm</td>
<td>RF (Reduction factor)</td>
<td>0.93</td>
<td>0.66 to 0.75</td>
<td>1.24 to 1.41</td>
<td>(Korolevych and Kim 2013)</td>
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</tr>
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<td>Alfalfa leaves and stems</td>
<td>TFWT</td>
<td>OBT-to-HTO ratios</td>
<td>0.78</td>
<td>0.72</td>
<td>1.08</td>
<td>(Diabaté and Strack 1993)</td>
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<tr>
<td>Barley</td>
<td>TFWT</td>
<td>OBT-to-HTO ratios</td>
<td>0.73</td>
<td>0.73</td>
<td>1</td>
<td>(Kim and Baumgärtner 1994)</td>
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<td></td>
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<td>Maize</td>
<td>TFWT</td>
<td>OBT-to-HTO ratios</td>
<td>0.64</td>
<td>0.73</td>
<td>0.88</td>
<td>(Kim and Baumgärtner 1994)</td>
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<tr>
<td>Barley</td>
<td>TFWT</td>
<td>Rstable</td>
<td>0.55</td>
<td>0.75</td>
<td>0.73</td>
<td></td>
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<tr>
<td>Maize</td>
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<td>Rstable</td>
<td>0.60</td>
<td>0.73</td>
<td>0.82</td>
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<td>Komatsuna leaves</td>
<td>TFWT</td>
<td>OBT/HTO ratio in plants</td>
<td>0.06-0.24</td>
<td>0.68</td>
<td>0.09-0.35</td>
<td>(Amano and others 1995)</td>
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<td>Radish leaves</td>
<td>TFWT</td>
<td>OBT/HTO ratio in plants</td>
<td>−0.5</td>
<td>0.70&lt;sup&gt;2&lt;/sup&gt;</td>
<td>−0.71</td>
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<tr>
<td>Radish roots</td>
<td>TFWT</td>
<td>OBT/HTO ratio in plants</td>
<td>−0.125</td>
<td>0.67</td>
<td>−0.19</td>
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<tr>
<td>Tomato leaves</td>
<td>TFWT</td>
<td>OBT/HTO ratio in plants</td>
<td>−0.35</td>
<td>0.70</td>
<td>−0.50</td>
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<td>Tomato leaflets</td>
<td>TFWT</td>
<td>OBT/HTO ratio in plants</td>
<td>−0.22</td>
<td>0.70&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.31</td>
<td></td>
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<tr>
<td>Wheat leaves</td>
<td>TFWT after exposition (night conditions)</td>
<td>relative OBT concentration</td>
<td>0.4% - 0.8%</td>
<td>0.70</td>
<td>0.57% - 1.14%</td>
<td>(Diabaté and Strack 1997)</td>
<td></td>
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</tr>
<tr>
<td>Wheat leaves</td>
<td>TFWT after exposition (day conditions)</td>
<td>relative OBT concentration</td>
<td>1.0% - 1.8%</td>
<td>0.70</td>
<td>1.43% - 2.57%</td>
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<tr>
<td>Rice plant</td>
<td>HTO atm</td>
<td>relative OBT concentration</td>
<td>0.7% - 1.2%</td>
<td>0.71&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1.0% - 1.7%</td>
<td>(Choi and others 2002)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice leaves</td>
<td>TFWT leaf water at the end of exposure</td>
<td>TLI</td>
<td>0.1% - 0.2%</td>
<td>0.70</td>
<td>0.1% - 0.3%</td>
<td>(Galeriu and others 2013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice grains</td>
<td>TFWT</td>
<td></td>
<td>0.10% - 0.14%</td>
<td>0.71</td>
<td>0.14% - 0.20%</td>
<td></td>
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</tbody>
</table>
### Animals

#### Environmental data

<table>
<thead>
<tr>
<th>Nature of the analysed dry matter</th>
<th>Water taken as reference</th>
<th>Name of the ratio as termed in the reference</th>
<th>ratio as in the text</th>
<th>Correction factor</th>
<th>Corrected ratio</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBT in urine (acute exp.)</td>
<td>HTO in urine</td>
<td>0.001 (2.5 day after exposure)</td>
<td>0.45&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.0022</td>
<td></td>
<td>(Trivedi and others 1995)</td>
</tr>
<tr>
<td>OBT in urine (acute exp.)</td>
<td>HTO in urine</td>
<td>0.09 (100 days after exposure)</td>
<td>0.45&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whelk</td>
<td>TFWT</td>
<td>ratios OBT/HTO</td>
<td>1.9</td>
<td>0.73</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Limpet</td>
<td>TFWT</td>
<td></td>
<td>1.1</td>
<td>0.77&lt;sup&gt;6&lt;/sup&gt;</td>
<td>1.43</td>
<td>(Lebaron-Jacobs and Renaud 2009)</td>
</tr>
<tr>
<td>Lobster</td>
<td>TFWT</td>
<td></td>
<td>0.8</td>
<td>0.77</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>Plaice</td>
<td>TFWT</td>
<td></td>
<td>0.8</td>
<td>0.77</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>Sole</td>
<td>TFWT</td>
<td></td>
<td>0.8</td>
<td>0.76</td>
<td>1.05</td>
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<tr>
<td>Wrasse</td>
<td>TFWT</td>
<td></td>
<td>0.9</td>
<td>0.76</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>Animal</td>
<td>HTO atm</td>
<td>OBT/air HTO ratios</td>
<td>0.18-0.45</td>
<td>0.78&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.23-0.58</td>
<td>(Davis and others 2005)</td>
</tr>
<tr>
<td>Terrestrial mammals</td>
<td>HTO in media water</td>
<td>SAR</td>
<td>0.25</td>
<td>0.69&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.36</td>
<td>(Galeriu and others 2005)</td>
</tr>
</tbody>
</table>

#### Chronic exposure in controlled conditions

<table>
<thead>
<tr>
<th>DNA of different organs</th>
<th>TFWT</th>
<th>Relative specific activity</th>
<th>SAR</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zooplankton</td>
<td>HTO in media water</td>
<td>0.76 (from 0.66 to 1.07)</td>
<td>0.83</td>
<td>(Commerford and others 1977)</td>
</tr>
<tr>
<td>Molluscs</td>
<td>HTO in media water</td>
<td>0.3</td>
<td>0.77&lt;sup&gt;3&lt;/sup&gt;</td>
<td>(Galeriu and others 2005)</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>HTO in media water</td>
<td>0.25</td>
<td>0.75&lt;sup&gt;4&lt;/sup&gt;</td>
<td>(Galeriu and others 2005)</td>
</tr>
<tr>
<td>Fish</td>
<td>HTO in media water</td>
<td>0.25</td>
<td>0.79&lt;sup&gt;7&lt;/sup&gt;</td>
<td>(Galeriu and others 2005)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>TFWT</td>
<td>0.19</td>
<td>0.80</td>
<td>(Kim and others 2013)</td>
</tr>
</tbody>
</table>

<sup>*</sup>: when possible, ratios were calculated by the formula:

$$0.75 \cdot A + 0.95 \cdot B + 0.64 \cdot C + 0.70 \cdot D$$  \hspace{1cm} (6)

Where A, B, C and D are respectively the fraction of proteins, fats, sugar and fiber in the dry matter of the studied matrix (Spector 1956; U.S. Department of Agriculture 2012). The coefficients (0.75, 0.95, 0.64 and 0.70) are deduced from data in Table 1.

<sup>1</sup>: unfound data on amount of exchangeable hydrogen. The default value of 0.22 was used to approximate the correction
<sup>2</sup>: unfound data for radish leaves. The ratio used is the mean of data from leaves of different species (sd = 0.017)
<sup>3</sup>: unfound data for tomato leaflets. The ratio was taken to be equal to that of mature tomato leaves.
<sup>4</sup>: ratio estimated from the relative weights of the different organs of rice plants (Choi and others 2002) and from the average data for straw of different species
<sup>5</sup>: data calculated from (Putnam 1971)
<sup>6</sup>: mean ratio of other molluscs
<sup>7</sup>: mean ratio of other fish
<sup>8</sup>: calculated ratio from data on rabbits
In Table 2, the average value of the estimated correction factor is 0.71 (standard deviation: 0.035) for plants and 0.74 (standard deviation: 0.10) in animals (0.77 if values for urine are not taken into account). In environmental data, average RAS of plants are 1.16 (standard deviation: 0.67) and 1.69 (standard deviation: 0.98) respectively without or with correction. For animals, average values are 0.85 (standard deviation: 0.46) and 1.12 (standard deviation: 0.36). When organisms are chronically exposed in controlled conditions, RAS of plants are 0.46 (standard deviation: 0.24) (without correction) and 0.64 (standard deviation: 0.32) (with correction). The values are 0.36 (standard deviation: 0.09) and 0.45 (standard deviation: 0.13) for animals. It is noticeable that RAS measured in organisms chronically exposed in controlled conditions are significantly lower than in samples collected in nature as also mentioned in (Jean-Baptiste and others 2009). These differences can be explained by the fact that the kinetic of free (with short half-lives) and bound tritium (which integrates tritium on long time scale) are very different and, in variable conditions, as in environment, the two compartments may present significantly different isotope ratios (Davis and Galeriu 2012). RAS in animals is systematically lower than RAS of plants.

In (Jean-Baptiste and others 2009), the authors show how seasonal variations of atmospheric HTO partially explain the differences in ratios neOBT/TFWT measured in the wild or in controlled conditions. Their explanations are based on the fact that TFWT in plants reaches equilibrium with atmospheric HTO very rapidly, while, as described above, neOBT integrates the tritium during the synthesis of organic matter. Two kinds of variations of atmospheric HTO are described in their paper: a rhythmic variation that occurs along the year (the maximum is reached in the middle of May and the minimum in mid November) and random noise. By playing only on these two parameters and considering that there is no isotopic fractionation, the authors simulated neOBT/TFWT ratios ranging from 0.8 to 4.0 (mean: 1.45). This illustrates how sampling date, time, and general context may induce a significant bias in the quantification of isotopic fractionation.

### 4.3 Dynamic incorporation of tritium from HTO to neOBT is not isotopic fractionation

Another way to express the OBT formation in an animal or plant exposed continuously to tritium is to calculate the transfer rate (also termed OBT production rate (Amano and others 1995), OBT formation (or loss) rate (Kim and others 2013) or conversion rate (Boyer and others 2009a)) according to equation (5) (Atarashi-Andoh and others 2002):

\[
\frac{dC_{\text{OBT}}}{dt} = v \times C_{\text{HTO}} \tag{7}
\]

Where:
- \(C_{\text{OBT}}\) (Bq L\(^{-1}\) of combustion water or equivalent water) is the OBT concentration in the different parts of the organism.
- \(v\) (hr\(^{-1}\)) is the conversion rate from HTO (TFWT, atmospheric HTO or soil HTO) to OBT.
- \(C_{\text{HTO}}\) (Bq L\(^{-1}\)) is TFWT, atmospheric HTO or even soil HTO.
In the case of an organism (or the part of the organism that is being studied) which has a constant mass during exposure, this rate gives a good indication of the turnover rate of the global organic matter in the organism or in a specific class of its molecules.

For example, the data presented in (Choi and others 2002) allow \( v \) to be evaluated at different stages, for straw (leaves + stem) and for whole plants. Note that during the first day after exposure, \( v \) for a whole plant is significantly lower than for straw. This can be explained by the fact that straw loses neOBT by conversion and translocation whereas \( v \) for the whole plant expresses only net loss of neOBT.

These comparisons are relevant because the mass of straw and plants are considered constant during the period of measurement. In (Choi and others 2002), the lack of \( v \) is particularly significant for the whole plant between 240 h and 1000 h after exposure since it is \(-0.38 \% h^{-1}\) (lower than for straw) whereas the activity per shoot remains constant during this period. This negative value of \( v \) is only due to plant growth: its mass doubles during the considered period.

When the studied organism grows during a chronic exposure, we suggest to modify (7) in order to take into consideration the growth rate of the organism. Two cases are then to be considered: first, when data of neOBT is available, it is thus possible to evaluate fractionation using (8). If only data of tOBT is available, an additional correction is necessary to take account of the exchangeable fraction of hydrogen (9). These formulas model the time course of tOBT or approximate the fractionation effect due to tritium. These relationships are relevant if the metabolism is known to integrate water into biomolecules.

\[
C_{\text{CW,ne},i+1} = \frac{m_{d,i}}{m_{d,i+1}} C_{\text{CW,ne},i} + \frac{m_{d,i+1} - m_{d,i}}{m_{d,i+1}} \tau_{ne} \cdot f \cdot \bar{C}_{\text{HTO}}.
\]  \tag{8}

\[
C_{\text{CW,t},i+1} = \left( \frac{m_{d,i}}{m_{d,i+1}} C_{\text{CW,ne},i} + \frac{m_{d,i+1} - m_{d,i}}{m_{d,i+1}} \tau_{ne} \cdot f \cdot \bar{C}_{\text{HTO}} \right) + \left( 1 - \tau_{ne} \right) f' C_{\text{HTO},i+1}.
\]  \tag{9}

Where:
- \( C_{\text{cw}} \) is the specific activity of the combustion water (Bq L\(^{-1}\)),
- \( i, i+1 \): time steps,
- \( \text{ne} \): non-exchangeable
- \( t \): total
- \( m_d \): the dry matter mass of shoot,
- \( \tau_{ne} \): non exchangeable fraction of hydrogen (dimensionless),
- \( C_{\text{HTO}} \): is the specific activity of TFWT, atmospheric HTO or even soil HTO at the end of the considered period
- \( \bar{C}_{\text{HTO}} \) is the average specific activity of TFWT, atmospheric HTO or even soil HTO during the considered period (Bq L\(^{-1}\)),
- \( f \) (dimensionless) is the fractionation factor from free water to neOBT. (it can be, depending on experiments, TFWT, atmospheric HTO or soil HTO),
- \( f' \) (dimensionless is the fractionation factor from free water to eOBT).

When data exist for both soil and air inputs, the balance between the two has to be assessed. The fraction of free water originating from soil is often assessed as between 30\% and 50\%.

For example, Fig. 3 presents the time course of tOBT in wheat grains during their growth in the vicinity of a nuclear research centre. Data are extracted from (Guétat and others 2013),
except the mass of the grains which are approximated with the modelled growth presented in (Dupont and Altenbach 2003). Modelled tOBT is calculated by means of (9), f and f' were both set equal to 1. This latter point is discussed in Guétat and others (2013).

Fig. 3: Measured and modelled time course of tOBT in wheat grains vs specific activity of atmospheric water vapour and growth of wheat grain

Relations (8) and (9) nevertheless do not perfectly reflect the processes which take place in a given part of an organism. This is because the different fluxes of matter (the translocation of OBT from leaves to fruits and roots, the respiration of the organ, etc) modify the balance between water and neOBT in the organ considered and shift the time evolution of neOBT in it. The same can be noted in (Choi and others 2002): ears and seeds have quite constant concentrations of neOBT (or slightly increasing concentrations) after the one hour exposure while their dry weight increases after exposure to tritium (except in the case of the last exposure) confirming that some tritium is continuously incorporated by ears and seeds, even long after exposure.

.5 Isotopic fractionation in translocation and secondary biosynthesis

In the case of tritium in plants, translocation can be defined as the biochemical process by which molecules (essentially sugars) synthesised in leaves (Belot 1986) incorporate tritium and are then transported to other organs of the plant, as grain for example after anthesys. The rate of translocation to an organ depends on the stage of development of the plant or on the studied organ: the higher the growth rate of the considered organ immediately after exposure to tritium, the higher the concentration of tritium in that organ (Arai and others 1985; Diabaté and Strack 1993; Diabaté and Strack 1997; Spencer 1984; Strack and others 1995).

To quantify the translocation of OBT to grain, Diabaté and Strack defined the Translocation Index (TLI) as the ratio of the neOBT in grains at harvest (in Bq L\(^{-1}\) of combustion water) to the TFWT concentration in leaves (in Bq L\(^{-1}\)) at the end of exposure (Diabaté and Strack
This ratio can be used to compare the efficiency of translocation of tritium in different plants without considering the type of photosynthesis (C3, C4 or CAM) of the species concerned or its efficiency when exposure occurs at different stages of development. Nevertheless, TLI does not provide information on fractionation during the different stages of metabolism that occur from incorporation of HTO to the “final” OBT. Considering the numerous metabolic pathways and the variable contribution to freewater of water from soil or atmosphere, measuring fractionation effects during translocation seems possible only in well-defined laboratory conditions where HTO in soil water and in atmospheric water is controlled.

In animals, the difficulties to measure the specific activity of given molecules synthesized after consumption of tritiated food (OBT) (see for example (Arai and others 1985; Jean-Baptiste and others 2009; Komatsu and others 1990; Putnam 1971) or of identified tritiated compounds (see (Takeda 1991; Taylor 2008)) explain why there is only bulk data on synthesized or excreted OBT. It is thus impossible to evaluate the fractionation in the different metabolic pathways involved. In fact, considering the numerous routes of tritium dilution and excretion, even with chronic exposure and in equilibrium conditions, evaluating the fractionation effect cannot be done globally in the organism or in an organ. Considering each metabolic step, one by one, seems to be the sole way to really measure a fractionation effect. Otherwise, comparisons of tritiation between organs (Kirchmann and others 1977) or molecules (as in (Commerford and others 1977) or (Commerford and others 1983)) is another way to evaluate the relative concentration or dilution of tritium by the organism.

### 6 Conclusion

Isotopic fractionation of hydrogen constitutes a major issue in radiation protection and needed some radioecological inputs and explanations. Its evaluation indicates whether the possibility of concentration (in an organelle, an organ, an organism or an ecosystem) exists or not. Nevertheless, to be relevant, care must be taken for such an evaluation because hydrogen (and tritium) is ubiquitous, because hydrogen interacts in different ways (with different kinetics) and because hydrogen’s sources are often multiple with relative contributions that are difficult if not impossible to evaluate precisely.

Even though tritium has almost the same chemical properties as protium and deuterium, isotopic fractionation does occur. It is for example well known that during phase transition of water, enrichment in tritium of the condensed phase varies with thermodynamic conditions (temperature, pressure). Such a phenomenon is for example well documented in geochemistry but is also observable in plants; see for example (Kim and Baumgärtner 1994) and in distillation processes (Le Goff and others 2014).

Particular attention should therefore be paid when determining the size of the compartment in which tritium is measured (or its growth rate when kinetics is studied), whenever it is HTO or OBT. It seems for example that the RSA does not provide a good approximation of isotopic fractionation, systematically underestimating it by a factor ranging from 5 % to 50 %, depending of the fraction of labile hydrogen, as detailed in the text. The reason is that neOBT is measured on combustion water which contains both the labile and carbon-bound hydrogen of the studied matrix.

Some models also use a factor (generally around 0.6) to calculate the amount of neOBT synthesised from HTO (Davis and others 2005; IAEA 2003; Le Dizès 2004; Le Dizès 2005). This factor is generally presented as a discrimination factor where it seems to fit well with the
correction factor proposed above. In (IAEA 2009), a partition factor ($R_p$) is employed in the calculation: “The partition factor accounts for the reduction in dry weight (DW) concentration due to the presence of exchangeable hydrogen in combustion water, as well as for isotopic discrimination.” Nevertheless, as the correction factor would only replace the “discrimination factor” or the “partition factor”, there will be very little, if any, modification in the evaluation of impacts, simply a change in the way the phenomenon observed is represented and what it actually signifies.

The data presented in Table 1 (except the data measured after acute or short-term exposure) give a mean RSA of $0.95 \pm 0.58$ ($k=2$). Once corrected, the discrimination factor becomes $1.32 \pm 0.83$ ($k=2$). This latter mean seems to be in much better agreement with an average value of environmental data reported in (Jean-Baptiste and others 2009) ($1.92 \pm 1.42$ ($k=2$)). Even when corrected, the fractionation factor is still close to 1, which means that the fractionation effect should be moderate. Moreover, the size and the number of possible sources of hydrogen lead to a very fast dilution of tritium since the organism lives in an open environment. This dilution increases along the foodchain and therefore does more than just compensate for possible fractionation effects. Experiments in controlled conditions appear still necessary to better evaluate the net fractionation of tritium in organisms, in particular during biosynthesis. It is noticeable that experiments in animal are far less numerous than in plants although they are needed to better understand ways tritium evolves along animal metabolisms.

Another difficulty occurs when studying isotopic fractionation of tritium in the environment: the different ways in which data are presented and analysed in the literature. OBT means tOBT or neOBT, RSA is the ratio of (t or ne)OBT to HTO in the atmosphere, the soil or in plants and different units are used for OBT (Bq L$^{-1}$, Bq kg$^{-1}$ fresh weight or Bq kg$^{-1}$ of dry weight). Overall, these examples indicate numerous causes for confusion and potential sources of errors when gathering data. Vocabulary should therefore be rationalized once and for all to avoid future misunderstandings. We recommend the systematic use of a subscript (t, ne or e: total, non-exchangeable or exchangeable respectively) to clearly state which part of the OBT is being considered. RSA could be used in most cases to compare specific activities. By using a subscript, the terms of the ratio should also be clarified: for example $RS_{neOBT/TFWT}$ or $RS_{OBT/atmHTO}$. Difficulties in the separation of the different forms of tritium (in particular neOBT from eOBT) show the necessity of cooperation to make more robust analytical methods.

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