Variation of conversion rate from tissue free water tritium to organically-bound tritium in lettuces continuously exposed to atmospheric HT and HTO

Cécile Boyer[1,2,3], Laurent Vichot[1], Thomas Boissieux[1], Yvan Losset[1], Christophe Mavon[2], Fabienne Tatin-Froux[3], Michel Fromm[2] and Pierre-Marie Badot[3]

[1] Commissariat à l'Energie Atomique, CVA/DSTA/SPR/LMSE, 21120 Is-sur-Tille, France. [2] Laboratoire de Microanalyses Nucléaires A. Chambaudet, UMR CEA E4, UFR Science et Techniques, 16 route de Gray, 25030 Besançon cedex, France. [3] Laboratoire Chrono-Environnement, UMR 6249, UFC/CNRS usc INRA, Place Leclerc, F-25030 Besançon cedex, France.

INTRODUCTION

Because of its long retention time in living organisms, importance of Organically-Bound Tritium (OBT) has been proved for the tritium monitoring around nuclear facilities. Major processes of tritium transfer between environmental compartments (Diabaté and Strack, 1993) and global parameters for tritium assimilation into plants (Murphy, 1993) have been reported. It is well-known that tritiated water vapour (HTO) easily enters into water pools of plants as Tissue Free Water Tritium (TFWT), and then could be converted into OBT by biological mechanisms, mainly photosynthesis. Nevertheless, a better quantification of OBT integration in regards of tritium exposure and involved metabolic processes is required, especially since very few experimental data are available to support models offered in literature. Most of the time, the accuracy of such models depends on the assessment of a global conversion rate from TFWT into OBT; however, some recent studies point out the variation of this coefficient with the growth rate (Atarashi-Andoh *et al.*, 2002). This study has been carried out to evaluate the uptake of tritium due to continuous atmospheric exposure of lettuces (*Lactuca sativa* L.) and investigate the evolution of the resulting conversion rate (from TFWT to OBT) at the different stages of plant development.

MATERIALS AND METHODS

Plants cultivation and sampling procedures

Experiments were led 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 dedicated to the tests was a plot exposed to the prevailing winds at 360 m from a tritium emitting source.
Soil used for culture was a medium-textured stagnic luvisol came from the experimental farm *La Bouzule* (Lorraine, France), sieved in particles smaller than 5 mm. Properties of this loamy soil have already been described in the literature (Boivin *et al.*, 2005). Professional seeds of leaf lettuce (*Lactuca sativa* L., provided by Rijk Zwaan) were chosen as clones with identical genotype. Plants were studied from seeding to senescence, during 2 series of culture carried out from April to June and from August to October 2007. No fertilizer was used but the pots were maintained at 80% of field capacity during the whole time of experiments.
Germination and earlier stages of plant development were conducted in a controlled-climate chamber. Globally, temperature and relative humidity in the chamber sat in the range [18 – 24°C] and [45 - 95%] respectively. Two solar lamps of 400W provided 12 h of daytime. The resultant average of Photosynthetically Active Radiation above plant canopy amounted to 75 $\mu mol.m^{-2}.s^{-1}$. When the plants had 4 completely developed leaves, they were dibbled in 2.0 L pots of soil and placed outdoor at the dedicated place. To focus on foliar uptake, plants were

protected against precipitations by a greenhouse and by an additional vinyl film on each pot for the autumn experiment. The ambient conditions (temperature, relative humidity and light intensity) in the chamber and outdoor were collected every 2 min by a specific recording device (Kistock KH200, Kimo). Wind direction and speed were recorded every 25 min by a close meteorological station in order to estimate the atmospheric tritium level using the GASCON model. This model established by the CEA (Iooss *et al.*, 2006) is based on released activities and equations of atmospheric dispersion. Atmospheric HT and HTO were weekly monitored by a bubbling system (MARC 7000, SDEC) at the same time.
Every week, from the start of tritium exposure to the plants senescence, about 5 lettuces were chosen in regards of their average size (to be representative of the global population) and sampled for tritium measurement. Roots were removed and all leaves were analysed together.

Analytical methods for tritium monitoring

The classical methodology for the extraction and analysis of each ^{3}H fraction was followed (Pointurier *et al.*, 2003). TFWT was extracted of fresh samples by freeze-drying (Lyolab 3000, HETO) during 48 hours.
Exchangeable OBT (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) during 3 days to insure isotopic exchange.
Non-exchangeable OBT extraction methods involve a combustion apparatus. In our device (Eraly), the dehydrated samples were burned under pure and dry oxygen flow and resultant combustion water was condensed in a cold trap. Background level was determined by analysing tritium in washing water before each assay. Furnace has been washed until this background did not exceed 5 $Bq.L^{-1}$. The yield of combustion was typically about 99%.
Tritium in TFWT fraction and combustion water was measured using ^{3}H liquid scintillation counters Tri-Carb (Tri-Carb 2900 TR and Tri-Carb 2750 TR/LL). Samples were mixed with Packard 'Ultima Gold LLT' cocktail in the proportions of 10 mL of water and 10 mL of cocktail. The detection limit was 5.4 $Bq.L^{-1}$ with a background of 3 cpm and for a counting time of 200 min. A quench correction was applied systematically. Samples activities and uncertainties were calculated as described by Pointurier (Pointurier *et al.,* 2003).

RESULTS AND CONCLUSIONS

Climatic conditions and tritium exposure

The main information about outdoor climatic data is presented in Table 1. Minimum, maximum and averaged values for temperature and relative humidity account for the whole considered periods. Values for light stand for averaged measurements at plant canopy level on daytime. The atmospheric tritium concentration at the place of cultivation varied slightly during the 2 periods of plant cultivation (cf. Table 2). However, measured values are all in the same range and in good agreement with the average calculated by the GASCON model for the corresponding periods.

Table 1. Climatic conditions during the two periods of 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)		3317			1266	

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

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

Growth of the plants

The growth curves (dry weight vs time) show the same pattern of sigmoid development, but final dry weight was higher for the spring experiment than for the autumn one. To get an accurate description of plants growth whatever the period of culture, an exponential model based on time, light and temperature ($lux.°C.days^2$) was used instead of a model only based on time. Measured values and calculated ones are in good agreement for both experiments sets.

Calculation of conversion rate

At first, global ratio ν of integrated OBT concentration (C_{OBT}) to TFWT concentration (C_{TFWT}) have been established from start of exposure to plant harvest, according to equation *(1)* (Atarashi-Andoh *et al.*, 2002) :

$$\frac{dC_{OBT}}{dt} = \nu \times C_{TFWT} \qquad (1)$$

The results averaged 0.16 $\%.h^{-1}$ for both experiments, which is consistent with the order of magnitude given for many vegetables in the literature (Atarashi-Andoh *et al.*, 2002), and with previous results obtained by the laboratory : 0.20 and 0.24 $\%.h^{-1}$ for experiments made on lettuces "Rouge de Grenoble" in 2006 (Vichot *et al.*, 2007). The slight differences of final value may be explained by the changes in lettuces variety and in environmental conditions.

Secondly, we consider instantaneous conversion rates in regards to the plants growth rate. Calculated dry masses from the growth model have been used for data processing. The expression of results according to the received energy since the germination is plotted on Fig.1. According to the spring experiment curve, the period of higher OBT integration is strongly linked to the exponential growth stage. This result was already observed in experiments led in 2006 (Vichot *et al.*, submitted). For the autumn experiment, the global energy received by the plants was very lower than in the spring period for the same duration of the experiment. Plants maturity has not been attained, and consequently, the conversion rate has followed a continuous increase without reaching a peak.

To conclude, the conversion rate from TFWT to OBT depends on the lettuces growth stage, and could be linked to the global energy received by the plants.

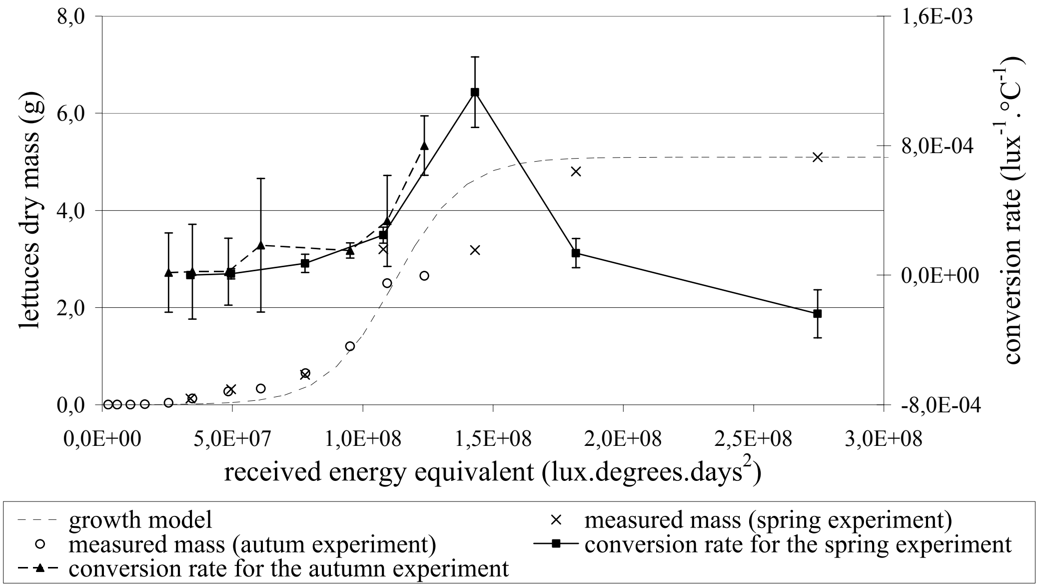

Figure 1. Conversion rates of TFWT to OBT calculated from model growth data and associated computed uncertainties values ($k = 1$).

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