## POLYCHLORINATED DIBENZO-P-DIOXINS, DIBENZOFURANS, AND COPLANAR PCBS IN SILAGE CORN GROWN IN A FULL-SCALE PRODUCTION FIELD-CHANGES IN CONCENTRATION DURING GROWTH STAGE, CHANGES OVER THREE YEARS, AND CHANGES DURING SILAGE FERMENTATION

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### Introduction

There is growing concern over food prepared from farm animals contaminated with polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated-dibenzofurans (PCDFs), and coplanar PCBs (Co-PCBs) (these chemical groups are collectively called dioxins in this paper). In 1998, it was reported that 90 % of the total intake of dioxins by humans is through food, of which 30 % originates from farm animals<sup>1</sup>). In addition, in 2001, the Japanese Ministry of Health, Labour and Welfare announced that, of dioxins ingested with food, about 27 % comes from food produced from farm animals<sup>2</sup>). To reduce the intake of these chemicals contaminating human food, we must minimize the original contamination in animal feed.

Corn silage, made by an anaerobic fermentation process, is now the most common preserved cattle feed in many countries, including Japan. However, we have poor knowledge of the dioxin contamination of corn grown in open fields. Moreover, the concentrations of chemicals that evolve in the silage during the fermentation process have been unclear. It has been reported that dioxins contaminate corn via the atmosphere<sup>3</sup>). Uegaki et al. reported<sup>4</sup>) that dioxin concentrations and homologue profiles detected in corn leaves were different from the dioxin concentrations and homologue profiles of soils.

In the present study, we examined the concentrations of dioxins in corn grown in open fields and the changes in dioxin concentrations during silage fermentation.

#### **Materials and Methods**

Experiment 1: The 'Dea' variety of silage corn (Pioneer Hi-Bred Japan Co. Ltd, Tokyo, Japan) was used in this experiment. Corn seed was sown on May 15, 2001 in an experimental field in Ibaraki, Japan. The corn was harvested 13 times: on June 1, June 12, June 19, June 26, July 3, July 10, July 17, July 24, July 31, August 7, August 14, August 21, and August 28. The leaf and stem parts of the plants were chopped and mixed thoroughly and then subjected to dioxin analysis.

Experiment 2: The 'Nasuhomare' variety of silage corn (bred by the National Institute of Livestock and Grassland Science) was grown in an experimental field in northern Kanto, Japan, in 1999, 2000, and 2001, and harvested at the yellow-ripe stage (August). The whole plants were chopped and mixed thoroughly and then subjected to dioxin analysis.

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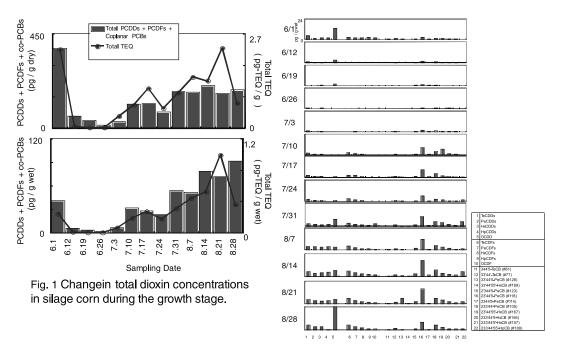


Fig. 2 Concentrations of each homologue in silage corn during the growth stage.

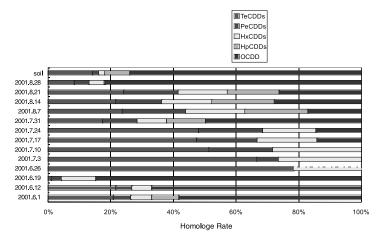


Fig.3 Proportions of PCDD homologues detected in each sample.

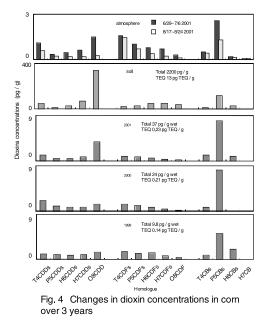
Experiment 3: This experiment tested the relationship between silage fermentation and dioxin concentrations. (1) Silage corn (Nasuhomare) was harvested at the yellow-ripe stage and used to make silage. Approximately 100-g portions of forage material were chopped into about 10-mm lengths and packed into plastic film bags (Asahikasei, Hiryu KN type, Tokyo, Japan) that were then vacuum sealed. These bags were kept at 25 °C, and they were opened after 1 year of fermentation for analysis of dioxin

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concentrations. (2) Two varieties of silage corn, 'Nasuhomare' and 'Snow Dent SH3980' (Snow Brand Seed Co. Ltd. ), were used for silage material. The corn was harvested by harvester at the yellow-ripe stage, chopped into about 20-mm lengths, and packed into a sealed drum.

Dioxin Analysis (Experiment 1): Analysis of dioxins was done as follows: Plant samples (100 g) were crushed with dry ice, added to hexane/acetone (150 mL/150 mL), and filtered through a glass fiber filter. The filtrate was treated with sulfuric acid, and the organic layer was then evaporated, purified, and separated. Purification and separation were carried out using silica gel and activated-carbon column chromatography. All samples were analyzed by HRGC/HRMS (HP6890 / VG Autospec ULTIMA, Micromass Ltd., Manchester, UK.) equipped with an SP-2331 column (Supelco) and a DB-5 capillary column (J & W Scientific).

Dioxin Analysis (Experiments 2 and 3): We commissioned the Japan Food Research Laboratory (JFRL), Tokyo, Japan, to analyze the concentrations of dioxins.



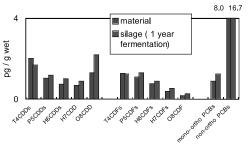
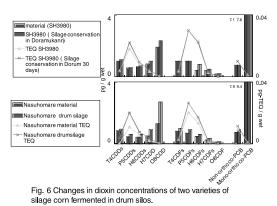


Fig. 5 Changes in dioxin concentrations in long-term fermentation silage. (Stored for 1 year.)



#### **Results and discussion**

Experiment 1: The results are shown in Fig.1. The lower chart shows wet concentrations, and the upper chart shows dry concentrations. The concentrations of each homologue are shown in Fig.2, and the proportions of PCDD homologues are shown Fig.3. The corn was sown on May 15, heading stage was around July 29, and yellow-ripe stage was around July 31. High concentrations of dioxins were

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detected in the June 1 sample in the early growth stage. We thought that this was a result of the soil stuck, because the dioxin homologue profile was almost the same as that of the soil (Fig. 3). After that, while corn was in the vegetative growth stage dioxin concentrations decreased, mainly as a result of dilution due to the increased biomass of the plant. A dramatic increase in dioxin concentrations was observed from early July. This is thought to be because biomass did not increase further as corn went from vegetative growth stage into reproductive growth. In addition, the Kanto area in Japan was very hot during July 2001; we think that this also influenced dioxin concentrations. We will have to explain a Relationship dioxins concentrations and biomass, concentrations and temperature. Around July 31, the corn reached the yellow-ripe stage—the best time for use as livestock feed. After this time, the total dioxin concentrations did not greatly change; however, the toxicity equivalence quotients (TEQ) increased. It is possible to think that harvesting at the most suitable time is important to control dioxin concentrations. The August 28 plant samples were almost completely dried up, and the dioxin isomers detected in this sample were similar to those of the soil (Fig.3).

Experiment 2: This experiment was carried out over 3 years in the same experimental field located in a dairying area in northern Kanto. Therefore, the meaning of the result of the experiment (Fig.4) is important. Total dioxin concentrations tended to increase—octachloro-dioxins were the isomers most responsible for this increase. On the other hand, PCDFs decreased.

Experiment 3: (1) This experiment tested whether long-term silage preservation influenced dioxin concentrations (Fig. 5). Silage fermentation was good, and there were no fungi. Although some changes were seen, the dioxin concentrations and homologues were almost all similar. (2) This experiment was done with two varieties of silage corn (Nasuhomare and Snow Dent SH3980) preserved in drum silage. Drum silage is similar to that made in an actual silo. Both varieties of corn silage showed good fermentation—the V-score was > 95 points. Both varieties of corn silage showed very similar results (Fig. 6). From these results, normal silage fermentation does not influence dioxin concentrations or homologues in silage corn. These results are similar to the results reported  $2001^{4}$ .

### References

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