§10. Environmental Behavior of Tritium and Biological Effects and Assesment of Tritium

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The main results of this program were as follows.

(1) Effect of low dose tritium on human leukemic cell line Molt-4 DNA and Burkitt lymphoma DNA estimated by comet assay

This paper deals with low dose effect of HTO on human leukemic cell line Molt-4 and Burkitt lymphoma DNA (in vitro irradiation) estimated by the comet assay. The leukemic cell line Molt-4 and Burkitt lymphoma embedded in agarose gel just after mixed with HTO. After leukemic cell line Molt-4 and Burkitt lymphoma were exposed to 0.93-4.58mGy/h of HTO, the agarose gel slides were washed to remove HTO and cell lysis treatment on the slides was conducted before electrophoresis. The individual comets on stained slides after electrophoresis were analyzed using imaging software. No significant DNA damages were observed. (Y. Ichimasa, K. Otsuka, S. Maruyama, K. Watabe, H. Tauthi, T. Uda and M. Ichimasa)

(2) Heavy water release experiments for tritium dose assessment purpose: Translocation of deuterium into orange (*Citrus unshiu* Marc.)

Heavy water (D_2O) release experiments were carried out in a greenhouse to determine uptake and loss kinetics of D_2O in leaves of Japanese mandarin orange (*Citrus unshiu* Marc.) and formation, translocation and retention of organically bound deuterium (OBD) in orange fruit in daytime and nighttime release experiments held at different growth process. Potted oranges were exposed D_2O vapor in the greenhouse for 8 h in the daytime or nighttime. The same kinds of parameters were obtained in D_2O supply experiments in soil of orange pots to estimate the contribution of contaminated soil. Although translocation rates of soil water D_2O to leaves were less than one-third those of atmospheric D_2O to leaves, those of leave D_2O derived from soil D_2O to OBD in orange fruit at harvest were more than those of leave D_2O derived from atmospheric D_2O to OBD in orange fruit at harvest. (Y. Ichimasa, H. Makihara, H. Tauchi and M. Ichimasa)

(3)Subcellular distribution of organically bound tritium in the rat liver after ingestion of tritiated water and some tritiated organic compounds

The experiments of single ingestion of tritiated water and several tritiated organic compounds showed that the subcellular distribution of OBT was dependent on the chemical form of the ingested tritium and on the time after the ingestion. (H. Takeda, K. Miyamoto and S. Fuma)

(4) Mutation induction by low dose rate tritium radiation: Development and application of a novel hypersensitive detection system

Genetic effect of low dose rate tritium radiation is a critical issue to assess the safety of a nuclear fusion reactor. Because a sensitive biological detector was necessary to test the low dose rate effects using cultured cells, we developed a novel hyper-sensitive detection system to detect Hprt-deficient mutations using Hprt deficient hamster fibroblast cells which carry a normal human X-chromosome. The system has been found to be 100-fold more sensitive for detecting mutations than the conventional system which uses an internal *Hprt* gene. Using the system, we first tested the mutation frequency induced by 1 Gy of tritium radiation at different dose rates (10 - 1.8 cGy/h), and observed a slight increase of mutation frequency when the dose rate was reduced to 1.8 cGy/h.

Then we reduced the total dose down to 0.3 Gy. No significant differences in mutation frequencies were observed within the range of dose rates used, suggesting that if a reverse dose-rate effect exists, it may not be observable with tritium radiation at dose rates between 80 cGy to 0.18 cGy/h. Interestingly, molecular analysis of the *Hprt* locus in Hprt-deficient mutants induced by tritium showed that deletion sizes observed in the hamster cell's human X-chromosome under these conditions are much smaller in cells exposed at less than 1.8 cGy/h than in cells exposed at 80 cGy/h. This phenomenon seems to be specific for tritium radiation because it was not apparent after exposure to γ-rays. Our novel hypersensitive detection system used here is useful for analysis of the mutagenic effects of low doses of tritium radiation delivered at low dose rates. (H. Tauchi and K. Komatsu)