COMPARISONS OF DIOXIN LEVELS OF CUCUMBER VARIETIES AND CULTIVATION PERIODS

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Introduction

According to a 2001 survey on Daily Intake in Japan[1], the total dioxins intake of a person in Japan amounts to 1.68 pg-TEQ per kg of body weight/day on average. The sharing a percentage of agricultural crops was about 3% in Japanese TDI amounts. However, all cases collect samples from supermarkets to estimate TDI of dioxins, which make it difficult to understand under what environment, food samples were pretreated and cultivated. Especially, TDI values in agricultural crops are probably dependent on the collection situation.

In the agricultural environment, some reports indicate that dioxins have accumulated in paddy and upland in Japan due to the past use of some types of agricultural chemicals, which contained dioxins as impurities [2,3,4]. Furthermore, taking the atmospheric pathway, for example, dioxins in the ambient air are associated with particulate matter and fall to the ground, contaminating agricultural land. Thus, it has been emphasized that dioxin compounds have a possibility to have adverse effect on vegetables. However, there is little understanding related to this fact in the country and worldwide.

Empirical studies conducted in Japan and abroad have reported low residual concentrations of dioxins in cucumbers *(Cucumis sativus L.)*, a member of the gourd family. However, there have been extremely few instances of research involving overall comparisons of cultivation environments.

Thus, we studied the persistence of dioxins in cucumbers of different varieties cultivated outdoors over varying cultivation periods, conducting thorough experiments to elucidate the actual conditions and mechanisms of dioxin contamination of cucumbers.

Methods and Materials

Method of Cultivation

The experiment was conducted in the fields of the Saitama Prefecture Agriculture and Forestry Research Center Horticultural Laboratory (Kuki). The soil used for the experiment consisted of fine-textured brown lowland soil (with a dioxin concentration of 10 pg-TEQ/g). We planted three types of "Sharp 1" cultivar seedlings: those grafted onto pumpkin rootstocks ("Ultra" blooming rootstock and "Hikari Power" bloomless rootstock) and ungrafted seedlings. Cultivation involved planting out the seedlings into 15-cm rows covered with silver-and-black plastic mulch. We planted on June 7, with seedlings set in rows 150 cm in width the seedlings in each row being spaced 50 cm apart. We fertilized the entire bed surface on June 5 with chemical fertilizer (NPK: 8-8-8) at a rate of 0.5 kg/ha. We pruned the plants on July 2, pinching the young vines at the second node so that two leaves and one fruit remained. For pest and disease control, we sprayed the plants with DDVP (for aphids) on June 13, Sumithion emulsion (for aphids) and Daconil 1000 flowable (for powdery mildew) on June 22, and DDVP and Yuparen (for powdery mildew) on June 27. We began harvesting fruits on July 5, with the last harvest on August 21. We analyzed 2 portions of the samples, the fruits and the stems and leaves. We harvested the fruit samples from July 11 to August 20, harvesting at approximately 10-day intervals starting July 11. We collected the leaves and stems on August 21.

Analytical procedures

Analysis of the cucumbers was based on the "Provisional Guidelines for Measurement of Dioxins and Coplanar PCBs in Food Products" (Ministry of Health and Welfare, Japan), analysis of the air was based on the "Toxic Air Pollution Substances Measurement Manual" (Ministry of Environment, Japan), and analysis of the soil was based on the "Manual for Soil Study with Regard to Dioxins" (Ministry of Environment, Japan). After treatment with concentrated sulfuric acid, the crude extracts were cleaned up using a multiplayer silica column (silver nitrate/acid/base-modified silica gel) and activated carbon column chromatography. All samples were spiked with 17 kinds of 13C-labelled analogues of 2,3,7,8-Cl-substituted congeners and 12 kinds of 13C-labelled mono/non-ortho-PCBs (IUPAC no. #123, 118, 114, 105, 167, 156, 157, 189 and #81, 77, 126, 169). Values below minimum detectable levels were calculated as zero.

HRGC-HRMS

Detection of PCDD/Fs were determined by of HRGC (6890 plus, Hewlett Packard, US) with a DB 5MS column (J&W Scientific, US) and an SP 2331 column (Supelco, INC., US), coupled to a HRMS (AutoSpec-Ultima, Micromass, UK) operation on a resolution of 10,000 using a positive electron ionization source and operating in the selected ion monitoring (SIM) mode. Verification of resolution in the working mass range was obtained by measuring perfluorokerosene (PFK) reference peaks. The current trap was 500 µA and the ionization energy was 30 eV. Ion source and injector temperatures were 260 °C. The samples were injected in the splitless mode.

Results and Discussion

We examined the persistence of dioxins in different cucumber cultivars grown in open fields during summer, a season in which cucumbers show vigorous growth. We used three types of cucumber plants: ungrafted seedlings of the "Sharp 1" cultivar, and "Sharp 1" seedlings grafted to two types of pumpkin rootstock ("Ultra" and "Hikari Power").

The compositional distribution of dioxins in the air where the outdoor cucumber experiment was conducted (Fig.1 (a)) shows relatively high concentrations of Co-PCBs and tetrachlorinated dioxins, with a total dioxin concentration in the air of 15 pg/m^3 (0.09 pg-TEQ/m^3). While each type of cucumber was grown under basically similar environmental conditions, the dioxin concentration was 15 pg-TEQ/g dry for the grafted cucumbers (on Ultra rootstock), 13 pg-TEQ/g dry for the grafted cucumbers (on Hikari Power rootstock), and 10 pg-TEQ/g dry for the ungrafted cucumbers (Fig.1 (b)). In particular, the compositional distributions of dioxins in the soils in which each type of cucumber was grown were extremely similar, with octachlorinated dioxin accounting for more than half of the total dioxin concentration. We harvested the fruits of the cucumbers grown is this environment at 10-day intervals and measured the amounts of residual dioxins in the fruits.

Figures 2 show the dioxin concentrations (as TEQ) in the fruits of cucumbers grown using the different rootstocks. Compared to the cucumbers grown using the Ultra and Hikari Power pumpkin rootstocks, the fruits from the ungrafted cucumber plants had a lower dioxin concentration. Comparing the time of harvest, concentrations in the ungrafted cucumbers and cucumbers grown on the Hikari Power rootstock were highest at the second harvest, after which the concentrations declined. In contrast, the dioxin concentration in cucumbers grown on the Ultra rootstock gradually increased from the first harvest. However, it is difficult to interpret any of these ranges in concentration as exhibiting any significant differences, and we could not observe any influence on dioxin concentration on the basis of different rootstock. The relationship between the weather conditions (average temperature, daily rainfall, and hours of sunlight) constituting the cultivation environment and cucumber were investigated. Even when the obtained results are compared with the concentration distributions for each type of outdoor-cultivated cucumber, we could not discover any clear correlation between the time of harvest for each of the cucumbers and their respective dioxin concentrations. Additionally, the dioxin concentrations in the stems and leaves collected on August 21 was 14.3 pg/g wet (0.059 pg-TEQ/g wet) for the ungrafted plants, 28.68 pg/g wet (0.05 pg-TEQ/g wet) for the plants grafted onto Hikari Power rootstock, and 52.24 pg/g wet (0.073 pg-TEQ/g wet) for the plants grafted onto Ultra rootstock, and while there were variations in the concentrations measured, there were no large differences after calculating TEQ. We found no differences in concentrations or compositions of dioxins in cucumbers grown outdoors in open fields significant enough to suggest that these differences were due to the variety of cucumber or the time of cultivation. In other words, we found the changes in residual dioxin concentrations according to the variety and time of harvest to be extremely minor.

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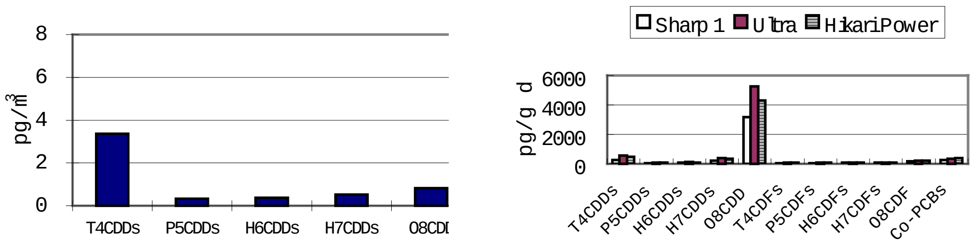

a) air (b) soils

Fig. 1. Homologue profiles of dioxins in cultivation environmental air (a) and cucumber cultivation soils (b).

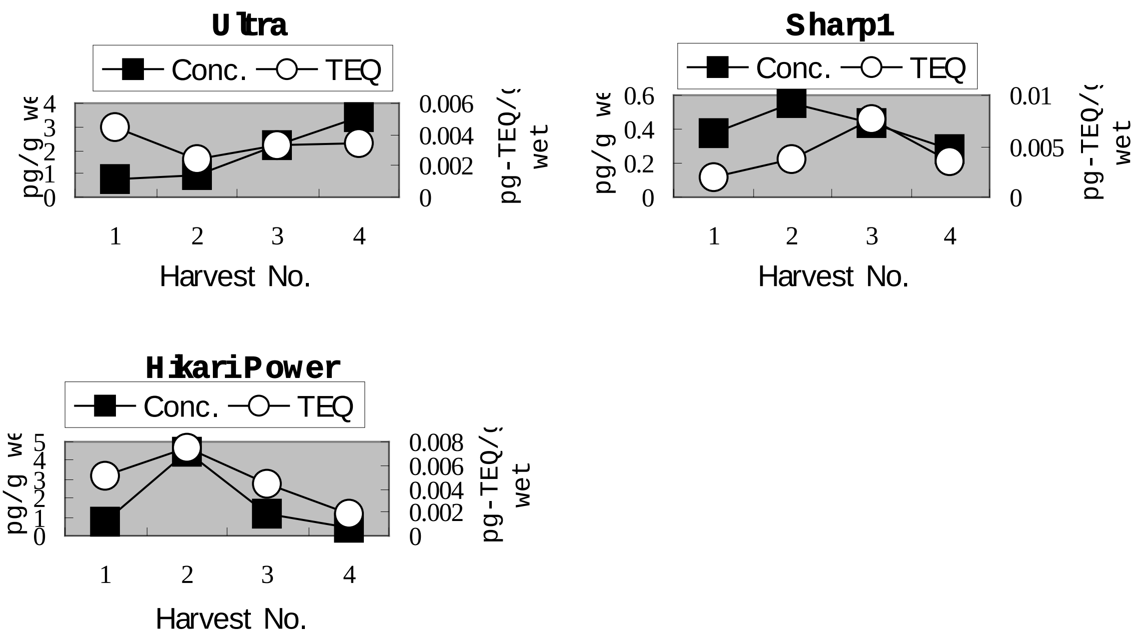

Fig. 2. Comparing the time of harvest, dioxins concentrations (as TEQ) in cucumbers