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Variations of conversion rate from Tissue Free Water Tritium to Organically-Bound Tritium in lettuces continuously exposed to atmospheric HT and HTO

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Abstract. To document the tritium uptake following chronic atmospheric exposure, potted lettuces (*Lactuca sativa* L.) were cultivated outdoors in the vicinity of a nuclear facility. During two sets of experiments performed in spring and autumn, the plants were continually exposed to low tritium levels throughout growth. These experiments factored in meteorological data, tritium monitoring in air (HT and HTO) and analysis of Tissue Free Water Tritium (TFWT) and Organically-Bound Tritium (OBT) in biological material. The global conversion rate of HTO to OBT in plants averaged $0.16\% \cdot h^{-1}$ over the growing period, but marked variations were observed during growth. In particular, a significant increase appeared at the exponential growth stage. Consequently, the usual predictive models (for cases of accidental or chronic exposure to tritium) should factor in the conversion rate according to the biological stage. Moreover, as plant growth is closely correlated to total energy received, meteorological parameters (temperature, light intensity) may be considered reliable indicators of growth.

1. INTRODUCTION

Because of its long retention time in living organisms, the importance of Organically-Bound Tritium (OBT) has been proved for the monitoring of tritium near nuclear facilities. Major processes of tritium transfer between environmental compartments [1] and global parameters for tritium integration into plants [2] have been reported. For instance, it is now well known that tritiated water from the soil and atmosphere easily enters into water pools of plants as Tissue Free Water Tritium (TFWT), and then may be converted into OBT by biological mechanisms, mainly photosynthesis. Nevertheless, there is little experimental data available on chronic and long-time tritium exposure to support models offered in literature, especially for plants grown in natural conditions. Moreover, the accuracy of such models largely depends on the global conversion rate from TFWT into OBT. Furthermore, some recent studies have pointed out the variations in this coefficient during the growth stage [3, 4]. Our study sets out to investigate the uptake of tritium due to continuous atmospheric exposure of lettuce (*Lactuca sativa* L.) and to determine the resulting conversion rate at different stages of plant growth.

2. MATERIALS AND METHODS

Experiments were performed in the vicinity of a nuclear facility to provide a convenient environment for the study of low-level tritium transfer to the plants. The place of testing was exposed to the prevailing winds 360 metres from a tritium emitting source.

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2.1 Plants cultivation and sampling procedures

Seeds of leaf lettuce (*Lactuca sativa* L.) were supplied by Rijk Zwaan ("Kitare" variety) and were chosen as clones with identical genotype. Soil used was a medium-textured stagnic luvisol (loamy soil) taken from the experimental farm *La Bouzule* (Lorraine, France) and was sieved in particles smaller than 5 mm to ensure effective aeration. Soil properties have been already described in the literature [5, 6].

Germination and early stages of plants development (first two weeks) were conducted in a controlled-climate chamber (night/day: 12 h/12 h, Photosynthetically Active Radiation (PAR) above plants: $0/75 \pm 10\,\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, temperature: $18\,^{\circ}\text{C}/24\,^{\circ}\text{C} \pm 2\,^{\circ}\text{C}$, relative humidity range: 45–95%). When the plants displayed four fully developed leaves, they were transferred into plastic containers with 2.0 litres of soil and placed outdoors at the dedicated place. Since our study focused on foliar uptake, the plants were placed in a greenhouse to protect them against precipitation. No fertilizer was used and the soil was maintained at 80% of its field capacity for the duration of the experiments. Plant growth was studied from germination to senescence. Two experiments were carried out, one from April to June 2007 (spring experiment) and the other from August to October 2007 (autumn experiment).

Tritium concentration in the air around the plants was monitored by weekly measurements taken with a bubbling system (MARC 7000 provided by SDEC). To compare, the atmospheric tritium concentration was estimated by using the GASCON model. This model, dedicated to chronic atmospheric releases and dose estimation, has been developed by the French Commissariat à l'Energie Atomique (CEA) and is used to assess the safety of facilities [7]. It is based on Doury's equations [8] i.e. a Gaussian puff model. GASCON includes a specific code for tritium, and thus can be used to estimate HTO and HT activity in the atmosphere due to identified releases and meteorological data. Therefore, wind direction and speed were recorded every 25 min by a meteorological station, and ambient conditions (temperature, relative humidity and light intensity) in the chamber and outdoors were recorded every 2 min with a specific device (Kistock KH200, Kimo).

Lettuces were sampled every week for tritium testing until the final harvest. The collected plants were chosen to be representative of the global population. Fresh and dry weights were measured for every plant collected. Roots were removed and all leaves of the batch were pooled before analysis.

2.2 Climatic conditions and tritium exposure

The minimum, maximum and mean values for temperature and relative humidity are given in Table 1. Daylight values are the average values over the whole period of each experiment, at plant canopy level.

Table 1. Outdoor climatic conditions (temperature, relative humidity and daylight) during the experiments.

	April to June experiment			August to October experiment		
	minimum	maximum	average	minimum	maximum	average
Temperature (°C)	5	41	21	6	33	17
Relative humidity (%)	21	88	65	41	94	70
Daylight (lux)	0	10000	3317	0	10000	1266

The atmospheric tritium concentration varied slightly during both experiments (Table 2). However, all the readings fall within the same range and tally with the average calculated by the GASCON model for the corresponding periods.

2.3 Analytical methods for tritium monitoring

Extraction and analysis of each ^{3}H fraction such as Tissue Free Water Tritium, exchangeable and non-exchangeable Organically-Bound Tritium (OBT) was done as described in literature [9].

Table 2. Comparison between measured and calculated (GASCON model) atmospheric tritium concentrations. Uncertainties are given with a 95% confidence level (k = 2).

April to June experiment				August to October experiment			
time from start of lettuces exposure (h)	HTO Bq/m^3	HT Bq/m^3	total ± uncertainty Bq/m^3	time from start of lettuces exposure (h)	HTO Bq/m^3	HT Bq/m^3	total ± uncertainty Bq/m^3
264	4.3	1.3	5.6 ± 1.1	792	3.6	0.8	4.4 ± 2.2
648	9.0	2.0	11.0 ± 3.0	960	6.3	0.7	7.0 ± 2.6
768	16.3	3.1	19.4 ± 3.6	1128	12.7	1.4	14.1 ± 4.8
960	8.7	2.5	11.2 ± 2.1	1296	14.4	2.8	17.2 ± 3.9
1128	11.8	1.4	13.2 ± 3.0	1464	4.4	0.9	5.3 ± 1.1
1296	4.1	0.5	4.6 ± 1.8	1632	3.7	0.8	4.5 ± 0.9
1464	8.5	0.8	9.3 ± 3.1	1824	3.1	0.7	3.8 ± 1.0
1632	14.4	1.8	16.3 ± 3.0	1968	4.9	1.0	5.8 ± 1.5
1800	18.9	5.6	24.5 ± 4.2	2136	3.8	0.8	4.6 ± 1.3
2160	18.3	2.8	21.1 ± 7.2	-	-	-	-
average on the period	12.5	2.3	14.8 ± 4.4	average on the period	5.9	0.9	6.8 ± 3.1
estimated concentration (GASCON)			17	estimated concentration (GASCON)			8

Samples were stored in a freezer at −20 °C before analysis. TFWT was extracted from samples by freeze-drying methods (Lyolab 3000 ETO) for 48 hours. Total elimination of TFWT was confirmed by weighing residual samples.

Exchangeable organic tritium (linked to atoms by weak covalent binding) was removed considering its quick turnover in organisms. Dried samples were mixed with a sufficient volume of tritium free water (50 mL for 1 g of dry matter) for 3 days to insure isotopic exchange.

Non-exchangeable OBT extraction methods involve a combustion apparatus. The dehydrated samples were burned under pure and dry oxygen flow ($60\,L \cdot h^{-1}$), and the resulting combustion water was condensed in a cold trap. Background level was determined by analysing tritium in washing water before each assay. The combustion efficiency was determined by the amount of liquid recovered after combustion of a known quantity of water, and was typically 99%.

Tritium in TFWT fraction and combustion water was measured using ^{3}H liquid scintillation counters (Tri-Carb 2900 TR and 2750 TR/LL). Samples were mixed with Packard 'Ultima Gold LLT' cocktail (proportions: 10 mL of water/10 mL of cocktail). The detection limit was $5.4\,Bq \cdot L^{-1}$ for a counting time of 200 min with a background of 3 cpm. A quench correction was systematically applied. Activities and uncertainties were calculated as described by Pointurier et al. [9].

3. RESULTS AND DISCUSSION

3.1 Growth of the plants

For each experiment, the growth curves show the same sigmoid pattern, but final dry weight was higher for the spring experiment than for the autumn one. To provide a description of plant growth regardless of the period of cultivation, a model based on equivalent energy delivered around the plants was used instead of a model based only on time. This equivalent of energy is defined mathematically as a function of time, light and temperature recorded near the lettuces and is expressed in lx.°C · days^2. This unit is not very accurate, because it factors in the whole solar spectrum, whereas the plants convert only part of the light into available chemical energy. Moreover, this equivalent of energy does not correlate light with temperature. However, it allows one to normalize the dry weight of the lettuces using the equation below (1), thereby enabling one to compare the results of the two sets of experiment.

$$W(E) = \frac{W_m}{1 + \dfrac{W_m - W_o}{W_o} \cdot \exp(-r \cdot E)} \qquad (1)$$

Where: r is the growth rate ($lx^{-1} \cdot {}^{\circ}C^{-1} \cdot days^{-2}$),

E is the global recorded energy near the plants,

$W(E)$ is the dry weight of lettuces (g); W_0 is stand for the first measurement, W_m is the maximal dry weight reached.

The coefficients for the modelling curve were determined from the data of both experiments by the least square method, which gives the following values:

$Mo = 0.001$ g, $K = 5.095$ g, and $r = 7.6 \times 10^{-8}\, lx^{-1} \cdot {}^{\circ}C^{-1} \cdot days^{-2}$.

Experimental measured values of dry mass and those calculated with the model tally for both experimental data sets, as shown in Figure 1.

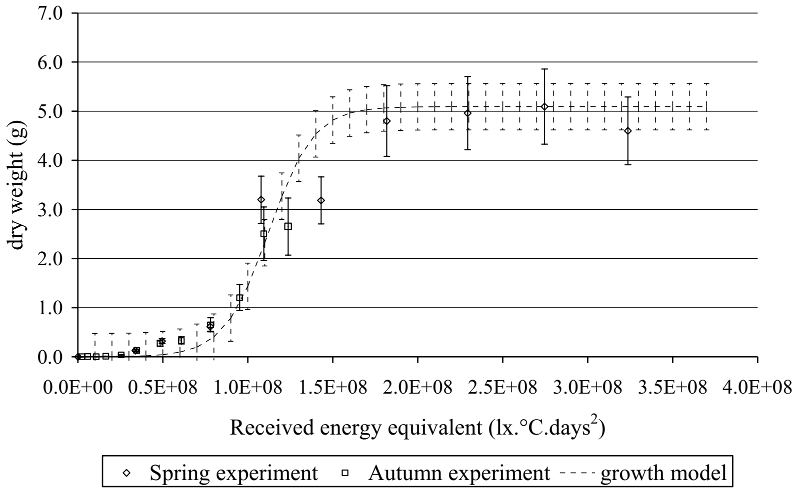

Figure 1. Growth of lettuces expressed in dry weight (g) vs. heat and solar radiation unit ($lx \cdot {}^{\circ}C \cdot days^2$). Experimental and model values are plotted with their associated computed uncertainty coefficient ($k = 1$).

3.2 Calculation of conversion rate

Initially a global ratio ν ($\% \cdot h^{-1}$) of integrated OBT (C_{OBT}) to TFWT (C_{TFWT}) was estimated on the basis on concentrations in $Bq \cdot L^{-1}$ and as a function of time, according to equation (2), described by Atarashi-Andoh [4]:

$$\frac{dC_{OBT}}{dt} = \nu \times C_{TFWT} \tag{2}$$

Ratio ν was calculated from the beginning of the exposure to plant harvest, disregarding biological parameters such as lettuces weight. The results averaged $0.16\% \cdot h^{-1}$ in both experiments, which is consistent with the order of magnitude for many vegetables as stated in the literature [4]. Moreover, it agrees with previous results obtained by our laboratory: 0.20 and $0.24\% \cdot h^{-1}$ for experiments on "Rouge de Grenoble" lettuces [10, 11]. The slight differences in the final conversion rates between our previous experiments and this study can be accounted for by the changes in lettuce variety and in some environmental conditions (climatic data, soil nature, etc.).

Figure 2 plots the curves of instantaneous conversion rate as a function of equivalent energy since germination for the spring experiment. This figure shows that experimental and model data produce the same pattern. In both cases an increase in conversion rate appears at the stage of higher growth rate, which means that the importance of OBT integration depends on the biological stage of the plants.

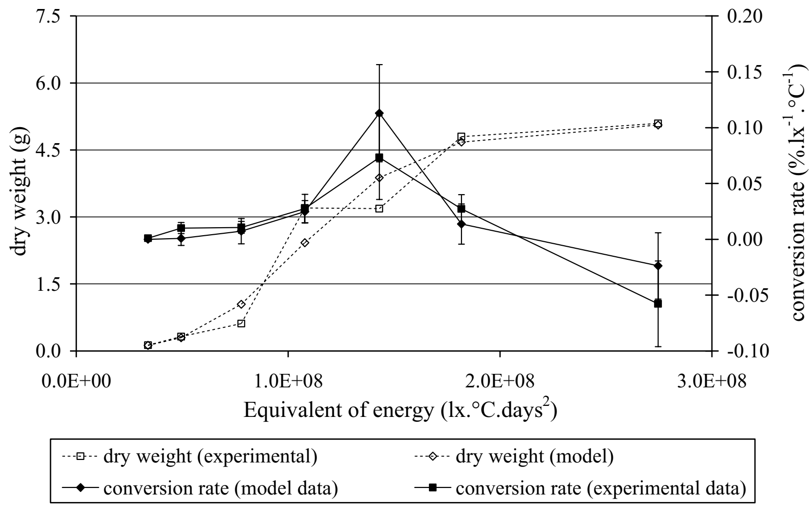

Figure 2. Dry weight (g) and instantaneous conversion rates as a function of received energy equivalent since germination for the spring experiment, for experimental and model data. Uncertainties are given with a 95% confidence level ($k = 2$).

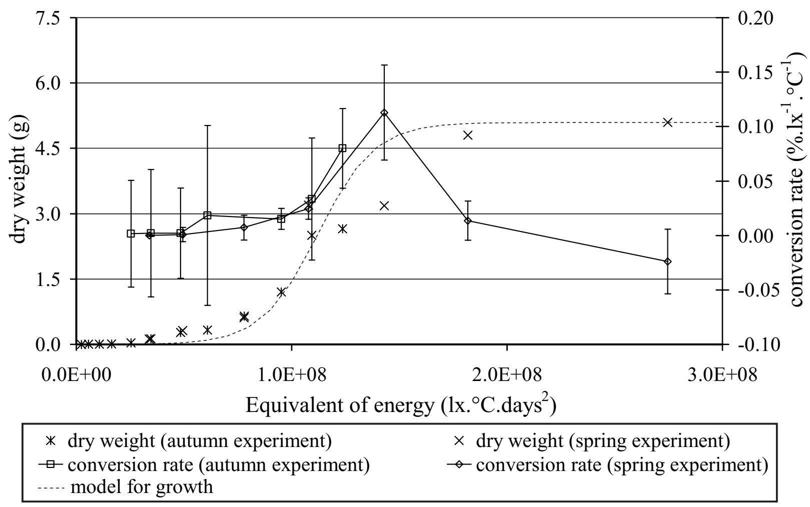

Figure 3. Conversion rates from TFWT to OBT calculated from model growth data and associated computed uncertainties values ($k = 2$).

This result is consistent with experiments conducted by Atarashi-Andoh [4] on young and old leaves of komatsuna and cherry tomato, and with previous work done by our laboratory [11].

The model described earlier was applied to compare the results of the two experiments (Figure 3). For experiments of fairly similar durations (about 1800 h for the spring experiment, and 2150 h for the autumn one), the global energy around the lettuce during the second set of experiments (August to October) was lower than in the first period (April to June) and not enough to reach the exponential growth stage. The conversion rate calculated for the autumn experiment follows the same pattern of continuous increase but without reaching the peak observed in the spring experiment, as shown in Figure 3.

Besides, tritium concentration in air was twice as high in the spring experiment as in the autumn one, yet the values of the conversion rate are the same in both cases. This observation leads us to conclude that the conversion rate from TFWT to OBT does not depend on the level of tritium exposure but primarily depends on the growth stage, and consequently, on the total energy received by the crops. However, the model for energy available to the plants used in our study is quite rough and has to be improved.

4. CONCLUSION

The foliar uptake of tritium in lettuces continuously exposed to atmospheric HT and HTO was experimentally studied from the earlier growth stage to plant senescence. The conversion rate from TFWT to OBT in plants leaves was calculated from weekly measurements and was observed to vary significantly during the lettuces growth. As the biological stage is closely linked to the available energy by the lettuces, the expression of this rate according to meteorological parameters (like temperature and light intensity) seems to be more relevant than as a function of the age of the plants only. Moreover, at this juncture of our research, the conversion rate would appear not to depend on the level of tritium exposure.

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References

[1] S. Diabaté and S. Strack, *Health Physics* **65**, 698–712 (1993).
[2] C.E. Murphy, *Health Physics* **65**, 683–697 (1993).
[3] Y.H. Choi, M.K. Lim, W.Y. Lee, H.G. Park, G.S. Choi, D.K. Keum, H. Lee, S.B. Kim and C.W. Lee, *J. Environ. Radioact.* **84**, 79–94 (2005).
[4] M. Atarashi-Andoh, H. Amano, M. Ichimasa and Y. Ichimasa, *Fusion Technology* **41**, 427–431 (2002).
[5] A. Boivin, S. Amellal, M. Schiavon and M.T. van Genuchten, *Environ. Pollut.* **138**, 92–99 (2005).
[6] F. Jacquin and L. Florentin, in *Atlas des sols de Lorraine*, edited by Presses Universitaires de Nancy, 54–58 (1988).
[7] B. Iooss, F. Van Dorpe and N. Devictor, *Reliab. Eng. Syst. Saf.* **91**, 1241–1251 (2006).
[8] A. Doury, *J. Loss Prev. Proc. Ind.* **1**, 156–163 (1988).
[9] F. Pointurier, N. Baglan, G.C. Alanic and R. Chiappini, *J. Environ. Radioact.* **68**, 171–189 (2003).
[10] L. Vichot, C. Boyer, T. Boissieux, Y. Losset and D. Pierrat, *Fusion Technology,* accepted for publication in special issue. (11/08/2007).
[11] L. Vichot, C. Boyer, T. Boissieux, Y. Losset and D. Pierrat, *J. Environ. Radioact.,* accepted for publication. (05/21/2008).