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Tritium concentration in soybean plants exposed to atmospheric HTO during nighttime and daytime

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Abstract Potted soybean plants were exposed to evaporate HTO for 1 h inside an exposure box at two different growth stage (flowering and podding stage, filling stage) during nighttime and daytime. The concentration of tissue-free water tritium (TFWT) and organically bound tritium (OBT) in leaves and beans was measured at the end of the exposure. The results indicated that the quasi-equilibrium between the TFWT concentrations in the soybean leaves and the HTO concentration of the ambient air moisture required more than 1 h to be reached. The relative TFWT concentrations in the nighttime represent about 1/2 to 2/3 of the concentrations obtained in the day in the leaves compared to about 1/2 to 4/3 in the beans. The relative OBT concentrations under night conditions were about 2/5 to 1/2 of those under day conditions in the leaves, contrary to 1/2–7/10 for the beans. By developing tritium concentration assessment model with a short-term release of atmospheric HTO, we comprehensively considered the plant growth stage and the environmental conditions.

Keywords TFWT · OBT · Soybean · Nighttime · Daytime

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1 Introduction

Tritium, a beta-emitting radionuclide, is the only radioactive isotope of hydrogen and can potentially be hazardous to human health. Tritium can enter the body by ingestion of foods containing tritium and can also be drunk, inhaled, and absorbed through the skin if tritiated water (HTO) is present [1–3]. It pre-dominantly exists under the form of water and is widely dispersed in the environment [4–9]. In order to estimate the risks for humans due to environmental tritium, it is necessary to understand the behavior of tritium in different ecological compartments.

Recently, an increased interest of the fate of tritium in vegetation generated a series of dedicated tritium research because photosynthesis is the first necessary step for the production of organic matter [2, 10–13]. The existing studies mainly focus on the routine release and short atmospheric HTO exposure at daytime [4, 11–24]. Little information is currently available regarding the tritium uptake and subsequent formation of organically bound tritium (OBT) in plants exposed to elevated levels of atmospheric tritium during the nighttime [24]. Up to now, some typical tritium concentration assessment models have been developed such as ETMOD code [25], UFOTRI code [26, 27], and TAS code [28, 29]. However, it was founded that these models do not perform well in plants for the nighttime release. So, it is important to understand the tritium behavior of the plant at nighttime.

There are considerable differences in the physiological and biochemical characters for most plants between day and night. In the daytime, atmospheric HTO can enter the plant directly through the stomata and is easily incorporated into the biological organisms as tissue-free water tritium (TFWT) [2]. Some portions of tritium are taken up in the water source of the plant and are incorporated as organic matter as OBT mainly through photosynthesis [2, 10]. In contrast, most plants close their stomata during nighttime, and the uptake of HTO from the atmosphere has been found to slow down [2]. The formation of organic matter in the plant follows different mechanisms at day and at night. In order to accurately estimate the tritium concentration in the plants, it is important to study the uptake of HTO, the behaviors of TFWT and OBT in the plant for short-term release of atmospheric HTO during the night.

Soybean is the major grain foodstuffs in the world. Experiments involving soybean in direct exposure to atmospheric HTO at daytime have been carried out in laboratories by Japanese and Korean researchers [1, 15, 19]. However, experimental studies with soybean at nighttime are very limited to our knowledge. This study was carried out to investigate the difference of HTO uptake and OBT formation in soybean following a short atmospheric HTO release at nighttime and daytime conditions. To this end, potted soybean plants were acutely exposed to HTO vapor for 1 h at two different growth stages (the flowering and podding stage, and the filling stage) during nighttime and daytime, respectively. The concentration of TFWT and OBT in leaves and beans was measured at the end of the exposure. The study provides some experimental data and parameters to improve and develop tritium dose assessment model for acute atmospheric releases.

2 Experimental

2.1 Plants culture

The soybean seeds were sown in plastic pots (30 cm × 30 cm) placed outdoors. The pots were filled with a medium loam soil. A few days before sowing, 5 g of composite fertilizer ($N{:}P_2O_5{:}K_2O = 14{:}17{:}14$) was supplied to each pot and fully mixed with the soil.

A total of 12 pots were prepared for exposure to HTO, the soybean seeds were randomly sown into each pot, and the final number of seedlings was 3. The pots were watered one or two times per week to ensure a wet soil for plant growth. Appropriate amounts of the composite fertilizer were supplied twice during plant growth.

2.2 Exposure to HTO vapor

Potted soybean plants were exposed to evaporated HTO in a transparent Plexiglas box [1.2 m (length) × 1 m (width) × 1.2 m (height)] at two different growth stages (the flowering and podding stage, and the filling stages) at night and the following day. The exposure box was composed of a bottom plate and an upper part. An electric furnace, a fan, and a hygrothermograph were placed on the inside, base of the box. There was a lid on the top of the upper part.

For each exposure, three pots with plants were mounted on the bottom plate and then the upper part was fitted onto the plate. A glass beaker containing 20 mL of HTO solution (7.65×10^3 Bq mL^{-1} in distilled water) was placed into the electric furnace. The exposure experiment was carried out for 1 h exposure in daytime and nighttime (from 9:00 to 10:00 for daytime and from 21:00 to 22:00 for nighttime). All the openings were sealed with silicon rubber. After heating for about 20 min, almost the entire volume of the HTO solution was evaporated. The surface soil of the pots was covered by vinyl paper during the exposure in order to prevent tritium deposition into the soil.

Temperature and relative humidity were recorded every 5 min. The fan was operated at the beginning of the heating to prevent the build-up of an HTO concentration gradient inside box. The lid was opened, and fresh air was blown into the box for 15 min immediately after the end of the exposure duration and directly followed by the removal of the upper part of the box. Following the exposure, the plants were removed from the box and cultivated outdoors as usual.

2.3 Sampling and measurement

The air moisture in the exposure box was collected with dry silica gel. The soybean plants exposed were sampled twice at the end of each exposure and at harvest. At each sampling, three exposed plants were randomly selected from three pots. The samples were immediately divided into leaves and seeds, which were then cut into small pieces. The three samples were pooled together to form a compound sample put into zip-lock bags before storage in a freezer at −20 °C.

The air moisture contained in the silica gel can be obtained through a distillation system, and the amount of water was collected and recorded. The tissue-free water was extracted by a freeze-drying method and trapped using liquid nitrogen for the frozen samples. The plant samples were oven-dried at 60 °C for 2–3 days after being freeze-dried. The dry samples were pulverized with mortar and pestle, and the powdered samples were then combusted using a combustion apparatus. Then, the water generated from the combustion was collected in 20 ml polyethylene vials. The combustion apparatus consisted of a furnace composed of two heating regions and a quartz tube. The sample was held in a quartz boat enclosed in the quartz tube in the first heating region which was movable. The second heating region was fixed, and the quartz tube inside the second heating region contains a catalyst. The temperatures of both regions were independent and adjustable.

The samples pyrolysis combustion performed in the first region, and the combustion gases were oxidized by the catalyst in the second one. The water vapor was trapped in a U-tube immersed in a cold trap. Finally, the OBT concentration could be determined by measuring the tritium concentration in the water generated from the combustion.

All prepared water samples were mixed with the scintillation cocktail (Ultima Gold LLT), and tritium concentration in the water samples was measured by liquid scintillation counter (TRI-CARB 3170TR/SL). The water samples were mixed in the proportion of 5 mL of water and 15 mL of cocktail for TFWT measurement and 1 mL of water and 19 mL of cocktail for OBT measurement. The detection limit was 1.2 Bq L^{-1} with a background of 2–3 cpm and a count efficiency of about 33 %. Twenty-milliliter glass vials (low potassium borosilicate glass) were used in the experiments for tritium measure.

3 Results and discussion

3.1 Conditions in the exposure box during exposures

The meteorological conditions in the exposure box during each exposure are shown in Table 1.

As shown in Table 1, at the end of the exposure, the temperature rose by about 4–5 °C in the nighttime and was about 6 °C higher than at the beginning in the daytime. The temperatures ranged from 30.7 to 35.6 °C in the nighttime and from 31.6 to 39.1 °C in the daytime. The increase in temperature of the box resulted mainly from the heat generated by the electric furnace.

The changes of relative humidity were in the range of about 8–17 % in the night and about 8–11 % in the day. The mean relative humidity of air ranged from 51.4 to 54.4 % during the night and from 56.7 to 58.9 % during the day. At the beginning of the exposure, the relative humidity increased and then gradually decreased about 30 min later.

Table 2 shows the mean HTO concentrations in air moisture inside the exposure box during each exposure. There were some differences in the mean HTO concentration in the air moisture in the exposure box which mainly depended on the HTO evaporation rate, the content of moisture in the air, the degree of air moisture condensation on the inner walls, and the amount of the HTO uptake by plant and the absolute humidity in the box.

3.2 HTO uptake by the soybean leaf

The tissue-free water tritium (TFWT) concentrations in the soybean leaves were measured, and the results are given in Table 2.

The leaf TFWT concentration at equilibrium and steady condition can be described by the following Eq. (1) on the assumption that the soil HTO concentration is zero [12, 13]:

$$C_{\mathrm{TFWT}} = A \times R_{\mathrm{CH}} \times C_{\mathrm{air}}, \tag{1}$$

where C_{TFWT}, the TFWT concentration in the plant leaf, is at equilibrium (Bq L^{-1}); A is the ratio of the specific activity of tritium (T/H) in the water to that in the vapor at equilibrium (1.1); R_{CH} is the relative humidity; C_{air} is the HTO concentration in the air moisture (Bq L^{-1}). In order to calculate C_{TFWT}, an approximation was made by using the mean values for R_{CH} in the Eq. (1).

The percentages of the leaf TFWT concentrations at the end of exposure to the estimated equilibrium concentrations were 50 % for nighttime conditions and 80 % for daytime ones at two different growth stages. The TFWT concentrations at the end of the exposure were considerably lower than the estimated equilibrium concentrations in the nighttime condition.

These results indicate that it usually takes longer than 1 h for the TFWT concentrations in the soybean leaves to reach quasi-equilibrium with the HTO concentration in the ambient air moisture. This phenomenon is in agreement with the experimental results for wheat, Chinese cabbage, and radish. Wheat plants were exposed to HTO vapor for 2 h in two different light conditions of about 120 and 550 W m^{-2} and a relative humidity in the range of 85–90 % during the early to middle phase of the rapid seed growth stage. The results showed that the leaf TFWT concentration reached 75–86 % of the HTO concentration in air moisture at the end of exposure [11]. The potted Chinese cabbage and radish plants were exposed to HTO vapor under semi-outdoor conditions at daytime for 1 h in different meteorological conditions with mean temperatures in

Table 1 Meteorological conditions in the exposure box during plant exposure to HTO vapor at nighttime and daytime

Time	Date	Stage	Temperature (°C)		Relative humidity (%)	
			Range	Mean	Range	Mean
Nighttime	7.21	Flowering and podding	30.7–35.6	33.6	42.3–59.5	54.4
	8.5	Filling	30.8–34.5	32.5	48.3–56.2	51.4
Daytime	7.22	Flowering and podding	31.6–37.9	34.3	52.3–60.7	56.7
	8.6	Filling	33.4–39.1	36.6	50.5–61.3	58.9

Table 2 Average concentrations of HTO in the air moisture inside the operating exposure box and the TFWT concentration in the soybean leaf at the end of the exposure at nighttime and daytime

Time	Stage	Mean HTO concentration in air moisture (Bq L^{-1}) ($\times 10^5$)	Equilibrium concentration of leaf TFWT (Bq L^{-1}) ($\times 10^5$)	Leaf TFWT at the end of exposure (Bq L^{-1}) ($\times 10^5$)	The percent ratios of the TFWT measured to calculated[a] (%)
Nighttime	Flowering and podding	4.12	2.47	1.26 ± 0.03	51
	Filling	9.56	5.40	2.66 ± 0.13	49
Daytime	Flowering and podding	2.47	1.54	1.18 ± 0.04	77
	Filling	7.54	4.89	4.02 ± 0.02	82

[a] The values are given in the percent ratios of the TFWT concentrations to the estimated leaf TFWT concentrations at equilibrium condition

the range of 15.2–34.5 °C, a mean light intensity in the range of 22.9–60.5 klx, and a mean relative humidity in the range of 67.9–89.0 %. The results indicated that the percentages of the leaf TFWT concentration at the end of exposure to the estimated equilibrium concentration were in the range of 9.4–68.0 and 28.9–57.7 % for Chinese cabbage and radish, respectively [13].

Experimental reports on grape leaves, potato, and rice leaves showed that the time to reach quasi-equilibrium was shorter than 1 h. The TFWT concentration in the leaves reached a theoretical plateau level after about 30 min when the grape plants were exposed to HTO vapor under a controlled outdoors conditions during the daytime in June. A field experiment concerning potato and grape plants exposed to HTO vapor in a clear chamber for 4 h during the daytime resulted in the TFWT concentrations in the leaves of both plants reaching an apparent plateau 45 min after the start of the exposure. Potted rice plants were exposed to atmospheric HTO in a box outdoors for 1 h at 9 different times from booting to yellow-ripe stages. Inside the box during each exposure, the means of relative humidity, temperatures, and solar radiations were in the range of 61.4–94.2 %, 25.1–34.8 °C, and 234–678 W m^{-2}, respectively. It is indicated that the leaf TFWT concentration may reach equilibrium within 1 h at clear weather [12].

The discrepancy between these experimental results is mainly due to the difference of physiological characteristics of the leaves, the exposure times, the meteorological conditions during the exposure, and the leaves age during the exposure.

The experimental results show that the absorption of atmospheric HTO by soybean leaves is much slower at night than during the day. This is in agreement with the experimental results obtained by others [1, 11]. It is well known that plant uptake of HTO vapor is closely related to the stomatal movement. The stomata open when stimulated by sunlight in the day and are more or less closed at night. Thus, the plant leaf can easily absorb HTO vapor from the atmosphere under daylight, but uptake is slower in the dark for various plants. The closure of the stomata partly prevents HTO vapor uptake by soybean leaves.

3.3 TFWT concentration in the soybean plant at the end of the exposure in the nighttime condition and in the daytime conditions

The TFWT and OBT concentration in the leaf and beans of soybean at the end of exposure are summarized in Table 3.

To compare the difference of HTO transfer from air to leaves and from leaves to bean between nighttime and daytime, the TFWT concentrations in soybean leaves and beans at the end of the exposure were normalized to the relative values defined as the ratio between TFWT at the end of the exposure and the average HTO concentrations of the air moisture during the exposure as referenced by others [13].

Figure 1a, b shows the relative TFWT concentrations in the soybean leaves and beans at the end of the exposure to HTO vapor in nighttime and daytime at the flowering and podding stage and at the filling stage.

At the flowering and podding stage of the experiment, the relative HTO concentration in leaves and young pods during nighttime conditions was about 0.31 and 0.09, but the relative HTO concentration during daytime conditions was 0.48 in the leaves and 0.06 in the young pod. The relative TFWT concentration at the end of the exposure in soybean leaves under the nighttime conditions was 63.66 % of the daytime conditions. Whereas the relative TFWT concentration in young beans during HTO vapor released at the nighttime was 1.37 times higher than that during the daytime exposure at the end of the exposure. The discrepancy between leaves and beans is probably due to physiological differences. The high relative TFWT concentration observed for young beans during the night at the flowering and podding stage can be attributed to the fact that the soybean was in the early stages of its

Table 3 TFWT and OBT concentrations in the leaf and beans of soybean at the end of the exposure

Stage	Time	Concentration (Bq L^{-1})			
		Leaf		Beans	
		TFWT ($\times 10^5$)	OBT ($\times 10^4$)	TFWT ($\times 10^5$)	OBT ($\times 10^4$)
Flowering and podding	Nighttime	1.26 ± 0.03	1.21 ± 0.008	0.36 ± 0.015	0.94 ± 0.009
	Daytime	2.66 ± 0.13	2.13 ± 0.04	0.16 ± 0.017	1.23 ± 0.03
Filling	Nighttime	1.18 ± 0.04	2.30 ± 0.02	1.30 ± 0.19	1.68 ± 0.01
	Daytime	4.02 ± 0.02	8.58 ± 0.02	1.77 ± 0.27	5.16 ± 0.05

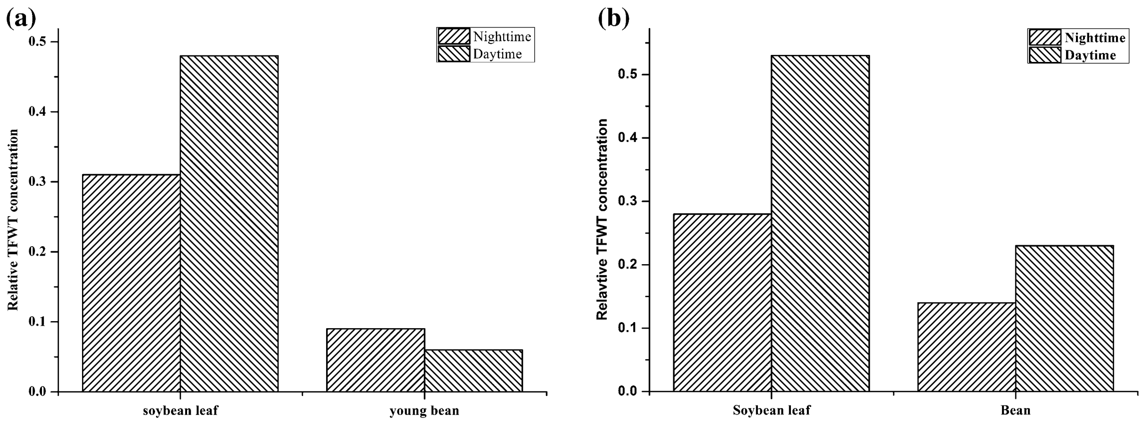

Fig. 1 Relative TFWT in soybean plants at the end of the exposure to HTO vapor under nighttime and daytime at the flowering and podding stage (**a**) and filling stage (**b**)

development. The green bean could have also absorbed the HTO vapor, and the loss of TFWT via moisture exchange and transpiration might have taken place at a much lower rate in the nighttime than during daytime.

The filling stage is a crucial time for the determination of the future soybean yield. As shown in Fig. 1b, in the night condition, a lower HTO vapor uptake and conversion to TFWT in soybean leaves and beans were observed compared to the day condition. The relative TFWT concentration under nighttime conditions is 0.28 in leaves, 0.14 in beans, 0.53 in leaves, and 0.23 in beans under daytime conditions. The relative TFWT concentration in leaves in dark conditions represented 52.3 % of that under light conditions and was of 57.8 % in beans. The percent ratio of the relative TFWT concentration in leaves between night and day at the end of exposure was not as large as that for the beans. This is likely because at the filling stage the soybean pods gradually become drier and the ability to uptake HTO from the air is reduced. Therefore, the HTO was mainly absorbed by leaves during the night and then transferred to the pods. Simultaneously, the water content was decreased and dry matter was increased in the beans.

The results indicate that during nighttime conditions, the ability of foliage to uptake HTO from the ambient environment during the flowering and podding stage was greater than that at the filling stage. This may be attributed to the leaves' strength during the early–middle stage of growth. In this study, the amount of HTO uptake in the leaves during the nighttime conditions is higher than that reported in experiments carried out with D_2O releases in soybean and in experimental data obtained by wheat [1, 11, 19]. This phenomenon can be explained by the difference in the growth stage exposed, the environmental conditions, and the crop characteristics and so on.

3.4 OBT concentration in soybean plants at the end of the exposure during the nighttime and daytime conditions

In order to compare the difference of OBT formation in soybean leaves and beans at the end of the exposure for the nighttime conditions and daytime conditions, the OBT concentrations at the end of the exposure in soybean leaves and seeds were normalized in order to obtain relative values. These values were calculated by relating the OBT concentrations in plant in each of the plant's organs to the leaves TFWT concentrations at the end of the exposure. This is based on the knowledge that the plant's OBT is mainly produced in leaves and then translocated to other organs.

The relative OBT concentrations in leaves and beans of the soybean plant after its exposure to atmospheric HTO at the flowering and podding stage and at the filling stage are given in Fig. 2a, b.

During the flowering and podding stage, the relative OBT concentration at the end of the exposure was 0.096 at nighttime and 0.18 at daytime in the leaves, whereas in beans, the obtained values were 0.075 and 0.1, respectively. At the filling stages, the relative OBT concentrations under night conditions were 0.087 in leaves and 0.063 in beans at the end of the exposure; under day conditions, the values were 0.21 and 0.13, respectively.

At the end of exposure, the relative OBT concentration levels in soybean leaves and beans are higher than those in rice leaves (0.7–1.2 %), and wheat leaves (1–1.2 %) during the day. However, these values are comparable to 5–25 % in various crop plants from the experiments conducted by Guenot, Belot, and Indeka [12]. Under night conditions, the relative OBT concentrations observed in leaves and beans are higher than that observed in white leaves and grains. This discrepancy may be attributed to the difference of the time of exposure, the development stage, and the plant species.

As shown in Fig. 2, whether exposure experiments are carried out at the flowering and podding stage or at the filling stage, the amount of OBT formation in soybean leaves and beans in the nighttime conditions was less than that in the daytime conditions. During the flowering and podding stage, the relative OBT concentration in leaves at the end of the exposure during the night represented 1/2 of the daytime's value compared to the value in beans which represented 7/10 of the daytime values. During the filling stage, the corresponding values were 2/5 and 1/2, respectively. These values are slightly higher than those found in the experiments with wheat and rice [1, 11, 19].

Table 4 gives the ratios of OBT concentrations to TFWT concentrations in the soybean plants at the end of exposure.

The TFWT concentration was one to eleven times higher than the OBT concentration depending on the time of experiments and the plant part. The OBT-to-TFWT ratio in the leaf and bean for the daytime conditions was about 2–3 times higher than that for the nighttime conditions. In case of soybean plants acute exposure to HTO vapor, if the beans were immediately ingested at the end of exposure, most of the ingestion dose would be generally given by TFWT because of the low OBT-to-TFWT ratios in spite of the high dry matters contents in the beans. The values obtained from our experiment are much higher than the published ratios in the leaves of the mature cabbage plants exposed to HTO vapor for 1 h and were considerably

Table 4 The ratios between OBT and TFWT in the soybean plants at the end of the exposure

Stage	Time	Ratio of OBT-to-TFWT	
		Leaf	Beans
Flowering and podding	Nighttime	0.1 ± 0.001	0.26 ± 0.012
	Daytime	0.18 ± 0.007	0.79 ± 0.071
Filling	Nighttime	0.09 ± 0.005	0.13 ± 0.001
	Daytime	0.21 ± 0.001	0.29 ± 0.004

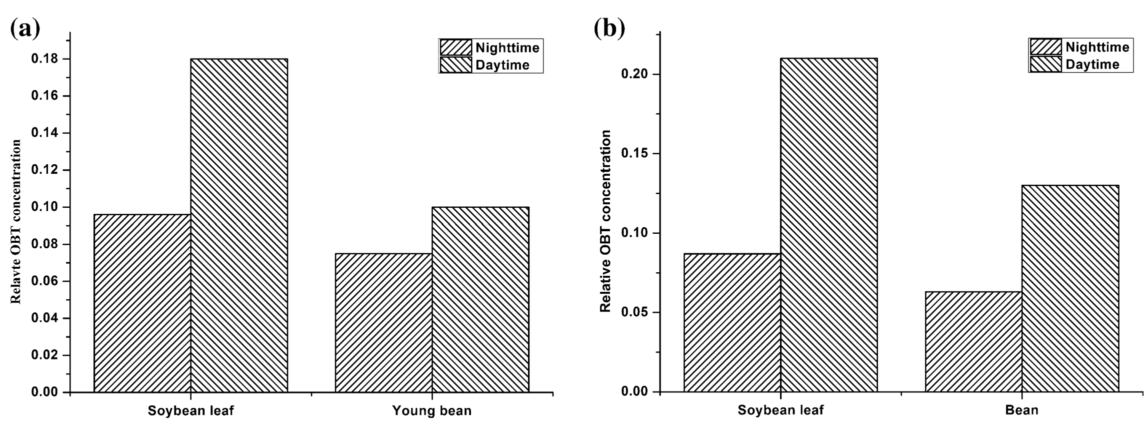

Fig. 2 Relative OBT in soybean plants at the end of the exposure to HTO vapor under nighttime and daytime at the flowering and podding stage (**a**) and at the filling stage (**b**)

different from the result following long-term exposure [21].

4 Conclusion

The foliar direct uptake of atmospheric HTO, incorporation from atmospheric HTO into tissue water, and OBT by soybean plants under nighttime and daytime conditions were experimentally simulated by a short-term release of airborne HTO during the flowering and podding stage and during the filling stage.

The experimental results show that quasi-equilibrium between the TFWT concentrations in the soybean leaves and the HTO concentration from the ambient air moisture at nighttime and daytime required longer than 1 h. In the night experiments, a lower TFWT uptake in leaves and beans was found compared to the experiments in the day.

Under nighttime conditions, the transformation of leaves and beans into TFWT was lower than that under daylight conditions, except for the experimental results of beans carried out during the flowering and podding stage. The quantity of OBT produced in leaves and beans during the night is less than that produced during the day, which is mostly due to the lower uptake of HTO during in the night. Nevertheless, the difference between nighttime conditions and daytime conditions was minimal. Consequently, under nighttime conditions, the TFWT and OBT concentrations in plants at the end of the exposure cannot be ignored. The TFWT and OBT concentrations in the leaves and beans at the end of the exposure were highly dependent on the meteorological conditions and on the growth stage during the exposure to HTO vapors.

In establishing tritium concentration assessment model for a short-term release of atmospheric HTO, the plant growth stage, the environmental conditions, and the crop characteristics should be comprehensively considered. In addition, the TFWT and OBT formation at night cannot be avoided in the safety assessment. The understanding of the night process must be depended in order to develop tritium concentration assessment models.

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